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Non-conventional Risk and Prognostic Factors in Coronary Heart Disease

Studies on Heart Rate Variability, Alcohol Consumption, Inflammation and Depression

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To my parents

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PUBLICATIONS

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

I Janszky I, Ericson M, Blom M, Georgiades A, Magnusson J-O, Alinaghizadeh H, Ahnve S. Wine drinking is associated with increased heart rate variability in women with coronary heart disease. Heart 2005;91:314-8.

II Janszky I, Ericson M, Lekander M, Blom M, Buhlin K, Georgiades A, Ahnve S. Inflammatory markers and heart rate variability in women with coronary heart disease. J Intern Med 2004;256:421-8.

III Janszky I, Lekander M, Blom M, Georgiades A, Ahnve S. Self-rated health and vital exhaustion, but not depression is related to inflammation in women with coronary heart disease. Brain, Behavior and Immunity. (in press)

IV Janszky I, Mukamal KJ, Orth-Gomér K, Romelsjö A, Schenck-Gustafsson K, Svane B, Kirkeeide RL, Mittleman MA. Alcohol consumption and coronary atherosclerosis progression – the Stockholm Female Coronary Risk Angiographic Study. Atherosclerosis 2004;176:311-9.

V Janszky I, Ericson M, Mittleman MA, Wamala S, Al-Khalili F, Schenck-Gustafsson K, Orth-Gomér K. Heart rate variability in long-term risk assessment in middle-aged women with coronary heart disease: The Stockholm Female Coronary Risk Study. J Intern Med 2004;255:13-21.

VI Janszky I, Ahlbom A, Hallqvist J, Ahnve S. Severe depression is associated with an increased risk for myocardial infarction, not explained by lifestyle, lipids, coagulation and inflammation – the SHEEP study. (submitted)

ABSTRACT

Background and aims. Although, there has been a drop in age-specific coronary heart disease (CHD) incidence, and its prognosis has improved considerably in most parts of the industrialized world, CHD is still by far the leading cause of death in industrialized countries. The established, conventional risk factors, i.e. hypertension, hypercholesterolemia, smoking, diabetes mellitus, obesity, and physical inactivity, are only partly responsible for the development of CHD. Recently, many relatively novel risk and prognostic factors had been proposed. Heart rate variability (HRV), alcohol consumption, inflammation and depression are among the most important novel factors. In this thesis we investigated their interrelations and their relation to CHD.

Methods. We used the corresponding data of three large population based studies, that of the Healthier Female Heart (HFH) Study, the Stockholm Female Coronary Risk (FemCorRisk) Study, and the Stockholm Heart Epidemiology Program (SHEEP). Cross-sectional relationships between the non-conventional risk factors were investigated in the HFH study (paper I-III). The HFH study included consecutive women patients who were hospitalized for acute myocardial infarction, and/or underwent percutaneous transluminal coronary angioplasty or coronary artery bypass grafting. We examined these patients in a stable phase, one year and five months after their index event. Ambulatory 24-hour ECG recordings were analyzed, and HRV was calculated. Self-reported consumption of individual alcoholic beverage types was assessed using a standardized questionnaire. Circulating levels of inflammatory markers were determined. Depression, vital exhaustion, and self-rated health were assessed by questionnaires. We examined the association between alcohol consumption and progression of coronary artery atherosclerosis (paper IV) using serial quantitative coronary angiography (QCA) in the FemCorRisk Study, which included middle-aged women patients who were hospitalized with acute myocardial infarction or unstable angina pectoris. We also assessed the long-term prognostic importance of HRV on mortality in these women (paper V), i.e. in a patient population, which was largely neglected in previous research. We examined if depression increases the risk for first myocardial infarction in the case-control SHEEP study. Depression was defined as history of hospitalization for the clinical diagnosis based on the data of the computerized Swedish hospital discharge registry (paper VI).

Results and Conclusions. *HRV*. We found that wine intake was associated with increased HRV independently of potential confounding factors and intake of other beverages in women with CHD. In contrast, consumption of beer, spirits or the total amount of alcohol did not relate to any of the HRV parameters (paper I). Concentration of IL-6 showed an inverse relation to HRV even after adjustment for potential confounding factors (paper II). HRV parameters predicted all-cause and cardiovascular mortality in a 9-year follow-up even after controlling for established prognostic factors (paper V). *Alcohol consumption*. Our finding that wine intake is associated with HRV suggests that HRV may be an important linking factor between CHD and wine drinking (paper I). We also demonstrated that moderate alcohol consumption is inversely associated with progression of coronary atherosclerosis regardless of the beverage type (paper IV). *Inflammation*. The inverse association between HRV and IL-6 suggests that increased inflammatory activity might represent a new auxiliary mechanism linking autonomic dysfunction, as reflected by decreased HRV, to poor prognosis in CHD (paper II). Our results do not suggest that inflammation is a major mediator between depression and CHD (paper III, VI). However, self rated health and vital exhaustion, constructs also referring to one's subjective well-being, showed an inverse relation to circulating levels of inflammatory markers (paper III). *Depression*. In the SHEEP study we found that hospitalization for depression, especially if repeated, was a considerable risk factor for AMI, and was also associated with poor short-term prognosis after the coronary event. Socio-economic position, lifestyle factors, lipid profile, coagulation, inflammatory and other factors could only partly explain our findings (paper VI).

LIST OF ABBREVIATIONS

ADH3	Alcohol dehydrogenase type 3
AMI	Acute myocardial infarction
AP	Angina pectoris
ApoA	Apolipoprotein A
ApoB	Apolipoprotein B
BDI	Beck depression inventory
BMI	Body mass index
BRS	Baroreflex sensitivity
BPV	Blood pressure variability
CABG	Coronary artery by-pass grafting
CAD	Coronary artery disease
CHD	Coronary heart disease
CI	Confidence interval
CRP	C-reactive protein
CVR	Cardiovascular reactivity
ECG	Electrocardiogram
FemCorRisk	Stockholm Female Coronary Risk Study
HDL-C	High-density-lipoprotein cholesterol
HF	High frequency power
HFH	Healthier Female Heart Study
HR	Hazard ratio
HRT	Heart rate turbulence
HRV	Heart rate variability
HRT	Hormone replacement therapy
ICD	International Classification of Diseases
IL-1ra	Interleukin-1 receptor antagonist
IL-6	Interleukin-6
IMT	Intima-media thickness
LDL-C	Low-density-lipoprotein cholesterol
LF	Low frequency power
OR	Odds ratio
MMPI	Minnesota multiphasic personality inventory
PTCA	Percutaneous transluminal coronary angioplasty
QCA	Quantitative coronary angiography
r-MSSD	Square root of the mean of the squared differences of RR intervals
RR	R wave to R wave interval (on ECG)
SD	Standard deviation
SDNN	Standard deviation of the mean of all RR intervals
SDNN index	Mean of the SDs of all normal to normal intervals for all 5-minute segments of the entire recording
SE	Standard error
SHEEP	Stockholm Heart Epidemiology Program
TNF-α	Tumor necrosis factor-alpha
UAP	Unstable angina pectoris
ULF	Ultra low frequency power
VLF	Very low frequency power

1 INTRODUCTION

Cardiovascular disease is the leading cause of death worldwide. According to the estimations of WHO, nearly 17 million people died due to cardiovascular disorders in 2002, which accounts for 29% of all deaths. The most common cardiovascular disorder is coronary heart disease (CHD), which occurs when the supply of blood to heart muscle cells is hampered due to the narrowing of the coronary vessels, and responsible for 13 % of deaths worldwide (1).

Recently, there has been a drop in age-specific CHD incidence and its prognosis has improved considerably in most parts of the industrialized world (2,3). In the European Union, for instance, CHD mortality in men dropped from 163 per 100 000 in 1965-69 to 99/100 000 in 1995-98, that is, it declined by 39%. Among women, the corresponding decline was 36% (4). However, despite this favorable change, CHD is still by far the leading cause of death in industrialized countries.

The drop in CHD incidence and prognosis is partly attributable to extensive research and increased understanding of the causes and mechanisms of the disease and the application of research findings in practice when designing and conducting primary and secondary prevention. An even greater decline could have been achieved by a more systematic adaptation of evidence-based strategies. For example, in the United States blood pressure is still not controlled in 45% of patients with hypertension, and only 40% of the eligible patients receive beta-blockers after an acute myocardial infarction (AMI) (5). On the other hand, according to Braunwald (5), 50% of CHD patients do not have any of the established, conventional risk factors, i.e. hypertension, hypercholesterolemia, smoking, diabetes mellitus, marked obesity, and physical inactivity. This may have been an underestimation though as suggested by other authors (6), it is clear that there is still much to learn about the mechanisms of CHD, and additional research is needed to establish the role of other, non-conventional CHD risk and prognostic markers.

Many relatively novel risk indicators have been suggested to predict CHD incidence and prognosis independently of the conventional markers. Heart rate variability (HRV), alcohol intake, inflammatory and psychosocial factors are probably among the most important relatively novel ones. The interrelationship of these non-conventional risk indicators, and their mechanisms are poorly understood. This thesis is aimed at contributing to the disentanglement of the complex network of mechanisms leading to CHD (Figure 1.). Focus is on these four novel risk indicators, and the corresponding data of three, large population based studies, that of the Healthier Female Heart Study (HFH), the Stockholm Female Coronary Risk Study (FemCorRisk), and the Stockholm Heart Epidemiology Program (SHEEP), are analyzed.

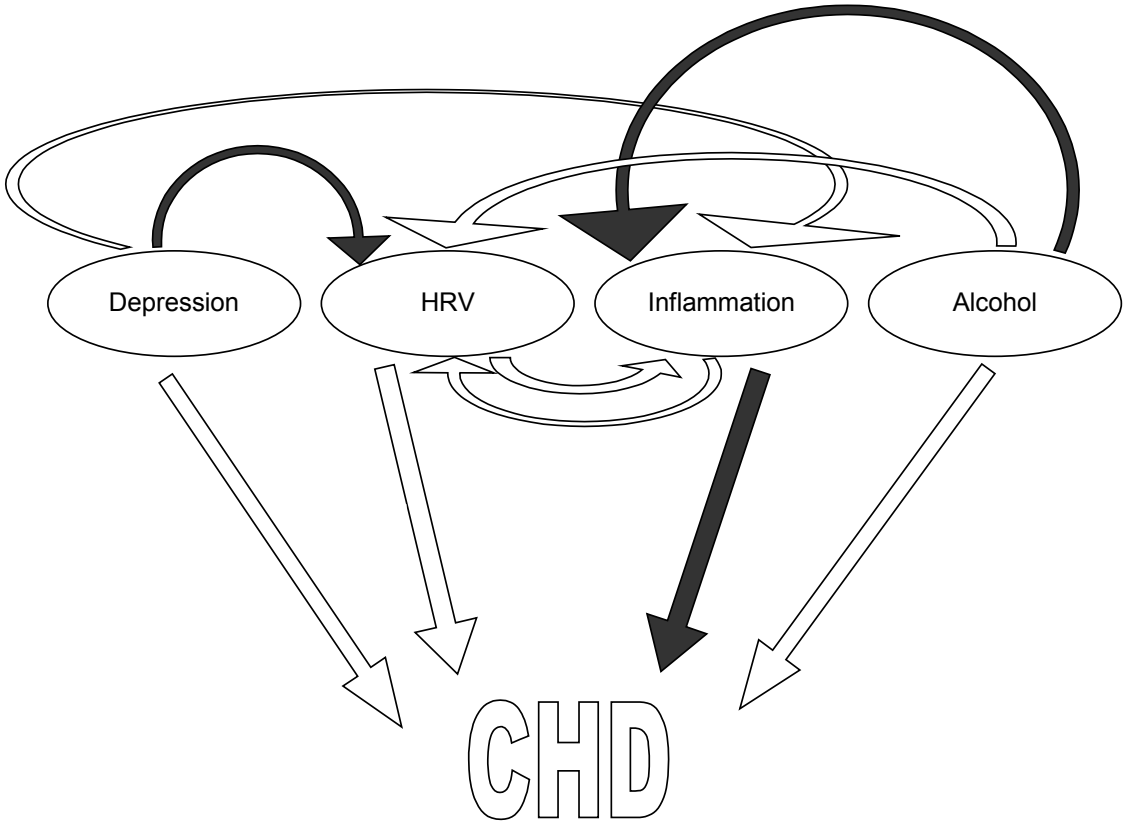

Figure 1. Suggested interrelationships between HRV, alcohol consumption, inflammation and depression and their relationship with CHD. Empty arrows refer to associations proposed and investigated in this thesis. Black arrows indicate established relationships, which are not examined here.

1.1 Heart rate variability and autonomic dysfunction

This thesis is concentrating most on heart rate variability (HRV) among the novel risk and prognostic factors of CHD. HRV is defined as the amount of fluctuation of the heartbeat-to-heartbeat differences. This fluctuation has been in focus since the discovery in the mid-19th century of the respiratory sinus arrhythmia, that is, that the heart beats more frequently during inhalation and slows down during exhalation (7).

HRV reflects the neurohumoral regulation of the heart. The cardiac parasympathetic influence is manifested by the short-term fluctuations of the beat-to-beat differences. The sympathetic regulation is somewhat slower, and the slowest regulation is organized by humoral factors, such as the renin-angiotenzin system (8). The analysis of HRV provides a tool to examine the regulatory mechanism and the ability of the heart to respond to these regulatory impulses.

Decreased HRV has been observed in many pathological conditions, such as CHD (9), heart failure (10), essential hypertension (11), hypertensive cardiac hypertrophy (12), renal failure (11), diabetic neuropathy (13), depression (14) and panic disorder (15). Large population-based studies found that decreased HRV is an independent predictor of mortality from all causes (16,17). Very high HRV was also suggested as a risk factor for all cause mortality in the elderly. However, their lower heart rate, which itself is associated with increased HRV, is possibly at least partly responsible for this finding (18).

1.1.1 Assessment of HRV and other methods to examine autonomic influence on the heart

HRV can be evaluated in both time and frequency domains. The time domain indices are based on the amount of time in the normal beat-to-beat intervals. The normal beat-to-beat interval is defined as the time in milliseconds between the normal R to R waves on an ECG. HRV can be analyzed both from short ECG recordings and from long-term Holter monitoring data. The most frequently used time domain parameters are the standard deviation of the mean of all RR intervals (SDNN), the mean of the standard deviations of the means of all RR intervals for different, usually for 5-minute, time epochs (SDNN index), and the square root of the mean of the squared differences of RR intervals (r-MSSD). These parameters refer to the absolute amount of the heart rate fluctuation.

Frequency domain measures, which identify the underlying frequencies and their distribution of this fluctuation, are calculated mostly by Fourier spectral analysis or by autoregressive method. High frequency power (HF: 0.15-0.40Hz) refers to the parasympathetic control of the heart and is connected with breathing and intermediated by the direct influence of medullary respiratory neurons on cardiomotor center, and indirectly by thoracic, atrial and arterial stretch receptors. Low frequency power (LF: 0.04-0.15 Hz) reflects both to the sympathetic and to the parasympathetic control by the baroreflex activity. HF/LF ratio is often considered as an indicator of the sympathovagal balance. Little is known about the very low frequency power (VLF: 0.003-0.04 Hz) or about the even slower fluctuations (ultra low frequency power, ULF: >0.003 Hz). The thermoregulation system, the renin-

angiotensin system or other humoral factors seem to be responsible for these frequencies (8,11).

Recently, a potential alternative method has emerged, the non-linear analysis of HRV, based on chaos theory and fractal mathematics. Although, the full scope of these methods has not yet been assessed, some data suggests that applying these methods may provide information beyond the generally used time and frequency domain indices (19,20).

Other methods available to assess autonomic influence on the heart are closely related to HRV:

Baroreflex sensitivity (BRS). In healthy subjects, the increase in the systemic blood pressure increases the excitation of the baroreceptors, which are located in the sinus caroticus. The excitation of the afferent nerves from the baroreflex augments the vagal tone, which slows the sinus rate and acts against the blood pressure increase. In experimental settings, the blood pressure is typically increased by iv. phenylephrine. The increase in sinus cycle length per millimeter of mercury of blood pressure increase is a measure of BRS (21,22).

Heart rate turbulence (HRT) is a new concept closely related to BRS. HRT is the physiological response of the sinus node to premature ventricular contractions. It consists of a short initial acceleration followed by a deceleration of the heart rate and can be quantified by two parameters, by the turbulence onset and the turbulence slope. The underlying mechanisms of HRT have not been fully identified, but HRT is most probably an autonomous baroreflex. Both the turbulence onset and the turbulence slope have remarkable prognostic importance (23).

Cardiovascular reactivity (CVR) refers to the difference in physiological outcomes (blood pressure, heart rate, stroke volume, total peripheral resistance etc.) between the baseline (rest) period and during the exposition to a stressor, i.e. during the sympathetic activation and parasympathetic withdrawal caused by the stressor. Stressors can be both psychological (e.g., mental task, public speech) and physiological (e.g., cold pressor test, exercise, caffeine). The supposed role of elevated cardiovascular reactivity as one of the mediators leading to CHD and to a worsened prognosis after a coronary event is based on the hypersensitivity hypotheses. According to this hypothesis, individuals who show exaggerated response to the stressful stimuli during an experiment show similar responses in their real life. The frequent and elevated long-term sympathetic activation as a consequence leads to the progression of atherosclerosis and CHD. Supporting this hypotheses both Allen et al. (24) and Veit et al. (25) found high over-time stability for the systolic blood pressure and heart rate response components of CVR suggesting that the reactivity to stressors is a stable individual characteristic. Positive family history of essential hypertension predicts the higher CVR (26,27). Longitudinal studies (28–30) showed an association between high CVR and subsequent hypertension.

1.1.2 HRV and CHD

Since Wolf et al. (31) and Kleiger et al. (32) revealed that decreased HRV is associated with increased mortality among AMI survivors, many researchers have investigated the predictive power of different time and frequency domain parameters as well as non-linear dynamic characteristics of HRV (19,20,22,33–37).

All these studies showed that HRV parameters add clinically relevant prognostic information. The effect of decreased HRV is largely independent of the left ventricular function and their predictive value is comparable (32,36). The decrease in the slower fluctuations was suggested to be more predictive for adverse outcomes than the decrease in faster oscillations (33).

Most investigators measured HRV around the hospital discharge period after AMI. HRV marked decreases immediately after the AMI, with most of the recovery period within the first six months (38). However, according to Bigger et al. (1993), who performed HRV analysis one year after AMI, HRV-parameters remain good predictors long after the coronary event.

Less is known about the role of HRV in other manifestations of CHD. Huang et al. (39) reported a reduced HRV in patients with UAP (unstable angina pectoris). In patients with UAP who stabilized after hospital admission, HRV started to increase within 48 hours of monitoring, and low HRV predicted poor prognosis (39). HRV is also a prognostic marker in stable AP (40).

The aforementioned studies on HRV and prognosis in CHD were carried out in predominantly male patient populations. Though women are at lower risk for AMI (41), at younger ages, women are known to have poorer post-AMI prognosis than men even after a careful adjustment for comorbidity (42). Before age 50, women have more than twice the mortality of men after an AMI. The difference disappears above age 74 (43). Women are also found to have different cardiac autonomic patterns than men. The BRS and HRV parameters - except for those reflecting the cardiac vagal activity - are lower in women (44–48).

However, because there were relatively few women enrolled in prior clinical studies on HRV and survival following acute coronary syndromes, extrapolation of these results to women can be misleading. One of the aims of this thesis, therefore, was to assess the role of HRV parameters in the long-term prognosis of middle-aged women surviving an acute coronary event (paper V).

1.1.3 Determinants of HRV and its relation to other cardiovascular risk factors

Several risk and prognostic indicators for CHD and other factors have been associated with HRV measures in the literature. Table 1 presents the different factors that have been associated with HRV according to previous studies.

Table 1. Determinants of HRV (+ indicates positive, - indicates negative relationship)

Age (-)
Gender (high frequency oscillations are higher in women, lower frequency oscillations in men)
Physical activity (+)
BMI (-)
Metabolic syndrome (-)
Smoking (-)
Diet
Coffee consumption (-)
Thyroid hormones (-)
Chatecholamins (-)
Plasma renin activity (-)
Insulin (-)
Triglycerides (-)
HDL cholesterol (+)
Leukocyte count (-)
Genetic factors
Personality

Based on references (8,44–57)

One aim of this thesis was to expand our knowledge on determinants of HRV. We investigated the associations between HRV, alcohol consumption and inflammatory markers.

1.1.4 Possible explanatory mechanisms

It has not been established yet whether decreased HRV is part of the mechanism of increased CHD mortality or is merely a marker of high risk and poor prognosis (8). Several potential pathways have been suggested to explain the strong association between decreased HRV and CHD events.

The decreased HRV after an AMI partly reflects the sympathetic overdrive and/or vagal withdrawal due to poor ventricular performance. However, depressed vagal activity itself has a role in the pathogenesis of ventricular arrhythmias and sudden cardiac death (8). In post-AMI patients low HRV shows the strongest relation to arrhythmic events (58). Nevertheless, HRV is also associated to non-arrhythmic deaths (58) and to progression of atherosclerosis (59).

Sloan et al. (60) suggests that the effects of the autonomic dysfunction on development and prognosis of CHD are mediated by the increased blood pressure variability (BPV), i.e. by high fluctuations in blood pressure. According to this hypothesis HRV serves to blunt the changes of blood pressure, that is, HRV stands in the causal pathway for developing atherosclerosis and CHD, and it is not only a marker of prediction. Both the breathing and the baroreflex related component of HRV (HF power and LF power, respectively) have proven to smooth the arterial blood pressure curve. Denervation of the baroreceptor in experimental animals and in humans is followed by markedly increased blood pressure variability even if the mean blood pressure is not changing (61,62), and decreased BRS was found to be predictive in experimental models and in humans for CHD events (22,63). On the other hand, it should be mentioned that according to some animal studies the development of atherosclerosis precedes alterations in BRS (64). Cardiac transplantation has often results in an unusually accelerated and diffuse form of obliterative coronary arteriosclerosis (65). Though, there are many potential explanations for this phenomena, one can hypothesize that the very reduced HRV with no definite spectral components (8) can stand in the causal path as well. Frequent and elevated sympathetic activation to real-life stressors (i.e. exaggerated CVR) also leads to frequent fluctuations in blood pressure. According to Manuck et al. (66), the blood pressure response to the mental stress test is higher among post-AMI patients who later suffer from a recurrent event than in those who did not. However, there are negative findings on the results of the CVR test and subsequent CHD risk as well (67). Figure 2 summarizes the possible model of HRV, BRS, and CVR as causal factors in the development and prognosis of CHD, based on the hypothesis of Sloan et al (60).

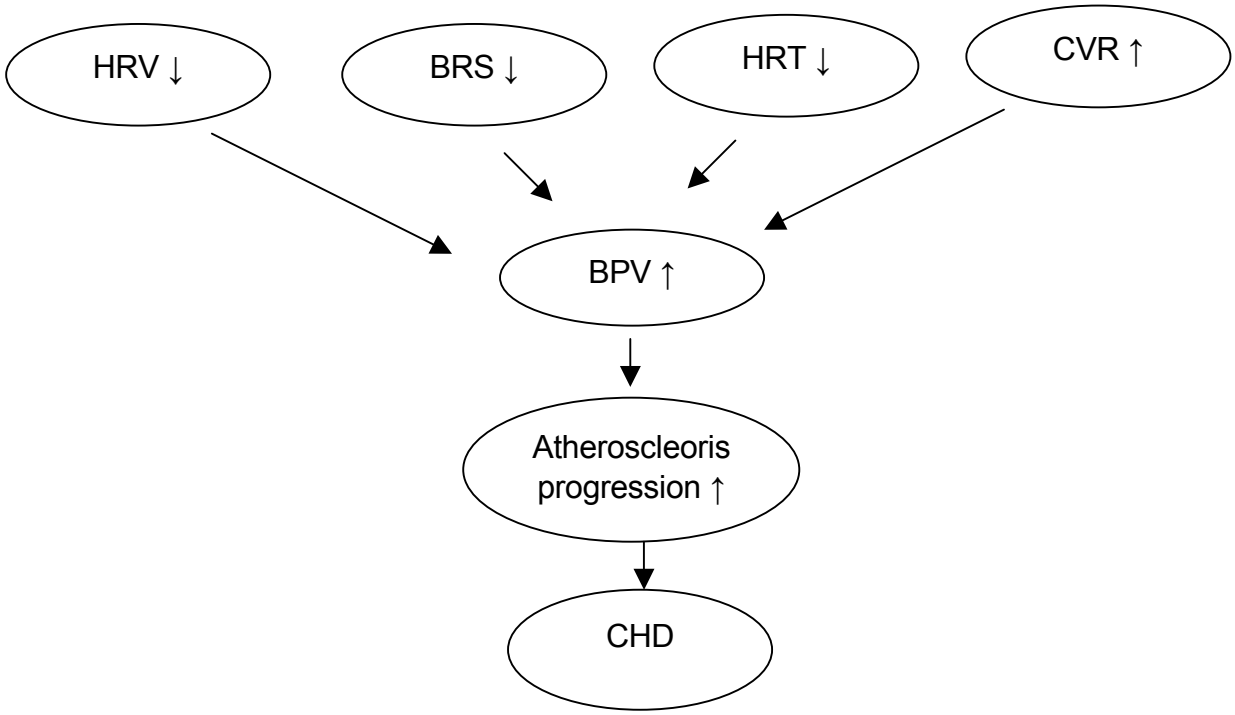

Figure 2. A supposed model for a causal pathway from the indicators of autonomic dysfunction, i.e. decreased HRV, BRS, HRT and increased CVR to CHD (based on Sloan et al. (60))

1.2 Alcohol consumption

The benefits and dangers of alcoholic beverages have been debated since the dawn of time. Although, the acute and chronic effects of alcohol are apparently responsible for many lost lives and disabilities, it seems that moderate drinking may have a considerable health benefit as far as CHD is concerned.

1.2.1 Alcohol consumption and risk and prognosis of CHD

Already in the early 1900s, an inverse relationship between alcohol consumption and CHD incidence was reported (68). It is now quite well documented that the descending leg in the U or J shaped curve for the relation between alcohol consumption and mortality from all causes results from a decreased risk of cardiovascular disease among moderate drinkers (69). Actually, this decreased CHD risk in moderate drinkers is one among the most consistent findings in epidemiology. Many studies with several different designs have led to the same conclusion. Ecological studies have shown a strong inverse correlation between alcohol intake, especially for wine, as assessed by import, export and sales, and CHD mortality across countries (70).

The most well known example is the so-called French Paradox. In France, despite of the high dietary intake of cholesterol and saturated fat and high rates of cigarette smoking, the CHD death rate is low. This phenomenon was described as the French Paradox in the 1980s by French epidemiologists, who suggested the relatively higher alcohol intake, especially of wine, as an explanation (71).

Case-control and even more importantly large prospective cohort studies have also investigated the CHD-alcohol relationship and consistently confirmed the protective effect of alcohol. Based on these large prospective studies when taking the other known CHD risk factors into account, the average reduction in CHD risk by drinking two drinks/day was 30-40% for men. Higher levels of consumption yielded minimal additional benefit (70). The definition of a 'drink' varies across the studies but it generally corresponds to 10-15 g of ethanol. In women, due to the differences in body weight and alcohol metabolism, the same risk reduction was observed for one standard drink/day. Prospective studies also concluded that moderate alcohol consumption is a positive prognostic factor after acute coronary events (72,73).

Experimental studies have been carried out as well, both in animals (74) and humans. However, the human studies were designed to study the short-term effects of alcohol on the cardiovascular system and on CHD risk markers rather than the association of average alcohol consumption and the clinical outcome. A randomized controlled trial on alcohol consumption and long-term clinical outcomes is not likely in the near future. Apart from practical problems like the high cost, it is impossible to blind participants to alcohol exposure, that is, we are unable to prevent participants from knowing whether they receive alcohol or not. Such a trial would also raise serious ethical considerations in light of the damage caused by the well-known 'side-effects' of alcohol in the treatment group, or the possibility that some participants instructed to consume alcohol would eventually misuse it or even become alcohol dependent (75).

Despite the consistent finding in the epidemiological studies, the lack of randomized trials makes it more difficult to conclude that there is a truly causal relationship between drinking and CHD risk. Several sources of confounding were suggested, as drinkers and abstainers may differ in many respects in addition to the alcohol consumption itself. Many abstainers may have chosen to forsake alcohol intake because of adverse experiences with alcoholic family members, which may also influence the underlying risk of heart disease (75). Furthermore, most studies, while carefully controlling for the conventional CHD risk factors, did not take into account the possible role of social factors. Difference in socioeconomic status, social networks, and personality among the different drinking groups can be responsible for the observed relationship. Skog (76) argued that in the Western world, both heavy drinking and abstention or very light drinking are deviant modes and associated with social disadvantages.

However, Murray et al. (77) found that adjustment for social integration failed to alter the inverse relation between CHD risk and alcohol consumption. Shaper et al. (78) suggested another form of a systematic error potentially able to explain the U shaped curve based on the dynamic relationship between ill-health and drinking behavior. According to these authors, some of the abstainers are actually former drinkers who stopped drinking due to illness and the pre-existing disease of these "sick quitters" could explain the protective effect observed among the drinking ones. Supporting this hypothesis in the prospective study of working Scottish men Hart et al. (79) did not confirm that moderate alcohol drinking has a favorable effect on CHD. The authors argued that a possible explanation for their unexpected findings could be that these results were not confounded by the inclusion of former heavy drinkers, and of subjects with illnesses that had lead them to be non-drinkers. However, Fuchs et al. (80) confirmed the U shaped curve even when former heavy drinkers who reported current abstinence were excluded and only the last eight years of the 12-year follow up were analyzed. The authors argued that the group of long-term non-drinkers could include participants who refrained from drinking because of early symptoms of disease. Thus, the higher mortality among non-drinkers might be due in part to undiagnosed, preexisting disease, resulting in higher rates of death in the earlier years of follow-up. This possible source of confounding would be expected to diminish in later years of observation.

In summary, though residual confounding cannot be ruled out and some controversy still exists, based on the strong epidemiological findings and biological plausibility (see the section below), a direct protective effect from alcohol on CHD is likely. This is in accordance what was concluded in a recent study by Hines et al. (81) on the polymorphism in the gene for alcohol dehydrogenase type 3 (ADH3), alcohol consumption, and the risk of myocardial infarction. The authors found an effect of the functional ADH3 polymorphism on the relation between moderate consumption of alcohol and the risk of myocardial infarction. As ADH3 genotype is unlikely to be modified by confounders as lifestyle or socioeconomic conditions, alcohol may have a causal relation to CHD.

1.2.2 Possible mechanisms

The relatively long follow-up time of the prospective studies makes it reasonable to presume that alcohol consumption is associated with the atherosclerotic process. However, despite the epidemiological evidence demonstrating lower rates of CHD among moderate drinkers than abstainers, the relationship of moderate alcohol use to coronary atherosclerosis is less well established.

Most of the studies on alcohol use and atherosclerosis have been limited by cross-sectional designs and the findings were inconsistent. In cross-sectional

analyses of CHD patients, Barboriak and colleagues (82) reported less atherosclerosis among moderate drinkers than abstainers undergoing coronary angiography. In contrast, alcohol consumption was not associated with carotid artery wall thickness or distensibility in the Atherosclerosis Risk in Communities Study (83). Few prospective studies have examined alcohol use and progression of atherosclerosis, and they have relied on carotid intima-media thickness (IMT), rather than direct arteriography (84,85). In the Bruneck Study, alcohol consumption had a J-shaped relation with five-year change in carotid IMT, with a lower risk found among consumers of 1-50 grams of alcohol (up to four drinks) per day but higher risk with heavier consumption (84). In a study of Finnish men, binge drinking was associated with greater four-year progression in carotid IMT, but the effect of more regular consumption was not reported (85). We know of no epidemiological studies that have examined alcohol use and progression of atherosclerosis directly in coronary arteries neither in men nor in women. Therefore, in this thesis, we investigated the association between alcohol consumption and progression of coronary artery atherosclerosis using serial quantitative coronary angiography (QCA).

Several biologically plausible mechanisms were suggested for how alcohol and other contents in alcoholic beverages can slow the atherosclerotic process and therefore protect from CHD. These include increased HDL-cholesterol levels (86,87), improved coagulation profile (88,89), lower levels of inflammation (90), greater insulin sensitivity (91), reduced endothelin-1 synthesis (92), LDL oxidation (93) and smooth muscle proliferation (94,95). However, there are pathways where alcohol could be potentially harmful. For example alcohol consumption raises the blood pressure (96), levels of triglycerides (87), and homocysteine (97), i.e. factors that have an unfavorable effect on cardiovascular health.

1.2.3 Is wine more beneficial than other beverage types?

There is still much controversy surrounding this issue. In a recent review, Gronbaek (69) concludes that wine drinkers are at a decreased risk of mortality from cardiovascular disease compared to non-wine drinkers, while other meta-analyses found that wine drinking confers no particular benefit (98,99). While findings from the ecological studies favor the hypothesis of a superior effect for wine, the prospective cohort studies provided little evidence for it. However, experimental studies showed some positive cardiovascular effects for wine only, like the reduced endothelin-1 synthesis (92), and LDL oxidation (93), though the clinical significance of these findings is not clear.

1.3 Inflammation

Pathologists described the presence of inflammatory cells in the atherosclerotic arterial wall as early as the middle of the 19th century. Moreover, the famous German pathologist, von Virchow assumed a primary role for inflammation already at that time (100). However, the presence and the role of the immune cells in the atherosclerotic lesions were largely neglected for a long period. It was only relatively recently, that the view of the process of atherosclerosis has changed considerably, with the acknowledgement that atherogenesis is much more complex than merely an accumulation of lipids and therefore degeneration of the artery wall. As Russell Ross (101) stated: "in fact, the lesions of atherosclerosis represent a series of highly specific cellular and molecular responses that can best be described, in aggregate, as an inflammatory disease".

1.3.1 Pathophysiological mechanisms

The immune system plays a central role in all stages of atherosclerosis. At the early stage the reaction of the immune system is evoked by potentially "offending" factors, probably mainly by elevated, "trapped" and oxidatively modified LDL, but also factors such as free radicals as well as microbial pathogens. First, the so-called innate, or memory-independent immunity reacts. This non-specific part of the immune system constitutes the first line of the immune defense and depends mainly on macrophages. The role of the macrophages is in part the removal and sequestration of the "offending" antigens. These macrophages become foam cells when internalizing and accumulating the LDL or the oxidized LDL particles, and the lipid-laden foam cells constitute the initial pathological manifestation of the atherosclerotic process, the fatty streak formation. Macrophages also facilitate the more sophisticated, memory-dependent, so-called adaptive immune system. Key components in the adaptive immune system are the T and B lymphocytes. The function of T lymphocytes is either acting directly on the antigens or regulating the action of the B cells through different cytokines. The B cells produce antibodies which attack the antigens (102,103). If the elimination or neutralization of the antigen is not complete, the inflammatory process continues and the atherosclerotic lesion develops further. The initial fatty streak transforms, with the contribution of the immune cells, to an advanced atheromatous lesion or plaque, which consists of lipid pool protected by a fibrous cap. If the intrinsic capacity of the arteries to compensate is exhausted, the atheromatous lesion can intrude into the lumen and alter the blood flow causing clinical symptoms (101,103,104). The atherosclerotic plaque can cause clinical symptoms by facilitating thrombosis as well. The surface of the plaque is itself thrombogenic and the rupture of the plaque also facilitates thrombosis. Immune activity may facilitate both the rupture of the plaque and thrombosis

by digestion of the fibrous cap or by changing the haemostatic properties of the blood (105).

1.3.2 Clinical significance of the immune origin of atherosclerosis

Prediction of CHD risk and prognosis. A large and rapidly increasing number of markers connected to the inflammatory activity, different cytokines, adhesion molecules, matrix metalloproteinases, heat shock proteins are associated with CHD risk and prognosis. For current practice, the C-reactive protein (CRP) is the most important among these markers given its strong relationship with CHD and the well-standardized widely available assay for its assessment (104). CRP is suggested to be a reliable measure of the underlying systemic inflammation and therefore of the activity of atherosclerosis from the early symptom free to the late clinical stages. It is associated with increased cardiovascular morbidity and mortality among general population cohorts (106,107), and with poor prognosis among survivors of acute coronary events (108–111).

Autoimmune disorders, chronic and acute infections, infectious agents and atherosclerosis. Several studies indicate that patients with autoimmune disorders like rheumatoid arthritis and systemic lupus erythematosus are at increased risk for CHD and this is not fully explained by conventional risk factors, and probably is associated with the increased inflammatory activity in these patients (112–114). An accelerated atherosclerotic process has been described in connection with chronic inflammatory disorders of many other origins. One of the most important is periodontitis given its high public health burden (115,116). How chronic inflammation leads to CHD is not clear. However, the possible role of microbial infectious agents is in focus since Fabricant and colleagues (117) demonstrated in 1978 that herpes virus infection could provoke gross atherosclerotic lesions in cholesterol-fed chickens. Since then several other viruses (as cytomegalovirus, Hepatitis A) and bacteria (as Chlamydia pneumoniae, Helicobacter pylori) were candidate culprits. Zhu J et al. (118) and Epstein et al. (119) suggested that the impact of microbes on atherogenesis is related to the total pathogen burden, i.e. the aggregate number of infectious pathogens to which an individual has been exposed. Other studies confirmed that the total pathogen burden predicts the progression of the carotid atherosclerosis and the CHD risk (120,121). It has also been proposed that acute infections are also associated with - a transient - increase in the risk of cardiovascular events (122).

Possible therapeutic consequences. The immune mechanisms involved in the progression of atherosclerosis are potentially new therapeutic targets. Based on the supposed role of the different microbial pathogens, antibiotic therapy was supposed to be beneficial in CHD patients. However, the results so far are not promising for a strong effect of anti-infectious agents (101,123). Other

approaches are based on the modulation of the immune system. Injection of immunoglobulins or anti-inflammatory cytokines, immunization with oxidized LDL and immunosuppressive treatment showed promising results in animal studies (102).

1.4 Depression

Several psychosocial factors have been associated with CHD incidence and prognosis. Hemingway and Marmot (124) concluded in their systematic review of prospective cohort studies that evidence exist for depression, anxiety, social support, and somewhat to a lesser extent type A/hostility and psychosocial work characteristics to be risk factors, and for depression, anxiety, and social support to be prognostics factors for CHD. Similarly, Rozanski et al. (125) stated in their review that there is a clear and convincing evidence that psychosocial factors, especially depression, anxiety, personality and character traits, social isolation, and chronic life stress, contribute significantly to pathogenesis and expression of CHD. However, other authors are not so enthusiastic about the evidence supporting a major role of the psychosocial factors in CHD (126).

Among the psychosocial factors, this thesis concentrates on the role of depression and subjective well-being in CHD and attempts to explore possible pathways.

1.4.1 Review of the previous studies on depression and CHD risk

Definition of depression, follow-up time, sample size. The majority of the previous studies with a prospective nature on CHD risk and depression used *questionnaires* to assess depressive symptoms (127–141). Some studies attempted to deal with the unspecificity of the symptoms. For example Barefoot et al. (137) excluded the somatic items from the MMPI questionnaire. Four of the studies using questionnaires had a relatively long follow-up time: Barefoot et al. (137) followed 730 individuals for 27 years, Vogt (129) had a follow-up time of 15 years on 2573 individuals, Anda et al. (128) followed 2832 subjects for a mean follow-up of 12.4 years, and Hallstrom et al. (127) followed 795 women for 12 years. The follow-up time for the rest of the studies ranged from 3-10 years.

In some investigations, *diagnostic interviews* were used in order to define depression. Aromaa et al. (142) had specially trained nurses interviewing 5355 subjects, who were then followed for a mean of 6.6 years. However, only age-adjusted associations were presented for depression and CHD mortality. In the study by Pratt et al. (143), depression was diagnosed by interviewers with 1-2 weeks of training but no clinical experience. The 1551 participants were followed for 13 years, and self-reported AMI served as outcome. Penninx et al. (144) followed 2847 individuals for four years and defined minor depression with

a questionnaire, major depression with an interview among those who scored high on the questionnaires.

Cohen et al. (145) defined depression as *self-reported history* of treatment for depression based on a single question. In the Johns Hopkins Precursors Study (146), depression was measured by *mailed surveys* with direct questions on occurrence of depression and associated treatment in 1190 male participants who entered The Johns Hopkins Medical School classes. Self-reports of depression were reviewed by a committee of physicians. The median time from the first episode of major depression to the first CHD event was 15 years, 1-44 years.

Hippisley-Cox et al. (147) studied the prospective association between the *general practitioner's diagnoses* of depression and CHD within one general practice.

Some studies examined future CHD events among *psychiatric patients with depression*; however these studies had no control groups, analyzed no CHD risk factors, and relied only on vital statistics concerning overall and CHD mortality (148–151).

Association between depression and CHD. Concerning the results of the aforementioned studies, Vogt et al. (129) found no relation between CHD and depression, while Hallstrom et al. (127) and Sykes et al. (140) reported associations only with angina. In some other studies, significant association was reported only with a combined CHD end-point including angina (134,135,141,147). Angina as an end-point was questioned as depressed individuals more often report angina-like symptoms in the absence of any stenotic coronary artery, and it may reflect merely a personality trait: those who over-report their depressive symptoms may over-report their cardiac symptoms like angina as well (140,152). However, the rest of the studies found an association between some forms of depression and fatal or non-fatal CHD excluding angina.

Recurrence of depression. Among studies where depression was measured more than once (131,134,136,137,143,146) or history of depression was considered (145,147) only three evaluated the effect of recurrent depression. Wassertheil-Smoller et al. (131) found that baseline depressive symptoms were not related to subsequent CHD events, but an increase in depressive symptoms later on was of prognostic importance. Similarly, Penninx et al. (136) measured three times the depressive symptoms and found that only newly depressed cases, i.e. depressed only at the last assessment, were at increased risk for CHD, the chronically depressed ones were not. Ariyo et al. (134) concluded that baseline depression scores were not as predictive as cumulative mean depression scores.

Gender issue. The aforementioned studies have shown varying results concerning the gender effect on the association between depression and CHD.

Some concluded that depression is a risk factor only in men but not in women (136,147), while others found the opposite (138).

Limitations. Previous research on depression and CHD has been subjected to several potential limitations. In most studies, depression was assessed at one point in time only, although depression is more known to have an episodic nature (153,154). Consequently, these studies may underestimate the relationship between depression and future CHD events. On the other hand, other possible sources of methodological shortcomings can lead to an overestimation of the true effect of depression. Instruments used assessing depression in previous investigations may not be specific for depression, but rather reflect a general distress (153,155). Thus, it can be difficult to separate depressive symptoms measured by these instruments from symptoms of a physical illness (156). Moreover, as most often the average length of follow-up was less than 10 years, individuals free from clinical CHD may not be free from coronary atherosclerosis, which in turn could facilitate depressive symptoms (157,158). Furthermore, the aforementioned reports inadequately controlled for possible confounding factors.

1.4.2 Depression and CHD prognosis

Depressive symptoms predicted the outcome in post-AMI (159–161) and in unstable angina patients (162). However, as the severity of CHD could not have been completely ruled out as a confounder, and moreover, trials on antidepressive treatment after AMI could not demonstrate increased survival, causality remains controversial (163).

1.4.3 Self-rated health and vital exhaustion – two other measures of subjective well-being

Two other constructs referring to one's subjective well-being, and therefore overlapping with the concept of depression, have been related to increased CHD morbidity and mortality in population-based studies and with adverse outcomes in existing CHD. These are self-rated health (164,165), and a relatively new construct, that of vital exhaustion which is characterized by a state of unusual fatigue, loss of energy, increased irritability, and feelings of demoralization (166–168).

1.4.4 Possible explanatory mechanisms

There are several potential routes by which depression may impact upon CHD and the underlying atherosclerotic process. Depression or poor subjective well-being may lead to an unhealthy lifestyle with low physical activity, obesity, and smoking and therefore increase the risk of CHD (158). Some studies suggested

that the effect is mediated by lipids, as depressed or vitally exhausted patients showed an unfavorable lipid profile (169,170). Hypercoagulability was also suggested as a potential pathway (171). Bondy et al. (172) proposed that CHD and major depression may have a common genetic background. Increasing evidence suggests that depressed patients have a markedly lower HRV and BRS, therefore autonomic dysfunction may also explain the increased CHD risk (171).

Recently, there has been much focus on inflammatory activity as a possible link between depression and CHD. Infectious or autoimmune diseases, as well as administration of cytokines, induce a symptomatology often referred to as "sickness behavior" – characterized by fatigue, loss of energy, anorexia, difficulties to concentrate, and anhedonia – that bears a strong resemblance to depression (173–175). This effect of cytokines can be prevented by antidepressant treatment (176). It was also suggested that the administered cytokines induce changes in the neuroendocrine and central neurotransmitter systems reminiscent of those implicated in depression (177,178). Furthermore, antidepressants have immunmodulatory properties (179), and successful treatment of depression can be accompanied by a decrease in inflammation (180). Moreover, depressed or vitally exhausted individuals show elevated levels of circulating (181–187) and stimulated cytokines (188), as well as reduced glucocorticoid sensitivity of monocyte IL-6 production (189).

However, much less attention has been paid to the link between depression or vital exhaustion and inflammation in CHD patients (190), and no studies with a prospective design ever examined if the observed effect of depression on CHD is actually mediated by inflammatory activity. Similarly, the effect of adjustment for coagulatory factors, HRV or genetic factors on the strength of the prospective association between depression score and CHD has not been reported yet. Data on lifestyle and lipids is also sparse in this respect. Therefore, despite of the several proposed plausible mechanisms there is still no clear explanation for the observed association between CHD and depression. In this thesis we examined the prospective association between the clinical diagnosis of depression and CHD risk, and investigated several possible explanatory mechanisms. We especially focused on increased inflammation as a potential pathway between depression and other measures of subjective well-being, and CHD.

1.5 Aims

General aim

To investigate the prognostic importance of HRV, inflammation, alcohol consumption and depression in CHD, and reveal the possible explanatory mechanisms.

Specific aims

- to assess the interrelationship between the four non-conventional risk indicators, i.e. HRV, alcohol consumption, inflammation, and depression
- to investigate HRV as a prognostic factor in middle aged women with CHD
- to assess the effect of alcohol consumption on atherosclerosis progression in human coronary arteries
- to assess the role of depression in CHD

2 METHODS

2.1 Study populations and designs

2.1.1 Healthier Female Heart (HFH) Study

We included patients from a randomized controlled intervention trial for the cross sectional analyses presented in papers I-III. The intervention comprised a rehabilitation program specifically designed for women with CHD. The program focused on providing information about well-established risk factors, including psychological ones and how to deal with them (191). The original study population consisted of 247 women that had survived acute myocardial infarction (AMI) or undergone a revascularization procedure, either percutaneous transluminal coronary angioplasty (PTCA) or coronary artery bypass grafting (CABG) and were hospitalized at Karolinska University Hospital at Huddinge or St Göran's Hospital in Stockholm, Sweden, between August 1996 and February 2000. The diagnosis of AMI was based on WHO criteria of typical enzyme patterns and chest pain and/or diagnostic electrocardiographic changes. Consecutively, all eligible women below 75 years were offered to participate in the study; subsequently, all those who agreed to participate were randomly assigned to either the control (128 patients) or to the intervention group (119 patients). Randomization took place during hospitalization, 2-4 days after the index event. Finally, out of the originally randomized 247 patients, 12 (6 from the intervention group, 6 from the control group) did not participate in the study, resulting in 235 eligible patients.

Analyses included to this thesis used the data derived from the examination of patients one year and five months (±2.5 months) after randomization, i.e. in a stable phase of their disease.

The Ethics Committee of Karolinska Institutet at Karolinska University Hospital approved the study and all patients gave their informed consent.

2.1.2 Stockholm Female Coronary Risk (FemCorRisk) Study

Data of the FemCorRisk study were used for papers IV and V. Consecutive women patients below 65 years, who were admitted between February 1991 and February 1994 for an acute CHD event (AMI or UAP) at any of the ten coronary care units in Stockholm were asked to participate in the FemCorRisk study (192). We enrolled 292 women, comprising 87% of the women identified during that time period. The study was approved by the Karolinska Ethics Committee, and all patients gave their informed consent.

The diagnosis of AMI was based on the WHO criteria of typical chest pain, enzyme patterns and/or diagnostic ECG changes. Electrocardiographic changes were classified according to the Minnesota code. Unstable angina was defined as

new onset of severe angina pectoris, or deterioration of known stable angina pectoris during the last four weeks before admission according to the criteria described by Braunwald (193). A clinical examination took place three to six months after the hospital discharge.

In paper IV we used a subgroup of the original patient population. The FemCorRisk Angiographic Study included 131 patients who underwent QCA on protocol within 3 months of the clinical examination and 106 women underwent also a second QCA evaluation an average of 3.25 years (range 2-5 years) later. Enrolment to the angiographic sub study was not based on clinical symptoms, and similarly, the participation in the follow-up QCA was offered to everyone who already participated in the first one, irrespective of symptoms or events during the follow up. The standard risk factors and geographical distribution did not differ between participants of the sub study and the rest of the cohort (194).

In paper V we analyzed the prospective association between HRV at the clinical screening and mortality. The centralized health care system in Sweden provides virtually complete follow-up information for all patients by matching their unique ten digit person identification numbers to the death and hospital discharge registers. The follow-up was terminated on 22nd November 2000. The median follow-up period, from baseline examination, was 9 years, range 7.5-10.5 years. Four patients had emigrated from Sweden during the follow up time, where the date of emigration was used as censoring time. Apart from that, no patient was lost to follow-up. All-cause mortality was used as a primary end-point. Patients were also followed for cardiovascular mortality, non-fatal AMI and revascularization procedures, PTCA or CABG.

2.1.3 Stockholm Heart Epidemiology Program (SHEEP)

Paper VI was based on the data of the SHEEP study, which had a population-based case-control design (195). The study base comprised all Swedish citizens living in the Stockholm County who were 45-70 years of age (calendar years) and free of previous clinically diagnosed AMI. Male cases were identified during a two-year period (1992-93) and female cases during 3 years (1992-94). During the period January – October 1992, the upper age limit was 65 years; from 1 November 1992 and onwards it was 70 years.

Cases were identified at (1) the coronary and intensive care units at the departments of internal medicine at all the emergency hospitals within the Stockholm County area (2) the hospital discharge register for the Stockholm County area and (3) death certificates from the National Register of Death Causes at Statistics Sweden. Criteria for AMI included (i) certain symptoms according to case history information, (ii) specified changes in blood levels of the enzymes CK and LD, (iii) specified ECG-changes and (iv) autopsy findings. The diagnosis of AMI required two of the criteria (i-iii) to be met, *or* that autopsy

findings showed myocardial necrosis of an age compatible with the time of disease onset. The age, sex and hospital catchment area matched controls were selected on line from the computerized register of the Stockholm County population within 2 days from case incidence.

The study was approved by the Karolinska Ethics Committee, and all patients gave their informed consent.

2.2 Measurement of the study variables

2.2.1 HRV in the HFH and FemCorRisk Study, papers I, II, V

A two channel ECG recording (Spacelab 90205, Spacelab Inc., Redmond, WA) was performed for a 24-hour period during normal daily activities. The ECG electrodes were attached at the position of CM-V5 (left anterior axillary line sixth rib) and CS-V1 (fourth rib at the sternal border). Patients were asked to continue their usual medications.

The 24-hour Holter tape recordings were digitized and QRS-labeled using a commercially available PC-based system (Aspect Holter System, Daltek, Borlänge, Sweden). An automatic analysis of arrhythmias was made and the QRS complexes classified. The consecutive RR intervals were expressed in centiseconds and analyses were made in units of 5-minute epochs by custom-made software. To be accepted for additional analysis, we required at least 96% of the QRS-complexes to be classified as normal by the Aspect system (196). The time series of RR intervals were resampled at a frequency of two samples per second. Gaps in the time series due to non-normal RR intervals (QRS-labeled by the Aspect System classification as noise or ectopic beats) were filled with values calculated by linear interpolation between adjacent normal RR intervals. Misclassified dropped beats deviating more than 3.0 SD from the normal RR interval of each epoch were also automatically checked.

Both time and frequency domain measures were analyzed. The mean of the SDs of all normal-to-normal intervals for all 5-minute segments of the entire recording (SDNN index, in msec) was obtained from the time series of normal RR intervals. Frequency domain parameters were calculated using an autoregressive method (197): HF power: 0.15-0.40 Hz, LF power: 0.04-0.15 Hz, VLF power: 0.0033-0.04 Hz and total power (in $msec^2$).

Patients were not included if they had non-sinus rhythm, or less than 50% of the original ECG recording was available for analysis.

In the HFH study (papers I and II), HRV was measured one year and five months (±2.5 months) after the randomization. In the FemCorRisk study (paper IV), the HRV assessment took place at the time of the clinical examination, i.e. three to six months after hospital discharge.

2.2.2 Alcohol consumption, papers I, IV, VI

In all three studies, consumption of alcoholic beverages was assessed by the corresponding items of the Willett food frequency questionnaire (198). This questionnaire has shown an excellent correlation to alcohol consumption as measured by four 1-week diet records (obtained 3-4 months apart) in a Swedish female cohort (193). The usual frequency and quantity of intake of five beverage types - regular beer, strong beer, wine, light spirits (e.g., liqueur, vermouth, and port) and spirits were asked. The estimated alcohol content of these beverages was 3.5%, 4.9%, 11%, 19%, and 39%, respectively. Average daily alcohol intake was calculated in grams. In paper I and IV daily alcohol consumption was categorized as follows: 0 (abstainers), >0-5 grams/day (light drinkers – up to half a standard drink per day), and >5 grams/day (moderate drinkers – over half a standard drink per day). Daily consumption of alcohol from regular beer, strong beer, wine, light spirits, and spirits was also calculated. Because overall intake of strong beer and light spirits was low (mean intake 0.14 and 0.22 grams/day), we grouped these beverages with regular beer and regular spirits in beverage-specific analyses, respectively. In paper I binomial categorical variables were also created indicating if patients consumed beer, wine or spirits at all.

2.2.3 Quantitative Coronary Angiography, paper IV

In the FemCorRisk Angiographic Study 25 patients out of 131 who underwent a baseline QCA were not available for repeat quantitative angiographic evaluation. Three patients died between the baseline QCA assessment and follow-up QCA assessment, one refused a second angiographic evaluation, 13 had poor quality angiograms at baseline and eight had poor follow-up angiograms. The repeat angiograms of three patients were not available for quantitative evaluation of progression/regression. This resulted in 103 patients with valid and comparable repeat QCA measurements.

Selective arteriography was performed at the Department of Thoracic Radiology, Karolinska University Hospital in Solna. Judkins' technique was used. Standard clinical angiographic procedure was followed using 7 French non-nylon catheters. After engaging the coronary segment under study with the injection catheter, the angiographic view was optimized with short test injections. During a breath hold, the filming started before contrast injection to show the catheter. Next, dye was injected to opacify the segments of interest for at least 3 cardiac cycles. Imaging conditions (angiographic view angles, catheter size, and field size) were recorded in an arteriography procedure log. The QCA catheterization laboratory was calibrated initially and then twice yearly to maximize the comparability of assessments. All angiograms were recorded as cine films at a rate of 25 frames per second.

The Angiographic Image Processing Laboratory of the Division of Cardiology, University of Texas performed computer assisted quantitative

evaluations of angiographic films (200). For each angiogram, absolute luminal diameter (in mm) was assessed in up to ten pre-defined coronary segments: (1) left main coronary artery, (2) proximal left anterior descending artery (LAD), (3) mid LAD, (4) first diagonal branch of the LAD, (5) proximal left circumflex artery (LCX), (6) mid LCX, (7) first obtuse marginal branch of the LCX, (8) proximal right coronary artery (RCA), (9) mid RCA, and (10) distal RCA. This classification was similar in other studies (201). The average segment diameter was calculated as the mean of all diameters measured along a given segment.

Special procedures were used to replicate the biologic and imaging conditions of the original angiogram during the follow-up angiogram. The baseline angiogram was reviewed, and a copy of the baseline arteriography procedure log was taken to the catheterization laboratory as the template for the follow-up angiogram. Catheters of the same size and type were used in the follow-up and baseline assessments. The filming sequence used in the baseline angiogram was exactly replicated. For each view, the image intensifiers were restored to their positions from the baseline angiogram. Patients were positioned at the second angiogram to ensure that the coronary arteries were seen in the same place on the x-ray monitor as in the baseline angiogram. Non-ionic, low osmolarity contrast medium was used containing >300 mg I/ml and standard intracoronary nitrates to provide comparable arterial tone at both assessments. The primary measure of progression of coronary atherosclerosis was the difference in the average segment diameters between the baseline and follow-up in all analyses. In the case of total occlusion or when the area distal to occlusion could not be visualized, the segment was excluded from further analyses.

2.2.4 Inflammatory markers in the HFH study, paper II, III

Blood samples for analysis of circulating levels of cytokines were taken from the patients one year and five months (±2.5 months) after randomization. Blood samplings were conducted at 10 am ± 1 hour. Levels of high-sensitivity CRP were measured by nephelometry using N-dilutent for Nephelometry, Behring OUMT 61 (Dade Behring GmbH, Marburg, Germany). Interleukin-6 (IL-6) and interleukin-1 receptor antagonist (IL-1ra) concentrations were determined by enzyme-linked immunoassay (R & D Systems, Abingdon, UK). For IL-6, high sensitivity (IL-6hs) kits were used in order to accurately determine low cytokine levels. We used single samples to measure IL-6 and CRP, and double samples for IL-1ra. The intra-assay coefficient of variation, for CRP, IL-6 and IL-1ra, respectively, varied between 2.0-2.4%, 3.8-11.1% and 3.1-6.2%. The inter-assay coefficient of variation varied between 2,9-3.4%, 9.9-16.0% and 4,4-6,7%, respectively. The repeat determinations on the same plasma sample were highly correlated (r >0.9).

2.2.5 Depression and subjective well-being

Questionnaires measuring subjective well-being in the HFH Study, paper III

In assessing vital exhaustion, we used the Maastricht Questionnaire (202), consisting of 21 items with each item rated on a scale 0-2. To evaluate depressive symptoms the Beck Depression Inventory (BDI) (203) was used, which has 21 items rated on a 0-3 score. The concept of vital exhaustion is partially overlapping with depression, the magnitude of the shared variance is estimated between 25-50% depending on the method used to assess both constructs (204). Beck Depression Inventory overlaps with the Maastrich Questionnaire regarding the questions related to tiredness, listlessness, hopelessness, irritation, crying, sleep problems, loss of libido, but not on loss of appetite or weight, indecisiveness, self-dissatisfaction, self-accusation or suicidal ideation, while the vital exhaustion scale concentrated more on loss of vigor and fatigue. The Maastricht Questionnaire has an adequate internal consistency (Chronbach´s α=0.89).

In assessing self-rated health, patients were asked to grade their general condition during the past five years as (1) healthy, (2) reasonably healthy, (3) temporarily ill, (4) seriously ill, or (5) never being totally healthy.

Hospitalization for depression of the SHEEP study participants

The centralized Swedish health care system provides virtually complete information on all hospitalizations and corresponding diagnoses. Depression was defined as being ever hospitalized with either psychotic or neurotic depression. To identify hospitalizations with depression, we matched the unique ten digit person identification numbers of the SHEEP participants to the hospital discharge registers since 1968 until the index event hospitalization or the corresponding inclusion time for controls. The codes of International Classification of Diseases, Eighth Revision (ICD-8), from 1968 to 1986, and Ninth Revision (ICD-9), from 1986, were considered: (i) psychotic depression: all codes with 296 except for 296A, (ii) neurotic depression: 300,40 or 300E.

2.2.6 Covariates

HFH Study

Educational attainment was classified into three levels – mandatory school only, completion of high school, and college or university. Menopausal status was categorized as premenopausal, postmenopausal on hormone replacement therapy, and postmenopausal without hormone replacement therapy. Smoking status was categorized as never, current, or former smoker. History of diabetes mellitus was also assessed. Height and weight were measured, and body mass index (BMI) was calculated. All variables were obtained in a stable phase, one year and five months (±2.5 months) after randomization, except for history of diabetes mellitus and educational and menopausal status, which were assessed two months after randomization.

FemCorRisk Study

All covariates were measured during the clinical examination, that is three to six months after the hospital discharge. Menopausal status (pre-, post- or postmenopausal with hormone replacement therapy), educational attainment (mandatory school only, completion of high school, and college or university), smoking status (current, previous or non-smoker) and physical activity (active vs. sedentary, the latter one was defined as no physical exertion greater than casual activity) were assessed. History of diabetes, hyperlipidemia and hypertension, and family history of CHD were registered. Blood pressure, height and weight was measured, and body mass index (BMI) was calculated. Triglycerides, HDL, and total cholesterol were determined from fasting venous blood samples (192,194,205).

SHEEP Study

History of diabetes and hypertension, smoking (current, previous or non-smoker), physical activity (active vs. sedentary), and education (mandatory school only, completion of high school, and college or university) were assessed by questionnaires among 4069 participants (1754 cases and 2315 controls). For other covariates a health examination took place at the outpatient clinics of the 10 emergency hospitals on 2880 participants (1267 cases and 1613 controls). The appointed SHEEP nurses measured blood pressure, height and weight and collected blood samples after overnight fasting. Lipids, coagulation and inflammatory factors were determined among others (195). For cases the examination was undertaken at least 3 months after the AMI onset. For controls the examination time was as close as possible to that of 'his/her' case, to avoid biases due to seasonal variation in the blood parameters. Consequently, clinical covariate data are generally not available for those who ceased within 28 days.

2.3 Statistical Methods

2.3.1 Cross-sectional analyses (paper I, II, III - the HFH study)

Variables were logarithmically transformed if they showed skewed distribution (heart rate variability, inflammatory markers). General linear models were performed to assess uni- and multivariate associations. The multivariate models included potential confounders. The inclusion of these potentially confounding covariates was based on previous knowledge about their relationship with the variables in focus. Stratified analyses were performed as well to assess possible effect modification. To test the robustness of our findings continuous/ordinal variables were also analyzed after categorization. For the analyses, SAS 8.02 and SPSS 10.0 or 11.5 for Windows were used.

2.3.2 Longitudinal analyses (paper IV, FemCorRisk study)

When testing the association between alcohol consumption and coronary atherosclerosis progression we performed segment-specific analyses with a multi-level approach. Multi-level or mixed linear models are used to describe relationships in hierarchical data structures (206). We have two levels when working with coronary atherosclerosis progression/regression as assessed by QCA: the patient-level and the segment-level. Segments are nested within a given subject, consequently segment data in a specific subject are statistically dependent. We implemented these analyses by Proc Mixed in SAS 8.02 for Windows (207). In these models, change in the mean segment diameter served as dependent variable and we treated alcohol and the clinical covariates we adjusted for as fixed, and segments as random effect to account for the statistical dependence between segment data within an individual. We performed age- and fully-adjusted analyses, controlling for age, current smoking, BMI (in quartiles), educational status (in three levels), index event (AMI versus UAP), diabetes mellitus, sedentary lifestyle, history of hyperlipidemia, and menopausal status (in three categories). In analyses of potential intermediates on the causal pathway between alcohol use and coronary atherosclerosis, we further adjusted for history of hypertension and triglycerides (potential mediators of higher progression among drinkers) and HDL and fibrinogen concentrations (potential mediators of lower progression among drinkers) and performed stratified analyses to assess possible effect modification.

2.3.3 Prospective analyses (paper V, FemCorRisk study)

Cox proportional hazard model was used to assess the relative importance of HRV parameters in predicting mortality. First, univariate analyses were performed. Each parameter of HRV was logarithmically transformed and

entered separately as a continuous variable. Hazard ratios with 95% confidence intervals were computed for each 25% decrease of the HRV parameters.

Stepwise Cox multivariate survival analysis was performed with the following clinical variables: age, menopausal status (pre-, post- or postmenopausal with hormone replacement therapy), left ventricular function, HDL, triglycerides, total cholesterol, BMI, systolic blood pressure, smoking status (current, previous or non-smoker), use of beta-blockers, and the diagnosis at index event (AMI or UAP). Variables with $p < 0.10$ were entered and variables with $p > 0.15$ were removed from the model using the forward selection method.

The variables in the final equation served as significant determinants of mortality in the assessment of the predictive power of HRV parameters. Indices of HRV measures were tested again using the Cox model after adjustment for the aforementioned variables and age. The hazard ratios with 95% confidence intervals were recalculated for each HRV parameter.

As the proportional hazards assumption was in general not satisfied when tested for the HRV parameters, hazard ratios were also calculated separately for the first five years and for the rest of follow up using extended Cox model.

2.3.4 Case-control analyses (paper VI, SHEEP study)

Estimates of relative risks were based on odds ratios from unconditional logistic regression (SAS 8.02). The matching criteria, i.e. sex, age (in five-year age groups) and the hospital catchment area (the latter two as dummy variables) were adjusted for in all analyses. Further control was performed with socio-economic position. For the rest of the covariates we cannot differentiate between a confounder and a mediating variable, consequently when adjusting for the lifestyle factors, history of hypertension and diabetes, lipid profile, coagulation, inflammation and other covariates, we could just test if the relationship between depression and CHD is independent from these variables. We also performed stratified analyses to assess possible effect modification. In addition, we calculated odds ratio for dying within 28 days after AMI onset among the cases.

3 RESULTS

3.1 Cross-sectional results from the Healthier Female Heart Study

3.1.1 Heart Rate Variability and alcohol consumption (paper I)

Out of the 235 patients enrolled in the study three had died between randomization and present assessment, all from the control group, leaving 232 eligible patients, 113 in the intervention group, and 119 controls. One hundred and sixty six women underwent Holter monitoring, 124 patients had analyzable ECG recordings. Forty tapes were excluded having less than 50% of the original ECG recording; furthermore we excluded two patients having non-sinus rhythm. Among the 124 patients, 102 reported complete information about alcohol use, which formed the actual study population of this report (47 control, 55 treated patients). The mean age of this population was 64.4±8.1 years, somewhat higher than for the rest of the cohort (61.8±9.1, p=0.035), statin therapy (77.5% vs. 64.9%, p=0.043) was also more common among these women, while CABG (38.2% vs. 25%, p=0.032) was more, and AMI (45.1% vs. 67.7%, p<0.001) was less frequent as an inclusion diagnosis. No other study parameters were statistically different between those patients having both analyzable ECG recording and complete information about alcohol use and the rest of the cohort.

In Table 2 we present the HRV parameters and mean RR interval in the group of abstainers, light (>0-5 grams/day of alcohol) and moderate (>5 grams/day) drinkers before and after adjustment. Though, HRV parameters appeared to be higher with increasing alcohol intake, we found no statistically significant differences. However, mean RR interval was significantly differently distributed among the alcohol consumption categories; the highest values were registered among light drinkers.

Table 2. Heart rate variability parameters and mean RR interval according to daily alcohol use.

	Daily Alcohol Consumption(g/day)			P-value
	0 **n = 20**	**0-5.0** **n = 76**	**>5.0** **n = 6**	
Ln SDNN index (SE)				
Unadjusted	3.68 (0.11)	3.83 (0.06)	3.91 (0.38	0.48
Adjusted*	3.65 (0.18)	3.83 (0.13)	3.89 (0.25)	0.44
Ln Total Power (SE)				
Unadjusted	6.90 (0.23)	7.19 (0.13)	7.31 (0.83)	0.57
Adjusted	6.87 (0.39)	7.20 (0.28)	7.31 (0.56)	0.54
Ln VLF Power (SE)				
Unadjusted	6.19 (0.21)	6.39 (0.12)	6.49 (0.63)	0.73
Adjusted	5.99 (0.36)	6.27 (0.26)	6.41 (0.51)	0.58
Ln LF Power (SE)				
Unadjusted	5.57 (0.25)	5.91 (0.13)	6.06 (0.94)	0.50
Adjusted	5.59 (0.42)	5.95 (0.30)	6.08 (0.59)	0.54
Ln HF Power (SE)				
Unadjusted	5.33 (0.26)	5.68 (0.15)	5.72 (0.99)	0.57
Adjusted	5.47 (0.45)	5.85 (0.33)	5.79 (0.65)	0.58
Mean RR (SE)				
Unadjusted	884 (28)	912 (14)	775 (51)	0.04
Adjusted	850 (39)	872 (28)	730 (55)	0.03

Adjusted for age, menopausal status, body-mass index, smoking habits, educational status, history of diabetes mellitus and treatment status (intervention vs. control).

Table 3 presents the HRV parameters and mean RR interval according to the use of the three beverage types. Most of the HRV measures were significantly higher among women drinking wine in the unadjusted as well as in the adjusted models. Heart rate variability parameters also tended to be higher among beer-drinkers and spirit-drinkers when compared to those women who did not drink beer or spirits, but these differences did not reach the level of statistical significance. These findings remained essentially unchanged after further adjustment for β blockers and Ca-channel blockers medication (ln SDNNI index was 3.89 vs. 3.59 in the adjusted model for wine intake, p=0.019). Though, mean RR interval was longer among wine drinkers (p=0.026 and p=0.064 for the unadjusted and adjusted models, respectively), adjusting for mean RR interval when testing the relationship between wine drinking and HRV parameters showed similar results (for example ln SDNNI was 3.92 vs. 3.68, p=0.043 in the model with further adjustment for mean RR). Furthermore, we examined maximum heart rate as an indicator for physical activity during the 24-hour recording period and found no relationship with wine intake and virtually no effect of adjustment.

To evaluate in more detail the relation of HRV to specific beverage intake, we also categorized consumption of beverage types as non-drinkers, drinking below the median and drinking above the median. Ln SDNN index was 3.58 versus 3.96 versus 3.80 (p=0.025, adjusted model) in these three groups of wine drinking, respectively. The other HRV parameters also differed significantly among the three groups, except for HF power, which was only marginally significant. However, there were no significant differences in HRV measures among the corresponding categories of consumption of spirits or beer.

Furthermore, we performed stratified analyses in selected clinical subgroups to ensure that our results were consistent. The results were not materially different when analyses were restricted to the control or to the intervention group, to patients ≥65 years or to patients below that age, to those included with the diagnosis of AMI or those who underwent CABG or PTCA.

We also tested the hypothesis that patients drinking beer or spirits may have a trend for higher HRV only due to their higher prevalence of wine drinking. When we simultaneously controlled for intake of other beverage types, wine intake remained significantly associated with HRV (ln SDNN index among wine drinkers was 3.61, among non-drinkers 3.89, p=0.041, adjusted model). However, the tendency toward higher HRV among the consumers of beer and spirits was considerably attenuated in the simultaneous analyses (ln SDNN index 3.76 vs. 3.74, p=0.85, and 3.76 vs. 3.73, p=0.86, for drinkers versus non-drinkers for beer and spirits, respectively).

Table 3. Heart rate variability parameters and mean RR interval according to use of wine, beer and spirits.

	Consumption of Alcoholic Beverages								
	Wine		P value	Beer		P value	Spirits		P value
	Yes n = 69	No n = 33		Yes n = 52	No n = 50		Yes n = 54	No n = 48	
Ln SDNN index (SE)									
Unadjusted	3.88 (0.07)	3.65 (0.07)	0.025	3.87 (0.07)	3.74 (0.07)	0.07	3.86 (0.07)	3.74 (0.07)	0.16
Adjusted*	3.89 (0.12)	3.59 (0.15)	0.014	3.87 (0.14)	3.76 (0.13)	0.30	3.88 (0.14)	3.74 (0.13)	0.21
Ln Total Power (SE)									
Unadjusted	7.30 (0.15)	6.81 (0.16)	0.049	7.29 (0.16)	6.99 (0.16)	0.10	7.24 (0.17)	7.03 (0.16)	0.36
Adjusted	7.33 (0.28)	6.72 (0.33)	0.023	7.31 (0.31)	7.06 (0.29)	0.31	7.30 (0.30)	7.05 (0.30)	0.31
Ln VLF Power (SE)									
Unadjusted	6.50 (0.14)	6.06 (0.14)	0.062	6.43 (0.14)	6.28 (0.15)	0.24	6.46 (0.15)	6.24 (0.15)	0.18
Adjusted	6.41 (0.25)	5.80 (0.30)	0.014	6.30 (0.28)	6.21 (0.27)	0.66	6.40 (0.28)	6.11 (0.27)	0.22
Ln LF Power (SE)									
Unadjusted	6.02 (0.16)	5.49 (0.16)	0.035	6.02 (0.17)	5.67 (0.17)	0.07	5.98 (0.18)	5.70 (0.17)	0.32
Adjusted	6.09 (0.29)	5.44 (0.35)	0.024	6.08 (0.32)	5.79 (0.31)	0.25	5.77 (0.32)	6.07 (0.32)	0.27
Ln HF Power (SE)									
Unadjusted	5.78 (0.17)	5.27 (0.19)	0.089	5.84 (0.19)	5.38 (0.19)	0.06	5.67 (0.19)	5.56 (0.18)	0.75
Adjusted	5.96 (0.32)	5.37 (0.39)	0.059	6.05 (0.35)	5.61 (0.34)	0.12	5.89 (0.35)	5.72 (0.35)	0.56
Mean RR (SE)									
Unadjusted	918 (15)	857 (22)	0.026	906 (18)	890 (18)	0.51	891 (18)	906 (19)	0.55
Adjusted	872 (29)	821 (34)	0.064	871 (31)	849 (30)	0.39	852 (31)	864 (31)	0.63

Adjusted for age, menopausal status, body-mass index, smoking habits, educational status, history of diabetes mellitus and treatment status (intervention vs. control).

Summary

Intake of wine, but not spirits or beer showed a positive association with HRV parameters in women with CHD. These results were not materially different after multivariate adjustment.

3.1.2 HRV and inflammatory markers (paper II)

Of 124 patients with analyzable Holter ECG, 121 women were included in the evaluation of inflammatory markers, which formed the actual study population of this report (61 control, 60 treated patients). Their mean age was 63.7±8.6 years, while the rest of the cohort (n=111) was 62.0±8.9 years old (p=0.14). Percutaneous transluminal coronary angioplasty was more (37.2% vs. 24.3%, p=0.034), AMI was less frequent as an inclusion diagnosis (49.6% vs. 64.0%, p=0.027), respectively. No other study parameters were statistically different between those patients having both analyzable ECG recording and inflammatory markers and the rest of the cohort.

As shown in Table 4 IL-6 had a significant univariate inverse relation with all HRV parameters, except for HF power. The univariate relation between IL-6 and HF power and the relations between the levels of CRP and IL-1ra levels to HRV indices were also inverse, but weaker and non-significant. Controlling for the potential confounding factors did not attenuate the significant inverse relation between IL-6 and HRV measures. Further adjustment for β blocker, Ca-channel blocker, statin, ACE-inhibitor or aspirin medication yielded similar results.

Moreover, we performed stratified analyses in selected subgroups to ensure that our results were consistent. The results were not materially different when analyses were restricted to the intervention or to the control group, to patients ≥65 years or to patients below that age, to those included with the diagnosis of AMI or to those who underwent CABG or PTCA, respectively.

We also tested the hypothesis that the observed non-significant inverse relation between CRP and IL-1ra levels with HRV is explained by the high intercorrelation between the inflammatory markers. When we simultaneously controlled for the other two inflammatory markers, the inverse relation of IL-6 values remained similar in essence, e.g. with SDNN index: beta coefficient=-0.20, p=0.05, adjusted model.

However, the inverse relation with HRV indices was attenuated for CRP and IL-1ra in the simultaneous analyses, e.g. with SDNN index: B=-0.01, p=91; B=-0.03, p=0.79, for CRP and IL-1ra, respectively.

Table 4. Linear relation between inflammatory markers and heart rate variability indexes (N=121).

	Cytokines					
	Ln CRP		Ln IL6		Ln IL1-ra	
	Beta Coefficient (SE)		Beta Coefficient (SE)		Beta Coefficient (SE)	
	Unadjusted	Adjusted*	Unadjusted	Adjusted	Unadjusted	Adjusted
Ln SDNN index	-0.07 (0.04)	-0.06 (0.05)	-0.21 (0.07)	-0.21 (0.09)	-0.12 (0.08)	-0.09 (0.10)
P value	0.08	0.23	0.004	0.02	0.14	0.38
Ln Total Power	-0.13 (0.09)	-0.10 (0.11)	-0.43 (0.17)	-0.41 (0.20)	-0.24 (0.18)	-0.17 (0.22)
P value	0.18	0.39	0.01	0.04	0.20	0.44
Ln VLF Power	-0.14 (0.08)	-0.13 (0.10)	-0.47 (0.15)	-0.48 (0.18)	-0.21 (0.17)	-0.19 (0.20)
P value	0.09	0.20	0.002	0.009	0.23	0.36
Ln LF Power	-0.15 (0.10)	-0.10 (0.12)	-0.53 (0.17)	-0.46 (0.21)	-0.29 (0.20)	-0.20 (0.24)
P value	0.12	0.42	0.003	0.03	0.14	0.40
Ln HF Power	-0.08 (0.11)	-0.05 (0.13)	-0.34 (0.19)	-0.32 (0.24)	-0.26 (0.22)	-0.17 (0.26)
P value	0.46	0.68	0.09	0.18	0.23	0.50

Adjusted for age, menopausal status, body-mass index, smoking habits, educational status, history of diabetes mellitus and treatment status (intervention vs. control).

Summary

Concentration of IL-6 showed a negative association with HRV parameters in women with CHD even after controlling for potential confounding factors.

3.1.3 Inflammatory markers and subjective well-being (paper III)

Of the 232 eligible patients, 164 women had completed the questionnaires on depression, 168 on vital exhaustion, and 193 on self-rated health. Among these women, we also had missing values for inflammatory markers. Table 5 shows the numbers of women available for the analyses of the relationship between inflammatory markers and psychological factors.

We compared our original study population with the group of patients included for the assessment of the relation between subjective well-being and inflammation. In general, there were more diabetics among women having both valid scores for psychological factors and assessment of inflammatory markers. For instance, among women having both CRP values and depression scores, out of 157 patients there were 32 diabetics, while only 6 were diabetics from the rest of the cohort, that is out of 75 patients ($p=0.02$). Moreover, the 184 patients included in the analyses of the relationship between inflammatory markers and self-rated health were older than the others were (63.5, SD=8.6 years vs. 60.6, SD=9.0 years, $p=0.04$). However, none of the other study parameters was statistically different between those patients having both valid scores for psychological factors and inflammatory markers and the rest of the cohort.

As presented in Table 5, both CRP and IL-6 correlated significantly with vital exhaustion and self-rated health in univariate analyses. Their correlations with depression were weaker and not significant. Interleukin-1 receptor antagonist levels did not correlate significantly to any of the psychological factors.

Table 5 also summarizes the multivariate linear regression analyses for the relation between inflammatory markers and psychological factors. After controlling for the potential confounding factors, significant relations were found between IL-6 levels and vital exhaustion and IL-6 levels and self-rated health. The associations between CRP and vital exhaustion, and between CRP and self-rated health became borderline significant. Other correlations remained non-significant.

Moreover, we performed stratified analyses in selected subgroups to ensure that our results were consistent. The results were not materially different when analyses were restricted to the control or to the intervention group, to patients ≥65 years or below that age, to those included with the diagnosis AMI or those who underwent CABG or PTCA, respectively.

Table 5. Linear relation between inflammatory markers and depression, vital exhaustion and self-rated health.

	Ln CRP		Ln IL-6		Ln IL-1ra	
	Unadjusted	Adjusted*	Unadjusted	Adjusted	Unadjusted	Adjusted
	Standardized Regression Coefficients		Standardized Regression Coefficients		Standardized Regression Coefficients	
Depression	0.08	0.02	0.09	0.04	0.002	-0.03
P value	0.34	0.84	0.24	0.64	0.98	0.78
N	157	155	156	154	156	154
Vital exhaustion	0.20	0.16	0.24	0.21	0.09	0.09
P value	0.01	0.07	0.002	0.02	0.24	0.31
N	161	160	160	159	160	159
Self-rated health	0.16	0.12	0.21	0.24	0.12	0.11
P value	0.03	0.14	0.004	0.004	0.10	0.18
N	184	182	183	181	183	181

Adjusted for age, menopausal status, body-mass index, smoking habits, educational status, history of diabetes mellitus and treatment status (intervention vs. control), use of beta-blockers, Ca-channel blockers, statins, ACE-inhibitors or aspirin.

We also tested the association between cytokine levels and vital exhaustion when in addition we adjusted for depression in the multivariate models. The strength of the association decreased somewhat for IL-6, standardized beta=0.13, p=0.04. Similarly, it decreased and remained non-significant for IL-1ra. However, for CRP, the association became somewhat stronger, the standardized beta=0.16, p=0.008. Adjustment for depression also moderately decreased the strength of association between self-rated health and inflammatory markers. Furthermore, when both self-rated health and vital exhaustion were in the same model, their association with inflammatory markers decreased which indicates some overlapping of their effects. The standardized regression coefficient decreased to 0.12 (p=0.15) for vital exhaustion. However, the association between self-rated health and IL-6 levels remained significant even in this case (beta=0.18, p=0.04).

We tested the robustness of our findings when psychological factors were categorized into tertiles and tested against the inflammatory markers. We obtained essentially similar results as with the continuous approach. The following multivariate models were significant: the relationship between vital exhaustion and CRP levels (p=0.003, least square means (LSM) of ln CRP levels across the tertiles of vital exhaustion: 1.06, 0.69-1.43; 0.62, 0.22-1.01; 1.30, 0.93-1.67), vital exhaustion and IL-6 (p=0.03, LSM of ln IL-6: 1.03, 0.80-1.27; 0.98, 0.73-1.23; 1.30, 1.07-1.54), and self-rated health and IL-6 (p=0.03, LSM of ln IL-6: 0.97, 0.75-1.19; 1.09, 0.87-1.32; 1.25, 1.05-1.46).

Summary

There was a positive correlation between IL-6 levels and vital exhaustion and poor self-rated health, even after controlling for potential confounding factors. The corresponding correlation with depression was considerably weaker.

3.2 Longitudinal analyses of coronary atherosclerosis progression and alcohol consumption in the FemCorRisk Angiographic Study (paper IV)

Among the 103 patients with valid and comparable repeat QCA measurements, 93 reported complete information about alcohol use, 14 reported no alcohol consumption, 55 light drinking, and 24 moderate drinking. Among the moderate drinking women, three consumed 19-25 grams per day, two consumed more than 25 grams (i.e., two drinks) per day, and no woman consumed more than 41 grams per day. Wine was the beverage consumed in the greatest amount.

In total, we studied 649 individual coronary segments among the 93 women. Before adjustment, we found comparable progression among light drinkers and abstainers, with slight regression among moderate drinkers (Table 6).

Multivariate adjustment strengthened the inverse association, with the highest level of progression among abstainers, a similar degree of progression among light drinkers, and modest regression among moderate drinkers (Figure 2).

We performed additional sensitivity analyses to ensure our results were robust. To avoid the possibility that abstainers included women who had stopped drinking due to illness, we compared light drinkers to moderate drinkers. The multivariate-adjusted difference in progression between light and moderate drinkers was 0.146 mm (95% confidence interval, 0.075 – 0.217; $p<0.001$).

Adjusting for hypertension and triglycerides, two potential mediators of increased progression among drinkers, did not alter our results (Table 6). Additional adjustment for vitamin use yielded similar results to the base model, as did replacing hyperlipidemia with LDL level as a covariate.

We hypothesized that moderate drinkers might have less progression of atherosclerosis due to their higher levels of HDL and lower levels of fibrinogen. Models that adjusted for these factors suggested that a modest portion of the inverse association between alcohol intake and atherosclerotic progression could be attributed to HDL and fibrinogen (Table 6). For example, we found that controlling for HDL and fibrinogen concentrations attenuated the difference between abstainers and moderate drinkers by 13% and the difference between light and moderate drinkers by 12%.

We also performed stratified analyses in selected clinical subgroups to ensure our results were consistent. The relation of alcohol consumption and progression of coronary atherosclerosis was roughly inverse regardless of age, index event, body-mass index, history of hypertension, smoking status or vitamin use.

When looking separately for the different alcohol beverage types, both wine, spirits and beer consumption were inversely associated with progression of coronary atherosclerosis, with somewhat weaker results for beer than wine or spirits. These relationships were similar in analyses that simultaneously controlled for intake of other beverage types.

Summary

Moderate alcohol consumption was inversely associated with progression of coronary atherosclerosis, even after controlling for potential confounders. This association was consistent across beverage types.

Table 6. Progression of coronary artery luminal narrowing among 93 women in the FemCorRisk study, according to daily alcohol use, adjusted for potentially mediating factors.

	Daily Alcohol Consumption			P-value
	0 g/day	>0-5.0 g/day	>5.0 g/day	
No of segments analysed	73	398	178	
Unadjusted progression (mm)	0.090	0.117	-0.017	<0.001
95% CI	(0.001, 0.180)	(0.079, 0.156)	(-0.074, 0.041)	
Age-Adjusted Progression (mm)	0.089	0.117	-0.015	<0.001
95% CI	(0.000, 0.179)	(0.078, 0.155)	(-0.073, 0.042)	
Base Model-Adjusted Progression (mm)	0.123	0.127	-0.029	<0.001
95% CI	(0.013, 0.234)	(0.049, 0.205)	(-0.123, 0.064)	
Base Model + HDL -Adjusted Progression (mm)	0.120	0.124	-0.015	0.001
95% CI	(0.010, 0.230)	(0.047, 0.202)	(-0.111, 0.081)	
Base Model + Fibrinogen -Adjusted Progression (mm)	0.114	0.117	-0.033	<0.001
95% CI	(0.002, 0.226)	(0.039, 0.196)	(-0.126, 0.061)	
Base Model + Triglyceride -Adjusted Progression (mm)	0.115	0.129	-0.012	<0.001
95% CI	(0.005, 0.226)	(0.052, 0.207)	(-0.107, 0.082)	
Base Model + Hypertension -Adjusted Progression (mm)	0.122	0.127	-0.030	<0.001
95% CI	(0.011, 0.233)	(0.049, 0.205)	(-0.123, 0.064)	

Base model includes age, index event, smoking (never/former versus current), educational status (in 3 levels), diabetes mellitus, sedentary lifestyle, BMI (in quartiles), history of hyperlipidemia, and menopausal status (in 3 categories).

3.3 HRV and long-term risk assessment in the FemCorRisk Study (paper V)

During the 9-year follow-up period, there were 33 deaths including 20 from cardiovascular causes among the 251 patients who had analyzable ECG recordings at baseline. In the entire cohort of 292 patients, there were a total of 40 deaths including 23 from cardiovascular causes.

Among those whose ECG was not acceptable (17 patients) 5 had died. In more detail: 3 deaths occurred among 7 patients excluded due to more than 10% non-sinus rhythm, 2 among the 8 patients having less than 50% of the original ECG recording. In addition, there were 2 deaths among the 24 patients who did not undergo ambulatory ECG monitoring at baseline. Cox survival analyses of HRV parameters as continuous variables are shown in Table 7: the hazard ratios for each 25% decrease in SDNN index, total power, VLF power, LF power, HF power, LF/HF ratio are presented. All parameters were statistically significant all-cause mortality predictors for the whole follow up and for the first five years with the strongest effect for SDNN index. For the period after the first five years there was only a nonsignificant trend toward increased risk with heart rate variability decrease.

Using the stepwise selection method, left ventricular function (dysfunction vs. normal function), triglycerides and use of beta-blockers remained in the model as independent clinical predictors of all-cause mortality. Adjusting for these selected variables and for age, Cox regression analysis on the HRV measures was repeated (Table 7). SDNN index, total power, VLF, LF and HF power remained statistically significant predictors for the whole period and for the first five years and there was a moderate increase in their hazard ratios. LF/HF ratio was statistically significant only for the first five years. Mean RR interval was not a significant predictor in any of the models (adjusted HR for the whole period: 1.38, 0.63-3.04).

Since non-fatal cardiovascular events that occurred during the follow-up time may influence prognosis, we repeated our analyses censoring our cases at the date of a non-fatal AMI or a revascularization procedure. Fourteen deaths were preceded by any of these events resulting in 19 uncensored death cases. The results were essentially the same as without censoring, though with less statistical power. The multivariable adjusted hazard ratios for each 25% percent decrease were: 1.46, 1.00-2.14 (SDNN index), 1.17, 1.00-1.36 (total power), 1.18, 1.01-1.37 (VLF power), 1.14, 0.99-1.31 (LF power), 1.16, 0.99-1.36 (HF power), 1.14, 0.81-1.62 (LF/HF ratio).

The results were not materially different when analyses were restricted to cardiovascular mortality. However, the hazard ratios were slightly higher with somewhat wider confidence intervals. After controlling for the independent, significant predictors of cardiovascular mortality the hazard ratios for each 25% decrease in HRV parameter were, SDNN index (HR: 1.65, 95% CI= 1.10-2.47),

total power (HR: 1.24, 95% CI= 1.04-1.47), VLF power (HR: 1.26, 95% CI= 1.07-1.49), LF power (HR: 1.21 95% CI= 1.04-1.40), and HF power (HR: 1.18, 95% CI= 1.00-1.38).

Table 7. Hazard Ratios for All-Cause Mortality for each 25% decrease of the Heart Rate Variability Measures (Cox Regression)

HRV parameters		<5 years	>=5 years	whole period
SDNN index	unadjusted HR* (95% CI):	2.05 (1.38-3.04)	1.23 (0.90-1.66)	1.46 (1.15-1.86)
	adjusted† HR (95% CI):	2.11 (1.38-3.23)	1.27 (0.87-1.84)	1.56 (1.19-2.05)
Total power	unadjusted HR (95% CI):	1.35 (1.15-1.58)	1.08 (0.95-1.23)	1.17 (1.06-1.29)
	adjusted HR (95% CI):	1.36 (1.16-1.63)	1.10 (0.94-1.29)	1.21 (1.08-1.35)
VLF power	unadjusted HR (95% CI):	1.39 (1.20-1.61)	1.07 (0.93-1.24)	1.19 (1.07-1.31)
	adjusted HR (95% CI):	1.38 (1.18-1.61)	1.09 (0.92-1.29)	1.22 (1.09-1.36)
LF power	unadjusted HR (95% CI):	1.28 (1.13-1.47)	1.09 (0.97-1.22)	1.15 (1.06-1.25)
	adjusted HR (95% CI):	1.32 (1.14-1.52)	1.09 (0.95-1.26)	1.18 (1.07-1.30)
HF power	unadjusted HR (95% CI):	1.27 (1.06-1.53)	1.06 (0.94-1.20)	1.12 (1.01-1.24)
	adjusted HR (95% CI):	1.33 (1.09-1.63)	1.10 (0.95-1.28)	1.18 (1.05-1.33)
LF/HF ratio	unadjusted HR (95% CI):	1.63 (1.13-2.34)	1.25 (0.96-1.63)	1.36 (1.10-1.69)
	adjusted HR (95% CI):	1.59 (1.07-2.37)	1.01 (0.71-1.44)	1.21 (0.93-1.58)

*HR=hazard ratio for each 25% decrease of the HRV parameters, †=adjusted for age, left ventricular function, triglycerides and use of β blockers

Summary

Low HRV is a predictor of long term mortality among middle-aged women with CHD when measured 3-6 months after hospitalization for an acute coronary syndrome, even after controlling for established clinical prognostic markers.

3.4 Hospitalization for depression and AMI risk in the SHEEP study (paper VI)

Forty-seven patients and 22 controls were ever hospitalized with either psychotic and/or neurotic depression. The time between the first hospitalization for depression and the AMI varied between 113-9059, median=5553 days, i.e. 15

years and 2 months. The time from last hospitalization for depression to AMI ranged from 90 to 7591, median=4776 days. The risk of having AMI among depressed individuals was 2.9 times that of non-depressed (95 percent confidence interval, 1.7-4.8), after adjustment for the matching criteria, i.e. age, gender and hospital catchment area. In comparison, the odds ratio for diabetes was 3.6 (2.9-4.5), for hypertension 2.2 (1.9-2.5), for current 3.2 (2.7-3.7) and for former smoking 2.3 (1.9-2.7) when compared to never smokers.

Depression was associated with increased risk for AMI in a dose dependent manner. Odds ratios increased with increasing number of hospitalizations for depression (OR for a single depressive episode=2.5, 1.2-4.8; OR for 4 or more hospitalizations= 6.8, 1.5-31.3).

Depression was also associated with higher risk for death within 28 days after AMI. Fifteen cases died within this period among the 47 depressed cases, and 358 among the 1752 non-depressed cases. The odds ratio associated with depression for dying within 28 days was 1.7 (0.9 to 3.3).

As patients long before the AMI may have subclinical CHD, which in turn may facilitate depressive symptoms, we also analyzed the risk for AMI associated with depression in relation to the dates of hospitalizations for depression. We found that those who had their first hospitalization for depression before the median time between the first depression diagnoses and index event (5553 days, i.e. approximately 15 years and 2 months), were at similar risk for AMI as those hospitalized first after the median time: the matching criteria adjusted ORs were 2.8 (1.4-5.8) and 2.9 (1.4-6.0), respectively.

Table 8. presents the adjusted analyses. Additional adjustment for socio-economic position provided an essentially similar odds ratio to the only matching criteria adjusted OR: 2.9 (1.8-4.9). Further adjustment for lifestyle-related covariates, lipids, coagulation and inflammatory factors, or other variables showed only a moderate influence on the association between depression and CHD. When testing if the association between depression and AMI remains after adjustment for the well-established risk factors we extended the base model with smoking, obesity, alcohol, physical activity, triglycerides, HDL and total cholesterol, PAI-1, fibrinogen, hypertension and diabetes. These data were simultaneously available for 2536 individuals. The number of depressed cases and controls were 26 (2.4%) vs. 16 (1.1%), and the odds ratio remained elevated: 2.1 (1.1-4.2). Inflammatory factors were available in fewer subjects. The extension of the aforementioned model with inflammatory markers provided similar results, for example the odds ratio after the extension with high sensitivity (hs) CRP was 2.1 (0.9-4.5), simultaneously available for 1898 individuals. The number of depressed cases and controls were 19 (2.4%) vs. 13 (1.2%), respectively. Further extension with homocysteine levels, available for the same number of individuals as hsCRP values, provided an identical odds ratio and confidence intervals.

We also performed stratified analyses in selected clinical subgroups to ensure our results were consistent. We found roughly similar associations between depression and nonfatal AMI risk among men and women, in the six age categories, among individuals with a BMI value over and below 30 kg/m^2, among physically active and inactive subjects, never-, current-, and former smokers, and among individuals having total cholesterol ≤6.5 mmol/L and above that value.

We performed additional sensitivity analyses to ensure our results were robust. The cases and controls were recruited from Stockholm. However, before the index event many of them moved to the capital from other regions. To control for the possible effect of the different geographical regions we performed an analyses among those cases (1425) and controls (1905) who were living continuously in Stockholm since 1968, i.e. during the time we have data from the hospital discharge register. The restricted analyses showed that the risk associated with depression (matching criteria adjusted OR=2.6, 1.5-4.5) among individuals living always in Stockholm is essentially similar to that of the whole study population.

Patients hospitalized with clinical depression may get hospitalized for other reasons more frequently, which in turn can be associated with CHD risk. Therefore, as a sensitivity measure, we repeated our analyses when restricting to cases and controls that were never hospitalized for other reasons than depression or delivery. There was no indication for a decreased association between depression and AMI in this group (base model OR=5.3, 0.5-59.9).

Summary

Depression was associated with increased risk for AMI. Adjustment for potential explanatory factors just moderately attenuated the association.

Table 8. Association between depression and AMI, adjusted for potentially mediating factors

	N (%) of depressed among cases /N(%) of depressed among controls	OR (95% CI)
Base Model (adjustment for age, sex, hospital catchment area and education)	45 (2.6) / 22 (1.0)	2.9 (1.8-4.9)
Lifestyle factors:		
Base Model + smoking adjusted	45 (2.6) / 22 (1.0)	2.7 (1.6-4.6)
Base Model + alcohol	42 (2.5) / 22 (1.0)	2.8 (1.7-4.8)
Base Model + physical activity	44 (2.6) / 22 (1.0)	2.6 (1.5-4.4)
Base Model + overweight (bmi≥30)	44 (2.6) / 22 (1.0)	2.9 (1.7-4.8)
Base Model + smoking, alcohol, physical activity, overweight	41 (2.5) / 22 (1.0)	2.3 (1.3-3.9)
Lipids		
Base Model + total cholesterol	27 (2.2) / 17 (1.1)	2.3 (1.2-4.3)
Base Model + HDL	27 (2.3) / 17 (1.1)	2.4 (1.2-4.5)
Base Model + LDL	27 (2.3) / 17 (1.1)	2.4 (1.3-4.5)
Base Model + ApoA	27 (2.2) / 17 (1.1)	2.4 (1.3-4.6)
Base Model + ApoB	27 (2.2) / 17 (1.1)	2.3 (1.3-4.4)
Base Model + LP(a)	27 (2.3) / 17 (1.1)	2.4 (1.3-4.5)
Base Model + TG	27 (2.2) / 17 (1.1)	2.2 (1.2-4.1)
Base Model + total cholesterol+HDL+TG	27 (2.3) / 17 (1.1)	2.4 (1.3-4.6)
Coagulation factors		
Base Model + Fibrinogen	26 (2.3) / 16 (1.1)	2.3 (1.2-4.4)
Base Model + PAI	26 (2.3) / 16 (1.1)	2.3 (1.2-4.3)
Base Model + tPA/PAI complex	20 (2.3) / 11 (0.9)	2.4 (1.1-5.1)
Base Model + von Willebrand factor	20 (2.2) / 13 (1.1)	1.9 (0.9-4.0)
Inflammatory markers		
Base Model + hsCRP	20 (2.3) / 13 (1.1)	2.1 (1.0-4.3)
Base Model + IL6	17 (2.2) / 11 (1.3)	1.8 (0.8-3.9)
Base Model + TNFα	18 (2.2) / 13 (1.3)	2.0 (1.0-4.3)
Base Model + Homocysteine	20 (2.3) / 13 (1.1)	2.2 (1.1-4.5)
Base Model + Diabetes	45 (2.6) / 22 (1.0)	2.7 (1.6-4.5)
Base Model + Hypertension	44 (2.6) / 22 (1.0)	2.8 (1.7-4.8)

4 DISCUSSION

In these studies we identified and confirmed low HRV, lack of alcohol consumption, and depression as determinants of CHD. We also examined possible pathways and the interrelations between these three non-conventional risk indicators and inflammatory activity. As discussed below some of our findings are in agreement with most of the previous studies, in other cases conflicting results exist or the information is sparse in the literature.

4.1 Heart Rate Variability

We found that wine intake was associated with increased HRV independently of the potential confounding factors and of the intake of other beverages in women with CHD. In contrast, consumption of beer, spirits or the total amount of alcohol did not relate significantly to any of the HRV parameters (paper I).

According to the previous observations acute alcohol intake decreases HRV, especially the indexes of the vagal activity (208–211). It has also been demonstrated that chronic excessive intake of alcohol is associated with decreased HRV (212,213).

However, studies on usual daily alcohol use and HRV have provided varying and apparently opposite effects. Ryan and Howes (214) reported a negative relationship between HRV and usual alcohol intake in apparently healthy men. In the Framingham study, Tsuji et al. (52) found a positive association between HRV and alcohol intake, but the association remained no longer statistically significant after controlling for potential determinants of HRV. In line with this finding alcohol was not a significant independent predictor in the studies of Virtanen et al. (56) and Stolarz et al. (51) According to Kupari et al. (215) alcohol use is positively related to HRV in multivariate models in women, but not in men. All these studies used apparently healthy subjects or population samples, except for the investigation of Virtanen et al. (55), where newly diagnosed hypertensive patients were included in addition to healthy subjects.

Christensen et al. (54), in a study population similar to our one, showed that use of wine, but not beer, was associated with higher HRV in patients referred to coronary angiography. However, this effect was not independent of polyunsaturated fatty acids derived from fish, which was strongly related to HRV.

We also found an inverse relation between IL-6 concentration and HRV even after adjustment for potential confounding factors in women patients with CHD. C-reactive protein and IL-1ra had a non-significant inverse relation

with the HRV indices, but the strong correlation of CRP and IL-1ra to IL-6 largely explained these relations (paper II).

To the best of our knowledge our study was the first that assessed the relation of pro-inflammatory cytokines and HRV indices in CHD. Few other studies have examined this relationship in other populations. After our work was submitted, in a very recent study, Sajadieh et al. in 2004 (216) found that reduced heart-rate variability was associated with increased inflammatory activity in healthy middle-aged and elderly subjects. Aronson et al. (217) demonstrated an inverse association between IL-6 levels and long-term HRV indices in patients with decompensated heart failure, while TNF-α levels did not correlate with any of the HRV measures. In a similar study, however, there was a significant inverse association between levels of TNF-α and HRV indices in healthy subjects and in patients with mild-to-moderate heart failure (218). Serum IL-6 levels were inversely correlated with HRV in patients with metabolic syndrome (219). Moreover, subcutaneous treatment with interleukin-2 decreased HRV in patients with renal cell carcinoma (220). Furthermore, Jensen-Urstad et al. (49) showed an inverse relation between HRV and leukocyte count in healthy men, but not in women.

We also found HRV parameters to be predictors of all-cause and cardiovascular mortality in a 9-year follow-up even after controlling for established prognostic factors among middle-aged women following hospitalization for an acute coronary syndrome, in a patient population largely neglected in previous research (paper V). Our results are in accordance with previous investigations of the prognosis of post-AMI patients (19,22,33–37) and patients with unstable angina (39,221), studies, which had predominantly male populations. Furthermore, we performed the ECG monitoring in a stable phase 3 to 6 months after the admission to the hospital, while other investigators measured HRV around the hospital discharge period. This is an important difference because, as mentioned in the introduction, HRV decreases markedly after the AMI with most of the recovery period within the first 6 months (38). In patients with UAP, who are stabilized soon after hospital admission, HRV starts to increase within 48 hours of monitoring (39). Our results support those from Bigger et al. (34), who performed the HRV analysis one year after the myocardial infarction and found that HRV-parameters remain good predictors long after the coronary event.

Previous studies have examined the association of HRV with mortality for up to three years. In our study women were followed for nine years. Though, the effect of HRV on survival was stronger for the first five years, it was significant for the whole study period. However, it should be mentioned that the mortality in our study was lower than that of the previous studies. The long time interval between the acute coronary event and the HRV assessment can account for this difference.

4.2 Alcohol consumption

As mentioned in the previous section, in paper I we described a positive association between wine intake and HRV not explained by the traditional risk factors. Thus, our results, suggesting that HRV may be an important linking factor, may contribute to the understanding of the complex relation of alcohol consumption with CHD. As mentioned in the introduction, many other factors were previously suggested to be responsible for the observed positive effect of the alcoholic beverages, including increased HDL-cholesterol levels, improved coagulation profile, lower levels of inflammation, greater insulin sensitivity, reduced endothelin-1 synthesis and LDL oxidation and smooth muscle proliferation. Some positive cardiovascular effects were suggested for wine only, like the reduced endothelin-1 synthesis (92), and LDL oxidation (93). Though, it is still debated whether the preventive effect of wine is really superior to that of the other beverages (67,98,99), our results showed that wine was the only independent determinant of HRV among the alcoholic beverages, suggesting that only wine may have favorable effects mediated by this pathway.

We have also demonstrated that moderate alcohol consumption was inversely associated with progression of coronary atherosclerosis (paper IV). To the best of our knowledge this was the first study to examine alcohol consumption and coronary atherosclerosis using serial QCA analysis. The few other studies that have assessed alcohol intake and progression of atherosclerosis have done so using change in carotid intima-media thickness, which correlates only modestly with change in coronary atherosclerosis (222). Our results support the hypothesis that moderate alcohol consumption can slow progression of coronary atherosclerosis. Although direct comparison is difficult, the effect of moderate alcohol intake on mean coronary diameter in this study appears comparable with that of dietary changes or lipid-lowering therapy. We found that the multivariate-adjusted difference in progression between light and moderate drinkers was 0.146 mm (0.075 – 0.217 mm). In the Simvastatin/Enalapril Coronary Atherosclerosis Trial, mean luminal diameter progressed by 0.07 mm among subjects treated with simvastatin and 0.014 mm among subjects treated with placebo over four years (223). In the St Thomas' Atherosclerosis Regression Study, mean segment width increased by 0.003 mm with dietary intervention alone, increased by 0.103 mm with dietary intervention and cholestyramine, and decreased by 0.201 mm among the controls (224).

Interestingly, we found no substantial difference in this association in unadjusted or adjusted analyses among different beverage types. As mentioned in the introduction, previous studies suggest that HRV is most strongly associated with arrhythmic events. In the FemCorRisk study we failed to demonstrate an association between HRV and atherosclerosis progression. One can thus hypothesize that on one hand the alcohol content of the beverages is mainly responsible for slowing the atherosclerotic process, while the non-alcohol

substances present in wine can also protect from atherosclerosis-independent pathophysiological mechanisms in CHD, and this effect is partly mediated by HRV. However, further studies with larger sample sizes are needed to confirm this hypothesis.

Among the aforementioned proposed mechanism mediating the effect of alcohol on atherosclerosis we investigated the role of lipids and coagulation. Surprisingly, adjusting for the HDL and fibrinogen levels attenuated the association of moderate use and atherosclerosis by only 12-13%. Epidemiological studies indicate that HDL levels mediate approximately half of the relationship of alcohol use with incident CHD (86). On the other hand, experimental animal models suggest that alcohol intake may slow atherosclerosis predominately through non-HDL pathways (74).

4.3 Inflammation

As presented earlier, concentration of IL-6 showed a negative, independent association with HRV in women with CHD. Thus, increased inflammatory activity might represent a new auxiliary mechanism linking autonomic dysfunction, as reflected by decreased HRV, to poor prognosis in CHD. The most plausible explanation for the inverse association between HRV and IL-6 levels would be the interaction between the autonomic and immune systems. On one hand, autonomic nervous system is activated by cytokines, on the other it controls the release of cytokines (225). Concerning IL-6 levels, adrenergic stimulation has been found to facilitate, while vagal activity seems to inhibit IL-6 release (226,227). As described in the introduction, HF power is determined predominantly by the parasympathetic activity, while LF power is modulated by both the parasympathetic and sympathetic system (11,228). However, we found that the inverse association with IL-6 was somewhat more pronounced for LF than for HF power. The association with VLF power was even stronger. The origin of VLF power is not entirely clear, the thermoregulatory and the renin-angiotensin systems or other humoral factors may be responsible for these slower fluctuations in heart rate (11,228). The decrease in VLF power was suggested to be more predictive for adverse outcomes than the decrease in faster oscillations (33).

During the immune response IL-6 is known to induce the release of IL-1ra and CRP, while IL-1ra inhibits IL-6 release (229). However, only IL-6, but not CRP or IL-1ra showed a significant independent association to HRV indices in our investigation. The role of IL-6 seems to be more complex, than just being implicated in the peripheral regulation of inflammation. Interleukin-6 is also involved in hypothalamic-pituitary-adrenal axis activation and regulation of lipid and glucose metabolism; moreover IL-6 stimulates the secretion of growth hormone and arginine vasopressin and suppresses thyroid-stimulating hormone

(230,231). Furthermore, IL-6 produced by neurons and glial cells was proposed as a possible neuromodulator and neuroprotective agent (232).

We have also investigated the relationships of CRP, IL-6 and IL-1ra levels to three related constructs which assess an individual's subjective well-being in CHD (paper III). Vital exhaustion and self-rated health showed an independent association with IL-6. Their relation to CRP was weaker and only marginally significant in most of the multivariate models. There was no evidence for a relation between depressive symptoms as measured by the Beck Depression Inventory and inflammatory markers.

As mentioned in the introduction growing evidence implicates pro-inflammatory cytokines in the determination of subjective well-being, and depressed or vitally exhausted individuals show elevated cytokine levels. However, among other reports, a recent relatively well-powered study on volunteers drawn from the Whitehall II epidemiological cohort failed to document an association between depression and inflammatory markers, including circulating levels of IL-6, IL-1ra and CRP (233). Less information is available about inflammation and self-rated health. Cohen et al. (234) reported a significant, positive correlation between circulating levels of IL-6 and poor self-rated health in a community-dwelling elderly population. In addition, Lekander et al. (235) observed positive, independent correlations between poor self-rated health and circulating levels of IL-1ß, IL-1ra, and TNF-α in a primary health care population, suggesting that subjective health perceptions may be affected by cytokines as part of a generalized sickness response.

From an evolutionary perspective, it was hypothesized that behavioral changes induced by cytokines, often referred to as 'sickness behavior', represent a widespread, and highly conserved adaptive strategy. During sickness there is a need for reorganizing one's priorities, that is, to save energy for coping with the infectious pathogens and reducing the risk of predator exposure or other challenges when being in a weakened state. In this process, the immune system acts as an interoceptive sensory organ, providing information about viral or bacterial challenges interpreted by the brain as 'sickness signals' (174,175,178).

Given the fundamental role that inflammation plays in the pathogenesis of atherosclerosis, it was suggested that the observed link between depression and CHD is mediated by the increased inflammatory activity (171,190,236,237). However, very few studies examined the relationship between subjective well-being and inflammation in patients with existing CHD, and the results are conflicting. To the best of our knowledge, our is the first investigation to examine the association between self-rated health and inflammatory markers in a CHD population. Appels et al. (238) investigated 15 vitally exhausted and 15 non-exhausted patients who underwent PTCA due to severe angina. Exhausted individuals showed higher circulating TNF-α and IL-1ß levels than non-exhausted ones, and the difference in IL-6 levels was borderline significant in the same direction. Moreover, IL-1ß and IL-6 levels were significantly higher

among depressed patients than among the rest of the study population. However, when investigating CHD patients, Lyness et al. (239) found no association between IL-1ß levels and severity of depressive symptoms whether or not controlled for potential confounders. Lesperance et al. (240) found that soluble intercellular adhesion molecule 1 was the only inflammatory marker significantly related to current major depression in patients two months after hospitalization for an acute coronary syndrome. Depression was not related to IL-6, however, the authors observed an interaction between depression and statin therapy for levels of CRP. Depressed patients not taking statins had markedly higher C-reactive protein levels than did non-depressed patients. We could not detect such an interaction in our study.

We demonstrated an association between increased inflammatory activity and vital exhaustion, but not with depression. Even though vital exhaustion shows a high overlap with depression and it is not clear as to what extent it represents a distinct state, there are data indicating that the two constructs are not entirely redundant as psychosocial risk factors for CHD (241). Moreover, in a prospective population-based study of 3877 middle-aged men, Appels et al. (242), found that only feelings of fatigue, but not depressed mood or irritability, had an independent relation to incident myocardial infarctions. Interestingly, in a recent study, interferon alpha treatment of patients with chronic active C-hepatitis resulted in an increase of expressed and unexpressed sadness, irritability, insomnia, loss of appetite, and asthenia; but pessimistic or suicidal thoughts and anhedonia did not increase significantly as measured by the Montgomery Asberg Depression Rating Scale. In other words, the items more closely related to the vital exhaustion construct than to depression showed an increase in response to interferon alpha treatment (243).

In paper VI, where we defined depression as ever being hospitalized with the clinical diagnosis of depression, we found no evidence for an intermediatory effect for inflammatory markers, like IL-6, CRP and TNF-α. Though, this is in accordance with the cross-sectional results concerning depression, due to the marked differences concerning the definition of depression and the subject population, direct comparison of the two studies is difficult.

4.4 Depression

Despite of the abundant research, which was reviewed in the introduction, depression is still not accepted as a major risk factor for CHD (126,154) (paper VI). However, our data suggest that the relative risk associated with a previous hospitalization for depression is comparable to the risk of smoking. The relation between depression and AMI had a dose dependent manner, as the risk for AMI was associated with the frequency of hospitalizations for depression. In addition, depression was also associated with mortality related to AMI as

patients with a history of hospitalization for depression had a tendency toward increased risk to die within 28 days of their AMI.

As summarized in the introduction, previous research on depression and CHD has been subjected to several potential limitations. In most studies depression was assessed at one time point only, although depression is more known to have an episodic nature. Instruments used assessing depression in previous investigations may not be specific for depression, but rather reflect a general distress, thus, it could be difficult to separate depressive symptoms measured by these instruments from symptoms of a physical illness. Moreover, individuals free from clinical CHD may not be free from coronary atherosclerosis, which in turn could facilitate depressive symptoms, which makes the interpretation of the results from studies with short follow-up time difficult. Furthermore, previous reports inadequately controlled for possible confounding factors. Ideally, a study aiming to address the overall issue as to whether or not depression is associated with increased CHD risk, would have to follow a large cohort for a long period and monitor the depressive symptoms continuously and relate the severity and recurrence of depression to CHD risk. Professionals able to distinguish between depressive symptoms and similar symptoms caused by somatic disorders should preferably carry out the monitoring. An ideal study is also supposed to detect and control for potential confounders. While such a study is unlikely to be conducted in the near future, as an attempt to approximate the continuous monitoring by professionals we used the data of the Swedish Hospital Discharge Register and we defined depression in a severe and specific form, i.e. hospitalization for clinical depression, and assessed the effect of cumulative exposure to depressive symptoms long before AMI. Our definition seems to be very specific reflecting a truly severe state of depression. It implies that non-psychiatric causes of symptoms reminiscent to depression were probably carefully excluded. We believe that due to this definition and due to the long time between the hospitalization for depression and the coronary event it is unlikely that depression in our study could simply reflect a poor physical status or a subclinical CHD.

In our study, depressed individuals even 15 years after their initial diagnosis were at increased risk for AMI. Due to this long time interval it is reasonable to presume that depression is associated with the atherosclerotic process. Among others, two recent studies with large sample sizes investigated the relationship between atherosclerosis and depression. O'Malley et al. (244) found no correlation between depressive symptoms assessed by a questionnaire and coronary calcification score as measured with electron-beam computed tomography. However, Jones et al. (245) assessed lifetime history of depression by structured interviews and found that recurrent major depressive episodes were associated with carotid atherosclerosis, while there was no such association for a single major depressive episode.

As described in the introduction there are several potential routes by which depression may impact upon CHD and the underlying atherosclerotic process. Our study extensively included covariates potentially able to explain the observed relation between AMI and depression. Among the well-established risk factors, most of the previous studies on depression and CHD risk considered some of the lifestyle factors, especially smoking. Relatively high proportion of the studies measured at least one of the lipids (128,134–137,140,141,145,146). Though, hypercoagulability was suggested as a potential pathway (171) only Ariyo et al. (134) evaluated coagulation factors in their prospective study, and found a positive correlation between depressive symptoms and fibrinogen. However, the effect of adjustment for fibrinogen on the strength of the prospective association between depression score and CHD was not reported. Moreover, none of the aforementioned studies on CHD and depression measured inflammatory factors.

In our study, adjusting for the potential mediators just moderately attenuated the association between CHD risk and depression, suggesting that lifestyle, lipids, coagulation, inflammation and other factors could only partly explain the observed relationship. As mentioned in the introduction other proposed explanatory mechanisms are decreased HRV (171) or a common genetic background (172), which were not evaluated in the SHEEP study, neither were these variables assessed in any of the previous studies on depression and CHD risk.

4.5 Limitations

Several potential limitations of our findings need to be considered. Some limitations apply to all these studies, some are more specific ones.

4.5.1 General limitations

All these studies had an observational nature. As with any observational study, unevenly distributed characteristics associated with the variables of interest could lead us to an over- or underestimation of the true associations. Though, we report results of multivariate adjustments for the potential confounders measured in our studies, we cannot exclude the possibility of residual confounding. At the same time, a remaining confounder would need to be associated with the variables in interest and generally unrelated to the factors included in our multivariate analyses.

Generally, our results are limited by the small sample sizes. Though, several associations were statistically significant at these levels of statistical power, caution is needed for interpreting a lack of statistical evidence as a negative finding. Moreover, the small number of participants restricted the number of covariates we could control for in our multivariate models and we could not use

extensive categorization of our variables in order to describe the nature of the relationships in more detail.

Except for the SHEEP study our data were collected only from rather specific populations of women patients with CHD, and generalization of our findings to men or other populations is not obvious. However, as mentioned earlier this group of patients was largely neglected in previous investigations.

Direction of causality cannot be inferred from cross-sectional studies, consequently interpretation of the interrelationships between the four non-conventional risk factors (papers I-III) is necessary speculative.

4.5.2 Specific limitations

HRV. When HRV is derived from Holter monitoring missing cases are quite frequent, mostly attributable to the inadequate quality of recording, and neither the HFH nor the FemCorRisk study were exceptions. Thus, HRV values were not available for many cases in our analyses. We believe that even the relatively large number of missing cases is not a probable cause of a bias as the lack of the appropriate attachment of the electrodes is unlikely to be associated with the factors investigated in our studies.

Alcohol consumption was self-assessed using a standardized questionnaire, which may lead to an under or over-estimation of the real intake. However, there is no reason to believe that for example patients with lower HRV or higher atherosclerosis progression would underestimate more their alcohol consumption. This questionnaire has also shown an excellent correlation to alcohol consumption as measured by four 1-week diet records (obtained 3-4 months apart) in a Swedish female cohort (199). Another potential limitation of the observed associations with alcohol is the possibility that some women ceased drinking in response to the severity of their illness. Since we have no information on drinking habits in the past, the 'sick quitter' hypothesis (78) could be an alternative explanation for our findings. Moreover, as alcohol consumption was rather modest among patients both in the HFH and in the FemCorRisk studies, we cannot extrapolate our results to heavier alcohol use.

Inflammatory activity was assessed in the HFH study by means of circulating levels of CRP, IL-6 and IL-1ra, and by means of circulating CRP, IL-6 and TNF-α levels in the SHEEP study. However, as mentioned in the introduction, there are many other indicators and methods to investigate inflammation, consequently we cannot exclude the possibility that different methods would lead to different results and conclusions.

Depression and subjective well-being. In paper III the lack of association between depression and inflammation could be attributed to our method measuring depression, and we can not exclude the possibility that using other

questionnaires than the Beck Depression Inventory, or defining depression with diagnostic interviews would lead to different results.

In paper VI we gathered information on previous hospitalization with depression by an automated search through computerized register data, excluding potential sources of different biases. We emphasize that in Sweden, the health care system is equally accessible to all citizens and also that the participation in the hospital registers is unavoidable. Consequently, all patients who sought help for severe depression or for AMI symptoms had the possibility to get adequate treatment and consequently being registered. However, we had no data before 1968 and even in 1968 the hospital discharge register did not cover the whole country. Nevertheless, it is important to recognize, that these potential limitations would tend to increase random misclassification of our data, which would lead to an underestimation of true effects. Furthermore, as a sensitivity measure we restricted our analyses to those study participants who were continuously residents of Stockholm since 1968, and found no substantial difference when comparing to the whole study group.

We defined depression as hospitalization for the clinical diagnosis of depression. Using this definition we can assume that those classified as depressed in our study, were truly depressed and physical causes behind depressive symptoms were most probably carefully excluded. On the other hand, as certainly many individuals were mildly or even severely depressed among the "non-depressed" group during the follow-up period or before 1968, our sensitivity seems to be quite low. However, one of the major limitations of the previous studies was the low specificity (153,154) and the effect of the low sensitivity would again lead more to an underestimation of the true effect than vice versa.

Information on the medication for the depressive episodes was not available, so consequently we cannot draw any conclusion about the effect of antidepressive medication. It is not clear whether the observed association between hospitalization for depression and CHD risk is attributable to depression itself or to the antidepressive medication. However, we found that the risk associated with depression was rather stable over the time. Those who had their first hospitalization for depression before the median time between the first depression diagnoses and index event (15 years and 2 months), were at the same risk for AMI as those hospitalized first after the median time. This does not support the role of the medication as during this period the antidepressant medication has changed substantially.

5 CONCLUSION

HRV, alcohol consumption, inflammation and depression are among the potentially most important novel non-conventional risk and prognostic factors for CHD. In this thesis we investigated their interrelations and their relation to CHD as summarized in Figure 3.

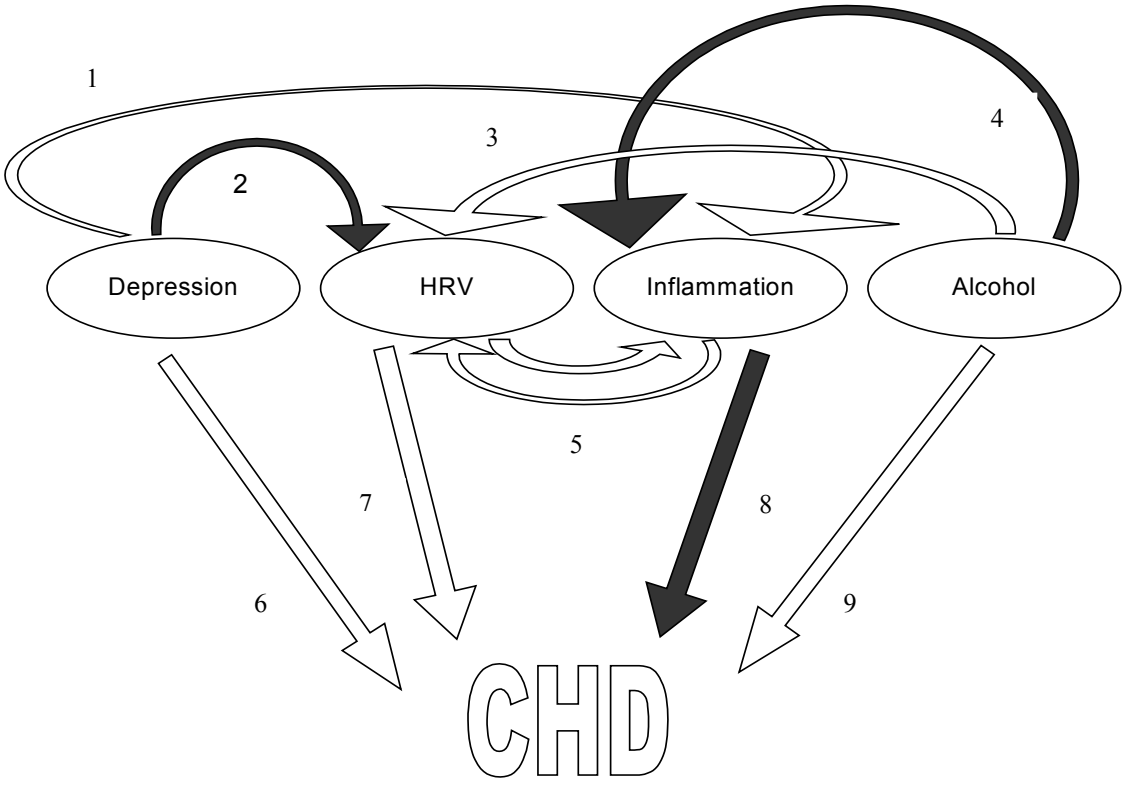

Figure 3. Suggested interrelationships between the non-conventional risk factors and their relation to CHD.

1. depression and inflammation: not confirmed by our results;
2. depression and HRV: not investigated in this thesis, but it is a well established relationship based on previous studies;
3. alcohol and HRV: supported by our findings (at least for wine drinking);
4 alcohol and inflammation: suggested by other studies, not investigated here;
5. interrelationship between inflammatory and autonomic activity: supported by our findings;
6. depression and CHD risk: supported by our findings;
7. autonomic activity and CHD prognosis: supported by our findings;
8. inflammation and CHD: not investigated in this thesis but solid finding in the literature;
9. alcohol and CHD: supported by our findings;

In the cross sectional analyses of the HFH study, we investigated the associations between HRV and alcohol, HRV and inflammatory markers, and inflammatory markers and indices of subjective well-being. We concluded that the protective effect of alcohol, or at least wine intake, on atherosclerosis progression and/or on arrhythmic events could be partly attributable to its positive association with HRV. Our results also suggested that the effect of HRV is partly mediated by the inflammatory activity and/or the increased inflammatory activity is acting through the alteration of the autonomic nervous system. Finally, we found that inflammatory activity, reflected by the IL-6 and CRP levels, is associated with vital exhaustion and self-rated health but we found no support for such an association with depressive symptoms as measured by the Beck Depression Inventory. Thus, on one hand, these results provide further evidence for a possible psychoneuroimmune link between mental state and CHD, and suggest that cytokine-induced sickness response in CHD may be better represented by constructs of vital exhaustion and self-rated health as compared to depression as defined by the Beck Depression Inventory. On the other hand, and this was also supported by our findings in the SHEEP study, inflammation is unlikely to be a major mediator between depression and CHD.

In the longitudinal FemCorRisk Angiographic Study we demonstrated that alcohol consumption at a moderate level is associated with slower progression of atherosclerosis in human coronary arteries. No beverage type appeared to confer particular benefit and coagulatory factors, lipids, history of hypertension could just partly explain the observed relationship. However, inflammation, and other potentially important explanatory factors were not assessed in the FemCorRisk study.

Analyzing the 9-year follow-up of the FemCorRisk study we concluded that the HRV parameters are long-term prognostic predictors of all-cause and cardiovascular mortality in middle-aged women surviving an acute CHD event. Since previous studies were carried out predominantly in men and results can hardly be extrapolated to women as women differ from men both in their cardiac autonomic control and in their CHD prognosis, our results may have direct clinical relevance.

In the SHEEP study we found that hospitalization for depression, especially if repeated, was a considerable risk factor for AMI, and was also associated with poor short-term prognosis after the coronary event. Socio-economic position, lifestyle factors, lipid profile, coagulation, inflammatory and other factors could only partly explain our findings. Confounding from poor physical conditions, subclinical CHD or other somatic causes of depressive syndromes are unlikely to account for our findings in these settings.

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