Increased Concentrations of C-Reactive Protein and IL-6 but not IL-18 Are Independently Associated With Incident Coronary Events in Middle-Aged Men and Women

Results From the MONICA/KORA Augsburg Case–Cohort Study, 1984–2002

Wolfgang Koenig, Natalie Khuseyinova, Jens Baumert, Barbara Thorand, Hannelore Loewel, Lloyd Chambless, Christa Meisinger, Andrea Schneider, Stephan Martin, Hubert Kolb, Christian Herder

Objectives—We performed a prospective case–cohort study in initially healthy, middle-aged men and women from the MONICA/KORA Augsburg studies conducted between 1984 and 2002 to assess the role of IL-18 in comparison with IL-6 and CRP in the prediction of incident coronary heart disease (CHD).

Methods and Results—Concentrations of IL-18 were measured in 382 case subjects with incident CHD and 1980 noncases. Mean follow-up was 11 years. Baseline concentrations of IL-18 were slightly higher in cases than in noncases (172.4 [1.0] versus 161.3 [1.0] pg/mL, respectively; \( P = 0.114 \)), but were clearly elevated for C-reactive protein (CRP) and IL-6 in cases compared with noncases. In multivariable analyses, accounting for classical cardiovascular risk factors and inflammatory markers, no statistically significant association was seen between increased concentrations of IL-18 and incident CHD in men (hazard ratio [HR] and 95% confidence intervals [CIs] comparing extreme tertiles, 1.20; 95% CI, 0.85 to 1.69), and in women (HR, 1.25; 95% CI, 0.67 to 2.34). However, in this population increased concentrations of CRP and IL-6 were found to be independent predictors of future CHD events, even after multivariable adjustment.

Conclusions—Elevated concentrations of CRP and IL-6, but not IL-18, were independently associated with risk of CHD in subjects from an area with moderate absolute risk. (Arterioscler Thromb Vasc Biol. 2006;26:0000-0000.)

Key Words: case–cohort study ■ coronary heart disease ■ IL-18 ■ inflammation ■ risk factors

Cytokine-mediated inflammation accompanies atherosclerosis from its initiation to the occurrence of clinical endpoints. Recently IL-18, a member of the IL-1 family of cytokines, has been suggested to play a central role in the regulation of both innate and adaptive immunity. IL-18 is widely expressed in monocytes/macrophages, dendritic cells, Kupffer cells, adipocytes, colon carcinoma cells, keratinocytes, and osteoblasts. It is synthesized as a 23-kDa biologically inert precursor, which is further cleaved by caspase 1 (or IL-1β-converting enzyme) to yield the mature and active 18.3-kDa glycoprotein. IL-18 induces interferon- \( \gamma \) with subsequent promotion of the Th1 immune response; it also enhances the expression of matrix metalloproteinases. These 2 abilities of IL-18 characterize it as a crucial and potent mediator of atherosclerotic plaque destabilization and vulnerability. Indeed, some experimental studies demonstrated significantly increased expression of IL-18 in human atheroma, especially in lesions prone to rupture, where it is localized mainly in plaque macrophages. Animal models further support the proatherogenic role of IL-18, demonstrating that endogenous inhibition of IL-18 by IL-18 binding protein reduced atherosclerotic plaque development and progression in apolipoprotein (apo) E-deficient mice. Similarly, IL-18/apoE double knockout mice exhibited reduced lesion size. In contrast, direct administration of IL-18 enhanced atherogenesis in an interferon- \( \gamma \)-dependent manner, even in the absence of T cells and promoted a switch to a vulnerable plaque phenotype by decreasing intimal collagen content and cap-to-core ratio.

Although the aforementioned experimental studies seem to be relatively consistent, the results of only few clinical studies remain somewhat controversial. While a number of recent cross-sectional studies favored a role of IL-18 in the development of plaque instability, showing significantly increased

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levels of this cytokine in patients with acute coronary syndrome (ACS).14-15 several other investigations were not able to demonstrate any meaningful relationship with coronary heart disease (CHD).16-17 Furthermore, in 1 prospective study IL-18 predicted cardiovascular events in patients with manifest CHD,18 and data in initially healthy subjects are scarce and need to be confirmed.19

Therefore, we conducted a prospective case–cohort study within the MONICA/KORA Augsburg platform to assess the role of IL-18 in the prediction of incident CHD (fatal and nonfatal myocardial infarction [MI] and sudden cardiac death) over a mean follow-up (FU) of 11 years.

## Methods

### Study Design

The design of this prospective case–cohort study conducted within the population-based MONICA/KORA Augsburg cohort has been recently described in detail.20 A combined endpoint that included incident fatal/nonfatal MI and sudden cardiac death occurring before the age of 75 years was used as the outcome variable, and was identified through the MONICA/KORA Augsburg coronary event registry and through FU questionnaires for subjects who had moved out of the study area. Because of the low incidence of CHD in those younger than 35 years, the present study was limited to 10,718 persons (5382 men and 5336 women) between 35 to 74 years at baseline who participated in at least 1 of the 3 MONICA/KORA Augsburg surveys (S), conducted in 1984/1985 (S1), 1989/1990 (S2), and 1994/1995 (S3).21 After exclusion of 1187 subjects with missing blood samples and 231 participants with self-reported prevalent CHD, the source population for the present study comprised 9300 subjects (4507 men, 4793 women).

For the case–cohort study, a random sample of the source population, called here the subcohort, containing 2163 subjects (1154 men, 1009 women) was selected stratifying by sex and survey. Participants with missing values for IL-18 or any of the covariates used in the present analysis were excluded leading to a subcohort of 2095 subjects (1101 men, 994 women). The final stratum-specific sample sizes were used together with the stratum-specific sizes of the cohort of interest to compute sampling fractions, and the inverse of the sampling fractions yielded the survey and sex-specific sampling weights: 3.01, 3.84, 6.25 for men, and 3.93, 4.76, 6.12 for women.

The number of cases of incident CHD until December 31, 2002 was 397 (307 men, 90 women). For 295 men and 87 women, complete information on all relevant variables was available. Because 92 male and 23 female cases were also part of the randomly drawn subcohort, the present analysis comprised a total of 2362 participants (295 men and 87 women with incident CHD, 1009 men and 971 women without incident CHD). For detailed information on the study design please see the online data supplement (http://atvb.ahajournals.org).

### Data Collection, Laboratory Measurements, and Statistical Analyses

Most aspects of data collection and laboratory measurements have been described before.21 For additional details and information on statistical analyses, please see the online data supplement.

### Results

Overall, 2362 participants (382 subjects with incident CHD and 1980 subjects without incident CHD) of the 3 population-based MONICA/KORA Augsburg surveys were included in this case–cohort study. The mean FU time (±SD) was 11.0 (±5.0) years for the study population and ranged from 0.05 to 18.2 years.

The baseline demographic, clinical, and laboratory characteristics of the study population are shown in Table 1. Subjects with incident CHD were older, more likely to be current or former smokers, were less active, and showed a higher body mass index and waist-to-hip ratio compared with noncases. Furthermore, cases more frequently reported a history of diabetes mellitus and actual hypertension, as well as a positive or unknown parental history of MI, whereas no significant differences were observed for educational levels and alcohol consumption habits between the 2 groups.

As expected, total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) concentrations, and TC/high-density lipoprotein cholesterol (HDL-C) ratio were considerably higher and HDL-C was considerably lower in cases compared with noncases.

Although plasma concentrations of IL-18 at baseline tended to be slightly higher in CHD cases than in noncases, they did not differ statistically significantly between subjects with incident CHD and those who remained free of an event during FU (weighted geometric means with antilog of standard errors of log means); 172.4 (1.0) versus 163.1 (1.0) pg/mL, respectively; P=0.114 (Table 1): Geometric means of IL-18 were 174.1 (1.1) pg/mL in cases and 162.4 (1.0) pg/mL in noncases for men (P=0.163) and 165.2 (1.1) pg/mL and 160.3 (1.0) pg/mL for women (P=0.712), respectively. Conversely, CRP and IL-6 concentrations were found to be significantly increased in subjects with incident CHD compared with healthy individuals and that held true for both genders (CRP concentration for men: 2.5 [1.1] mg/L in cases and 1.5 [1.0] mg/L in noncases; P<0.001), for women, 2.8 [1.1] versus 1.4 [1.0] mg/L (P<0.001), respectively; IL-6 concentration for men: 3.0 [1.1] pg/mL in cases and 2.1 [1.0] pg/mL in noncases; P<0.001 and for women, 3.4 [1.1] versus 1.9 [1.0] pg/mL; P<0.001, respectively.

Pearson correlation coefficients (R) between log IL-18 and inflammatory markers, lipid variables or conventional risk factors calculated in the randomly sampled subcohort of 2095 individuals, revealed a weak positive, but statistically significant, correlation between log IL-18 and log CRP (R=0.095, P<0.001), log IL-6 (R=0.085, P<0.001), and the TC/HDL-C ratio (R=0.067, P=0.006). Furthermore, a borderline significant positive correlation between log IL-18 and WHR (R=0.071, P=0.005); diastolic blood pressure (BP) (R=0.055, P=0.012) and a negative correlation with HDL-C (−0.063, P=0.004) were also observed. No statistically significant correlations were seen with age, body mass index, total, and LDL-C (data not shown).

Table 2 shows the results of Cox proportional hazards analysis, in which the association of baseline IL-18 concentrations with incident CHD was assessed. In age and survey adjusted analyses (Model 1), there was a statistically significant association between increased concentrations of IL-18 and incident CHD in men (HR and 95% CI comparing tertile extremes were 1.43; 95% CI, 1.05 to 1.96; P for trend 0.019, respectively), whereas the association of similar size in women (HR, 1.47; 95% CI, 0.87 to 2.48; P for trend 0.108, respectively) was not statistically significant. In multivariable analyses, accounting for traditional cardiovascular risk factors and TC/HDL-C ratio, this association was attenuated and...
TABLE 1. Baseline Demographic, Clinical, and Laboratory Characteristics of the Participants With and Without Incident CHD During Follow-Up

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CHD Cases</th>
<th>Noncases</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (women/men)</td>
<td>87/295</td>
<td>971/1009</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>57.3 (0.4)</td>
<td>52.3 (0.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Education (&lt;12 years)</td>
<td>78.8</td>
<td>76.7</td>
<td>0.361</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>40.1</td>
<td>23.7</td>
<td></td>
</tr>
<tr>
<td>Former smoker</td>
<td>31.4</td>
<td>27.7</td>
<td></td>
</tr>
<tr>
<td>Never smoker</td>
<td>28.5</td>
<td>48.6</td>
<td></td>
</tr>
<tr>
<td>Frequency of exercise</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Active</td>
<td>27.5</td>
<td>38.5</td>
<td></td>
</tr>
<tr>
<td>Inactive</td>
<td>72.5</td>
<td>61.5</td>
<td></td>
</tr>
<tr>
<td>Alcohol consumption†</td>
<td></td>
<td></td>
<td>0.195</td>
</tr>
<tr>
<td>0 g/d</td>
<td>28.0</td>
<td>31.7</td>
<td></td>
</tr>
<tr>
<td>&gt;0 to 39.9/19.9 g/d</td>
<td>41.6</td>
<td>42.1</td>
<td></td>
</tr>
<tr>
<td>≥40/20 g/d</td>
<td>30.4</td>
<td>26.3</td>
<td></td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>28.5 (0.2)</td>
<td>27.1 (0.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist to hip ratio¶</td>
<td>0.93 (0.005)</td>
<td>0.87 (0.002)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History of diabetes mellitus</td>
<td>18.1</td>
<td>4.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Parental history of MI</td>
<td></td>
<td></td>
<td>0.030</td>
</tr>
<tr>
<td>Positive</td>
<td>22.8</td>
<td>19.8</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>25.4</td>
<td>20.9</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>51.8</td>
<td>59.3</td>
<td></td>
</tr>
<tr>
<td>History of actual hypertension</td>
<td>65.5</td>
<td>41.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>142.6 (1.1)</td>
<td>133.5 (0.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure mm Hg</td>
<td>83.5 (0.6)</td>
<td>81.5 (0.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Current HRT‡</td>
<td>5.4</td>
<td>10.3</td>
<td>0.104</td>
</tr>
<tr>
<td>Current use of OC§</td>
<td>0.0</td>
<td>14.3</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>258.0 (2.5)</td>
<td>237.1 (1.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>170.0 (3.0)</td>
<td>148.8 (1.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>49.3 (0.8)</td>
<td>57.2 (0.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ratio TC/HDL cholesterol</td>
<td>5.7 (0.1)</td>
<td>4.5 (0.04)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C-reactive protein, mg/L**</td>
<td>2.6 (1.1)</td>
<td>1.4 (1.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Interleukin-6, pg/mL**</td>
<td>3.1 (1.1)</td>
<td>2.0 (1.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Interleukin –18, pg/mL**</td>
<td>172.4 (1.0)</td>
<td>161.3 (1.0)</td>
<td>0.114</td>
</tr>
<tr>
<td>Survey</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Data are weighted percentages for categorical variables, weighted means (standard errors) for normally distributed continuous variables and **weighted geometric means with (antilog of standard errors of log means) for skewed continuous variables.

†Only measured in participants of survey 2 and 3 (cases: n=234; noncases: n=1263).
‡Only for women aged <50 years (n=413) with no current hormone replacement therapy.
¶Only measured in participants of survey 2 and 3 (cases: n=234; noncases: n=1263).
§Only for women aged <50 years (n=234; noncases: n=1263).

no longer statistically significant in men (HR, 1.21; 95% CI, 0.86 to 1.69; P for trend 0.241) and remained nonsignificant in women (HR, 1.23; 95% CI, 0.67 to 2.28; P for trend 0.461) (Model 2). Additional adjustment for the inflammatory markers CRP and IL-6 (model 3) did not have a further impact on the relation between elevated IL-18 concentrations and risk of subsequent coronary events. Finally, when the prognostic value of IL-18 was assessed in the whole population (n=2362), the magnitude of such association was consistent with that observed in men. A statistically significant association between increased IL-18 concentration (T3 versus T1) and incident CHD was found only in age and survey adjusted analyses, whereas further adjustment for various traditional cardiovascular risk factors, TC/HDL-C ratio and inflammatory biomarkers led to a loss of the statistical significance of the association (HR for T3 versus T1 in the fully adjusted model including CRP and IL-6: 1.22, 95% CI, 0.91 to 1.64, P for trend 0.164) (Table ●●). Moreover, almost identical results have been obtained if risk estimates for the top tertile of the IL-18 distribution were compared with the combined first and second tertiles with a HR of 1.22 (95% CI, 0.95 to 1.57; P=0.11) in the fully adjusted model.

Taking into account that increased IL-18 concentrations might be related to more severe disease, we also assessed the association between this biomarker and fatal MI among 202 cases (157 male and 45 female) who died from MI during 11 years of FU and 1980 noncases. Although plasma concentrations of IL-18 tended to be slightly higher in fatal MI cases compared with nonfatal cases (175.8 versus 168.7 pg/mL), this difference was not statistically significant (P=0.59). Similarly, we found no statistically significant association between increased IL-18 concentrations and incident fatal MI in multivariable analysis, although the HRs were slightly higher compared with those obtained in the whole study population (HR, 1.39; 95% CI, 0.93 to 2.06; P for trend 0.11 for T3 versus T1 in the fully adjusted model including CRP and IL-6). Finally, no statistically significant associations were seen between IL-18 concentrations and future coronary events, if IL-18 was used as a continuous (untransformed and log-transformed) variable instead of tertiles (data not shown).

However, when we investigated the prognostic values of CRP and IL-6 in this population (Figure), we found an ≈2-fold increase in coronary risk in men associated with CRP levels in the top tertile compared with the lowest tertile after adjustment for a range of traditional cardiovascular risk factors and the TC/HDL-C ratio (HR, 1.89; 95% CI, 1.28 to 2.77; P=0.014, Model 2). This held true in further analyses taking into account a potent inflammatory marker such as IL-6 (HR, 1.87; 95% CI, 1.27 to 2.76; P=0.018, Model 3) (Figure, upper left panel). No statistically significant association was found in women with a multivariable adjusted HR of 1.35 (95% CI, 0.64 to 2.84; P=0.245) in Model 2 and a HR of 1.32 (95% CI, 0.62 to 2.81; P=0.283) in a model additionally adjusted for IL-6 (Figure, upper right panel). Conversely, increased levels of IL-6 revealed a strong and independent association with first-ever coronary events in women after multivariable adjustment (HR, 2.99; 95% CI, 1.38 to 6.49; P=0.009; Model 2) (Figure, lower right panel). Further inclusion of CRP in the model did not affect its
predictive ability in women (HR, 2.94; 95% CI, 1.31 to 6.62; 
P = 0.005; Model 3), whereas no statistically significant 
association was found in men, comparing the top tertile of the 
IL-6 distribution to the bottom tertile (HR, 1.42; 95% CI, 0.97 
to 2.07; P = 0.266; Model 3) (Figure, lower left panel).

Additional adjustment for IL-18, which was attempted in a 
final model for CRP and IL-6, as expected, had no impact on 
the observed HRs (data not shown).

Taking into account that IL-18 was previously considered 
as a short-term or intermediate-term risk marker, we exam-
ined the prognostic value of increased IL-18 concentrations in 
accordance with the occurrence of a first coronary events over 
different FU periods (1, 2, 3, 4, 5, and 6 years) and found that 
multivariable adjusted HRs estimated for various time points 
were similar to the HRs estimated for the full FU period (data 
not shown).

Discussion

Data from this large prospective case–cohort study did not 
show a statistically significant independent association be-
tween increased IL-18 concentrations and subsequent coro-
nary events in apparently healthy, middle-aged subjects. By 
contrast, increased concentrations of CRP in men and IL-6 in 
women remained strong and independent predictors of CHD 
risk in this population even after adjustment for traditional 
cardiovascular risk factors, and for IL-6 in case of CRP and 
vice versa and thus confirmed results from various cohorts.

Our data on IL-18 are in contrast to 2 prospective cohorts 
reported in the literature.18,19 Results from 1 large prospective 
study, conducted in 1229 patients with angiographically 
confirmed CAD showed that increased concentrations of 
IL-18 at baseline were associated with future cardiovascular 
(CV) death during a 3.9-year follow-up, even after adjust-

<table>
<thead>
<tr>
<th>TABLE 2. Hazard Ratios (95% CI) for the Risk of Incident CHD According to Baseline Concentration of IL-18</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tertiles of IL-18</strong></td>
</tr>
<tr>
<td><strong>T1</strong></td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td><strong>Men</strong></td>
</tr>
<tr>
<td>Median (IQR), pg/mL</td>
</tr>
<tr>
<td>No. cases/noncases</td>
</tr>
<tr>
<td>HR (95% CI) adjusted for age and survey</td>
</tr>
<tr>
<td>P</td>
</tr>
<tr>
<td>HR (95% CI) adjusted for age, survey, and traditional CV risk factors*</td>
</tr>
<tr>
<td>P</td>
</tr>
<tr>
<td>HR (95% CI) additionally adjusted for CRP and IL-6</td>
</tr>
<tr>
<td>P</td>
</tr>
<tr>
<td><strong>Women</strong></td>
</tr>
<tr>
<td>Median (IQR), pg/mL</td>
</tr>
<tr>
<td>No. cases/noncases</td>
</tr>
<tr>
<td>HR (95% CI) adjusted for age and survey</td>
</tr>
<tr>
<td>P</td>
</tr>
<tr>
<td>HR (95% CI) adjusted for age, survey, and traditional CV risk factors*</td>
</tr>
<tr>
<td>P</td>
</tr>
<tr>
<td>HR (95% CI) additionally adjusted for CRP and IL-6</td>
</tr>
<tr>
<td>P</td>
</tr>
<tr>
<td><strong>Total</strong></td>
</tr>
<tr>
<td>Median (IQR), pg/mL</td>
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<tr>
<td>No. cases/noncases</td>
</tr>
<tr>
<td>HR (95% CI) adjusted for age, gender, and survey</td>
</tr>
<tr>
<td>P</td>
</tr>
<tr>
<td>HR (95% CI) adjusted for age, gender, survey, and traditional CV risk factors*</td>
</tr>
<tr>
<td>P</td>
</tr>
<tr>
<td>HR (95% CI) additionally adjusted for CRP and IL-6</td>
</tr>
<tr>
<td>P</td>
</tr>
</tbody>
</table>

Hazard ratios were estimated by Cox proportional hazard model. Correction for standard errors was made by SAS macro ROBPHREG using the method by Barlow.

Tertiles of the weighted distributions of IL-18 in the subcohort, stratified for gender, were used: men 139.2 and 222.4; women 133.8 and 208.9.

*Adjustment for traditional cardiovascular (CV) risk factors includes adjustment for body mass index, smoking status, alcohol consumption, physical activity, systolic blood pressure, TC/HDL-C, parental history of MI, and history of diabetes.
ment for potential confounders (HR, 3.3; 95% CI, 1.3 to 8.4; \( P=0.01 \)). Such association held further true if the prognostic value of increased IL-18 on subsequent CV events was assessed separately in patients with stable and unstable patients on admission. However, when the FU was extended from 3.9 to 5.9 years, IL-18 concentrations were no longer predictive of outcome, thus questioning the value of IL-18 as an independent risk marker for future CV events.

These results, however, cannot be directly compared with our findings, because they were based on data from subjects with pre-existing CHD. Our data, by contrast, is based on a large prospective case–cohort study including initially healthy subjects from a population based sample.

To date, the prognostic value of elevated IL-18 for future coronary events in apparently healthy subjects has been investigated only in the PRIME study, representing a cohort of 10,600 middle-aged men without pre-existing CHD, recruited from 2 European populations (French and Irish populations). Three hundred thirty-five subjects who experienced at least one CHD event (nonfatal MI, coronary death, and angina pectoris) during a 5-year FU were compared with 670 age-matched controls. Baseline concentrations of IL-18 were significantly higher among cases compared with those who remained free of disease (225.1 versus 203.9 pg/mL, \( P=0.005 \)). Furthermore, elevated IL-18 concentrations were associated with a 2-fold increased risk for subsequent coronary events after multivariable adjustment, if the upper tertile of the IL-18 distribution was compared with the bottom tertile. However, such an association was statistically significant only when data from both populations were pooled for analysis. When the prognostic value of IL-18 was investigated separately in the French and the Belfast cohort, the statistical significance of the association between increasing IL-18 tertiles and future coronary events was completely lost in France (for both, combined endpoints and for coronary death/MI), whereas in the Irish population it even increased.

One possible explanation for such discrepancy could be because of the fact that the French and the Belfast PRIME cohorts represent heterogeneous populations. For instance, differences between these 2 cohorts with regard to the incidence of CHD are marked and have been reported previously.23–24 Additionally, the distribution of several cardiovascular risk factors in the Irish PRIME population was also different from the French cohort and thus might account, at least in part, for the excess risk of CHD seen in Belfast. Indeed, several studies demonstrated that the Belfast participants, as compared with the French, had much higher levels of triglycerides and LDL-C and significantly lower concentrations of HDL-C.23,25 In addition, increased concentrations of several inflammatory biomarkers such as CRP, fibrinogen, plasminogen activator inhibitor-1, IL-6, and IL-18 were also observed in Belfast as opposed to France.19,26–27 Therefore, the Irish PRIME population might represent a population with a high risk for CHD, whereas the French and the German cohorts represent populations with moderate or even low absolute CHD risk. Thus, such existing differences could explain the observed discrepancies between our cohort and the PRIME study.

Our study has several strengths that need to be mentioned. First, it represents a population-based prospective study conducted in initially healthy subjects. Second, we minimized the likelihood of survival bias because fatal and nonfatal coronary events were included in our study. Third, we carefully adjusted for conventional risk factors by multivariable methods.

The present study has also several limitations that should be considered. The mean FU period of 11 years is relatively long and might be responsible for the weakening of the association between the risk marker and the disease outcome. This, however, cannot completely explain the lack of a significant association between IL-18 and coronary risk, because increased concentrations of CRP and IL-6 were
strongly and independently associated with future CHD, as seen in other cohorts. Moreover, in the same cohort increased concentrations of IL-18 strongly and independently predicted incident type 2 diabetes mellitus over 10.8 years. Further, the lack of association between increasing concentrations of IL-18 and incident CHD in women should be interpreted with caution because of the relatively low number of CHD events in this group (n = 87). However, if multivariable analysis was performed in the total study population that had a power of 97% to detect a HR of 1.5, almost identical results were found, with similar hazard ratios as can be seen in Table 2. Moreover, testing for interaction of gender and IL-18 revealed \( P > 0.7 \) in all 3 models, indicating no modification of the association between IL-18 and CHD by gender. Another point that may be mentioned here is that IL-18 might be more suitable to predict short-term risk versus long-term risk. However, we did not find any significant association between increased concentrations of IL-18 and occurrence of a first coronary event during short or intermediate FU periods as well. Regarding the method of IL-18 detection, we used a Luminex assay with a somewhat higher interassay CV than the enzyme-linked immunosorbent assays used in previous studies, which might have attenuated the association between IL-18 concentrations and incident CHD slightly. Thus, although higher quality assays might have provided similar HRs, CIs most probably would be narrower. Another limitation of our study may result from the fact that only middle-aged men and women of German nationality were included. Thus, these data may not be extrapolated to other populations, ethnic/racial minorities and other age-groups. Despite these limitations, this study does not suggest an independent, clinically relevant association between increased concentrations of IL-18 and risk of future CHD in apparently healthy subjects from an area with moderate absolute risk of CHD. However, the lack of prognostic impact of increased IL-18 concentrations on the occurrence of future coronary events among apparently healthy subjects does not completely exclude the potential significance of IL-18–regulated mechanisms in the pathophysiology of atherosclerotic disease, especially taking into account potent plaque destabilizing properties of this molecule. Moreover, IL-18 might serve as a marker of future cardiovascular events in men with manifest CHD and/or in areas of high absolute risk of CHD. Thus, further studies are warranted to establish the role of IL-18 in the prediction of CHD risk.

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Disclosures

None.

References


Increased Concentrations of C-Reactive Protein and IL-6 but not IL-18 Are Independently Associated With Incident Coronary Events in Middle-Aged Men and Women. Results From the MONICA/KORA Augsburg Case-Cohort Study, 1984-2002

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ON-LINE SUPPLEMENT

Methods

Study Design

Based on data from the MONICA/KORA Augsburg studies we conducted a prospective case-cohort study in initially healthy, middle-aged men and women. The MONICA Augsburg project (Monitoring of Trends and Determinants in Cardiovascular Disease) was part of the multinational WHO MONICA project. Briefly, three independent population-based MONICA/KORA Augsburg surveys (S), with a total number of 13,427 participants (6,725 men, 6,702 women) aged 25-64 (S1) or 25-74 years (S2-S3), were conducted in 1984/85 (S1), 1989/90 (S2) and 1994/95 (S3). All subjects were prospectively followed within the framework of KORA (Kooperative Gesundheitsforschung in der Region Augsburg). The case-cohort design used in the present study has been recently described in detail.

A combined endpoint that included incident fatal/non-fatal MI and SCD occurring before the age of 75 years was used as the outcome variable, and was identified through the MONICA/KORA Augsburg coronary event registry and through FU questionnaires for subjects who had moved out of the study area. Until December 2000, the diagnosis of a major non-fatal MI event was based on the MONICA algorithm taking into account symptoms, cardiac enzymes and ECG changes. Since January 1, 2001 all patients with MI diagnosed according to ESC (European Society of Cardiology) and ACC (American
College of Cardiology) criteria were included\textsuperscript{5-6}. Deaths from MI were validated by autopsy reports, death certificates, chart review, and information from the last treating physician. Due to the low incidence of CHD under 35 years, the present study was limited to 10,718 persons (5,382 men and 5,336 women) between 35-74 years at baseline who participated in at least one of the three surveys. After exclusion of 1,187 subjects with missing blood samples and 231 participants with self-reported prevalent CHD, the source population for the present study comprised 9,300 subjects (4,507 men, 4,793 women).

For the case-cohort study, a random sample of the source population, called here the subcohort, containing 2,163 subjects (1,154 men, 1,009 women) was selected stratifying by sex and survey. Participants with missing values for IL-18 or any of the covariables used in the present analysis were excluded leading to a subcohort of 2,095 subjects (1,101 men, 994 women). The final stratum-specific sample sizes were used together with the stratum-specific sizes of the cohort of interest to compute sampling fractions, and the inverse of the sampling fractions yielded the survey and sex specific sampling weights: 3.01, 3.84, 6.25 for men, and 3.93, 4.76, 6.12 for women.

The number of cases of incident CHD until December 31\textsuperscript{st}, 2002 was 397 (307 men, 90 women). For 295 men and 87 women complete information on all relevant variables was available. Since 92 male and 23 female cases were also part of the randomly drawn subcohort, the present analysis comprised a total of 2,362 participants (295 men and 87 women with incident CHD, 1,009 men and 971 women without incident CHD).

**Data collection**

All participants completed a standardized questionnaire, including medical history, lifestyle, and drug history. In addition, all subjects underwent a medical examination. Systolic and diastolic blood pressures (BP), body height (m), body weight (kg), body mass index (BMI, kg/m\textsuperscript{2}), smoking behavior, history of diabetes, history of MI, alcohol consumption (g/d) were determined as described previously\textsuperscript{7}. Obesity was defined as a BMI ≥30 kg/m\textsuperscript{2}. Hypertension was defined as BP values ≥140/90 mmHg and/or use of
antihypertensive medication given that the subjects were aware of being hypertensive. The number of education years was calculated on the basis of the highest level of formal education completed. Leisure-time physical activity was assessed by a four-level graded scale assessing sports activities during summer and winter time (0, <1, 1-2, >2 hours/week). The study was approved by the local authorities. Written informed consent was obtained from all subjects.

**Laboratory methods**

A non-fasting venous blood sample was obtained from all study participants at baseline. All samples were stored at –80°C until further analysis.

Total (TC) and HDL cholesterol (HDL-C) were measured by routine enzymatic methods (CHOD-PAP; Boehringer Mannheim, Mannheim, Germany). Serum concentrations of IL-18 were determined in single measurements by Luminex technology using an antibody pair and recombinant IL-18 protein from MBL (Nagoya, Japan). The assay was based on a protocol of de Jager et al. The lower detection limit of IL-18 in this assay was approximately 9.8 pg/mL. In general, the intra- and inter-assay coefficients of variation (CV) of this assay are <10.0 and <25.0%, respectively. In this study, the median inter-assay CV was 14.4% based on duplicate measurements of 61 random serum samples of cases and non-cases.

Serum levels of IL-6 were determined using a previously described sandwich ELISA. In addition, CRP serum concentrations were determined by a high-sensitive immunoradiometric assay (IRMA) using a 5-point calibration with the WHO international reference standard 85/506 (range, 0.05-10 mg/L) (S1: men aged 45-64; S3) or a high sensitivity immunonephelometric assay (Dade Behring, Marburg, Germany) on a BN II analyzer (S1: men aged 35-44 and all women; S2); both methods gave similar results when the same samples were analyzed. The intra- and inter-assay CV for CRP and IL-6 were: CRP-IRMA: 4.0 and 12.0%, CRP immunonephelometry: 2.5 and 5.1%, IL-6: <10.0 and <10.0%. All analyses were run in a blinded fashion.

**Statistical analysis**
Means or proportions for baseline demographic and clinical characteristics were computed using the SAS procedures SURVEYREG or SURVEYFREQ which estimated standard errors appropriate to the sampling scheme. In case of non-normality, tests were carried out with log-transformed variables and results are presented as geometric means with antilogs of standard errors of the adjusted log means. Associations between continuous variables were assessed by weighted Pearson Correlations with p values obtained by SURVEYREG.

Cox proportional hazards analysis was used to assess the association between IL-18, CRP and IL-6 and incident CHD. Due to the case-cohort design, standard errors were corrected using a SAS macro with a "sampling weight" approach developed by Barlow et al\textsuperscript{1} The weighted tertiles of IL-18, CRP and IL-6 in the subcohort were used to classify subjects in different risk groups for each inflammation parameter. In multivariable analyses, we adjusted for the continuous variables age, BMI, BP, TC/HDL-C ratio, CRP, IL-6 and the categorical indicator variables sex, survey, smoking status (never smoker, former smoker, current smoker), alcohol consumption (men 0, 0.1-39.9, ≥40 g/d; women 0, 0.1-19.9, ≥20 g/d), physical activity (inactive vs. active, i.e. regular physical activity of ≥1 hour/week in both summer and winter), prevalent diabetes and parental history of CHD (negative, positive, unknown). Results are presented for each inflammation parameter tertile (coded as dummy variables with the first tertile as the reference category) as hazard ratios (HRs) and their 95% confidence intervals (CIs). P values are based on robust variance estimates using the Barlow method. For test for trends, tertiles were coded by their median values. Interactions between tertiles (coded as dummy variables) and other risk factors were examined by additionally including respective interaction terms in the model. The power estimation, computed by the method of Cai and Zheng\textsuperscript{13}, revealed a power of 84% to detect an HR of 1.35 and of 97% to detect an HR of 1.5 for subjects above the upper tertile of the IL-18 distribution compared to subjects below the upper tertile. For all statistical analyses p values were two-tailed and values lower than 0.05 were considered to be statistically significant. All statistical analyses were performed using the Statistical Analysis System (SAS) software package (Version 8.02 for Unix and Version 9.1 for Windows, SAS-Institute Inc., Cary, NC, USA).
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