C-reactive protein, depressed mood, and the prediction of coronary heart disease in initially healthy men: results from the MONICA–KORA Augsburg Cohort Study 1984–1998

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Aims C-reactive protein and depressive mood (DM) are novel risk factors for coronary heart disease (CHD). The goal of the present study was to assess possible combined effects of these factors on the prediction of a future fatal and non-fatal coronary event.

Methods and results Baseline highly sensitive (hs) C-reactive protein and DM were analysed in 3021 apparently healthy male subjects aged 45–74 from three subsequent population based surveys (1984–95) of the MONICA–KORA Augsburg Cohort Study. During a median follow-up period of 7.7 years (IQR = 6.9 years), 165 CHD events occurred. Risks of CHD were estimated from Cox proportional hazard models adjusted for age and survey and multiple risk factors. The age and survey adjusted interaction term of continuous hs-C-reactive protein by DM disclosed a significant effect (HR 1.03; 95% CI 1.00–1.06; P = 0.037). A stratified analysis of subpopulations with (n = 986) and without (n = 2035) DM revealed that high hs-C-reactive protein (>3 mg/L) was predictive in the group with DM (HR 2.69; 95% CI 1.32–5.47) but was not significant in the low-level depression group (HR 1.55; 95% CI 0.89–2.69). Relative to the low C-reactive protein/no depression subgroup (n = 712), high C-reactive protein/no depression (n = 565) did not significantly predict a future CHD event. However, combined high C-reactive protein and DM (n = 282) significantly predicted future CHD events (HR 2.91; 95% CI 1.25–2.18; P < 0.0001).

Conclusion In apparently healthy men, a DM substantially increases the power of elevated C-reactive protein to predict a subsequent myocardial infarction. Both conditions may share a common underlying mechanism.

KEYWORDS
Myocardial infarction; C-reactive protein; Prediction; Depression

Introduction
Depressive mood (DM) is an independent behavioural risk factor for subsequent coronary events in apparently healthy subjects.1,2 Among mechanisms responsible for the observed link between depression and coronary heart disease (CHD), immune correlates of depression3–7 with heightened expression of inflammatory markers implicated in the pathogenesis of CHD8–10 gained increasing attention over the last decade.

Growing evidence from population based surveys points to an association between DM states with elevated markers of low-grade inflammation.11–17 As for CHD patients, cross-sectional data confirm higher levels of cytokines and C-reactive protein in depressed and exhausted subjects.12,18,19 Pro-inflammatory cytokines and C-reactive protein also actively mediate illness-associated behavioural changes which are similar to a DM pattern.20

C-reactive protein is a highly sensitive acute phase reactant produced in response to tissue injury and inflammation and is synthesized in the liver by hepatocytes mainly following stimulation of pro-inflammatory cytokines.21 Prospective data from epidemiological studies demonstrate a significant relationship between C-reactive protein and the risk of future coronary events.8–10 A persisting acute phase response with generally modest elevations in plasma C-reactive protein points to a chronic inflammatory process in the artery or elsewhere in the body,21 which might reflect endothelial dysfunction caused by various atherogenic stimuli.23–26
To date, most population-based studies that examined the link between DM and inflammatory markers have been cross-sectional.11–17 Recently, a nested case–referent investigation of the Irish–French PRIME Study revealed that depression contributed significantly to the prediction of a future myocardial infarction (MI) and remained unchanged when adding inflammatory markers. 18 Using data from the prospective population based MONICA (monitoring trends and determinants in cardiovascular disease)–KORA Augsburg Cohort Study, we sought to investigate a possible interaction between C-reactive protein and DM and to explore whether a DM augments the capacity of C-reactive protein to predict a future coronary event in a large population based cohort of initially healthy male subjects.

Methods

Setting

The presented data were derived from the population based MONICA Augsburg studies (Germany) as part of the multinational WHO MONICA project. 27 Altogether, 13 427 persons (6725 men, 6702 women, response 77%) aged 25–74, randomly drawn from the general population, participated in at least one of three independent population based surveys, conducted in 1984–85 (S1), 1989–90 (S2), and 1994–95 (S3). The psychosocial data set followed recommendations given by the MONICA steering committee. 28 Psychosocial data from 13 265 subjects were available. Risk factor assessment was done at the time of the inclusion into the study. In the KORA follow-up study (www.gsf.de/kora), the vital status was assessed for all participants of the three MONICA surveys in 1998. During the observation period, 772 participants (531 men and 241 women) had died. Vital status could not be assessed for 56 persons (31 men and 25 women) who had moved to an unknown location.

Study group

The present analysis was restricted to male subjects aged 45–74 at baseline examination (n = 3819) because no C-reactive protein data were available for women. A total of 592 subjects had missing values on C-reactive protein or depression status. These subjects were older and had a lower educational level than subjects included into the study. However, the proportion of events in the missing value group did not differ from that in the study group (χ² test: P = 0.354). Participants with prevalent CHD or stroke (n = 158) or a history of cancer (n = 48) at baseline were excluded. Thus, 3021 subjects were included in the present analysis. Of these, 852 (28%) subjects were from survey S1 and 1052 (35%) from S2 and 1117 (37%) from S3.

Risk factor assessment

A non-fasting venous blood sample was collected from all participants in a supine resting position. Samples for measurement of C-reactive protein were stored at −80 °C until analysis. Serum C-reactive protein concentrations were measured using a highly sensitive immunoradiometric assay (range 0.05–10 mg/L) in S1 29 and a high sensitivity latex enhanced nephelometric assay on a BN II analyser in S2 and S3 (Dade Behring, Marburg, Germany). Both methods gave similar results when the same samples were analysed. 30 The intra- and inter-assay coefficients of variation of quality control test sera for C-reactive protein and cytokines were as follows: C-reactive protein-IRMA: 4.0 and 12.0%, C-reactive protein nephelometric assay: 2.5 and 5.1%, respectively. 30 Following a recent AHA/CDC scientific statement, 31 we stratified the study population into three categories of C-reactive protein concentration with cut-points of low risk (<1.0 mg/L), average risk (1.0–3.0 mg/L), and high risk (>3.0 mg/L). A total of 4.5% subjects exhibited C-reactive protein values of >10 mg/L.

Total serum cholesterol (mg/dL) was measured by enzymatic methods (CHOD-PAP, Boehringer Mannheim, Germany). A regular smoker was defined as a subject who smoked at least one cigarette per day. Body mass index (BMI) was calculated as weight in kilograms divided by height in meter square. Systolic blood pressure (SBP) was measured on the right arm in a sitting position using a Hawksley random-zero sphygmomanometer adhering to the WHO MONICA protocol. 27 Education level was categorized into ‘low’ (<12 years of schooling) and ‘high’ (≥12 years of schooling). Alcohol consumption was classified into three categories: non-drinkers (0 gal/day), intake of 0.1–39.9 gal/day, and intake of ≥40 gal/day. To assess physical activity, participants were classified as ‘active’ during leisure time if they regularly participated in sports and if they were active for at least 1 h/week in summer and winter.

Depressive symptomatology was assessed using a subscale from the von Zerssen affective symptom check list. 32 The subscale combines eight items (irritability, fatigue, nervousness, inner tension, loss of energy, difficulty in concentrating, irritability, and anxiety) ranging from 0 to 3, leading to a Likert-like scoring range of 0–24. The scale is normally distributed. In the absence of predefined cut-points, subjects in the top tertile (≥11.0) of the depressive symptom distribution (n = 986 vs. 2035) were considered as index group for subjects with a DM. 11

Study endpoints

The combined endpoint used in this study was incident non-fatal or fatal acute MI and sudden cardiac death. According to the MONICA manual, the diagnosis of a major non-fatal MI event was based on symptoms, cardiac enzymes (creatinine kinase, aspatate aminotransferase, and lactate dehydrogenase), and typical ECG changes. 27 Deaths from cardiovascular causes were validated by autopsy reports, death certificates, medical records, and information from the last treating physician 33 and were coded for the underlying cause of death using the 9th revision of the International Classification of Diseases (ICD-9, 410-414, 798). Cases were identified via the MONICA Augsburg coronary event registry, which covered the same population from which the survey participants had been sampled. They were censored at the 75th year of age. 34

Statistical analysis

Mean values or proportions for baseline demographic and clinical characteristics were computed for the study participants stratified by three levels of C-reactive protein. Significance of differences in mean values was tested with F-tests and significance of differences in proportions was tested by χ² test. All tests were performed two-sided.

Cox proportional hazard models 35 were applied to assess possible combined effects of continuous C-reactive protein and depression on the prediction of a future fatal and non-fatal coronary event. Next, two models were built for subjects with (n = 975) and without (n = 2018) depression. Cox proportional hazard models were run separately in these two subpopulations to obtain the effect of C-reactive protein. To examine the combined effects of categorical C-reactive protein and depression, we examined the hazard ratios for all five subgroup combinations derived from three levels of C-reactive protein (low, median, and high) and two levels of depression (highest tertile vs. low and medium tertiles) relative to low C-reactive protein/no-depression group.

The assumption of proportionality was assessed by fitting models stratified by risk factor categories, then plotting the log (–log) (survival) curves for each risk factor to check parallelism by visual inspection. The assumption of a linear relationship between a
continuous variable and the incidence of a coronary event was assessed by univariate Cox proportional hazard models including the variable in simple and square-rooted form in the regression equation. Except for BMI, the effects of square roots of all continuous variables (C-reactive protein, depression, age, cholesterol, and blood pressure) were not significant, indicating linearity of these variables. In contrast, linearity could not be assumed for BMI. Therefore, we chose to apply BMI as categorical variable (BMI ≥ 30, yes/no) in all analyses of the present investigation.

In each model, variables were entered simultaneously. Crude models were adjusted for age and survey. All other models were further adjusted for BMI, total cholesterol, smoking, SBP, education, alcohol consumption, and physical activity. Goodness-of-fit of the Cox models was tested by the \( \chi^2 \) criterion. \( P \)-values for the Cox models were based on Wald statistic. A \( P \)-value of less than 0.05 or more was considered significant. Statistical analyses were performed using SPSS (version 11.0).

### Results

The cohort of 3021 male subjects was followed for a median of 7.7 years (IQR = 6.9 years). During this observational period, 165 coronary events occurred; of which 88 events were fatal and 77 were non-fatal.

The baseline characteristics of the study participants stratified by three groups of C-reactive protein concentration are shown in Table 1: as expected, subjects in the high C-reactive protein group yielded the highest mean values in age, SBP, and BMI. Moreover, they had a higher cholesterol level, had smoked more, had a lower educational level, were less physically active, and alcohol consumption was more pronounced. In contrast, a DM was equally distributed across the three C-reactive protein groups. This finding was further confirmed by a very low correlation (Pearson's correlation coefficient \( r = 0.021 \)). As shown in Table 2, depressed

<table>
<thead>
<tr>
<th>CRP group</th>
<th>Non-depressed n = 2035</th>
<th>Depressed n = 986</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>57.1 (8.0)</td>
<td>57.5 (7.8)</td>
<td>0.195^a</td>
</tr>
<tr>
<td>Mean SBP</td>
<td>139.0 (18.2)</td>
<td>138.8 (18.7)</td>
<td>0.699^a</td>
</tr>
<tr>
<td>Mean BMI (kg/m²)</td>
<td>27.9 (3.5)</td>
<td>27.7 (3.4)</td>
<td>0.091^a</td>
</tr>
<tr>
<td>Mean total cholesterol (mg/dL)</td>
<td>242 (44)</td>
<td>246 (50)</td>
<td>0.043^b</td>
</tr>
<tr>
<td>Regular smoker, n (%)</td>
<td>480 (24)</td>
<td>235 (24)</td>
<td>0.881^b</td>
</tr>
<tr>
<td>Education &lt;12 years, n (%)</td>
<td>653 (32)</td>
<td>302 (31)</td>
<td>0.418^b</td>
</tr>
<tr>
<td>CRP group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-risk CRP (&lt;1.0 mg/L), n (%)</td>
<td>712 (35)</td>
<td>354 (36)</td>
<td>0.644^b</td>
</tr>
<tr>
<td>Average risk (1.0–3.0 mg/L), n (%)</td>
<td>758 (37)</td>
<td>350 (35)</td>
<td></td>
</tr>
<tr>
<td>High-risk CRP (&gt;3.0 mg/L), n (%)</td>
<td>565 (28)</td>
<td>282 (29)</td>
<td></td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0, n (%)</td>
<td>328 (16)</td>
<td>171 (17)</td>
<td>0.060^b</td>
</tr>
<tr>
<td>&gt;0.1–39 gal/day, n (%)</td>
<td>997 (49)</td>
<td>513 (52)</td>
<td></td>
</tr>
<tr>
<td>≥40 gal/day, n (%)</td>
<td>708 (35)</td>
<td>300 (31)</td>
<td></td>
</tr>
<tr>
<td>Physically active</td>
<td>840 (41)</td>
<td>336 (34)</td>
<td>&lt;0.001^b</td>
</tr>
</tbody>
</table>

^A-F-test.  
^B-\( \chi^2 \) test.
study participants had a higher mean value in total cholesterol level and were less likely to be physically active.

The interaction term of continuous C-reactive protein by depression disclosed a significant interaction effect in the age and survey adjusted model (log C-reactive protein x Depress = 1.03; 95% CI 1.00–1.06; P = 0.037) and lost significance in the model after multiple risk factor adjusting (1.02; 95% CI 1.00–1.06; P = 0.091). Lowering of significance in the fully adjusted model resulted from confounding effects of smoking (HR 2.0; 95% CI 1.4–2.8; \( P < 0.001 \)), total cholesterol (HR 1.004; 95% CI 1.001–1.008; \( P = 0.011 \)), and SBP (HR 1.009; 95% CI 1.001–1.017; \( P = 0.035 \)).

Next, we calculated the effect of C-reactive protein to predict a future coronary event separately in the non-depressed and depressed subpopulations. The findings shown in Table 3 disclose that C-reactive protein in the non-depression group was not significant, whereas the risk of a future event for high C-reactive protein levels in the depression group mounted to an HR of 2.69 (95% CI 1.32–5.47).

Figure 1 shows the relative risk (as HR) of all subgroup combinations derived from three levels of C-reactive protein and two levels of DM: relative to the subgroup with low level C-reactive protein and non-depression (n = 712), the combination of average C-reactive protein/depression in 350 men (HR 1.92; 95% CI 1.05–3.51; \( P = 0.035 \)), and high C-reactive protein/depression in 282 men (HR 2.91; 95% CI 1.67–5.10; \( P > 0.0001 \)) significantly predicted future CHD events. In contrast, the combination of high C-reactive protein/no depression (n = 565) (HR 1.60; 95% CI 0.93–2.75; \( P = 0.09 \)) was not predictive as was the combination of medium C-reactive protein/no depression (n = 758) (HR 1.12; 95% CI 0.64–1.96, n.s.). The risk of suffering from a cardiac event differed significantly \( (P < 0.009) \) in the high level C-reactive protein subgroups with (n = 282) and without (n = 565) depression.

### Discussion

Two lines of findings show that moderately raised serum C-reactive protein levels\(^8\)–\(^{10,22,31}\) and a DM state\(^1\)–\(^2\) are independently associated with an increased risk for a future first coronary event among apparently healthy men. The present study is the first to demonstrate that combined high C-reactive protein and DM exceeds considerably the relative risk of the top C-reactive protein tertile in subjects without apparent negative affectivity, which points to a possible synergistic effect of both conditions in the prediction of future CHD.

According to a consensus CDC/AHA statement\(^{31}\) and a recent meta-analysis,\(^{36}\) an average relative risk of 2.0 of the top tertile of C-reactive protein (>3 mg/L) in the prediction of future CHD is to be expected. The risk ratio after risk factor adjustment close to 3.0 for the combined high C-reactive protein and DM discloses the degree of the synergistic effect of both conditions on the prediction of a future CHD event. From these data, clinicians may be advised to interpret C-reactive protein on the background of a full knowledge of the clinical state of the patient, as ascertained by a thorough medical examination including the patients’ affective state.

So far, the only study which provided prospective data on the contribution of DM and inflammatory markers to CHD occurrence is a recent nested case–referent investigation within the PRIME study of healthy middle-aged men from Belfast and France.\(^{19}\) The authors found a stable association between depression and CHD outcome after adjustment of inflammatory markers and concluded that according to this finding, inflammation might not be primarily responsible for this association. In contrast, the present investigation disclosed a strong synergism. So far, reasons for the observed synergism are not known. However, it may be speculated that both conditions share a common underlying mechanism, which exerts a detrimental influence on the basic atherosclerotic disease process.

Studies in clinical populations with major depression revealed increased plasma concentrations of IL-1, IL-6, and acute phase proteins.\(^3\)–\(^7\) Higher levels of IL-1β and TNF-α were found in a depressed/exhausted sample of patients with angina pectoris when compared with angina patients without DM.\(^{18}\) Growing evidence from community-based samples points to a link between inflammatory markers and DM.\(^{11–17,19}\) Moreover, inflammation is often accompanied by behavioural effects, such as somnolence and decreased social exploration, referred to as ‘sickness behaviour’ in animals.\(^{20}\) In the present study, no evidence for a direct association between baseline C-reactive

### Table 3

<table>
<thead>
<tr>
<th>Stratification</th>
<th>Sample size</th>
<th>Events</th>
<th>Hazard ratio</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subgroup non-depression CRP (overall effect)</td>
<td>2018</td>
<td>94</td>
<td>Ref.</td>
<td>0.220</td>
<td></td>
</tr>
<tr>
<td>Low-risk CRP</td>
<td></td>
<td></td>
<td>1.12</td>
<td>0.64–2.00</td>
<td>0.697</td>
</tr>
<tr>
<td>Average-risk CRP</td>
<td></td>
<td></td>
<td>1.55</td>
<td>0.89–2.69</td>
<td>0.123</td>
</tr>
<tr>
<td>High-risk CRP</td>
<td></td>
<td></td>
<td>2.69</td>
<td>1.32–5.47</td>
<td>0.006</td>
</tr>
<tr>
<td>Subgroup depression high CRP (overall effect)</td>
<td>975</td>
<td>70</td>
<td>Ref.</td>
<td>0.016</td>
<td></td>
</tr>
<tr>
<td>Low-risk CRP</td>
<td></td>
<td></td>
<td>1.60</td>
<td>0.77–3.31</td>
<td>0.206</td>
</tr>
<tr>
<td>Average-risk CRP</td>
<td></td>
<td></td>
<td>2.69</td>
<td>1.32–5.47</td>
<td>0.006</td>
</tr>
</tbody>
</table>

\(^a\)Adjusted for age, survey, BMI, smoking, SBP, total cholesterol, physical activity, education years and alcohol consumption.
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protein concentrations and depression was found. Some studies did not confirm an association or the association was attenuated after controlling for cardiovascular risk factors. 7,12,14,37

Adiposity has been recently addressed to be among pathways through which depression may become associated with inflammation. 7,38,39 However, in the present analysis, the predictive value of C-reactive protein in the depression group remained significant even after adjustment for body mass and other risk factors which are known to affect C-reactive protein concentrations. 23–26

Limitations

The most advantageous aspect of the present study is its prospective population based design. The sample size allows controlling of confounding variables. The high sensitive measurement of C-reactive protein allowed classification following the recommendation of the CDC/AHA statement. 31

However, there are also limitations that need to be addressed. Depression was measured using a symptom rating scale which is among the less rigorous options to assess DM, although a recent re-examination of its validity and reliability is promising. 33 The results do not pertain to major depression as defined in international classification systems. Additionally, depression was measured at one time point, so that transient states of depression could not be distinguished from persistent states. Misclassification of exposure cannot be excluded because only one C-reactive protein measurement was available. Acute infections lead to temporarily increased C-reactive protein concentrations which do not represent the ‘true’ long-term basal C-reactive protein value. However, in this study, only 4.5% of subjects had C-reactive protein values > 10 mg/L. Patients with missing values were older and had a lower educational level. The exclusion of these subjects may underestimate the risk of future events, because both characteristics are likely to promote CHD risk. Although reasonable adjustments for major risk factors have been made, residual confounding through improved methods or other new risk factors remains possible (e.g., apolipoprotein B). The variable ‘physical activity’ focuses only on leisure time activity. Because leisure time physical activity accounts for a small proportion of the day and therefore for a small percentage of total energy output, non-leisure energy expenditure may also be important.

The present study is restricted to male subjects because no C-reactive protein data were available for women. Conflicting evidence surrounds the ability of depression in women to predict a future coronary event 40–42 and the association between inflammation and depression seems to be less pronounced in women. 13,15,17 Future investigations should evaluate the existence of a possible sex effect.

In conclusion, the present study demonstrates a significant interaction between C-reactive protein and depression, which resulted in a synergistic effect of moderately increased C-reactive protein in depressed but otherwise apparently healthy middle aged to older men to predict a subsequent first coronary event. Reasons for these interactions are largely unknown; however, it is likely that a depressive and emotional exhausted condition may set stage for activation of acute phase reactants by stimulating pro-inflammatory cytokines which, in a secondary loop, may foster low-grade inflammation. 18

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Conflict of interest: none declared.

References


