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Plasma levels of matrix metalloproteinase-9 are independently associated with psychosocial factors in a middle aged normal population.

Running title: MMP-9 and psychosocial factors

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Abstract

Objective: Psychosocial factors have been associated with inflammatory markers, and are of prognostic significance for coronary artery disease (CAD). The degrading enzyme matrix metalloproteinase-9 (MMP-9) is up-regulated in inflammatory processes and hypothesized to play a role in the rupture of atherosclerotic plaques. This study aimed at testing the association between psychosocial factors and circulating levels of MMP-9 in a normal population sample.

Methods: 402 participants (50 % women), aged 45 to 69 years, were randomly drawn from a normal population. Psychosocial instruments covered depression (CES-D), vital exhaustion, hostile affect, cynicism, mastery, self esteem, sense of coherence (SOC), emotional support, and social integration. Plasma MMP-9 was measured by an ELISA method. Linear regression models were adjusted for age, sex, known CAD, rheumatoid arthritis, cancer, cardiovascular risk factors including C-reactive protein and ongoing medication.

Results: After full adjustment, there were independent associations of elevated MMP-9 levels with CES-D (+2.9 ng/mL per SD, $p=.02$), hostile affect (+3.0 ng/mL per SD, $p=.02$), cynicism (+3.5 ng/mL per SD, $p=.006$) and SOC (-2.5 ng/mL per SD, $p=.046$). A principal component analysis extracted three components. The first was mainly extracted from CES-D, vital exhaustion, self esteem, mastery and SOC, and the second mainly from hostile affect and cynicism. Both were independently associated with MMP-9 ($p=.02$, $p=.04$) when run in the same model.

Conclusions: MMP-9 levels were associated with psychosocial factors in a middle aged normal population sample, independently of traditional risk factors. The findings may constitute a possible link between psychosocial factors and cardiovascular risk.

Key Words

depression; hostile affect; cynicism; sense of coherence; metalloproteinases; cardiovascular

Acronyms used in text

CAD = coronary artery disease

CES-D = Center for Epidemiological Studies Depression Questionnaire

CRP = C-reactive protein

EDTA-plasma = ethylene diamine tetraacetic acid treated blood plasma

HDL = high density lipoprotein

IL-6 = interleukin-6

IQR = interquartile range

LDL = low density lipoprotein

LSH-study = Life conditions, Stress and Health study

MMP-9 = matrix metalloproteinase-9

SOC = Sense of Coherence

SBP = systolic blood pressure

SD = standard deviation

Introduction

The prognostic significance of psychosocial factors for coronary artery disease (CAD) has been established in a substantial body of literature (1-8). In particular, the associations have been found for depression, both at clinical and sub-clinical levels (1-4), but also for cynicism (3-6) and lack of psychosocial resources such as low mastery and sense of coherence (3, 7, 8). As an inflammatory process in the arterial wall seems to be of crucial importance for the development of atherosclerosis (9-11), the association between psychosocial factors and inflammation are of plausible interest when explaining the epidemiologic findings of psychosocial factors and CAD (12). There are a number of studies demonstrating that psychosocial factors are independently associated with inflammation. More specifically, e.g. depressive symptoms, vital exhaustion, cynicism, hostile affect, mastery and self esteem have been linked to elevated levels of inflammatory markers such as interleukin-6 (IL-6), and C-reactive protein (CRP) (13-17). However, there is some inconsistency in the literature (18, 19). In a recent epidemiological study from the Whitehall II study on civil servants free from clinically validated coronary heart disease (n=6,396), Nabi and colleagues conclude that “Our prospective study suggests that major inflammatory markers such as pro-inflammatory cytokines (IL-6) and acute phase reactants (CRP and fibrinogen), do not mediate the association between psychosocial factors and coronary heart disease incidence” (19). This conclusion calls on the need for studies of other inflammatory markers, to further understand the role of inflammation in the link between psychosocial factors and CAD. In this context, it has been argued that stress and psychosocial factors might exert both direct effects on inflammation as well as an indirect effect via behavioral factors such as smoking, high alcohol intake, poor eating habits and poor physical exercise, where both pathways contribute to cardiovascular risk (20, 21). If so, a plausible marker should be associated both with traditional cardiovascular risk factors and psychosocial factors independently from each other.

In this study, we used the following five requirements to identify and examine a marker that could be of relevance in the exploration of this link: 1) Plausible to have a direct impact on plaque vulnerability (22-27). 2) Elevated in patients diagnosed with angina or myocardial infarction (24, 27-32). 3) Associated with cardiovascular risk factors before onset of disease (32-36). 4) Upregulated by pro-inflammatory cytokines (37-39). 5) Directly regulated by stress hormones (40-42). When matching requirements with a literature search over theories and empirical data, the enzyme family of matrix metalloproteinases (MMPs) emerged as a group of relevance. MMPs constitute a family of enzymes with the ability to degrade all components of the extra cellular matrix. At present, 23 different human MMPs have been categorized and described, with partially overlapping substrate specificity (31). MMPs are considered to be a key determinant of extra cellular matrix degradation, and are essential for various physiological processes such as matrix remodeling, cell migration and angiogenesis, under which the expression of MMPs is strictly regulated. The choice of MMP-9 as the main outcome was based on the fulfilling of each of the requirements above. Moreover, the substrate specificity to collagen type IV and other proteins in the basal lamina hints at a potential proximal role of vascular remodeling. It has been shown that unstable, inflammatory active plaques that are prone to rupture, have an increased expression of MMP-9 as compared to stable plaques, indicating a possible role of MMP-9 in fibrous cap degradation leading to plaque rupture and acute cardiovascular events (43-45). In accordance, it has been pointed out that circulating MMP-9 is an independent predictor of new events in patients with stable coronary disease (28). The synthesis and secretion of MMP-9 is induced during the inflammatory response, primarily by the pro-inflammatory cytokine interleukin-1 (IL-1) (37), or other pro-inflammatory pathways (38, 39). In a previous study from our group, it has been pointed out that there is a significant correlation between MMP-9 and CRP, but the fairly low

coefficient ($r=0.2$ $p<.001$) suggests that CRP and MMP-9, at least in part, may be markers of different physiological pathways, or different stages in an inflammatory process (32).

This study aimed to test if there is an association between psychosocial factors and circulating levels of MMP-9 in a middle aged normal population sample, independent of known diseases, cardiovascular risk factors including CRP and ongoing medication.

Methods

Study population and design

Participants were recruited from the Life conditions, Stress and Health (LSH) study, a prospective study aiming at testing if psychobiological pathways mediate the association between socio-economic status and incident cardiovascular disease. Data collection was conducted in late 2003 and early 2004, constituted by a brief health examination, collection of blood samples and questionnaires. The participants were randomly chosen within the county of Östergötland in the southeast of Sweden, with a response rate of 62.5 % (46). Exclusion criteria were self-reported severe disease that hindered the possibility to participate, e.g. terminal cancer, severe dementia and psychiatric disorders. The sample was representative for the population in terms of educational attainment, employment rates and immigrant status, and evenly distributed by sex and age, ranging from 45 to 69 years at enrolment. An *a priori* power calculation based on assumptions regarding the prevalence of CAD risk factors and MMP-9 distribution in a normal population was conducted, yielding an estimation of 400 participants to be sufficient in order to show significant associations between CAD risk factors and MMP-9. 402 individuals, randomly chosen within the first 1,000 participants in the LSH-study were included in this sub-study, without any stratification criteria such as sex, age or other properties.

The participants came to a Primary Health Care Centre where a brief health examination was conducted, and blood samples were collected. All samples were collected in the morning, in a fasting state. Systolic and diastolic blood pressure were measured in a sitting position in two minutes interval after five minutes of rest, using the mean of second and the third measurement (Omron M5-1 digital). For full information on data collection, see Hollman and Kristenson (46). The study design was approved by the ethical committee of the medical faculty, Linköping University, and written consent was obtained from all participants.

Data from questionnaires

Nine instruments covering psychosocial factors that either have been previously shown to be associated with risk of CAD events (1, 3, 4, 47-49), or have been associated to inflammatory markers (13, 16) were used. Psychosocial risk factors were measured by the following four instruments: Radloff's Center for Epidemiological Studies Depression (CES-D) questionnaire (50), a slightly modified version of the Maastricht Questionnaire for vital exhaustion (51), with 19 items instead of the original 21, and the two subsets of hostile affect and cynicism from Cook-Medley's Hostility scale (48). Five instruments were used for psychosocial resources: Pearlin's Mastery scale (52), Pearlin's Self esteem scale (52), Antonovsky's sense of coherence (53), Orth-Gomér's emotional support (54) and Orth-Gomér's social integration (54), respectively.

To capture any previous events of myocardial infarction, the question "Have you ever had any event of myocardial infarction diagnosed by a medical doctor? (Yes/No/Don't know)" was used. Questions regarding angina pectoris, stroke, diabetes, and cancer had a similar construct. Diagnose of rheumatoid arthritis was captured using the question "Have you ever had any other chronic or long term disease diagnosed by a medical doctor? (Yes/No/Don't know. If yes, please specify)"

Data on the behavioral factors smoking, alcohol intake, physical exercise and fruit intake was collected, described in detail elsewhere (32).

The participants were further asked to specify any ongoing medication for any disease, taken on a regular basis. All medications were listed and grouped by the Anatomical Therapeutic Chemical (ATC) classification system.

Biochemical analyses

Concentrations of MMP-9 were measured in EDTA-plasma (55) by human Biotrak ELISA systems (Amersham Biosciences, Uppsala, Sweden). The assay for MMP-9 measures MMP-

9, Pro-MMP-9 and the ProMMP-9/Tissue inhibitor of metalloproteinases (TIMP)-1 complex. The lower detection limit was 0.6 ng/mL, interassay coefficient of variance (CV) was 7.2 to 7.9 %. C-reactive protein (CRP) was measured in serum by a highly sensitive latex-enhanced turbidimetric immunoassay (Roche Diagnostics GmbH, Vienna, Austria) with a lower detection limit of 0.03 mg/L and CV of 1.7%. Plasma glucose and lipids were analyzed directly after sample collection, and LDL-cholesterol was calculated using Friedewald's formula (56). Aliquots of plasma and serum (0.5 mL) were stored in -70° Celsius approximately 18 months before laboratory analysis of MMP-9 and CRP.

Statistical analysis

Continuous data on MMP-9 was set as the main outcome. Outliers were identified as being more than 3 standard deviations higher than the included top level of MMP-9. Mean values with standard deviation, medians with interquartile range and regressions were calculated after exclusion of outliers. t-tests were performed for mean comparisons. Partial correlations between different psychosocial instruments were performed, adjusting for age and sex.

Regression models on possible confounders i.e. known diagnoses, cardiovascular risk factors and ongoing medication were run on continuous data where applicable or on categories (dummies or ordinal), respectively. Regression models on psychosocial instruments were run on continuous data and were performed in six steps. 1) A crude association was tested, adjusting solely for age and sex. Based on the results for possible confounders, regressions were then, apart from age and sex, adjusted for 2) self-reported diagnoses, 3) cardiovascular risk factors including CRP and 4) ongoing medication in an additional manner. To further eliminate diagnoses as a possible confounder for the crude associations, regressions were run 5) excluding participants with diagnoses that seem to be related to MMP-9 levels. In the last set of models, 6) both participants with self-reported diagnoses and ongoing medication were

excluded, thus adjusting for age, sex and cardiovascular risk factors including CRP in a population free of disease and without any ongoing medications.

To facilitate comparison of beta coefficients for different psychosocial instruments in the regression models, all beta coefficients were expressed as increase per standard deviation. Two different principal component analyses (unrotated and varimax rotation) were performed on the psychosocial instruments, extracting the instruments into components to see how many dimensions the nine psychosocial instruments were composed of.

A value of $p \leq .05$ was considered as statistically significant.

Analyses were performed in STATA statistical software, release 6.0, Stata Corporation, and SPSS for Windows statistical software, release 15.0, SPSS Inc.

Results

MMP-9 levels were detected in all participants. 2 outliers were identified and excluded (224.4 and 246.1 ng/mL, respectively). The mean and standard deviation for the remaining participants ($n=400$) were 39.2 ng/mL (SD 22.8 ng/mL), ranging from 2.9 to 143.9 ng/mL. Women had significantly lower levels than men (dif= -8.3 ng/mL, $p<.001$), whereas no significant associations could be found with age.

Descriptive statistics on self-reported diagnoses, individual characteristics and ongoing medication and their associations with MMP-9 are shown in table 1. There were in total 25 groups of medicines tested (grouped by ATC code classification), of which 16 groups were reported by five or more participants. All 16 groups were used in the same regression testing the associations with MMP-9, as shown in table 1. Only the groups with a p-value lower than 0.50 were listed in the table. Results from the other medication groups are not shown, as there was a supposedly low confounding effect on MMP-9 levels in this study.

The distribution of psychosocial instrument scores in the study population is presented in table 2. In a crude analysis, adjusting solely for age and sex, there were significant

Table 1: Descriptive statistics of the population sample. Regressions of MMP-9 levels adjusted for age and sex, apart from regressions on medications, adjusted for age, sex, myocardial infarction, angina pectoris, rheumatoid arthritis and cancer with ongoing treatment, and other medications. n=372 to 400. Beta coefficient expressed as increase of plasma levels of MMP-9 (ng/mL) per dichotomy, category or SD increment.

	n with data	Prevalence (%) or mean (SD)	Beta (95 CI)	p-value
Sex (women/men)	n=400	n=199 (50 %)	-8.3 (-12.8;-3.9)	<.001
Age (5 year categories)	n=400	57.1 (7.2)	-1.2 (-2.8;.4)	.12
Self-reported diagnoses				
Myocardial infarction (y/n)	n=390	n=4 (1 %)	34.0 (12.0;56.0)	.003
Angina pectoris (y/n)	n=389	n=13 (3 %)	13.6 (1.2;26.0)	.03
Stroke (y/n)	n=388	n=7 (2 %)	0.4 (-16.2;17.1)	.96
Rheumatoid arthritis (y/n)	n=400	n=7 (2 %)	12.2 (-4.7;29.2)	.16
Diabetes (y/n)	n=391	n=27 (7 %)	.0 (-8.9;9.0)	.99
Cancer, ever in lifetime (y/n)	n=387	n=12 (3 %)	-4.0 (-17.1;8.9)	.54
Cancer with ongoing treatment (y/n)	n=400	n=2 (1 %)	-22.6 (-54.0;8.7)	.16
Clinical and laboratory characteristics				
BMI (kg/m ²)	n=394	26.7 (4.3)	1.2 (-1.1;3.5)	.29
Systolic blood pressure (mm Hg)	n=394	133.4 (2.9)	3.4 (1.0;5.9)	.004
Diastolic blood pressure (mm Hg)	n=395	84.3 (11.9)	2.6 (.3;4.9)	.02
LDL (mmol/L)	n=394	3.4 (.8)	-0.3 (-2.6;1.9)	.78
HDL (mmol/L)	n=399	1.6 (.4)	-2.2 (-4.6;.2)	.07
Triglycerides (mmol/L)	n=399	1.3 (.8)	3.3 (1.0;6.0)	.004
CRP (mg/L, quartiles)	n=389	.8 (.3;2.2) ^a	3.7 (1.8;5.8)	<.001
Behavioral characteristics				
Smoking (y/n)	n=386	n=63 (16 %)	19.2 (13.2;25.0)	<.001
Alcohol intake (3 ordinal cat.)	n=395	n=34;46 (9;12%) ^b	6.7 (3.2;1.3)	<.001
Physical activity (3 ordinal cat.)	n=372	n=282;71 (76;19%) ^b	-6.7 (-11.7;-1.8)	.007
Veg and fruit intake (3 ordinal cat.)	n=396	n=287;54 (72;14%) ^b	-5.2 (-9.5;-9)	.02
Medication				
Antihypertensive medication (y/n)	n=400	n=65 (16 %)	3.6 (1.0;15.3)	.03
Cholesterol-lowering medication (y/n)	n=400	n=31 (8 %)	-8.5 (-19.2;2.1)	.11
Glucose-regulating medication (y/n)	n=400	n=13 (3 %)	-8.8 (-22.6;5.0)	.21
Bronchial dilatation (y/n)	n=400	n=14 (4 %)	7.1 (-7.2;21.4)	.33
Sex hormones (oestrogens) (y/n)	n=400	n=7 (2 %)	6.3 (-1.6;23.4)	.46
Acid inhibitor for gastric ulcer (y/n)	n=400	n=12 (3 %)	-15.2 (-29.7;-8)	.04
Anti-inflammatory medication (y/n)	n=400	n=23 (6 %)	6.7 (-4.0;17.5)	.22
Sedative medication (y/n)	n=400	n=15 (4 %)	-8.6 (-23.9;6.6)	.27
Antidepressive medication (y/n)	n=400	n=16 (4 %)	7.5 (-6.0;21.1)	.28

a CRP expressed as interquartile range

b The two top categories

c Sixteen groups of medication were used in the model. Medication with p<.50 shown in table. Medications for glaucoma, benign prostate hyperplasia, osteoporosis, allergies, rheumatoid arthritis, hypothyroidism and analgetica were also included in the model, data not shown.

associations to all psychosocial instruments tested, with the exception of vital exhaustion. The levels of MMP-9 are further illustrated in figure 1, showing the distribution of MMP-9 over quartiles of the nine instruments tested, adjusted for age and sex. In regression models running quartiles as continuous variables adjusted for age and sex, there were significant positive trends on CES-D (p=.004), hostile affect (p=.01), cynicism (p=.001) and a significant

Table 2: Psychosocial instruments used. I) Psychosocial risk factors II) Psychosocial resources. Regression on MMP-9 and psychosocial score as continuous data, adjusted for age and sex. Beta coefficient expressed as increase of MMP-9 in ng/mL per SD (95% CI).

Psychosocial instrument	a) n with data	b) Range in instrument	c) Range in study pop.	d) Median and IQR	e) Mean and SD	f) regression MMP-9 vs score, adj for age and sex
I. CES-D (Radloff)	n=381	0-60	0-47	7 (3;12)	8.4 (7.4)	3.1 (0.8;5.5), p=.007
Vital exhaustion (Appels)	n=391	19-57	19-56	29 (24;35)	29.8 (7.2)	1.0 (-0.5;3.2), p=.13
Hostile affect (Cook-Medley)	n=389	5-25	5-20	11 (9;13)	11.4 (2.8)	2.8 (0.6;5.2), p=.013
Cynicism (Cook-Medley)	n=391	12-60	12-52	32 (26;37)	31.3 (8.1)	3.8 (1.5;6.2), p=.001
II. Mastery (Pearlin)	n=381	7-28	7-28	23 (20;25)	22.7 (3.4)	-2.8 (-5.2;-0.5), p=.02
Self esteem (Pearlin)	n=377	10-40	17-40	33 (30;36)	32.4 (4.5)	-3.0 (-5.4;-0.7), p=.01
SOC (Antonovsky)	n=389	13-91	39-90	70 (63;76)	69.0 (4.5)	-2.7 (-5.0;-0.4), p=.02
Emotional support (Orth-Gomer)	n=389	0-6	0-6	6 (6;6) ^a	5.5 (1.1)	-2.4 (-4.7;-0.1), p=.04
Social integration (Orth-Gomer)	n=388	6-36	6-36	20 (17;25)	20.7 (5.8)	-2.7 (-5.0;-0.4), p=.02

a IQR not possible as 75 % of the study population had a score of 6 (maximum of scale).

Table 3: Partial correlations adjusted for age and sex. I) psychosocial risk factors. II) psychosocial resources. n=368 to 386.

	I.				II.				
	CES-D	Vital exhaustion	Hostile affect	Cynicism	Mastery	Self esteem	Sense of coherence	Emotional support	Social integration
Psychosocial instrument	r	r	r	r	r	r	r	r	r
I. CES-D	1.00	0.73 (p<.001)	0.17 (p<.001)	0.08 (p=.13)	-0.51 (p<.001)	-0.50 (p<.001)	-0.56 (p<.001)	-0.30 (p=<0.001)	-0.38 (p<.001)
Vital exhaustion		1.00	0.20 (p<.001)	0.11 (p=.03)	-0.55 (p<.001)	-0.55 (p<.001)	-0.64 (p<.001)	-0.21 (p<.001)	-0.34 (p<.001)
Hostile affect			1.00	0.42 (p<.001)	-0.25 (p<.001)	-0.25 (p<.001)	-0.38 (p<.001)	-0.16 (p=.001)	-0.17 (p=.001)
Cynicism				1.00	-0.14 (p=.005)	-0.20 (p<.001)	-0.30 (p<.001)	-0.12 (p=.02)	-0.13 (p=.002)
II. Mastery					1.00	0.71 (p<.001)	0.61 (p<.001)	0.18 (p<.001)	0.36 (p<.001)
Self esteem						1.00	0.61 (p<.001)	0.19 (p=.02)	0.38 (p<.001)
Sense of coherence							1.00	0.23 (p<.001)	0.40 (p<.001)
Emotional support								1.00	0.35 (p<.001)
Social integration									1.00

negative trend on emotional support ($p=.03$). Close to significant negative trends were found on self esteem (.07) and SOC ($p=.05$).

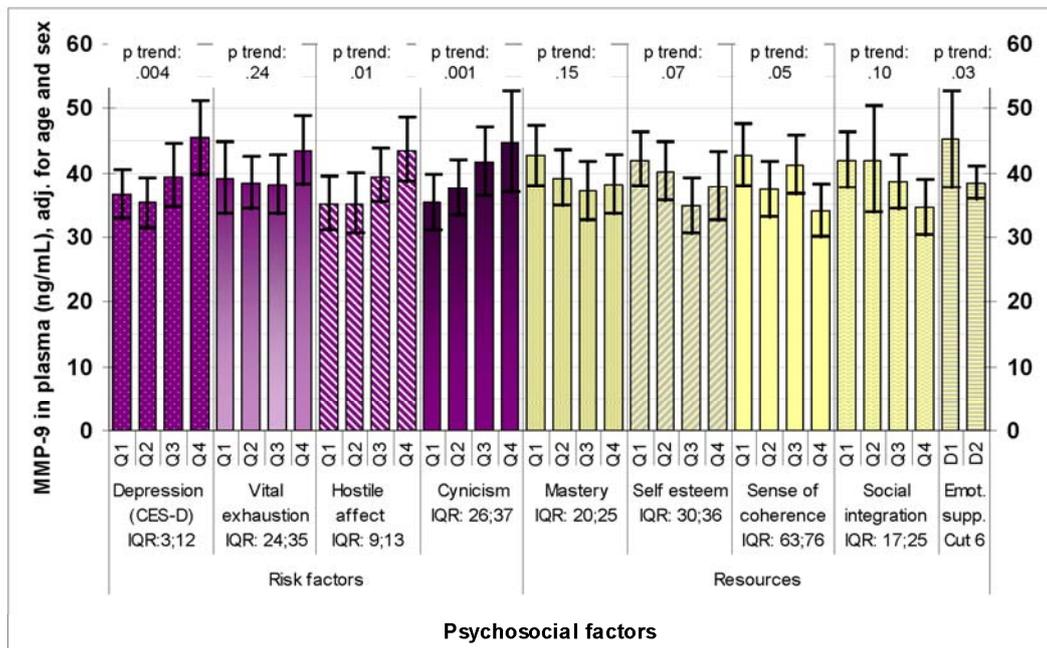


Figure 1. Distribution of MMP-9 levels over quartiles of the nine psychosocial instruments tested. $n=377-391$. Mean values and significance test of trend adjusted for age and sex.

The scores of psychosocial instruments were correlated, as presented in table 3. As expected, there were high correlations between several instruments with overlapping constructs.

Cynicism showed lowest correlations to other instruments tested, yet being significant to all but CES-D.

The main results are presented in Table 4, showing linear regressions on continuous scores for the psychosocial factors. Each of the psychosocial factors was adjusted for age and sex.

Further adjustments were made for possible confounders illustrated in table 1. Stroke ($p=0.96$), cancer ever in lifetime ($p=0.54$) and diabetes ($p=0.99$) were left out from the adjustment of diagnoses, as they seem to have no association to levels of MMP-9. LDL

Table 4: Linear regressions for MMP-9 as dependent variable. I) Psychosocial risk factors. II) Psychosocial resources. Beta coefficients are expressed as increase of MMP-9 in ng/mL per standard deviation. a) n=377-391 b) and c) n=325-338.

	a) age, sex and diagnoses ^a		b) age, sex, diagnoses, and CAD risk factors ^b		c) age, sex, diagnoses, CAD risk factors and medication ^c	
	Beta (95 % CI)	p-value	Beta (95 % CI)	p-value	Beta (95 % CI)	p-value
I. Psychosocial factors (SD)						
CES-D (7.4)	2.9 (0.6;5.3)	.01	3.2 (0.7;5.7)	.01	2.9 (0.4;5.4)	.02
Vital exhaustion, (7.2)	1.9 (-0.4;4.2)	.11	1.8 (-0.7;4.5)	.15	1.6 (-1.0;4.3)	.22
Hostile affect (2.8)	2.6 (0.4;5.0)	.02	2.7 (0.2;5.2)	.03	3.0 (0.5;5.6)	.02
Cynicism (8.1)	3.4 (1.1;5.8)	.004	3.1 (0.6;5.7)	.02	3.5 (1.0;6.2)	.006
II. Mastery (3.4)	-2.6 (-4.9;-0.3)	.03	-2.6 (-5.1;-0.2)	.03	-2.4 (-4.9;0.0)	.05
Self esteem (4.5)	-2.5 (-4.9;-0.3)	.03	-2.4 (-4.8;-0.2)	.048	-1.9 (-4.4;0.5)	.11
SOC (10.2)	-2.6 (-4.9;-0.3)	.03	-2.3 (-4.9;-0.2)	.07	-2.5 (-5.0;-0.0)	.046
Emotional support (1.1)	-1.8 (-4.2;0.5)	.11	-2.0 (-4.5;0.3)	.08	-2.0 (-4.5;0.3)	.08
Social integration (5.8)	-2.3 (-4.6;-0.0)	.045	-1.8 (-4.2;0.6)	.13	-1.3 (-3.8;1.0)	.28

^a Adjusted for age, sex, CAD, rheumatoid arthritis and cancer with ongoing treatment.

^b Adjusted as a), plus BMI, systolic and diastolic blood pressure, HDL, triglycerides, CRP, smoking, high alcohol intake, low physical activity and low fruit and vegetable consumption.

^c Adjusted as b) plus ongoing medication. The following nine groups of medication were used in the adjustments: Antihypertensive medication, cholesterol-lowering medication, glucose-regulating medication, bronchial dilatation, sex hormones, acid inhibitors for gastric ulcer, anti-inflammatory medication, sedative medication and antidepressive medication.

($p=0.78$) was left out from the adjustment of cardiovascular risk factors for the same reason. After adjustment for diagnoses, cardiovascular risk factors including CRP and ongoing medication, there were significant associations with MMP-9 of CES-D ($p=0.02$), hostile affect ($p=0.02$), cynicism ($p=0.006$) and SOC ($p=0.046$), as shown in table 4c . Close to significant associations were found to mastery ($p=0.05$) and emotional support (0.08). The crude regressions on the psychosocial factors tested (table2) had R^2 values ranging from 0.04 to 0.06. Adjusting for possible confounders (table 4c) increased the R^2 values, ranging from 0.27 to 0.30. Importantly, rerunning the analyses in table 4a, adjusting for age and sex but excluding participants with a history of CAD, rheumatoid arthritis or cancer with ongoing treatment, did not alter the conclusions. There were significant associations to the same seven psychosocial instruments tested as in table 4a. ($n=358$ to 369 , data not shown). The final model, rerunning table 4b, adjusting for age, sex and cardiovascular risk factors but both excluding participants with a history of CAD, rheumatoid arthritis or cancer with ongoing treatment, and participants with any ongoing medication, were heavily reducing the number of participants. Significant associations were still found in this healthy selection with hostile affect ($+4.1$ ng/mL per SD, $p=0.01$, $n=189$), and cynicism ($+3.5$ ng/mL per SD, $p=0.03$, $n=190$), whereas there were close to significant associations with CES-D ($+2.9$ ng/mL per SD, $p=0.10$, $n=188$) and mastery (-3.0 ng/mL per SD, $p=0.06$, $n=185$).

A principal component analysis (unrotated) extracted three components, explaining 70.2% of the variance in the nine psychosocial instruments. The first component had high loading of all instruments (lowest loading from cynicism, 0.31) but were mainly extracted from CES-D (0.77), vital exhaustion (0.80), self esteem (0.80), mastery (0.79) and SOC (0.84). The second component was mainly extracted from cynicism (0.79) and hostile affect (0.65). The third component was mainly extracted from social integration (0.77) and emotional support (0.49). An altered principal component analysis (varimax rotation) yielded the same three

components, with only slightly different loadings (data not shown). F-tests were performed in regression models (same variables as adjusted for in Table 4c) with and without the components. In both the unrotated principal component analysis and the varimax rotation, there were significant changes in the proposed models when adding the three components as one block ($p=.02$ for both principal component analyses). Testing all three components simultaneously in a regression model adjusting for age, sex, diagnoses, cardiovascular risk factors including CRP and ongoing medication ($n=291$), there were independent associations of MMP-9 with both the first component mainly extracted from CES-D, vital exhaustion, mastery, self esteem and SOC ($p=0.02$) and the second component mainly extracted from cynicism and hostile affect ($p=0.04$). The third component mainly extracted from social integration and emotional support were non-significant ($p=0.70$).

The independent associations between MMP-9 and the two extracted dimensions were further demonstrated when (the uncorrelated) scores of CES-D and cynicism were tested in the same model ($n=327$): Adjusting for age, sex, diagnoses, cardiovascular risk factors including CRP and ongoing medication, there were independent associations of MMP-9 with both CES-D ($+3.0$ ng/mL per SD, $p=0.02$) and cynicism ($+3.6$ ng/mL per SD, $p=0.006$).

Discussion

The role of inflammation as a potential mechanism linking psychosocial factors to CAD has been proposed in several studies (4, 12, 20, 21). However, as far as we know, this is the first study to demonstrate an association between MMP-9 and psychosocial factors.

In crude analyses, all psychosocial factors apart from vital exhaustion were associated with levels of MMP-9. The main finding in this study was that, after adjusting for possible confounders, significant positive associations remained with CES-D, hostile affect, cynicism and significant negative association with SOC. Importantly, the principal component analyses extracted two dimensions of psychosocial factors, the first mainly extracted from CES-D,

vital exhaustion, self esteem, mastery and SOC, and the other mainly extracted from cynicism and hostility, both of which were independently associated with MMP-9 after adjustment for possible confounders.

These findings have two main implications: 1) The explanatory mechanisms behind the epidemiological findings linking psychosocial risk factors to increased risk of CAD have been discussed for long (20, 21). Our data suggest an association between psychosocial factors and a novel marker of plausible relevance in plaque remodeling. Further, according to our data, the associations of MMP-9 to psychosocial factors are independent, and not merely mediated by behavioral factors such as smoking, alcohol intake, poor eating habits and poor physical exercise. 2) The question if depression and cynicism are unique as separate domains or if they both are proxies of a more consistent concept of negative emotions have been raised, i.e. if depression and cynicism have a unique or shared contribution to cardiovascular risk (57-59). Our data suggest that the association of MMP-9 with depression and cynicism, respectively, are independent from one another.

The exploration of MMP-9 is still in an early phase. The lack of prospective data evaluating the predictive value of MMP-9 for CAD is for now limiting the conclusions of these findings. However, the association between MMP-9 and total CAD risk load (based on blood lipid imbalance, hypertension, diabetes, obesity, smoking, high alcohol intake, low physical activity and low fruit and vegetables consumption) in a population free from known CAD, as previously reported by our group (32), in combination with these findings, indicate that MMP-9 could be a promising marker when explaining the epidemiological findings between psychosocial factors and CAD. The findings spur on a prospective follow-up, exploring the predictive value of MMP-9 for CAD.

Although the associations between MMP-9 and psychosocial factors were independent of the possible confounders tested, and that the R^2 values are reasonable in the fully adjusted models,

we can not exclude that some confounding remained in the analyses. The regulation of MMP-9 is complex and multi-factorial. Further information on e.g. arterial stiffness, (35, 60) genotypes (28, 61), and oxidative stress (62-64) would probably decrease possible remaining confounding and increase the understanding of the inter-individual variation of MMP-9. Moreover, as mentioned in the introduction, there are a number of experimental studies indicating direct effects of cortisol and norepinephrine on MMP-9 regulation (40-42). Of the factors not accounted for in the analyses, the stress hormones might be of particular relevance, as there are a number of studies linking both hostility and depression with a dysregulation of cortisol (65, 66). Extended measurements of stress hormones in population-based and clinical studies could thus be of interest when further studying the association between MMP-9 and psychosocial factors.

It should be noted that increased levels of MMP-9 have been demonstrated in several other pathophysiological conditions apart from CAD: In autoimmune diseases such as rheumatoid arthritis, MMP-9 activity is thought to increase the degradation of cartilage and joint tissues (67, 68). In cancer, MMP-9 is suggested to play a vital role in tumor-induced angiogenesis and tumor growth (69-71). It is also suspected to be elevated in diabetes, as MMP-9 has been shown to be up-regulated by hyperglycemia (72, 73). Thus, it is highly recommended to take these diagnoses into account when further studying MMP-9. However, the prevalence for these diagnoses was low as seen in table 1, why it was concluded that these diagnoses are unlikely to act as confounders to any major extent in this study. This was further strengthened in the analyses excluding participants with a history of CAD, rheumatoid arthritis or cancer with ongoing treatment, and participants with any ongoing medication. Although heavily reducing the number of participants, the associations were still pointing in the same direction with beta coefficients in the same range as analyses on the whole sample.

The power of this study was sufficient in relation to the aim set. This study shows an independent association between psychosocial factors and MMP-9, independent of known diseases, other cardiovascular risk factors including CRP and ongoing medication. One possible limitation was that the adjustments for known diagnoses were based on self-reported data. Even though the agreement between for instance self-reported diagnosis and medical record of myocardial infarction has been demonstrated to be high, both in terms of specificity and sensitivity (74), it cannot be excluded that there were a presence of symptomatic CAD or any other disease of relevance that could not be adjusted for. Nor can it be excluded that there were a number of participants that were using any kind of medication, being non-compliant to the questions on ongoing medication. However, even if there are a limited number of participants that should have been adjusted for or excluded according to the criteria, it is, based on our findings on possible confounding, unlikely that the influence of those would alter the conclusions to any major extent.

Conclusion

MMP-9 levels were associated with psychosocial factors in a middle aged normal population sample, independently of traditional risk factors. The findings may constitute a possible link between psychosocial factors and cardiovascular risk.

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References

1. Frasure-Smith N, Lesperance F: Reflections on depression as a cardiac risk factor. *Psychosom Med* 67 Suppl 1:S19-25, 2005
2. Glassman AH, Shapiro PA: Depression and the course of coronary artery disease. *Am J Psychiatry* 155:4-11, 1998
3. Rozanski A, Blumenthal JA, Kaplan J: Impact of psychological factors on the pathogenesis of cardiovascular disease and implications for therapy. *Circulation* 99:2192-217, 1999
4. Everson-Rose SA, Lewis TT: Psychosocial factors and cardiovascular diseases. *Annu Rev Public Health* 26:469-500, 2005
5. Matthews KA, Gump BB, Harris KF, Haney TL, Barefoot JC: Hostile behaviors predict cardiovascular mortality among men enrolled in the Multiple Risk Factor Intervention Trial. *Circulation* 109:66-70, 2004
6. Chang PP, Ford DE, Meoni LA, Wang NY, Klag MJ: Anger in young men and subsequent premature cardiovascular disease: the precursors study. *Arch Intern Med* 162:901-6, 2002
7. Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, McQueen M, Budaj A, Pais P, Varigos J, Lisheng L: Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet* 364:937-52, 2004
8. Surtees PG, Wainwright NW, Luben R, Khaw KT, Day NE: Mastery, sense of coherence, and mortality: evidence of independent associations from the EPIC-Norfolk Prospective Cohort Study. *Health Psychol* 25:102-10, 2006
9. Libby P, Theroux P: Pathophysiology of coronary artery disease. *Circulation* 111:3481-8, 2005
10. Hansson GK: Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med* 352:1685-95, 2005
11. Ross R: Atherosclerosis--an inflammatory disease. *N Engl J Med* 340:115-26, 1999
12. Gidron Y, Gilutz H, Berger R, Huleihel M: Molecular and cellular interface between behavior and acute coronary syndromes. *Cardiovasc Res* 56:15-21, 2002
13. Suarez EC: Plasma interleukin-6 is associated with psychological coronary risk factors: moderation by use of multivitamin supplements. *Brain Behav Immun* 17:296-303, 2003
14. Suarez EC: C-reactive protein is associated with psychological risk factors of cardiovascular disease in apparently healthy adults. *Psychosom Med* 66:684-91, 2004
15. Miller GE, Stetler CA, Carney RM, Freedland KE, Banks WA: Clinical depression and inflammatory risk markers for coronary heart disease. *Am J Cardiol* 90:1279-83, 2002
16. Sjogren E, Leanderson P, Kristenson M, Ernerudh J: Interleukin-6 levels in relation to psychosocial factors: Studies on serum, saliva, and in vitro production by blood mononuclear cells. *Brain Behav Immun*, 2005
17. Steptoe A, Hamer M, Chida Y: The effects of acute psychological stress on circulating inflammatory factors in humans: A review and meta-analysis. *Brain Behav Immun*, 2007
18. Kuo HK, Yen CJ, Chang CH, Kuo CK, Chen JH, Sorond F: Relation of C-reactive protein to stroke, cognitive disorders, and depression in the general population: systematic review and meta-analysis. *Lancet Neurol* 4:371-80, 2005

19. Nabi H, Singh-Manoux A, Shipley M, Gimeno D, Marmot MG, Kivimaki M: Do psychological factors affect inflammation and incident coronary heart disease: the Whitehall II Study. *Arterioscler Thromb Vasc Biol* 28:1398-406, 2008
20. Carney RM, Freedland KE, Miller GE, Jaffe AS: Depression as a risk factor for cardiac mortality and morbidity: a review of potential mechanisms. *J Psychosom Res* 53:897-902, 2002
21. Rozanski A, Blumenthal JA, Davidson KW, Saab PG, Kubzansky L: The epidemiology, pathophysiology, and management of psychosocial risk factors in cardiac practice: the emerging field of behavioral cardiology. *J Am Coll Cardiol* 45:637-51, 2005
22. Herrmann J, Lerman LO, Mukhopadhyay D, Napoli C, Lerman A: Angiogenesis in atherogenesis. *Arterioscler Thromb Vasc Biol* 26:1948-57, 2006
23. Newby AC: Do metalloproteinases destabilize vulnerable atherosclerotic plaques? *Curr Opin Lipidol* 17:556-61, 2006
24. Brown DL, Hibbs MS, Kearney M, Loushin C, Isner JM: Identification of 92-kD gelatinase in human coronary atherosclerotic lesions. Association of active enzyme synthesis with unstable angina. *Circulation* 91:2125-31, 1995
25. Hallenbeck JM, Hansson GK, Becker KJ: Immunology of ischemic vascular disease: plaque to attack. *Trends Immunol* 26:550-6, 2005
26. Kovanen PT, Kaartinen M, Paavonen T: Infiltrates of activated mast cells at the site of coronary atheromatous erosion or rupture in myocardial infarction. *Circulation* 92:1084-8, 1995
27. Fukuda D, Shimada K, Tanaka A, Kusuyama T, Yamashita H, Ehara S, Nakamura Y, Kawarabayashi T, Iida H, Yoshiyama M, Yoshikawa J: Comparison of levels of serum matrix metalloproteinase-9 in patients with acute myocardial infarction versus unstable angina pectoris versus stable angina pectoris. *Am J Cardiol* 97:175-80, 2006
28. Blankenberg S, Rupprecht HJ, Poirier O, Bickel C, Smieja M, Hafner G, Meyer J, Cambien F, Tiret L: Plasma concentrations and genetic variation of matrix metalloproteinase 9 and prognosis of patients with cardiovascular disease. *Circulation* 107:1579-85, 2003
29. Kai H, Ikeda H, Yasukawa H, Kai M, Seki Y, Kuwahara F, Ueno T, Sugi K, Imaizumi T: Peripheral blood levels of matrix metalloproteinases-2 and -9 are elevated in patients with acute coronary syndromes. *J Am Coll Cardiol* 32:368-72, 1998
30. Loftus IM, Goodall S, Crowther M, Jones L, Bell PR, Naylor AR, Thompson MM: Increased MMP-9 activity in acute carotid plaques: therapeutic avenues to prevent stroke. *Ann N Y Acad Sci* 878:551-4, 1999
31. Nagase H, Visse R, Murphy G: Structure and function of matrix metalloproteinases and TIMPs. *Cardiovasc Res* 69:562-73, 2006
32. Garvin P, Nilsson L, Carstensen J, Jonasson L, Kristenson M: Circulating Matrix Metalloproteinase-9 Is Associated with Cardiovascular Risk Factors in a Middle-Aged Normal Population. *PLoS ONE* 3:e1774, 2008
33. Sillanaukee P, Kalela A, Seppa K, Hoyhtya M, Nikkari ST: Matrix metalloproteinase-9 is elevated in serum of alcohol abusers. *Eur J Clin Invest* 32:225-9, 2002
34. Kang MJ, Oh YM, Lee JC, Kim DG, Park MJ, Lee MG, Hyun IG, Han SK, Shim YS, Jung KS: Lung matrix metalloproteinase-9 correlates with cigarette smoking and obstruction of airflow. *J Korean Med Sci* 18:821-7, 2003
35. Yasmin, McEniery CM, Wallace S, Dakham Z, Pulsalkar P, Maki-Petaja K, Ashby MJ, Cockcroft JR, Wilkinson IB: Matrix metalloproteinase-9 (MMP-9), MMP-2, and serum elastase activity are associated with systolic hypertension and arterial stiffness. *Arterioscler Thromb Vasc Biol* 25:372, 2005

36. Tayebjee MH, Nadar S, Blann AD, Gareth Beevers D, MacFadyen RJ, Lip GY: Matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in hypertension and their relationship to cardiovascular risk and treatment: a substudy of the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT). *Am J Hypertens* 17:764-9, 2004
37. Liang KC, Lee CW, Lin WN, Lin CC, Wu CB, Luo SF, Yang CM: Interleukin-1beta induces MMP-9 expression via p42/p44 MAPK, p38 MAPK, JNK, and nuclear factor-kappaB signaling pathways in human tracheal smooth muscle cells. *J Cell Physiol* 211:759-70, 2007
38. Lu Y, Wahl LM: Production of matrix metalloproteinase-9 by activated human monocytes involves a phosphatidylinositol-3 kinase/Akt/IKKalpha/NF-kappaB pathway. *J Leukoc Biol* 78:259-65, 2005
39. Opdenakker G, Van den Steen PE, Dubois B, Nelissen I, Van Coillie E, Masure S, Proost P, Van Damme J: Gelatinase B functions as regulator and effector in leukocyte biology. *J Leukoc Biol* 69:851-9, 2001
40. Aljada A, Ghanim H, Mohanty P, Hofmeyer D, Tripathy D, Dandona P: Hydrocortisone suppresses intranuclear activator-protein-1 (AP-1) binding activity in mononuclear cells and plasma matrix metalloproteinase 2 and 9 (MMP-2 and MMP-9). *J Clin Endocrinol Metab* 86:5988-91, 2001
41. Yang EV, Sood AK, Chen M, Li Y, Eubank TD, Marsh CB, Jewell S, Flavahan NA, Morrison C, Yeh PE, Lemeshow S, Glaser R: Norepinephrine up-regulates the expression of vascular endothelial growth factor, matrix metalloproteinase (MMP)-2, and MMP-9 in nasopharyngeal carcinoma tumor cells. *Cancer Res* 66:10357-64, 2006
42. Eberhardt W, Schulze M, Engels C, Klasmeier E, Pfeilschifter J: Glucocorticoid-mediated suppression of cytokine-induced matrix metalloproteinase-9 expression in rat mesangial cells: involvement of nuclear factor-kappaB and Ets transcription factors. *Mol Endocrinol* 16:1752-66, 2002
43. Galis ZS, Sukhova GK, Lark MW, Libby P: Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. *J Clin Invest* 94:2493-503, 1994
44. Galis ZS, Asanuma K, Godin D, Meng X: N-acetyl-cysteine decreases the matrix-degrading capacity of macrophage-derived foam cells: new target for antioxidant therapy? *Circulation* 97:2445-53, 1998
45. Nikkari ST, Hoyhtya M, Isola J, Nikkari T: Macrophages contain 92-kd gelatinase (MMP-9) at the site of degenerated internal elastic lamina in temporal arteritis. *Am J Pathol* 149:1427-33, 1996
46. Hollman G, Kristenson M: The prevalence of the metabolic syndrome and its risk factors in a middle-aged Swedish population - Mainly a function of overweight? *Eur J Cardiovasc Nurs*, 2007
47. Appels A, Kop W, Bar F, de Swart H, Mendes de Leon C: Vital exhaustion, extent of atherosclerosis, and the clinical course after successful percutaneous transluminal coronary angioplasty. *Eur Heart J* 16:1880-5, 1995
48. Barefoot JC, Dodge KA, Peterson BL, Dahlstrom WG, Williams RB, Jr.: The Cook-Medley hostility scale: item content and ability to predict survival. *Psychosom Med* 51:46-57, 1989
49. Surtees P, Wainwright N, Luben R, Khaw KT, Day N: Sense of coherence and mortality in men and women in the EPIC-Norfolk United Kingdom prospective cohort study. *Am J Epidemiol* 158:1202-9, 2003
50. Radloff L: The CES-D Scale. A Self-Report Depression Scale for Research in the General Population. *Applied Psychological Measures* 1:385-401, 1977

51. Appels A, Hoppener P, Mulder P: A questionnaire to assess premonitory symptoms of myocardial infarction. *Int J Cardiol* 17:15-24, 1987
52. Pearlin LI, Schooler C: The structure of coping. *J Health Soc Behav* 19:19, 1978
53. Antonovsky A: *Unraveling the mystery of health : how people manage stress and stay well*. San Francisco, Jossey-Bass, 1987
54. Orth-Gomer K, Unden AL: The measurement of social support in population surveys. *Soc Sci Med* 24:83-94, 1987
55. Gerlach RF, Demacq C, Jung K, Tanus-Santos JE: Rapid separation of serum does not avoid artificially higher matrix metalloproteinase (MMP)-9 levels in serum versus plasma. *Clin Biochem* 40:119-23, 2007
56. Friedewald WT, Levy RI, Fredrickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 18:499-502, 1972
57. Kubzansky LD, Cole SR, Kawachi I, Vokonas P, Sparrow D: Shared and unique contributions of anger, anxiety, and depression to coronary heart disease: a prospective study in the normative aging study. *Ann Behav Med* 31:21-9, 2006
58. Suarez EC: Joint effect of hostility and severity of depressive symptoms on plasma interleukin-6 concentration. *Psychosom Med* 65:523-7, 2003
59. Raynor DA, Pogue-Geile MF, Kamarck TW, McCaffery JM, Manuck SB: Covariation of psychosocial characteristics associated with cardiovascular disease: genetic and environmental influences. *Psychosom Med* 64:191-203; discussion 204-5, 2002
60. Kingwell BA, Medley TL, Waddell TK, Cole TJ, Dart AM, Jennings GL: Large artery stiffness: structural and genetic aspects. *Clin Exp Pharmacol Physiol* 28:1040-3, 2001
61. Cipollone F, Toniato E, Martinotti S, Fazio M, Iezzi A, Cucurullo C, Pini B, Ursi S, Vitullo G, Averna M, Arca M, Montali A, Campagna F, Uchino S, Spigonardo F, Taddei S, Viridis A, Ciabattini G, Notarbartolo A, Cucurullo F, Mezzetti A: A polymorphism in the cyclooxygenase 2 gene as an inherited protective factor against myocardial infarction and stroke. *Jama* 291:2221-8, 2004
62. Uemura S, Matsushita H, Li W, Glassford AJ, Asagami T, Lee KH, Harrison DG, Tsao PS: Diabetes mellitus enhances vascular matrix metalloproteinase activity: role of oxidative stress. *Circ Res* 88:1291-8, 2001
63. Kameda K, Matsunaga T, Abe N, Hanada H, Ishizaka H, Ono H, Saitoh M, Fukui K, Fukuda I, Osanai T, Okumura K: Correlation of oxidative stress with activity of matrix metalloproteinase in patients with coronary artery disease. Possible role for left ventricular remodelling. *Eur Heart J* 24:2180-5, 2003
64. Siwik DA, Pagano PJ, Colucci WS: Oxidative stress regulates collagen synthesis and matrix metalloproteinase activity in cardiac fibroblasts. *Am J Physiol Cell Physiol* 280:C53-60, 2001
65. Miller GE, Chen E, Zhou ES: If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. *Psychol Bull* 133:25-45, 2007
66. Pope MK, Smith TW: Cortisol excretion in high and low cynically hostile men. *Psychosom Med* 53:386-92, 1991
67. Ram M, Sherer Y, Shoenfeld Y: Matrix metalloproteinase-9 and autoimmune diseases. *J Clin Immunol* 26:299-307, 2006
68. Tchetverikov I, Lard LR, DeGroot J, Verzijl N, TeKoppele JM, Breedveld FC, Huizinga TW, Hanemaaijer R: Matrix metalloproteinases-3, -8, -9 as markers of disease activity and joint damage progression in early rheumatoid arthritis. *Ann Rheum Dis* 62:1094-9, 2003
69. Johansson N, Ahonen M, Kahari VM: Matrix metalloproteinases in tumor invasion. *Cell Mol Life Sci* 57:5-15, 2000

70. Hanemaaijer R, Sier CF, Visser H, Scholte L, van Lent N, Toet K, Hoekman K, Verheijen JH: MMP-9 activity in urine from patients with various tumors, as measured by a novel MMP activity assay using modified urokinase as a substrate. *Ann N Y Acad Sci* 878:141-9, 1999
71. Folkman J: Angiogenesis. *Annu Rev Med* 57:1-18, 2006
72. Derosa G, D'Angelo A, Scalise F, Avanzini MA, Tinelli C, Peros E, Fogari E, Cicero AF: Comparison between metalloproteinases-2 and -9 in healthy subjects, diabetics, and subjects with acute coronary syndrome. *Heart Vessels* 22:361-70, 2007
73. Shiau MY, Tsai ST, Tsai KJ, Haung ML, Hsu YT, Chang YH: Increased circulatory MMP-2 and MMP-9 levels and activities in patients with type 1 diabetes mellitus. *Mt Sinai J Med* 73:1024-8, 2006
74. Okura Y, Urban LH, Mahoney DW, Jacobsen SJ, Rodeheffer RJ: Agreement between self-report questionnaires and medical record data was substantial for diabetes, hypertension, myocardial infarction and stroke but not for heart failure. *J Clin Epidemiol* 57:1096-103, 2004