Plasma Levels of Matrix Metalloproteinase-9 are Independently Associated With Psychosocial Factors in a Middle-Aged Normal Population

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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°C 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 $p \leq 0.05$ 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.

<table>
<thead>
<tr>
<th></th>
<th>n with data</th>
<th>Prevalence (%) or mean (SD)</th>
<th>Beta (95 CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (women/men)</td>
<td>n=400</td>
<td>n=199 (50 %)</td>
<td>-8.3 (-12.8; -3.9)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Age (5 year categories)</td>
<td>n=400</td>
<td>57.1 (7.2)</td>
<td>-1.2 (-2.8; 4.4)</td>
<td>.12</td>
</tr>
<tr>
<td><strong>Self-reported diagnoses</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Myocardial infarction (y/n)</td>
<td>n=390</td>
<td>n=4 (1 %)</td>
<td>34.0 (12.0;56.0)</td>
<td>.003</td>
</tr>
<tr>
<td>Angina pectoris (y/n)</td>
<td>n=389</td>
<td>n=13 (3 %)</td>
<td>13.6 (1.2;26.0)</td>
<td>.03</td>
</tr>
<tr>
<td>Stroke (y/n)</td>
<td>n=388</td>
<td>n=7 (2 %)</td>
<td>0.4 (-16.2;17.1)</td>
<td>.96</td>
</tr>
<tr>
<td>Rheumatoid arthritis (y/n)</td>
<td>n=400</td>
<td>n=7 (2 %)</td>
<td>12.2 (-4.7;29.2)</td>
<td>.16</td>
</tr>
<tr>
<td>Diabetes (y/n)</td>
<td>n=391</td>
<td>n=27 (7 %)</td>
<td>0.0 (-8.9;9.0)</td>
<td>.99</td>
</tr>
<tr>
<td>Cancer, ever in lifetime (y/n)</td>
<td>n=387</td>
<td>n=12 (3 %)</td>
<td>-4.0 (-17.1;8.9)</td>
<td>.54</td>
</tr>
<tr>
<td>Cancer with ongoing treatment (y/n)</td>
<td>n=400</td>
<td>n=2 (1 %)</td>
<td>-22.6 (-50.0;8.7)</td>
<td>.16</td>
</tr>
<tr>
<td><strong>Clinical and laboratory characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>n=394</td>
<td>26.7 (4.3)</td>
<td>1.2 (-1.1;3.5)</td>
<td>.29</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>n=394</td>
<td>133.4 (2.9)</td>
<td>3.4 (1.0;5.9)</td>
<td>.004</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>n=395</td>
<td>84.3 (11.9)</td>
<td>2.6 (3.4;9.9)</td>
<td>.02</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>n=394</td>
<td>3.4 (8.8)</td>
<td>-0.3 (-2.6;1.9)</td>
<td>.78</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>n=399</td>
<td>1.6 (4.1)</td>
<td>-2.2 (-4.6;2.2)</td>
<td>.07</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>n=399</td>
<td>1.3 (8.8)</td>
<td>3.3 (1.0;6.0)</td>
<td>.004</td>
</tr>
<tr>
<td>CRP (mg/L, quartiles)</td>
<td>n=389</td>
<td>.8 (3.2;2.2)</td>
<td>3.7 (1.8;5.8)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td><strong>Behavioral characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking (y/n)</td>
<td>n=386</td>
<td>n=63 (16 %)</td>
<td>19.2 (13.2;25.0)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Alcohol intake (3 ordinal cat.)</td>
<td>n=395</td>
<td>n=34;46 (9;12%)</td>
<td>6.7 (3.2;1.3)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Physical activity (3 ordinal cat.)</td>
<td>n=372</td>
<td>n=282;71 (76;19%)</td>
<td>-6.7 (-11.7;-1.8)</td>
<td>.007</td>
</tr>
<tr>
<td>Veg and fruit intake (3 ordinal cat.)</td>
<td>n=396</td>
<td>n=287;54 (72;14%)</td>
<td>-5.2 (-9.5;-9)</td>
<td>.02</td>
</tr>
<tr>
<td><strong>Medication</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antihypertensive medication (y/n)</td>
<td>n=400</td>
<td>n=65 (16 %)</td>
<td>3.6 (1.0;15.3)</td>
<td>.03</td>
</tr>
<tr>
<td>Cholesterol-lowering medication (y/n)</td>
<td>n=400</td>
<td>n=31 (8 %)</td>
<td>-8.5 (-19.2;2.1)</td>
<td>.11</td>
</tr>
<tr>
<td>Glucose-regulating medication (y/n)</td>
<td>n=400</td>
<td>n=13 (3 %)</td>
<td>-8.8 (-22.6;5.0)</td>
<td>.21</td>
</tr>
<tr>
<td>Bronchial dilatation (y/n)</td>
<td>n=400</td>
<td>n=14 (4 %)</td>
<td>7.1 (-7.2;21.4)</td>
<td>.33</td>
</tr>
<tr>
<td>Sex hormones (oestrogens) (y/n)</td>
<td>n=400</td>
<td>n=7 (2 %)</td>
<td>6.3 (-1.6;23.4)</td>
<td>.46</td>
</tr>
<tr>
<td>Acid inhibitor for gastric ulcer (y/n)</td>
<td>n=400</td>
<td>n=12 (3 %)</td>
<td>-15.2 (-29.7;-8)</td>
<td>.04</td>
</tr>
<tr>
<td>Anti-inflammatory medication (y/n)</td>
<td>n=400</td>
<td>n=23 (6 %)</td>
<td>6.7 (-4.0;17.5)</td>
<td>.22</td>
</tr>
<tr>
<td>Sedative medication (y/n)</td>
<td>n=400</td>
<td>n=15 (4 %)</td>
<td>-8.6 (-23.9;6.6)</td>
<td>.27</td>
</tr>
<tr>
<td>Antidepressive medication (y/n)</td>
<td>n=400</td>
<td>n=16 (4 %)</td>
<td>7.5 (-6.0;21.1)</td>
<td>.28</td>
</tr>
</tbody>
</table>

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).

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

*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.

<table>
<thead>
<tr>
<th>Psychosocial instrument</th>
<th>CES-D</th>
<th>Vital exhaustion</th>
<th>Hostile affect</th>
<th>Cynicism</th>
<th>Mastery</th>
<th>Self esteem</th>
<th>Sense of coherence</th>
<th>Emotional support</th>
<th>Social integration</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CES-D</td>
<td>r</td>
<td>1.00</td>
<td>0.73 (p&lt;.001)</td>
<td>0.17 (p&lt;.001)</td>
<td>0.08 (p=.13)</td>
<td>-0.51 (p&lt;.001)</td>
<td>-0.50 (p&lt;.001)</td>
<td>-0.56 (p&lt;.001)</td>
<td>-0.30 (p&lt;.001)</td>
</tr>
<tr>
<td>Vital exhaustion</td>
<td>r</td>
<td>1.00</td>
<td>0.20 (p&lt;.001)</td>
<td>0.11 (p=.03)</td>
<td>0.42 (p&lt;.001)</td>
<td>-0.55 (p&lt;.001)</td>
<td>-0.55 (p&lt;.001)</td>
<td>-0.64 (p&lt;.001)</td>
<td>-0.21 (p&lt;.001)</td>
</tr>
<tr>
<td>Hostile affect</td>
<td>r</td>
<td>1.00</td>
<td>0.42 (p&lt;.001)</td>
<td>0.18 (p&lt;.001)</td>
<td>0.36 (p&lt;.001)</td>
<td>-0.25 (p&lt;.001)</td>
<td>-0.25 (p&lt;.001)</td>
<td>-0.38 (p&lt;.001)</td>
<td>-0.16 (p&lt;.001)</td>
</tr>
<tr>
<td>Cynicism</td>
<td>r</td>
<td>1.00</td>
<td>0.14 (p=.005)</td>
<td>0.20 (p&lt;.001)</td>
<td>0.35 (p&lt;.001)</td>
<td>-0.20 (p&lt;.001)</td>
<td>-0.30 (p&lt;.001)</td>
<td>-0.12 (p=.02)</td>
<td>-0.13 (p=.002)</td>
</tr>
<tr>
<td>II.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mastery</td>
<td>r</td>
<td>1.00</td>
<td>0.61 (p&lt;.001)</td>
<td>0.18 (p&lt;.001)</td>
<td>0.36 (p&lt;.001)</td>
<td>0.61 (p&lt;.001)</td>
<td>0.19 (p&lt;.001)</td>
<td>0.19 (p&lt;.001)</td>
<td>0.38 (p&lt;.001)</td>
</tr>
<tr>
<td>Self esteem</td>
<td>r</td>
<td>1.00</td>
<td>0.61 (p&lt;.001)</td>
<td>0.23 (p&lt;.001)</td>
<td>0.40 (p&lt;.001)</td>
<td>0.61 (p&lt;.001)</td>
<td>0.23 (p&lt;.001)</td>
<td>0.23 (p&lt;.001)</td>
<td>0.40 (p&lt;.001)</td>
</tr>
<tr>
<td>Sense of coherence</td>
<td>r</td>
<td>1.00</td>
<td>0.61 (p&lt;.001)</td>
<td>0.23 (p&lt;.001)</td>
<td>0.40 (p&lt;.001)</td>
<td>0.61 (p&lt;.001)</td>
<td>0.23 (p&lt;.001)</td>
<td>0.23 (p&lt;.001)</td>
<td>0.40 (p&lt;.001)</td>
</tr>
<tr>
<td>Emotional support</td>
<td>r</td>
<td>1.00</td>
<td>0.61 (p&lt;.001)</td>
<td>0.23 (p&lt;.001)</td>
<td>0.40 (p&lt;.001)</td>
<td>0.61 (p&lt;.001)</td>
<td>0.23 (p&lt;.001)</td>
<td>0.23 (p&lt;.001)</td>
<td>0.40 (p&lt;.001)</td>
</tr>
<tr>
<td>Social integration</td>
<td>r</td>
<td>1.00</td>
<td>0.61 (p&lt;.001)</td>
<td>0.23 (p&lt;.001)</td>
<td>0.40 (p&lt;.001)</td>
<td>0.61 (p&lt;.001)</td>
<td>0.23 (p&lt;.001)</td>
<td>0.23 (p&lt;.001)</td>
<td>0.40 (p&lt;.001)</td>
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</tbody>
</table>
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>
<thead>
<tr>
<th>I. Psychosocial factors (SD)</th>
<th>Beta (95 % CI)</th>
<th>p-value</th>
<th>Beta (95 % CI)</th>
<th>p-value</th>
<th>Beta (95 % CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CES-D (7.4)</td>
<td>2.9 (0.6;5.3)</td>
<td>.01</td>
<td>3.2 (0.7;5.7)</td>
<td>.01</td>
<td>2.9 (0.4;5.4)</td>
<td>.02</td>
</tr>
<tr>
<td>Vital exhaustion, (7.2)</td>
<td>1.9 (-0.4;4.2)</td>
<td>.11</td>
<td>1.8 (-0.7;4.4)</td>
<td>.15</td>
<td>1.6 (-1.0;4.3)</td>
<td>.22</td>
</tr>
<tr>
<td>Hostile affect (2.8)</td>
<td>2.6 (0.4;5.6)</td>
<td>.02</td>
<td>2.7 (0.2;5.2)</td>
<td>.03</td>
<td>3.0 (0.5;5.6)</td>
<td>.02</td>
</tr>
<tr>
<td>Cynicism (8.1)</td>
<td>3.4 (1.1;5.8)</td>
<td>.004</td>
<td>3.1 (0.6;5.7)</td>
<td>.02</td>
<td>3.5 (1.0;6.2)</td>
<td>.006</td>
</tr>
<tr>
<td>II. Mastery (3.4)</td>
<td>-2.6 (-4.9;-0.3)</td>
<td>.03</td>
<td>-2.6 (-5.1;-0.2)</td>
<td>.03</td>
<td>-2.4 (-4.9;0.0)</td>
<td>.05</td>
</tr>
<tr>
<td>Self esteem (4.5)</td>
<td>-2.5 (-4.9;-0.3)</td>
<td>.03</td>
<td>-2.4 (-4.8;-0.2)</td>
<td>.048</td>
<td>-1.9 (-4.4;0.5)</td>
<td>.11</td>
</tr>
<tr>
<td>SOC (10.2)</td>
<td>-2.6 (-4.9;-0.3)</td>
<td>.03</td>
<td>-2.3 (-4.9;-0.2)</td>
<td>.07</td>
<td>-2.5 (-5.0;-0.0)</td>
<td>.046</td>
</tr>
<tr>
<td>Emotional support (1.1)</td>
<td>-1.8 (-4.2;0.5)</td>
<td>.11</td>
<td>-2.0 (-4.5;0.3)</td>
<td>.08</td>
<td>-2.0 (-4.5;0.3)</td>
<td>.08</td>
</tr>
<tr>
<td>Social integration (5.8)</td>
<td>-2.3 (-4.6;0.0)</td>
<td>.045</td>
<td>-1.8 (-4.2;0.6)</td>
<td>.13</td>
<td>-1.3 (-3.8;1.0)</td>
<td>.28</td>
</tr>
</tbody>
</table>

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 R² values ranging from 0.04 to 0.06. Adjusting for possible confounders (table 4c) increased the R² 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.
Acknowledgement

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References


