IL-8 Plasma Concentrations and the Risk of Future Coronary Artery Disease in Apparently Healthy Men and Women

The EPIC-Norfolk Prospective Population Study

S. Matthijs Boekholdt, Ron J.G. Peters, C. Erik Hack, Nicholas E. Day, Robert Luben, Sheila A. Bingham, Nicholas J. Wareham, Pieter H. Reitsma, Kay-Tee Khaw

Objective—To study the role of IL-8 in predicting future coronary artery disease (CAD) in apparently healthy men and women.

Methods and Results—A nested case-control study was performed in the prospective EPIC-Norfolk population study. We measured baseline IL-8 concentrations among 785 apparently healthy individuals in whom fatal or nonfatal CAD developed during follow-up and 1570 matched controls. Baseline IL-8 concentrations were higher in cases than in matched controls (3.5 pg/mL versus 3.1 pg/mL, \( P=0.001 \)). The risk of future CAD increased with increasing quartiles of IL-8 (\( P \) linearity <0.0001). Among individuals in the highest IL-8 quartile, the unadjusted odds ratio for future CAD was 1.72 (95% CI, 1.34 to 2.21; \( P<0.0001 \)). The odds ratio for future CAD was still significant after adjustment for traditional risk factors (OR, 1.58; 95% CI, 1.19 to 2.09; \( P=0.002 \)) and after additional adjustment for C-reactive protein and white cell count (OR, 1.77; 95% CI, 1.21 to 2.60; \( P=0.001 \)).

Conclusions—We conclude that among apparently healthy men and women, elevated levels of IL-8 are associated with an increased risk of future CAD. These prospective data support a role for IL-8 in the development of CAD events.

Key Words: IL-8 • cytokines • coronary artery disease

Inflammation plays a key role in the initiation and progression of atherosclerosis.¹ For clinical purposes, C-reactive protein (CRP) is gradually gaining acceptance as the most useful inflammatory plasma marker.² However, the inflammatory processes that underlie atherosclerosis are mediated by a multitude of cytokines and are unlikely to be reflected by CRP levels alone. Prospective evidence on other cytokines in apparently healthy individuals is limited and exists only for IL-6³–⁶ and macrophage inhibitory cytokine-1.⁷

IL-8 is a proinflammatory cytokine that is produced by various cell types involved in atherosclerosis, including endothelial cells,⁸ peripheral blood monocytes,⁹ and vascular smooth muscle cells.¹⁰ The role of IL-8 in atherosclerosis may be through its chemoattractant and mitogenic effects on vascular smooth muscle cells.¹¹ In addition, IL-8 plays an important role in the immigration of monocytes into the subendothelial space, which is a crucial process in the early stages of atherosclerosis.¹² Evidence from murine atherosclerosis models suggests that initial leukocyte adhesion to the endothelium is mediated via KC, the murine equivalent of IL-8, whereas subsequent interaction between monocyte chemoattractant protein-1 (MCP-1) and its receptor CCR1 is essential for transendothelial diapedesis and recruitment into the subendothelial space.¹³ Experimental atherosclerosis can be largely prevented by eliminating the genetic expression of IL-8, MCP-1, or their leukocyte receptors.¹⁴–¹⁶ IL-8 plasma levels are higher in patients with unstable coronary artery disease (CAD) than in healthy controls,¹⁷–²⁰ but these measurements were all obtained after an acute coronary syndrome that may have affected these levels substantially. No prospective evidence exists on the relationship between IL-8 levels in individuals free of symptomatic cardiovascular disease and the risk of future CAD.

It was our objective to determine whether elevated plasma concentrations of IL-8 in apparently healthy individuals were associated with an increased risk of future CAD. In addition, we investigated whether this relationship was modified by other cardiovascular risk factors.

Received April 22, 2004; revision accepted May 6, 2004.

From the Department of Cardiology (S.M.B., R.J.G.P.) and the Laboratory for Experimental Internal Medicine (P.H.R.), Academic Medical Center, Amsterdam, the Netherlands; the Department of Public Health and Primary Care (N.E.D., R.L., N.J.W., K.T.K.), Institute of Public Health, University of Cambridge, Cambridge, United Kingdom; the Medical Research Council Dunn Nutrition Unit (S.A.B.), Cambridge, United Kingdom; and the Sanquin Research at the Central Laboratory of the Blood Transfusion Service (C.E.H), Amsterdam, the Netherlands.

Correspondence to Kay-Tee Khaw, Clinical Gerontology Unit, Box 251 Addenbrooke’s Hospital, University of Cambridge School of Clinical Medicine, Cambridge CB2 2QQ, United Kingdom. E-mail kk101@medschl.cam.ac.uk

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Arterioscler Thromb Vasc Biol. is available at http://www.atvbaha.org DOI: 10.1161/01.ATV.0000134294.54422.2e
Methods

Study Design

We performed a nested case-control study among participants of the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk study, a prospective population study of 30,466 men and women aged between 45 and 79 years, residents of Norfolk, UK, who completed a baseline questionnaire survey, and of whom 25,663 attended a clinic visit.21 Participants were recruited from age-sex registers of general practices in Norfolk as part of a 9-country collaborative study (EPIC) designed to investigate dietary and other determinants of cancer. Additional data were obtained to enable the assessment of determinants of other diseases.

The design and methods of the study have been described in detail.21 In short, eligible participants were recruited by mail. At the baseline survey between 1993 and 1997, participants completed a detailed health and lifestyle questionnaire. Blood was taken by venepuncture into plain and citrate bottles. Blood samples for assay were processed for assay at the Department of Clinical Biochemistry, University of Cambridge, or stored at −80°C. All individuals have been flagged for death certification at the UK Office of National Statistics, with vital status ascertained for the entire cohort. In addition, participants admitted to hospital were identified using their unique National Health Service number by data linkage with ENCORE (East Norfolk Health Authority database), which identifies all hospital contacts throughout England and Wales for Norfolk residents. Participants were identified as having CAD during follow-up if they had a hospital admission and/or died with CAD as underlying cause. CAD was defined as codes 410 to 414 according to the International Classification of Diseases 9th revision. We report results with follow-up up to January 2003, an average of ~6 years. The study was approved by the Norwich District Health Authority Ethics Committee and all participants gave signed informed consent.

Participants

For the present analysis, we only considered individuals who did not report a history of heart attack or stroke at the baseline clinic visit. Cases were 785 individuals in whom a fatal or nonfatal CAD developed during follow-up. Controls were study participants who remained free of CAD during follow-up. Two controls were matched to each case by sex, age (within 5 years), general practice, and date of visit (within 3 months).

Biochemical Analyses

Serum levels of total cholesterol, high-density lipoprotein cholesterol, and triglycerides were measured on fresh samples with the RA 1000 (Bayer Diagnostics, Basingstoke, UK), and low-density lipoprotein (LDL) cholesterol levels were calculated with the Friedewald formula.22 From 1994, full blood count was additionally measured on fresh EDTA samples using a Coulter Counter, and this measure was available for ~60% of the cohort. In 2003, plasma samples for cases and controls were retrieved from frozen storage, thawed, and the plasma concentration of IL-8 was measured by use of a validated cytometric bead array kit (BD Biosciences Pharmingen, San Diego, Calif; http://www.bdbioeurope.com/temp/497527.pdf) with slight modifications to extend the detection range in the lower part of the distribution. The specificity of the antibody pair was screened using recombinant protein, and no cross-reactivity or background detection was observed. The interassay and intra-assay variabilities were 4% in the appropriate range. The lower detection limit was 2.0 pg/mL, and the upper detection limit was 4000 pg/mL. CRP levels were measured with a sandwich-type enzyme-linked immunosorbent assay in which polyclonal rabbit anti-CRP antibodies were used as catching antibodies and biotinylated monoclonal antibodies against CRP (CLB anti-CRP-2) as the detecting antibody.23 Results were related to a standard consisting of commercially available CRP (Behringwerke AG, Marburg, Germany) and expressed as mg/L. The lower detection limit was 0.1 mg/L. Samples were analyzed in random order to avoid systemic bias. Researchers and laboratory personnel had no access to identifiable information and could identify samples by number only.

Statistical Analysis

Baseline characteristics were compared between cases and controls taking into account the matching between them. A mixed effect model was used for continuous variables, and conditional logistic regression was used for dichotomous variables. Because triglyceride and IL-8 levels had a skewed distribution, values were log-transformed before statistical analysis, but in the tables we show untransformed medians and corresponding interquartile range (triglycerides) or the distribution across quartiles (IL-8). Mean risk factor levels by IL-8 quartile were calculated to determine relationships between IL-8 and traditional cardiovascular risk factors. Conditional logistic regression analysis was used to calculate odds ratios (OR) and corresponding 95% confidence intervals (95% CI) as an estimate of the relative risk of incident CAD. IL-8 concentrations were analyzed as categorical variables after division into quartiles. The lowest quartile was used as reference category. ORs were adjusted for the following cardiovascular risk factors: age, sex, systolic blood pressure, total cholesterol, high-density lipoprotein cholesterol, body mass index, smoking (never, past, current), diabetes, and hormone replacement therapy. ORs were also estimated after additional adjustment for CRP, and for both CRP and white cell count. The interaction between sex and IL-8 was calculated to assess the validity of pooling sexes. Statistical analyses were performed using SPSS software (version 10.1). P<0.05 was considered significant.

Results

From the total number of 785 cases, 196 (25.0%) died with coronary heart disease as underlying cause, and 589 (75.0%) were nonfatal CAD events. Because of matching, age was comparable between cases and controls. As expected, both women and men in whom CAD developed during follow-up were more likely than controls to smoke and have diabetes (Table 1). Total cholesterol levels, systolic and diastolic blood pressure, body mass index, white cell count, and CRP were significantly higher in cases than controls, and high-density lipoprotein cholesterol levels were significantly lower in cases than controls. Among men, median IL-8 concentrations were higher in cases (3.5 pg/mL; quartile distribution, 23%, 24%, 23%, 31%) than in controls (3.1 pg/mL; quartile distribution, 28%, 24%, 23%, 25%, P=0.001). Among women, the IL-8 concentrations in cases (3.5 pg/mL; quartile distribution, 24%, 23%, 26%, 27%) and controls (3.0 pg/mL; quartile distribution, 28%, 24%, 25%, 23%) were significantly different (P=0.2). Baseline IL-8 levels were not significantly different between people with fatal and nonfatal CAD. Table 2 shows the distribution of cardiovascular risk factors by sex and IL-8 quartile. Among men, a linear relationship was observed between IL-8 quartile and age (P for linearity <0.0001). Among women, age also increased per IL-8 quartile but linearity was not statistically significant (P=0.1). No linear relationship was observed between IL-8 quartiles and any of the other cardiovascular risk factors.

Table 3 shows the unadjusted and adjusted ORs for future CAD by IL-8 quartile. For both the unadjusted and adjusted ORs, the interaction between sex and IL-8 was not statistically significant, and data for men and women were therefore pooled, although sex-specific ORs are also shown. Plasma concentrations of IL-8 were strongly related to the risk of future CAD, such that individuals in the highest IL-8 quartile had an unadjusted OR of 1.72 (95% CI, 1.34 to 2.21), compared with those in the lowest IL-8 quartile (P for linearity <0.0001). The relationship between IL-8 and risk of
CAD was not substantially changed on adjustment for traditional cardiovascular risk factors (OR for the highest IL-8 quartile, 1.58; 95% CI, 1.19 to 2.09; \( P = 0.002 \)) or on adjustment for traditional risk factors and CRP levels (OR, 1.56; 95% CI, 1.18 to 2.07; \( P = 0.002 \)). Interestingly, additional adjustment for white cell count had two effects on the relationship between IL-8 levels and future CAD. First, the CAD risk estimates per IL-8 quartile became higher. Second, the shape of the relationship appeared to change from a linear relationship to one with a threshold such that the risk of future CAD was significantly higher in the second IL-8 quartile compared with the lowest reference quartile (OR, 1.60; 95% CI, 1.09 to 2.34; \( P = 0.01 \)) and did not increase further in the third and fourth IL-8 quartiles. The assumption that the relationship was more compatible with a threshold model than with a linear one was underlined by the observation that

<table>
<thead>
<tr>
<th>TABLE 1. Baseline Characteristics of Study Participants</th>
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<tr>
<td></td>
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<tr>
<td>Controls (n=1028)</td>
</tr>
<tr>
<td>Age, y</td>
</tr>
<tr>
<td>Smoking</td>
</tr>
<tr>
<td>Never</td>
</tr>
<tr>
<td>Past</td>
</tr>
<tr>
<td>Current</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
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<tr>
<td>Total cholesterol, mmol/L</td>
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<tr>
<td>LDL cholesterol, mmol/L</td>
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<tr>
<td>HDL cholesterol, mmol/L</td>
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<tr>
<td>Triglycerides, mmol/L</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
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<tr>
<td>DBP, mm Hg</td>
</tr>
<tr>
<td>Diabetes</td>
</tr>
<tr>
<td>CRP, pg/mL</td>
</tr>
<tr>
<td>White cell count, *10⁵ cells/mm³</td>
</tr>
<tr>
<td>IL-8, mg/L</td>
</tr>
</tbody>
</table>

| Data are presented as mean±SD, median (interquartile range), or N (%). \( P \) values are for mixed effect model on continuous variables and for conditional logistic regression on dichotomous variables. Triglyceride and IL-8 concentrations were log-transformed before analysis, but untransformed medians are presented. BMI indicates body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HRT, hormone replacement therapy; LDL, low-density lipoprotein; HDL, high-density lipoprotein; CRP, C-reactive protein. |
and were not able to give a reliable estimate of the predictive value of these cytokines in our cohort.

### Discussion

The present study provides evidence that elevated plasma levels of IL-8 are associated with an increased risk of incident CAD in apparently healthy individuals, such that people in the highest IL-8 quartile had a fully adjusted OR of 1.77 (95% CI, 1.21 to 2.60) compared with those in the lowest quartile (P = 0.001). This relationship was independent of traditional cardiovascular risk factors and also independent of CRP levels.

Recruitment of peripheral blood monocytes into the arterial wall is one of the earliest steps in atherosclerosis. The vascular wall itself orchestrates this process by modulating the expression of a wide variety of cytokines that attract leukocytes, enable rolling leukocytes to adhere to the endothelium, and facilitate transendothelial immigration into the subendothelial space. Endothelial cells can be stimulated to express cytokines, such as IL-8 and MCP-1, by triggers such as high glucose, modified LDL cholesterol, homocysteine, and nitric oxide. If triggered by oxidized LDL cholesterol (oxLDL), then macrophages in the subendothelial space can also be a source of IL-8. In addition to its role in the initiation and progression of atherosclerosis, IL-8 may also increase the risk of cardiovascular events by destabiliz-
levels were found to be higher in patients with unstable CAD.

IL-8 plasma levels were found to be higher in patients with unstable CAD than in patients with stable CAD and controls. This is likely that differences in IL-8 plasma levels occurred as a result of the disease process.

The mechanism is provided by the observation that IL-8 plasma levels were found to be higher in patients with unstable CAD than in patients with stable CAD and controls. This is likely that differences in IL-8 plasma levels occurred as a result of the disease process.

**Table 3. Odds Ratios for Future Coronary Artery Disease Events by IL-8 Quartile and Sex**

<table>
<thead>
<tr>
<th>IL-8 Quartile</th>
<th>Cases/Controls</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartile Range, pg/mL</td>
<td>1 &lt;2.1</td>
<td>2 2.1–3.2</td>
</tr>
<tr>
<td>Men and women</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OR unadjusted</td>
<td>1.00</td>
<td>1.27 (0.99–1.65)</td>
</tr>
<tr>
<td>OR adjusted*</td>
<td>1.00</td>
<td>1.21 (0.91–1.61)</td>
</tr>
<tr>
<td>OR adjusted†</td>
<td>1.00</td>
<td>1.20 (0.90–1.60)</td>
</tr>
<tr>
<td>OR adjusted‡</td>
<td>1.00</td>
<td>1.60 (1.09–2.34)</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OR unadjusted</td>
<td>1.00</td>
<td>1.30 (0.94–1.81)</td>
</tr>
<tr>
<td>OR adjusted*</td>
<td>1.00</td>
<td>1.31 (0.91–1.88)</td>
</tr>
<tr>
<td>OR adjusted†</td>
<td>1.00</td>
<td>1.29 (0.89–1.85)</td>
</tr>
<tr>
<td>OR adjusted‡</td>
<td>1.00</td>
<td>1.93 (1.15–3.22)</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OR unadjusted</td>
<td>1.00</td>
<td>1.22 (0.80–1.86)</td>
</tr>
<tr>
<td>OR adjusted*</td>
<td>1.00</td>
<td>1.13 (0.71–1.82)</td>
</tr>
<tr>
<td>OR adjusted†</td>
<td>1.00</td>
<td>1.16 (0.72–1.86)</td>
</tr>
<tr>
<td>OR adjusted‡</td>
<td>1.00</td>
<td>1.53 (0.83–2.81)</td>
</tr>
</tbody>
</table>

*Odds ratios for the risk of CAD for men, women, and for sexes combined, adjusted for sex.
†Adjustment for age, SBP, LDL cholesterol, HDL cholesterol, BMI (continuous variables), smoking, diabetes, and HRT.
‡Adjustment for aforementioned variables and CRP.
P value for $\chi^2$ linear trend with 1 degree of freedom.

A number of issues have to be taken into account when interpreting the results of the present study. Plasma levels of IL-8 were determined in a single sample that was obtained at a nonuniform time of the day. Diurnal variation and variation over time could have affected the IL-8 concentrations. In addition, we cannot rule out that sample storage at $-80^\circ$C for 6 to 10 years may have affected the detection of IL-8. However, both these limitations would lead to increased random measurement error, which leads to an underestimation of any relationship, and therefore do not negate our findings. Although the current data are unable to establish whether the relationship between IL-8 and CAD is causal, it is unlikely that differences in IL-8 plasma levels occurred as a result of the disease process.
a consequence of cardiovascular events because individuals with symptomatic cardiovascular disease were excluded from our analysis. However, we cannot exclude the possibility that IL-8 concentration is a marker of advanced subclinical atherosclerosis. Of note, IL-8 concentrations were not related to other modifiable cardiovascular risk factors and, consistently, the OR for future CAD was not affected by adjustment for these risk factors.

We conclude that elevated plasma concentrations of IL-8 are associated with an increased risk of future CAD in apparently healthy individuals. This relationship appears to be stronger in men than in women and is independent of traditional cardiovascular risk factors and CRP. These prospective data support a role for IL-8 in the development of atherosclerosis. Of note, IL-8 concentrations were not related to outcome among individuals with symptomatic cardiovascular disease were excluded from our analysis.

Acknowledgments

We thank the participants, general practitioners, and staff in EPIC-Norfolk. We thank Angelique Groot and Anke Eerenberg for their expert help in the quantitation of the inflammatory cytokines. EPIC-Norfolk is supported by program grants from the Medical Research Council UK and Cancer Research UK, and with additional support from the European Union, Stroke Association, British Heart Foundation, Department of Health, Food Standards Agency, and the Wellcome Trust.

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Arterioscler Thromb Vasc Biol. 2004;24:1503-1508; originally published online June 3, 2004;
doi: 10.1161/01.ATV.0000134294.54422.2e
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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