

Myeloperoxidase is associated with incident coronary heart disease independently of traditional risk factors: results from the MONICA/KORA Augsburg study

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Abstract. Karakas M, Koenig W, Zierer A, Herder C, Rottbauer W, Baumert J, Meisinger C, Thorand B (University of Ulm Medical Center, Ulm; German Research Center for Environmental Health, Institute of Epidemiology II, Neuherberg; and Institute for Clinical Diabetology, Leibniz Center for Diabetes Research at Heinrich Heine University, Düsseldorf, Germany). Myeloperoxidase is associated with incident coronary heart disease independently of traditional risk factors: results from the MONICA/KORA Augsburg study. *J Intern Med* 2012; **271**: 43–50.

Aims. Oxidative stress plays a critical role in the initiation and progression of atherosclerosis. Myeloperoxidase (MPO) is a marker of oxidative stress. We prospectively investigated whether an increased serum concentration of MPO is associated with an increased risk of incident coronary heart disease (CHD).

Methods. We conducted a population-based case-cohort study in middle-aged, healthy men and women within the MONICA/KORA Augsburg studies. Serum levels of MPO were measured in 333 subjects with (cases) and 1727 without (noncases) incident CHD. Mean follow-up time was 10.8 ± 4.6 years.

Results. Baseline concentrations of MPO were higher in cases compared with noncases ($P \leq 0.001$ in men; $P = 0.131$ in women). After adjustment for major cardiovascular risk factors, the hazard ratio (HR) with 95% confidence interval (CI) comparing the top with

the two lower tertiles was 1.70 (95% CI, 1.25–2.30). After additional adjustment for markers of inflammation and endothelial dysfunction, the association was attenuated (HR 1.50; 95% CI, 1.08–2.09). There were no significant interactions of MPO with sex or increased weight on CHD risk.

Conclusions. Elevated concentrations of the oxidative stress marker MPO were independently associated with increased risk of incident CHD. This finding deserves detailed evaluation in further studies.

Keywords: case-cohort study, coronary heart disease, MONICA/KORA, myeloperoxidase.

Abbreviations: AUC, area under the curve; CHD, coronary heart disease; CRP, C-reactive protein; CVD, cardiovascular disease; HR, hazard ratio; IL-6, interleukin-6; KORA, Kooperative Gesundheitsforschung in der Region Augsburg; MI, myocardial infarction; MONICA, monitoring of trends and determinants in cardiovascular diseases; MPO, myeloperoxidase; ROC, receiver-operating characteristic; S1, MONICA/KORA baseline survey 1 conducted in 1984–1985; S2, MONICA/KORA baseline survey 2 conducted in 1989–1990; S3, MONICA/KORA baseline survey 3 conducted in 1994–1995; SCD, sudden cardiac death; sE-selectin, soluble E-selectin; sICAM-1, soluble intercellular adhesion molecule-1

Introduction

There is increasing experimental evidence that oxidation represents an important component of ath-

erosclerotic cardiovascular disease (CVD). However, despite the fact that oxidation is involved at all stages of the atherosclerotic process, from the initiation of fatty streaks to the development of plaque instability and rupture, the potential association between systemically measured oxidative biomarkers

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and coronary heart disease (CHD) is still a matter of controversy [1–3].

Myeloperoxidase (MPO), a member of the heme peroxidase superfamily, is a leucocyte-derived enzyme, which generates reactive intermediates, leading to oxidative damage of host lipids and proteins [4]. It has been shown that MPO is present within atherosclerotic plaque in human arteries and contributes to atherogenesis by catalysing oxidative reactions in the vascular wall [5, 6].

Although results from these experimental studies are fairly consistent, equivocal data have been reported from clinical and epidemiological studies. Several studies investigating the association between circulating levels of MPO and outcome in patients with acute coronary syndrome (ACS) have reported fairly strong associations [7, 8]; however, in initially healthy subjects, the results have been less clear [9, 10].

We sought to determine whether MPO is a predictor of incident CHD [fatal and nonfatal myocardial infarction (MI) and sudden cardiac death (SCD)] in a large prospective population-based cohort study of middle-aged men and women.

Methods

Study population

The Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA)/Cooperative Health Research in the Region of Augsburg (KORA) studies served as the database for this prospective case-cohort study in initially healthy, middle-aged men and women [11]. Briefly, three independent population-based MONICA Augsburg surveys (S), with a total number of 13 427 participants (6725 men and 6702 women) aged 25–64 (S1) or 25–74 years (S2 and S3), were conducted in 1984/85 (S1), 1989/90 (S2) and 1994/95 (S3), and all subjects were prospectively followed within the framework of the KORA study. The case-cohort design used in the present study has been described previously in detail [12].

Because of the low incidence of CHD before the age of 35, this study was limited to 10 718 individuals (5382 men and 5336 women) between 35 and 74 years of age at baseline who participated in at least one of the three surveys. 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 and 4793 women).

For the case-cohort study, a random sample of the source population, i.e. the 'subcohort', comprising 2163 subjects (1154 men and 1009 women) was selected by stratifying by sex and survey. Participants with missing values for MPO or any of the covariables used in the present analysis were excluded leading to a subcohort of 1819 subjects (900 men and 919 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: 4.63, 4.28 and 6.56 for men and 4.41, 5.06 and 6.45 for women. A variant of these weights was used for the calculations of weighted means and proportions based on the cohort random sample and additional incident CHD cases. A flow diagram is included in the supplemental data (Fig. S1) to illustrate the derivation of the study sample.

Assessment of risk factors for CVD

Trained medical staff collected information on sociodemographic variables, smoking habits, leisure time physical activity, alcohol consumption and parental history of CHD at baseline through standardized interviews. In addition, standardized medical examinations including the collection of a nonfasting venous blood sample were performed at baseline. All assessment procedures and standard laboratory methods have been described previously [13]. In 2008, serum samples stored at -80°C were used to analyse the baseline levels of MPO. Holvoet *et al.* convincingly showed that oxidative biomarkers can be measured in samples that have been stored for more than 15 years [14]. Serum levels of MPO were measured by enzyme-linked immunosorbent assay (ELISA; Mercodia, Uppsala, Sweden). The intra- and interassay coefficients of variation were $<10\%$. Serum levels of interleukin-6 (IL-6), soluble E-selectin (sE-selectin) and soluble intercellular adhesion molecule-1 (sICAM-1) were determined as previously described using commercially available ELISAs [15, 16]. C-reactive protein (CRP) concentration was measured with a high-sensitivity immunoradiometric assay [17] or a high-sensitivity latex-enhanced nephelometric assay using a BN II analyzer (Dade Behring, Marburg, Germany) as previously described in detail [18]. CRP levels and distributions were com-

parable using the two assays. All analyses were run in a blinded fashion.

Determination of CHD at follow-up

A combined end-point that included incident fatal/nonfatal MI and SCD before the age of 75 was used as the outcome variable and was identified through the MONICA/KORA Augsburg coronary event registry and through follow-up questionnaires for subjects who had moved out of the study area. Until December 2000, the diagnosis of a major, nonfatal MI event was based on the MONICA algorithm taking into account symptoms, cardiac enzymes and ECG changes. Since the beginning of 2001, all patients with MI diagnosed according to European Society of Cardiology (ESC) and American College of Cardiology (ACC) criteria were included in the study [19]. Deaths from MI were validated by autopsy reports, death certificates, chart reviews and information from the last treating physician.

A total of 397 incident cases of CHD (307 men and 90 women) were observed between the time participants entered the study and 31 December 2002. Because of missing information, only data from 333 subjects with incident CHD could be used for the present analyses.

Statistical methods

Means or proportions for baseline demographic and clinical characteristics were computed using the Statistical Analysis System (SAS) procedure SURVEYMEANS, which estimates standard errors appropriate to the sampling scheme. For categorical variables, tests were carried out using the Wald chi-square test based on the SAS procedure SURVEYFREQ. Associations between continuous variables were determined using *t* tests on regression coefficients based on the SAS procedure SURVEYREG. In case of non-normality, tests were carried out with log-transformed variables and results were presented as geometric means with antilogs of standard errors of the adjusted log means. Pearson correlation coefficients were calculated in the random cohort sample to assess univariate associations between inflammatory markers and continuous risk factors for CHD. Weighing was performed using the survey- and sex-specific sampling weights. Cox proportional hazard analyses were used to assess the association between MPO and incident CHD. Sex-specific tertiles of MPO were used for comparison of hazard ratios (HRs), with the lowest tertile as the reference. Because of the case-cohort design,

correction of the variance estimation is required. We used an approach developed by Barlow *et al.* based on the sampling weights to give a robust variance estimation [12]. Cox proportional hazard models with various degrees of adjustment were calculated (see Table footnotes). To test for trends, tertiles were coded by their median values. Results are presented for each tertile as HRs together with 95% confidence intervals (CIs). Interactions between tertiles of MPO and sex, smoking status and overweight were examined using likelihood ratio tests. Variance inflation factors were calculated to assess collinearity. Inflation factors were <5 in all models tested, indicating the absence of collinearity. The accuracy of the different models to assess 10-year CHD risk was estimated by three measures: (i) the area under the receiver-operating characteristic (ROC) curve [area under the curve (AUC); also known as C-statistic or C-index] using survival probabilities within 10 years estimated by a modified Kaplan–Meier method to account for censored observations and the weighting scheme appropriate to the case-cohort design (AUC differences between two models are given as Δ AUC) [20]; (ii) the integrated discrimination improvement (IDI) statistics that can be viewed as the difference in the R^2 statistic between two models, i.e. the difference in the proportion of variance explained by the two models [21]; and (iii) the net reclassification index (NRI) using the categories 0–3.0%, 3.1–8.0%, 8.1–15.0% and >15.0% [22]. A *P*-value <0.05 was considered to be statistically significant for all analyses. All statistical evaluations were performed using the SAS software package (Version 9.1, SAS-Institute, Cary, NC, USA).

Results

Overall, 2060 participants (333 subjects with (cases) and 1727 without (noncases) incident CHD) in the three population-based MONICA/KORA Augsburg surveys were included in this case-cohort study. The mean follow-up time ($\pm$ SD) was 10.8 ($\pm$ 4.6) years. The baseline demographic, clinical and laboratory characteristics of the study population are shown in Table 1. Subjects with incident CHD were older, were less active and had a higher BMI and waist-to-hip ratio compared with noncases. Furthermore, cases more frequently reported hypertension and diabetes, whereas differences in parental history of MI, number of never smokers and educational levels were only observed in men and significant differences in alcohol consumption between the two groups were only seen in women. As expected, the total cholesterol/HDL cholesterol ratio was considerably higher in cases than in noncases. In addition, concentrations of CRP,

Table 1 Baseline demographic, clinical and laboratory characteristics of men and women with (cases) and without (noncases) incident CHD during follow-up (total $n = 2060$)

Characteristic	Men			Women		
	CHD cases	Noncases	P^*	CHD cases	Noncases	P^*
Number	253	826		80	901	
Age*	57.0 (0.53)	52.0 (0.39)	<0.001	58.2 (0.75)	52.5 (0.35)	<0.001
Education (<12 years) %	77.1 (2.64)	66.8 (1.64)	0.001	86.5 (3.82)	85.1 (1.90)	0.741
Smoking status, %			<0.001			0.378
Current smoker	43.1 (3.11)	29.0 (1.58)		24.3 (4.79)	18.4 (1.29)	
Former smoker	38.1 (3.05)	41.6 (1.71)		18.4 (4.33)	16.6 (1.59)	
Never smoker	18.8 (2.46)	29.4 (1.59)		57.3 (5.53)	65.1 (1.60)	
Frequency of exercise, %			0.001			<0.001
Inactive	69.6 (2.89)	57.4 (1.72)		81.2 (4.37)	64.3 (1.60)	
Alcohol consumption ^a , %			0.180			0.038
0 g d ⁻¹	20.9 (2.55)	17.7 (1.33)		59.1 (5.50)	44.0 (1.65)	
<39.9/19.9 g d ⁻¹	43.4 (3.12)	50.1 (1.74)		27.5 (4.99)	35.7 (1.60)	
≥40/20 g d ⁻¹	35.7 (3.01)	32.2 (1.63)		13.4 (3.80)	20.3 (1.34)	
Body mass index, *kg m ⁻²	28.1 (0.24)	27.3 (0.13)	0.004	29.5 (0.58)	26.8 (0.15)	<0.001
Waist-to-hip ratio* ^c	0.95 (<0.01)	0.93 (<0.01)	<0.001	0.84 (0.01)	0.81 (<0.01)	<0.001
Parental history of MI, %			0.019			0.415
Positive	24.0 (2.68)	18.1 (1.34)		20.6 (4.52)	21.6 (1.37)	
Unknown	26.6 (2.78)	22.0 (1.44)		27.2 (4.98)	20.2 (1.34)	
Negative	49.5 (3.14)	59.8 (1.71)		52.2 (5.58)	58.2 (1.64)	
History of hypertension or actual hypertension, %	62.6 (3.04)	44.2 (1.73)	<0.001	73.5 (4.93)	38.5 (1.62)	<0.001
Systolic blood pressure, mmHg*	141.7 (1.26)	135.8 (0.64)	<0.001	147.2 (2.58)	131.7 (0.68)	<0.001
Diastolic blood pressure, mmHg*	83.6 (0.74)	83.4 (0.38)	0.819	83.9 (1.62)	79.9 (0.37)	0.014
Prevalent diabetes	16.9 (2.36)	5.5 (0.79)	<0.001	23.1 (4.71)	3.5 (0.61)	<0.001
Current HRT ^b , %		5.5 (2.54)	10.4 (1.02)	0.109		
Ratio TC/HDL*	5.83 (0.13)	5.07 (0.06)	<0.001	5.40 (0.29)	4.02 (0.04)	<0.001
C-reactive protein, mg L ^{-1**}	2.46 (1.07)	1.44 (1.04)	<0.001	2.79 (1.13)	1.44 (1.04)	<0.001
Interleukin-6, pg mL ^{-1**}	3.08 (1.06)	2.12 (1.04)	<0.001	3.40 (1.10)	1.90 (1.04)	<0.001
sICAM-1, ng mL ^{-1*}	869.7 (22.3)	785.3 (10.8)	0.001	879.8 (36.7)	729.8 (8.6)	<0.001
sE-selectin, ng mL ^{-1*}	66.1 (2.50)	59.8 (1.04)	0.020	65.1 (3.35)	51.2 (0.80)	<0.001
MPO, ng mL ^{-1**}	139.6 (1.03)	123.7 (1.02)	<0.001	133.3 (1.06)	122.7 (1.01)	0.131
MPO, pmol L ⁻¹	962.8 (7.1)	853.2 (7.04)		919.4 (7.31)	846.3 (6.97)	

Weights: cases = all cases/nonmissing cases; noncases = 1/sampling fraction, where sampling fraction = subcohort/full cohort without cases for each sex and survey.

CHD, coronary heart disease; HRT, hormone-replacement therapy; TC/HDL, total cholesterol/HDL cholesterol; MPO, myeloperoxidase; s-ICAM-1, soluble intercellular adhesion molecule-1; sE-selectin, soluble E-selectin; MI, myocardial infarction.

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

The t -test was used for continuous variables and χ^2 test for categorical variables.

^aMen: 0, >0–39.9 g d⁻¹, ≥40 g d⁻¹; women: 0, >0–19.9 g d⁻¹, ≥20 g d⁻¹.

^bOnly for women aged ≥50 years (cases: $n = 68$, noncases: $n = 517$) with no current use of OC.

^cOnly measured in participants of surveys 2 and 3 (cases: $n = 229$; noncases: $n = 1170$).

IL-6, sICAM-1 and sE-selectin were higher in cases with CHD than in noncases. The geometric mean MPO concentrations were 139.6 (1.03) ng mL⁻¹ for men and 133.3 (1.06) ng mL⁻¹ for women with incident CHD and 123.7 (1.02) and 122.7 (1.01) ng mL⁻¹, respectively, in noncases.

Pearson correlation coefficients between MPO and inflammatory markers revealed a positive, statistically significant correlation between log MPO and log IL-6, log CRP, sICAM-1 and sE-selectin (Table 2). None of these correlation coefficients reached an absolute value above 0.3.

Table 3 shows the results of Cox proportional hazard analysis, in which the association between baseline levels of MPO and incident CHD was assessed. In the basic model, which adjusted for the Framingham risk score as a continuous covariate and for survey (Model 1), there was a strong, statistically significant association between increased concentration of MPO and incident CHD (HR for top vs. bottom tertile, 1.70; 95% CI, 1.25 to 2.30; *P* for trend = 0.001). After further adjustment for CRP (Model 2), the association remained statistically significant (HR, 1.56; 95% CI,

1.14 to 2.14; *P* for trend 0.005). Further adjustment for markers of inflammation and endothelial dysfunction (IL-6, sICAM-1 and sE-selectin; Model 3) attenuated the relation between elevated MPO concentration and risk of subsequent coronary events, with the association being still significant (HR, 1.50; 95% CI, 1.08 to 2.09; *P* for trend 0.015).

Furthermore, Table 3 shows that the predictive accuracy in the diagnosis of incident CHD, as quantified by the AUC, was increased by MPO in all models, although the absolute incremental value in the fully adjusted model 3 was very small (0.838 vs. 0.841). This was confirmed by the other two measures for accuracy (IDI and NRI), showing only a marginal additional contribution of MPO in predicting CHD events.

Interaction analyses using likelihood ratio tests showed no significant interactions between MPO and sex and weight (data not shown); therefore, the presentation of Cox proportional hazard models is not stratified by sex and weight in the main part of the report, but sex-stratified analyses are provided in the online supplement. In women, analyses did not reach statistical significance, probably due to the small number of cases.

Interaction analyses of MPO with smoking status revealed significant interactions (see online supplement). This finding is in line with results from previous studies [10, 23].

Discussion

In this prospective, population-based study in initially healthy middle-aged men and women from the general population, we found an independent association between MPO and the risk of incident CHD after adjustment for various established cardiovascular

Table 2 Weighted Pearson correlation coefficients between MPO and selected biomarkers for CHD in the randomly selected subcohort (*n* = 1819) of men and women

Characteristics	Log MPO Men		Log MPO Women	
	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>
Log IL-6	0.254	<0.001	0.180	<0.001
Log CRP	0.163	<0.001	0.110	0.001
sE-selectin	0.041	0.223	0.094	0.005
sICAM-1	0.159	<0.001	0.083	0.011

CHD, coronary heart disease; CRP, C-reactive protein; MPO, myeloperoxidase.

Table 3 Hazard ratios (95% confidence intervals) for incident CHD according to baseline concentration of MPO (*n* = 2060)

	Tertiles of MPO				<i>P</i> for trend	AUC without MPO	AUC with MPO	ΔAUC	IDI	NRI
	T1	T2	T3							
	HR	HR (95% CI)	HR (95% CI)							
Model 1 ^a	1.0	1.19 (0.86–1.65)	1.70 (1.25–2.30)		0.001	0.745	0.756	0.011	0.004	0.015
Model 2 ^b	1.0	1.19 (0.86–1.64)	1.56 (1.14–2.14)		0.005	0.819	0.823	0.004	0.007	<0.001
Model 3 ^c	1.0	1.23 (0.88–1.72)	1.50 (1.08–2.09)		0.015	0.838	0.841	0.003	0.006	−0.013

AUC, area under the curve; CHD, coronary heart disease; CRP, C-reactive protein; HR, hazard ratio; IDI, integrated discrimination improvement; MPO, myeloperoxidase; NRI, net reclassification index.

^aAdjustment for Framingham risk score and survey.

^bAdditional adjustment for CRP.

^cAdditional adjustment for IL-6, sICAM-1 and sE-selectin.

risk factors. Although the value of the increase in AUC observed with MPO was small, it may, nevertheless, be clinically important given the relative insensitivity of the AUC for detecting moderate-sized effects. For example, even widely established cardiovascular risk factors such as systolic blood pressure and cholesterol are associated with small increases in the AUC for the prediction of cardiovascular events [24].

Clinicopathological role of MPO

What is the explanation for the observed predictive role of MPO? Recently, Ferrante *et al.* reported that high levels of systemic MPO are associated with coronary plaque erosion in patients with ACS [25]. Furthermore, by examination of culprit plaques from sudden coronary deaths, they could show that luminal thrombi associated with erosion contained a higher density of MPO-positive cells than thrombi superimposed on ruptured plaques. Sugiyama *et al.* identified a novel subset of macrophages containing MPO, which infiltrated the subendothelium at sites of coronary plaque erosion or rupture, with few neutrophils in the same coronary lesions in patients in whom death was later attributed to SCD [6]. In a subsequent study, the same authors were able to show a pathogenetic role of MPO in determining plaque erosion [26]. It is possible that elevated systemic levels of MPO reflect its accumulation in the subendothelial space, because of the presence of MPO-containing macrophages, and it is likely that deposition of MPO leads to plaque erosion with subsequent development of incident CHD.

Biomarkers of oxidative stress and risk of CHD

Here, we present a large single-centre study to assess the association between the oxidative biomarker MPO and incident CHD. In a previous, much smaller, prospective nested case-control study in initially healthy men, we compared another oxidative biomarker, plasma oxidized LDL (oxLDL) in 88 cases and 258 matched controls [27]. Baseline plasma oxLDL concentration was significantly higher in men who subsequently experienced a coronary event compared with matched controls, and the HR for future CHD events comparing the top tertile of the oxLDL distribution with the bottom tertile was 2.79 (95% CI, 1.21–6.42) after adjustment for traditional cardiovascular risk factors, CRP and conventional lipid markers. Despite several reports linking MPO to intima-media thickness and diabetes [28], peripheral arterial disease [29] and heart failure [30], there is a lack of further prospective data on the association

between MPO and subsequent coronary events in initially healthy subjects. Two studies in initially healthy subjects have reported positive associations between increased levels of MPO and incident CHD events. The EPIC-Norfolk prospective study, involving a total of 1138 CHD male and female cases, demonstrated an odds ratio of 1.27 (95% CI, 0.98–1.63) comparing extreme quartiles of MPO [10]. More recently, however, based on the same study, Rana *et al.* reported that plasma levels of MPO were predictive for CHD in 1002 cases and 1859 controls [9]. They reported a 17% increase in CHD per one standard deviation (SD) increase in MPO in men, although the association did not reach the level of significance in women. Nonetheless, adjustments were not made for important potential confounders, such as CRP, which the authors showed to be highly significantly correlated with MPO.

Previous studies have reported gender-specific differences, assuming that sex hormones, such as oestrogen, may cause differential MPO gene expression in men and women [31]. This may arise from competition for binding to adjacent sites in the MPO promoter between the oestrogen receptor (ER) and peroxisome proliferator-activated receptor γ (PPAR γ), a transcription factor that is expressed in foam cells and macrophages in atherosclerotic lesions and regulates genes involved in inflammation, including *CD36* and *MPO* [32]. Oestrogen blocks the induction of MPO expression by PPAR γ ligands, which upregulate MPO expression under inflammatory conditions [33].

Sex-specific Cox proportional hazard analyses (see supplemental data) did not reach statistical significance in women, which is most probably due to the small number of female cases. In addition, likelihood ratio tests showed no significant interactions of MPO with sex.

Our findings provide additional evidence for a significant role of oxidative stress in the pathophysiology of CHD. Even after extensive adjustment, including for markers of inflammation and endothelial dysfunction, the results of the present study in 2060 male and female subjects indicate that high levels of circulating MPO can predict future risk of CHD, thereby further strengthening the likelihood of a role of MPO in atherosclerosis.

Limitations and strengths of the study

This study has several limitations that need to be addressed. First, the number of female cases was

considerably lower than the number of male cases, which may explain the lack of significance in women. Second, total circulating MPO may reflect not only the extent of atherosclerosis but also oxidative processes in remote tissues, such as adipose tissue. However, interaction analyses using likelihood ratio tests showed no significant interactions between MPO levels and being overweight.

Our study has also several strengths. These include the population-based prospective design, inclusion of initially healthy subjects, a large number of incident cases, simultaneous measurement of several markers of endothelial dysfunction and inflammation besides MPO, a long follow-up period of >10 years, minimization of the likelihood of survival bias because fatal and nonfatal coronary events were both included and the careful adjustment in multivariable analyses for conventional as well as emerging risk factors. Finally, with regard to analytical methods, we used serum samples that were shown to give the best clinical correlates when allowed to stand at room temperature for approximately 60 min before centrifugation and subsequent storage at -80°C (Per Venge, Department of Medical Sciences, University of Uppsala, Uppsala, Sweden, personal communication).

Conclusion

In conclusion, in this large prospective case-cohort study, the oxidative stress marker MPO predicted future coronary events in apparently healthy, middle-aged subjects independently of lipid profile and other traditional cardiovascular risk factors and markers of inflammation and endothelial dysfunction. Thus, MPO may represent a potentially clinically useful marker of CHD risk and should be evaluated in further large-scale studies.

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Conflict of interest

No conflict of interest to declare.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Selection of case-cohort study participants.

Table S1. Hazard ratios (95% CI) for incident CHD according to baseline concentrations of MPO stratified by sex.

Table S2. Hazard ratios (95% CI) for incident CHD according to baseline concentrations of oxLDL stratified by smoking status.

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