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Serum Myeloperoxidase Levels Are Associated With the Future Risk of Coronary Artery Disease in Apparently Healthy Individuals

The EPIC-Norfolk Prospective Population Study

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Objectives We evaluated whether serum myeloperoxidase (MPO) levels are associated with the risk of future development of coronary artery disease (CAD) in apparently healthy individuals.

Background An enzyme of the innate immune system, MPO exhibits a wide array of proatherogenic effects. These include induction of oxidative damage to low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol and promotion of plaque vulnerability. Recent studies revealed that MPO independently predicts adverse outcomes in patients with chest pain or suspected acute coronary syndrome.

Methods Myeloperoxidase was measured in baseline samples of a case-control study nested in the prospective EPIC (European Prospective Investigation into Cancer and Nutrition)-Norfolk population study. Case subjects ($n = 1{,}138$) were apparently healthy men and women who developed CAD during 8-year follow-up. Control subjects ($n = 2{,}237$), matched for age, gender, and enrollment time, remained free of CAD.

Results The MPO levels were significantly higher in case subjects than in control subjects and correlated with C-reactive protein (CRP) ($\rho = 0.25$; $p < 0.001$) and white blood cell count ($\rho = 0.33$; $p < 0.001$). Risk of future CAD increased in consecutive quartiles of MPO concentration, with an odds ratio (OR) of 1.49 in the top versus bottom quartile (95% confidence interval [CI] 1.20 to 1.84; $p < 0.001$). After adjustment for traditional risk factors, the OR in the top quartile remained significant at 1.36 (95% CI 1.07 to 1.73). Elevated MPO levels (>728 pmol/l) similarly predicted increased risk of future CAD among participants with either LDL-cholesterol <130 mg/dl, HDL-cholesterol >50 mg/dl, or CRP <2.0 mg/l (OR 1.52 [95% CI 1.21 to 1.91], 1.59 [95% CI 1.24 to 2.05], and 1.42 [95% CI 1.14 to 1.77)], respectively).

Conclusion Elevated MPO levels predict future risk of CAD in apparently healthy individuals. This study suggests that inflammatory activation precedes the onset of overt CAD by many years. (J Am Coll Cardiol 2007;50:159–65)
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Inflammation plays a key role in the initiation and progression of atherosclerosis (1). For clinical evaluation, C-reactive protein (CRP) is gradually gaining acceptance as a marker of inflammation (2). Recent studies have also drawn attention to myeloperoxidase (MPO), one of the enzymes of the innate immune system, as a potential marker of cardiovascular disease (CVD) and a potential target for treatment (3).

Traditionally, MPO was considered to be a bactericidal agent (4), but recent studies have emphasized the impor-

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Abbreviations and Acronyms

ACS = acute coronary syndrome
BMI = body mass index
CAD = coronary artery disease
CRP = C-reactive protein
CVD = cardiovascular disease
HDL = high-density lipoprotein
LDL = low-density lipoprotein
MPO = myeloperoxidase
OR = odds ratio

tance of MPO in CVD progression. The principal sources of MPO are activated neutrophils and monocytes. Myeloperoxidase has been identified in human plaques (5) and exerts potent proatherogenic effects. These include oxidation of low-density lipoprotein (LDL), rendering it atherogenic (6), as well as oxidative modification of apolipoprotein (apo) AI, attenuating its capacity to promote cholesterol efflux (7,8). Myeloperoxidase activity also diminishes nitric oxide bioavailability, which leads to endothelial dysfunction (9–11). This combination of detrimental effects has culminated in the concept that MPO may be an active mediator of atherogenesis (3).

Moreover, MPO may play a role in the transition to unstable plaque. Myeloperoxidase-induced hypochlorous acid promotes endothelial cell apoptosis and detachment, causing superficial erosions (12). Indeed, MPO levels are higher in patients with coronary artery disease (CAD) and can predict future cardiovascular events in these patients and patients with chest pain even after correction for traditional risk factors and CRP (13–15).

However, in most of these clinical studies blood samples were obtained in an acute setting or when overt CAD was present. This may have affected MPO levels substantially. Thus far, data among individuals free of heart disease are absent. The purpose of the present study was to determine whether elevated concentrations of MPO in apparently healthy individuals are associated with an increased risk of future CAD and how this relates to other cardiovascular risk factors (e.g., LDL-cholesterol, high-density lipoprotein (HDL) cholesterol, and CRP). For this purpose, we determined serum MPO levels in a large prospective nested case-control study.

Methods

Study design. We performed a nested case-control study among participants of the EPIC (European Prospective Investigation Into Cancer and Nutrition)-Norfolk study, a community-based prospective population study. The EPIC study, a collaborative study of 9 countries in Europe, was designed to assess the determinants of cancer and other diseases. The EPIC-Norfolk cohort, which is part of the EPIC study, has been described in detail previously (16). In brief, investigators recruited 25,663 men and women between 40 and 79 years old, all residents of Norfolk, United Kingdom, from general practices and performed a baseline survey between 1993 and 1997. Nonfasting blood samples were obtained by venipuncture into plain and citrate bottles. Blood samples for assay were processed at the Department of Clinical Biochemistry, University of Cambridge, or stored at −80°C. All individuals have been flagged for death certification at the U.K. 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 the 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. Coronary artery disease was defined as code 410 to 414 according to the International Classification of Diseases-9th revision. We report results with follow-up to November 2003, an average of 8 years. The study was approved by the Norwich District Health Authority Ethics Committee, and all participants gave 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. Case subjects were 1,138 individuals in whom fatal or nonfatal CAD developed during follow-up. Control subjects (n = 2,237) remained free of CAD during follow-up. Two control subjects were matched to each case subject by gender, age (within 5 years), general practice, and date of visit (within 3 months).

Biochemical analyses. Serum levels of total cholesterol, HDL-cholesterol, and triglycerides were measured on fresh samples with the RA 1000 (Bayer Diagnostics, Basingstoke, United Kingdom). The LDL-cholesterol levels were calculated using the Friedewald formula. From 1994, full blood count was additionally measured on fresh EDTA samples using a Coulter counter. This measure is available for 60% of the cohort. The CRP levels were measured with an enzyme-linked immunosorbent assay (ELISA) in which polyclonal rabbit anti-CRP antibodies were used as capturing antibodies and biotinylated monoclonal antibodies against CRP (CLB anti–CRP-2, Sanquin, Amsterdam, the Netherlands) as the detecting antibodies. Results were related to a standard consisting of commercially available CRP (Behringwerke, Marburg, Germany). The lower detection limit of CRP was 0.1 mg/l. In 2005, serum samples for case and control subjects were retrieved from frozen storage and thawed, and serum concentration of MPO was measured by use of a commercially available ELISA (CardioMPO Test, Prognostix, Cleveland, Ohio). The interassay and intra-assay variabilities were 2% and 6%. The lower detection limit was 13 pmol/l, and the upper detection limit was 5,223 pmol/l. Samples were analyzed in random order to avoid systemic bias and in a blinded fashion.

Statistical analysis. The MPO, CRP, and triglyceride levels had a skewed distribution and were therefore log-transformed before being used as continuous variables in statistical analyses. Log transformation successfully normalized the distribution. In tables we show untransformed medians and corresponding interquartile range (IQR).

Baseline characteristics were compared between case and control subjects using a mixed effect model for continuous variables or conditional logistic regression for categoric variables, which takes into account the matching for gender, age, and enrollment time. Mean risk factor levels per MPO quartile were calculated. Associations between MPO quartiles and traditional risk factors were calculated using linear regression for continuous variables and the chi-square test for trend for categoric variables. In addition, Pearson correlation coefficients were calculated to assess the relationship between log-transformed MPO levels and other continuous risk factors. Conditional logistic regression analysis was used to calculate odds ratios (OR) and corresponding 95% confidence intervals (CI) as an estimate of the relative risk of incident CAD, taking into account the matching for gender, age, and enrollment time. The MPO concentrations were analyzed as categoric variables after division into quartiles based on the distribution in control subjects as well as a continuous variable. The lowest quartile was used as reference category. The ORs were adjusted for the following cardiovascular risk factors: systolic blood pressure, LDL-cholesterol, HDL-cholesterol, body mass index (BMI) (all as continuous variables), smoking, and diabetes mellitus. The ORs were also estimated after additional adjustment for CRP (as continuous variable). In addition, the area under the receiver-operating characteristic curve (AUC) was calculated for each risk factor to determine its discriminative capacity. To assess whether MPO levels had predictive value on top of the Framingham risk score, both variables were entered into a conditional logistic regression model (taking into account the matching for gender, age, and time of enrollment), and the cumulative AUC was calculated. The Framingham risk score was calculated using a previously reported algorithm, which takes into account age, gender, total cholesterol, HDL-cholesterol, systolic and diastolic blood pressure, smoking, and the presence of diabetes (17). Augmentation of the AUC has been put forward as the best way to evaluate the incremental value of a parameter (18). We used bootstrapping of receiver operating characteristic curves to calculate statistical significance of the differences between both AUCs (19,20). Statistical analyses were performed using SPSS software (version 12.0.2, SPSS Inc., Chicago, Illinois). A p value of <0.05 was considered to be statistically significant.

Results

Characteristics of participants. A total of 342 (30%) of 1,138 cases died of coronary heart disease. The remaining cases suffered nonfatal CAD events. Owing to matching, age was comparable between case and control subjects. As expected, individuals in whom CAD developed during follow-up were more likely than control subjects to smoke and have diabetes (Table 1). In line, cholesterol levels, systolic and diastolic blood pressure, BMI, leukocyte count, and CRP were significantly higher in case subjects than in control subjects, whereas HDL-cholesterol levels were significantly lower in case subjects than in control subjects.

Baseline serum MPO levels in case and control subjects. Median serum MPO levels were higher in case subjects (median 704 [IQR 492 to 1,021] pmol/l) than in control subjects (638 [IQR 454 to 951] pmol/l; $p < 0.001$) (Table 1). Furthermore, median serum MPO levels were significantly higher in men than in women (680 [IQR 481 to 1,013] pmol/l vs. 619 [IQR 448 to 904] pmol/l; $p < 0.001$). Among men, MPO levels in case subjects (734

Table 1 Characteristics of Study Participants

	Control (n = 2,237)	Case (n = 1,138)	p Value
Male, % (n)	63.1 (1,411)	63.7 (725)	matched
Age, yrs	65.3 ± 7.7	65.5 ± 7.8	matched
Diabetes, % (n)	1.8 (41)	6.6 (75)	<0.001
Smoking, % (n)			<0.001
Current	8.2 (181)	15.3 (172)	
Former	51.2 (1,138)	52.6 (592)	
Never	40.4 (894)	32.1 (361)	
Body mass index, kg/m^2	26.3 ± 3.5	27.3 ± 3.9	<0.001
Systolic blood pressure, mm Hg	139.1 ± 17.8	143.9 ± 18.9	<0.001
Diastolic blood pressure, mm Hg	83.6 ± 11.1	85.8 ± 12.0	<0.001
Total cholesterol, mg/dl	242 ± 45	251 ± 48	<0.001
LDL-cholesterol, mg/dl	157 ± 39	165 ± 40	<0.001
HDL-cholesterol, mg/dl	53 ± 16	49 ± 14	<0.001
Triglycerides, mg/dl	142 (106–204)	168 (124–248)	<0.001
CRP, mg/l	1.5 (0.7–3.1)	2.4 (1.1–5.0)	<0.001
White cell count, 10^3 cells/mm^2	6.5 ± 1.7	6.9 ± 2.1	<0.001
Serum MPO concentration, pmol/l	638 (454–951)	704 (492–1,021)	<0.001

Data are presented as mean ± SD, median (interquartile range), or % (n). Triglyceride, CRP, and MPO concentrations were log-transformed before analysis, but untransformed medians are presented.

CRP = C-reactive protein; HDL = high-density lipoprotein; LDL = low-density lipoprotein; MPO = myeloperoxidase.

Table 2 Distribution of CAD Risk Factors by MPO Quartile

	MPO Quartile						
	1	2	3	4	p Value*	R†	p Value‡
MPO range, pmol/l	<454	454–638	638–951	>951			
Case/control	219/559	276/560	318/559	325/559	<0.001		
Male/female, %	58/42	63/37	64/26	68/32	<0.001		
Age, yrs	64.7 ± 8.0	65.3 ± 7.5	65.8 ± 7.7	65.5 ± 7.8	0.05	0.036	0.04
Diabetes, % (n)	3.1 (24)	3.7 (31)	3.9 (34)	3.1 (27)	0.99		
Smoking, % (n)					<0.001		
Current	6.6 (51)	9.5 (79)	10.8 (93)	14.8 (130)			
Former	50.5 (389)	51.9 (430)	52.6 (453)	52.2 (458)			
BMI, kg/m^2	26.7 ± 3.6	26.4 ± 3.5	26.6 ± 3.7	26.8 ± 3.8	0.1	0.022	0.2
Total cholesterol, mg/dl	250 ± 44	243 ± 44	241 ± 45	241 ± 44	<0.001	−0.068	<0.001
LDL-cholesterol, mg/dl	162 ± 39	160 ± 40	158 ± 39	159 ± 39	0.1	−0.027	0.1
HDL-cholesterol, mg/dl	54 ± 16	52 ± 15	51 ± 15	50 ± 15	<0.001	−0.096	<0.001
Triglycerides, mg/dl	159 (106–221)	150 (106–204)	142 (106–204)	150 (106–204)	0.2	−0.048	0.005
Systolic blood pressure, mm Hg	140.4 ± 18.1	140.0 ± 17.9	140.1 ± 18.3	142.2 ± 18.7	0.04	0.034	0.05
Diastolic blood pressure, mm Hg	84.3 ± 11.2	83.9 ± 11.3	84.0 ± 11.5	85.2 ± 11.6	0.07	0.027	0.1
CRP, mg/l	1.3 (0.7–2.5)	1.5 (0.7–3.2)	1.8 (0.8–3.8)	2.7 (1.2–6.9)	<0.001	0.25	<0.001
White cell count, 10^3 cells/mm^2	5.9 ± 1.8	6.5 ± 1.6	6.8 ± 1.6	7.5 ± 2.1	<0.001	0.33	<0.001

Distribution of characteristics by MPO quartiles. Quartiles are based on values in control subjects. Data are presented as mean ± SD, median (interquartile range), or % (n). *Association between serum MPO quartiles and risk factors. †Pearson correlation between log-transformed serum MPO levels and risk factors. ‡p value corresponding to R.

BMI = body mass index; CAD = coronary artery disease; other abbreviations as in Table 1.

[IQR 516 to 1,061] pmol/l) were significantly higher than in control subjects (657 [IQR 464 to 985] pmol/l; $p < 0.001$). Among women, there was a trend toward higher MPO levels in case subjects (660 [IQR 456 to 952] pmol/l) vs. 607 [IQR 446 to 894] pmol/l; $p = 0.098$). In addition, baseline MPO levels were significantly higher in subjects with fatal compared with nonfatal CAD (789 [IQR 529 to 1,133] pmol/l vs. 681 [IQR 480 to 961] pmol/l; $p < 0.001$). Ethnic differences could not be addressed in this study.

Serum MPO levels and other CAD risk factors. Table 2 summarizes the distribution of CAD risk factors by MPO quartiles. The strongest linear positive associations with serum MPO were observed for CRP and white blood cell count (Table 2). For HDL-cholesterol, we identified a linear negative association with serum MPO. The MPO levels were also related to smoking habit. There was no association with other traditional risk factors.

Serum MPO levels and risk of future CAD. The risk of future CAD increased in consecutive MPO quartiles so that individuals in the top quartile had an OR of 1.49 (95% CI 1.20 to 1.84) compared with those in the bottom quartile ($p = 0.001$) (Model 1, Table 3). After adjustment for traditional risk factors, i.e., systolic blood pressure, LDL-cholesterol, HDL-cholesterol, BMI, smoking, and diabetes, a significant association between MPO quartiles and risk of CAD remained present (OR 1.36, 95% CI 1.07 to 1.73 for top vs. bottom quartile; $p < 0.001$) (Model 2, Table 3). Additional adjustment for CRP weakened the association, especially in the top quartile (OR 1.27, 95% CI 0.98 to 1.63 for top vs. bottom quartile; $p < 0.001$) (Model 3, Table 3). Analyses by MPO tertiles or quintiles did not essentially change the results.

Notably, the association was substantially stronger in participants who suffered from fatal CAD (OR 1.82, 95% CI 1.23 to 2.70, unadjusted; $p < 0.025$) compared with whose who suffered from nonfatal CAD (OR 1.35, 95% CI 1.04 to 1.74; $p = 0.013$) (Table 4). We did not identify a statistically significant interaction between gender and

Table 3 Odds Ratios for Future CAD Events by MPO Quartile and for MPO as Continuous Variable

	MPO Quartile						
	1	2	3	4	p Value*	Ln(MPO)†	p Value‡
MPO range, pmol/l	<454	454–638	638–951	>951			
Case/control	219/559	276/559	318/560	325/559			
Model 1	1	1.24 (1.00–1.54)	1.46 (1.18–1.80)	1.49 (1.20–1.84)	0.001	1.36 (1.19–1.56)	<0.001
Model 2	1	1.15 (0.90–1.46)	1.33 (1.05–1.69)	1.36 (1.07–1.73)	<0.001	1.30 (1.12–1.52)	0.001
Model 3	1	1.14 (0.89–1.46)	1.32 (1.03–1.68)	1.27 (0.98–1.63)	<0.001	1.25 (1.07–1.47)	0.006

Odds ratios and corresponding 95% confidence intervals calculated by conditional logistic regression, taking into account matching for age, gender, and enrollment time, per MPO quartile. CRP and MPO were log transformed before analysis. Model 1: unadjusted. Model 2: adjustment for LDL-cholesterol, HDL-cholesterol, systolic blood pressure, BMI (continuous variables), smoking, and diabetes. Model 3: adjustment for aforementioned variables and CRP (continuous variable). *Association between MPO quartiles and CAD risk. †Odds ratios and corresponding 95% confidence intervals calculated by conditional logistic regression, taking into account matching for age, gender, and enrollment time, for MPO as continuous variable. ‡p value corresponding to Ln(MPO).

Abbreviations as in Tables 1 and 2.

Table 4 Odds Ratios for Fatal and Nonfatal Future CAD Events and Gender-Specific Odds Ratios by MPO Quartile (Unadjusted)

	MPO Quartile				
	1	2	3	4	p Value
MPO range (pmol/l)	<454	454–638	638–951	>951	
Fatal	1	1.45 (0.96–2.18)	1.44 (0.95–2.18)	1.82 (1.23–2.70)	0.03
Nonfatal	1	1.17 (0.91–1.51)	1.47 (1.15–1.87)	1.35 (1.04–1.74)	0.01
Male MPO range (pmol/l)	<464	464–657	657–985	>985	
	1	1.34 (1.02–1.75)	1.54 (1.17–2.02)	1.61 (1.23–2.12)	0.003
Female MPO range (pmol/l)	<446	446–607	607–894	>894	
	1	0.91 (0.63–1.30)	1.20 (0.86–1.67)	1.17 (0.84–1.62)	0.3

Abbreviations as in Tables 1 and 2.

MPO. Therefore, data for men and women were pooled, although gender-specific analyses were also performed. The association of MPO with CAD was stronger in men (OR 1.61, 95% CI 1.23 to 2.12 for top vs. bottom quartile; p = 0.003) than in women (OR 1.17, 95% CI 0.84 to 1.62 for top vs. bottom quartile; p = NS) (Table 4). Further analyses in low CAD risk subgroups showed that elevated MPO levels (>728 pmol/l) were also associated with an increased risk of future CAD among subjects with either low LDL-cholesterol (<130 mg/dl, OR 1.52 [95% CI 1.21 to 1.91]), high HDL-cholesterol (>50 mg/dl, 1.59 [95% CI 1.24 to 2.05]), or low CRP levels (<2.0 mg/l, 1.42 [95% CI 1.14 to 1.77]) (Table 5). The cut-off of 728 pmol/l was determined using the Youden index and represents the MPO concentration at which (sensitivity + specificity − 1) is maximal.

Discriminative ability of MPO. The AUC for a regression model indicates the percentage of CAD events that could be predicted successfully using the risk factors in that model. For MPO alone, the AUC was 0.55 (95% CI 0.53 to 0.57). The CRP levels, as a single risk factor, yielded the highest AUC (0.60 [95% CI 0.58 to 0.62]). Traditional risk factors such as LDL- and HDL-cholesterol ranked in between (LDL 0.56 [95% CI 0.54 to 0.58], 1/HDL 0.59 [95% CI 0.57 to 0.61]). Addition of MPO to the Framingham risk score did not significantly change the AUC (Framingham risk score alone AUC 0.59 [95% CI 0.57 to 0.61], Framingham and MPO 0.60 [95% CI 0.58 to 0.62]).

Table 5 Odds Ratio and 95% Confidence Intervals for MPO in Subgroups Otherwise Associated With Low Risk

	MPO <728 pmol/l	MPO >728 pmol/l
LDL <130 mg/dl	1	1.52 (1.21–1.91)
LDL >130 mg/dl	1.42 (1.15–1.74)	1.81 (1.45–2.26)
HDL >50 mg/dl	1	1.59 (1.24–2.05)
HDL <50 mg/dl	1.80 (1.42–2.28)	2.22 (1.73–2.85)
CRP <2 mg/l	1	1.42 (1.14–1.77)
CRP >2 mg/l	2.15 (1.74–2.64)	2.36 (1.93–2.89)

Abbreviations as in Table 1.

Discussion

Myeloperoxidase can exert a plethora of proatherogenic effects, including oxidation of lipoproteins and induction of vascular dysfunction (6–11). In support of a proatherogenic role of MPO in vivo, expression of human MPO in macrophages of LDL receptor-deficient mice led to a 2-fold increase in atherosclerotic lesion size (21). These intriguing findings indicate that MPO may be a marker for CAD risk, a mediator of atherogenesis, and a potential target for CAD prevention. The present study is the first to show that elevated serum levels of MPO in apparently healthy individuals are associated with an increased risk of future CAD events, largely independent of traditional risk factors. These findings may indicate that leukocyte activation or "priming" appears to be increased many years before the onset of overt CAD.

In this study, MPO is associated with the future risk of CAD in a primary prevention setting, i.e. among individuals not known to have heart disease. However, the relationship between MPO and CAD in these individuals is weaker than has been reported in patients with acute coronary syndromes (ACS) (13–15). This may indicate that MPO level is a more potent marker of plaque instability than of future CAD risk and/or atheroma burden. Because MPO is predominantly derived from activated neutrophils and monocytes, increased MPO in ACS is likely to reflect influx and activation of these cells in the vicinity of the unstable plaque (22–24). Of note, in atherosclerotic lesions removed during vascular surgery, MPO colocalized predominantly with macrophages within the lesion without significant involvement of neutrophils (25). These findings indicate that the origin of MPO may differ between acute and chronic vascular disease. Despite these findings, the present epidemiologic study shows that MPO is associated with CAD in a primary prevention setting as well.

Interestingly, participants with high MPO levels were more likely to smoke. This observation is in line with a recent study that describes higher serum MPO levels and other markers of systemic inflammation in smokers without severe airway systems (26). The origin and the effect of this MPO release are still unclear.

The utility of MPO measurement in clinical practice can not be based on the present study alone. We evaluated 2 aspects of MPO measurements to address potential clinical relevance for predicting cardiovascular risk in apparently healthy individuals. First, the association between MPO and CAD was, in line with previous studies, largely independent of traditional risk factors but attenuated by CRP. Moreover, the association of MPO with future CAD among apparently healthy individuals was weaker than that of traditional cardiovascular risk factors and CRP. The unadjusted OR (top vs. bottom quartile) of the traditional risk factors in the EPIC study have been described elsewhere and vary between 1.7 for LDL-cholesterol to 3.9 for diabetes (27). The OR for CRP was 2.4, 1.66 after adjustment for traditional risk factors, compared with 1.49 and 1.36, respectively, for MPO. Second, we determined the ability of MPO to increase the AUC of cardiovascular event prediction. Receiver-operating characteristic analysis evaluates sensitivity and specificity at each possible cut-off point of a clinical test and is a widely used method to judge the discriminative ability, and therefore clinical utility, of novel risk factors (28). However, the methodology is debated, because it is relatively insensitive (29). We did not observe a significant increase in the AUC by adding MPO to the Framingham risk score. Additional analyses and studies will be helpful in assessing the clinical utility of routine measurement of serum MPO levels in community-based risk assessment of apparently healthy subjects. The MPO levels have been shown to have additional prognostic value in the acute setting (13–15).

Study limitations. Several aspects of the current study warrant attention. First, CAD events were ascertained through death certification and hospital admission data, which could lead both to underascertainment and misclassification of cases. However, previous validation studies in this cohort indicate high specificity of such case ascertainment (30). Second, serum levels of MPO and lipoproteins were determined in a single nonfasting sample that was obtained at a nonuniform time of the day. Diurnal variation, variation over time, e.g., temporarily increased levels due to infection and differences in the time span from the last meal, could have affected these variables. In this respect, measurement of specific MPO products, such as chlorotyrosine in HDL, may provide a better indication of MPO activity and MPO-induced damage (8,9). Furthermore, it has recently been suggested that MPO levels after heparin administration are a better reflection of subendothelial MPO (31). However, these analyses could not be performed in the present study. Third, sample storage at −80°C for 8 to 12 years may have affected the MPO concentration. However, this would affect samples of both cases and controls. This study does leave us to assume similar effects in samples containing low and high MPO. Random measurement error in both case ascertainment and time variations would lead to an underestimation of any relationships between risk factors and CAD risk. The extent of measurement error, however, is unlikely to differ from those for other risk factors or from other prospective studies. Finally, elevated MPO levels may also reflect subclinical atherosclerotic burden in participants who eventually developed symptomatic CAD.

Conclusions

Elevated serum concentrations of MPO are associated with an increased risk of future CAD in apparently healthy individuals. It is clear that elevation of inflammatory markers, such as MPO and CRP, and their interactions precede the onset of CAD by years. This underscores the potential relevance of exploration of antiinflammatory strategies.

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