American Heart Association | American Stroke Association

Peroxiredoxin 4, A Novel Circulating Biomarker for Oxidative Stress and the Risk of Incident Cardiovascular Disease and All-Cause Mortality

Ali Abbasi, MD; Eva Corpeleijn, PhD; Douwe Postmus, PhD; Ron T. Gansevoort, MD, PhD; Paul E. de Jong, MD, PhD; Rijk O. B. Gans, MD, PhD; Joachim Struck, PhD; Janin Schulte, PhD; Hans L. Hillege, MD, PhD; Pim van der Harst, MD, PhD; Linda M. Peelen, PhD; Joline W. J. Beulens, PhD; Ronald P. Stolk, MD, PhD; Gerjan Navis, MD, PhD; Stephan J. L. Bakker, MD, PhD

***Background*—**Oxidative stress has been suggested to play a key role in the development of cardiovascular disease (CVD). The aim of our study was to investigate the associations of serum peroxiredoxin 4 (Prx4), a hydrogen peroxide–degrading peroxidase, with incident CVD and all-cause mortality. We subsequently examined the incremental value of Prx4 for the risk prediction of CVD compared with the Framingham risk score (FRS).

***Methods and Results*—**We performed Cox regression analyses in 8141 participants without history of CVD (aged 28 to 75 years; women 52.6%) from the Prevention of Renal and Vascular End-stage Disease (PREVEND) study in Groningen, The Netherlands. Serum Prx4 was measured by an immunoluminometric assay in baseline samples. Main outcomes were: (1) incident CVD events or CVD mortality and (2) all-cause mortality during a median follow-up of 10.5 years. In total, 708 participants (7.8%) developed CVD events or CVD mortality, and 517 participants (6.3%) died. Baseline serum Prx4 levels were significantly higher in participants with incident CVD events or CVD mortality and in those who died than in participants who remained free of outcomes (both $P<0.001$). In multivariable models with adjustment for Framingham risk factors, hazard ratios were 1.16 (95% CI 1.06 to 1.27, $P<0.001$) for incident CVD events or CVD mortality and 1.17 (95% CI 1.06 to 1.29, $P=0.003$) for all-cause mortality per doubling of Prx4 levels. After the addition of Prx4 to the FRS, the net reclassification improvement was 2.7% ($P=0.01$) using 10-year risk categories of CVD.

***Conclusions*—**Elevated serum Prx4 levels are associated with a significantly higher risk of incident CVD events or CVD mortality and all-cause mortality after adjustment for clinical risk factors. The addition of Prx4 to the FRS marginally improved risk prediction of future CVD. **(*J Am Heart Assoc.* 2012;1:e002956 doi: 10.1161/JAHA.112.002956)**

Key Words: cardiovascular disease • epidemiology • mortality • oxidative stress • peroxiredoxin 4

Experimental and clinical studies suggest that oxidative stress plays a key role in the pathogenesis of cardiovascular disease (CVD).[1–4] Oxidative stress status is usually defined as overproduction of reactive oxygen/nitrogen species in imbalance with endogenous antioxidant defenses, which in turn result in increased oxidative damage.[5] Several biomarkers, including target oxidation products and antioxidants, have been proposed for assessment of the level of oxidative stress, but clinical data examining association between markers and CVD independent of common risk factors are limited.[5–7]

Recently, peroxiredoxin 4 (Prx4), which is a secretable and stable isoform of the Prx family of antioxidant peroxidases,[8] has been found in the circulation of humans. Prx4 can be precisely measured by a validated immunoassay.[9] Previous evidence has shown abundant cellular antioxidant activity of Prx4 and other Prx isoforms in all mammals protecting against oxidative stress.[10–13] So far, a limited number of small-scale studies have evaluated the association of the serum Prx4 with clinical data.[9,14] In these studies, serum level of Prx4 was increased in septic patients compared with that of healthy individuals and was positively associated with well-established inflammatory markers like procalcitonin, C-reactive protein

From the Departments of Epidemiology (A.A., E.C., D.P., H.L.H., R.P.S.), Internal Medicine (A.A., R.T.G., P.E.d.J., R.O.B.G., G.N., S.J.L.B.), and Cardiology (P.v.d.H.), University of Groningen, University Medical Center Groningen, Groningen, the Netherlands; Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, the Netherlands (A.A., L.M.P., J.W.J.B.); Department of Research, Thermo Fisher Scientific/BRAHMS GmbH, Hennigsdorf, Germany (J. Struck, J. Schulte).

Correspondence to: Ali Abbasi, MD, Department of Epidemiology, University Medical Center Groningen, University of Groningen, Hanzeplein 1, P. O. Box 30.001, 9700 RB Groningen, the Netherlands. E-mail: a.abbasi@umcg.nl

Received June 20, 2012; accepted September 4, 2012.

(CRP), and interleukin 6 (IL-6).[9,14] Recently, a study in patients presenting to emergency departments showed the incremental prognostic value of Prxr4 to predict 30-day survival beyond usual risk predictors.[15]

We aimed to investigate whether serum Prx4 is a predictor of CVD and all-cause mortality. For this study, we used data of a large-scale observational cohort of the general population and examined the association of Prx4 with incident CVD events or CVD mortality, and all-cause mortality. Because a major clinical application of a biomarker lies within risk stratification and guided preventive strategies,[16–19] we also evaluated the incremental predictive value of Prx4 above the Framingham risk score for the 10-year risk of CVD.

Methods

Study Population and Design

The study population was obtained from the Prevention of Renal and Vascular End-stage Disease (PREVEND) study, a Dutch cohort drawn from the general population (age range, 28 to 75 years) of the city of Groningen, the Netherlands, between 1997 and 1998. We have reported details of the study design and recruitment of participants elsewhere.[20,21] Briefly, 40 856 individuals (47.8%) completed a questionnaire on demographics, history of cardiovascular and metabolic outcomes, medication use and pregnancy before their first visit, and collecting an early-morning urine sample in a vial to measure urinary albumin concentration. Those who were unable or unwilling to participate, individuals using insulin, and pregnant women were excluded. The baseline PREVEND participants were recruited from a total of 6000 individuals with a urinary albumin concentration ≥10 mg/L and a random control sample of individuals with a urinary albumin concentration <10 mg/L (n=2592).

In the baseline cohort, serum Prx4 assay was missing for 370 participants, leaving 8222 for the baseline cross-sectional analyses. The PREVEND study was approved by the local medical ethics committee at the University Medical Center Groningen and conformed to the principles outlined in the Declaration of Helsinki. All participants gave written informed consent.

Clinical and Biomarker Measurements

In the baseline screening, study participants underwent 2 outpatient visits to assess demographics, anthropometric measurements, cardiovascular and metabolic risk factors, health behaviors, and medical family history and to collect two 24-hour urine samples on 2 consecutive days. Furthermore, information on medication use was substantiated with use of pharmacy-based data from all community pharmacies in the city of Groningen.[22] Smoking and alcohol use were based on self-reports.

Hypertension was defined on the basis of self-report of diagnosis by a physician, measured hypertension (≥140/90 mm Hg systolic/diastolic blood pressure), or the use of blood pressure–lowering agents. Prevalent cases of type 2 diabetes were ascertained if 1 or more of the following criteria were met: (1) a fasting plasma glucose ≥7.0 mmol/L (126 mg/dL) or a random sample plasma glucose of 11.1 mmol/L (200 mg/dL), or (2) self-report of diagnosis by a physician, or (3) use of glucose-lowering agents according to a central pharmacy registration.[23] Prevalent CVD was defined on the basis of self-reported physician diagnosis of cardiac, cerebral, and peripheral events by a physician. Kidney disease was defined on the basis of a history of kidney disease requiring dialysis or estimated glomerular filtration rate (eGFR) <60 mL/min per 1.73 m^2. We used the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation to calculate eGFR.[24]

In all participants, blood sample measurements for biomarkers were taken after an overnight fast. Serum Prx4 level was measured retrospectively in analogously stored baseline serum samples by a novel immunoluminometric assay, which was described previously.[9] The functional assay sensitivity (interassay coefficient of variation <20%) was 0.51 U/L. The intraassay coefficient of variation was <8% throughout the range of Prx4 levels.[9,14] Insulin was measured with an AxSym autoanalyzer (Abbott Diagnostics, Amstelveen, the Netherlands). Details on assays for total cholesterol, high-desnity lipoprotein (HDL) cholesterol, triglycerides, hs-CRP, and procalcitonin have been described previously.[25] These baseline assays were performed in EDTA–plasma aliquots that had been stored frozen at −80°C without previous thawing and refreezing. Twenty-four-hour urinary albumin excretion (UAE)—given as the mean of the two 24-hour urine excretions—was measured by nephelometry with a threshold of 2.3 mg/L and intra- and interassay coefficients of variation <2.2% and <2.6%, respectively (Dade Behring Diagnostic, Marburg, Germany). All technicians were blinded to the participants' characteristics.

Definition of Outcomes

In prospective data, we ascertained the main outcomes as follows: (1) incident CVD events, (2) incident CVD events or CVD mortality, and (3) all-cause mortality (up to January 1, 2009). Information (on hospitalization) for incident CVD events was obtained from PRISMANT, the Dutch national registry of hospital discharge diagnoses. The validity of this database has been shown to be good, with 84% of primary diagnoses and 87% of secondary diagnoses matching the diagnoses recorded in patients' charts.[26] Data were coded

according to the *International Classification of Diseases* (ICD), 9th revision, and the classification of interventions. The incident CVD events were classified as acute myocardial infarction (ICD code 410), acute and subacute ischemic heart disease (ICD 411), and occlusion or stenosis of the precerebral (ICD 433) or cerebral arteries (ICD 434), and the procedures including coronary artery bypass grafting or percutaneous transluminal coronary angioplasty and other vascular interventions, namely, percutaneous transluminal angioplasty or bypass grafting of aorta or peripheral vessels.

Data on mortality were obtained through the municipal registration. Cause of mortality was ascertained by linking the number of the mortality certificate to the primary cause of mortality as coded by the Dutch Central Bureau of Statistics. Survival time was defined as the period from baseline to the date of first incident CVD event, CVD mortality, date of death, or January 1, 2009. In the case when a person had moved to an unknown destination, the date on which the person was removed from the municipal registry was used as the censor date.[27]

Statistical Analyses

Data are shown as mean±standard deviation (SD) or median (quartiles 1 and 3 [Q1, Q3]) for continuous variables, which were compared by 1-way analysis of variance or the Kruskal–Wallis test as appropriate. Frequency was used for categorical variables, which were compared by χ^2 test across tertiles of Prx4. We calculated Spearman correlation coefficients of Prx4 with age, systolic blood pressure, body mass index (BMI), waist circumference, glucose, total cholesterol, HDL cholesterol, triglycerides, hs-CRP, procalcitonin, and 24-hour UAE. We used backward-elimination regression models to examine which of the clinical variables were independently associated with Prx4 as a dependent variable. The distribution of Prx4 was highly skewed. To normalize the distribution, we performed logarithmic transformation of the values of Prx4 before analyses. We used the logarithm base 2 ($\log_2$) to allow for interpretation of results per doubling of Prx4. Interpretation of results expressed per doubling of Prx4 seems more meaningful than interpretation per factor 10 change or per factor e change, which would have been the case if transformation according to base 10 or transformation according to the natural logarithm, respectively, would have been applied. We used Cox proportional hazards regression in crude and multivariable-adjusted models to examine the associations of Prx4 with incident CVD and all-cause mortality. We adjusted for age and sex in model 1. In model 2, we adjusted for Framingham risk factors including age, sex, smoking, systolic blood pressure, use of antihypertensive therapy, diabetes at baseline, total cholesterol, and HDL cholesterol.[28] We tested the assumptions of proportional hazards for Cox regression models by Shoenfeld's global tests. Finally, in stepwise adjustments, we included alcohol use, triglycerides, high-sensitivity CRP (hs-CRP), and 24-hour UAE in model 2.

To assess incremental value of Prx4 for the risk prediction of CVD, we examined improvement of the prediction of CVD compared with the Framingham risk score. To do so, we calculated 10-year general CVD risk on the basis of the Framingham Risk Score[28] and on the basis of a model with the Framingham Risk Score and $\log_2$ Prx4. Subsequently the models were compared in terms of the following measures, taking into account the time-to-event nature of the data[18,19,29,30]: (1) Harrell's C-statistic for the Cox proportional hazards regression to quantify the discrimination performance of the models (ability to distinguish between individuals with and without outcome); (2) net reclassification improvement (NRI) to examine if individuals with and without outcome were correctly reclassified (using the threshold values <6%, 6% to 20%, and ≥20% for categories of low-, medium-, and high risk, respectively[28,31]); and (3) integrated discrimination improvement (IDI), a continuous measure of reclassification.

Of the baseline sample of 8592 participants, 451 had a history of CVD. To do the prospective analyses, we first excluded these prevalent cases of CVD, leaving 8141 participants. For most baseline variables, fewer than 1% were missing; however, this was up to 8% for self-reported variables. We performed a single imputation with predictive mean matching for missing data. This method can be used for skewed data with <10% missingness, because it produces less biased estimates for a nonlinear model and imputations are in the metric of the observed data.[32,33]

Moreover, a weighted method was performed to compensate the baseline enrichment for the PREVEND participants with UAC ≥10 mg/L. Given the frequency of individuals with UAC ≥10 mg/L (24.4%) in our general population,[20,21,27] we calculated the weight by sampling fractions. Those with UAC ≥10 mg/L had a weight=0.35, and those with UAC <10 mg/L had a weight=2.51.

Subsequently, we performed secondary analyses to take into account residual confounding. To do this, we incorporated other covariates that might be confounding of the association between Prx4 and the risk of incident CVD in combination with the Framingham risk factors (model 2). First, we further adjusted for BMI or waist circumference in separate models. Second, we adjusted for family history of CVD and examined the effects of kidney disease on this association. Third, we calculated metabolic syndrome, which was defined according to the National Cholesterol Education Program's Adult Treatment Panel III report criteria.[25] And then we adjusted for metabolic syndrome or insulin in combination with variables in model 2. Next, we examined

the association of Prx4 with each component of CVD events or CVD mortality including myocardial infarction, cerebrovascular disease, and cardiovascular mortality. In addition, we performed another analysis that included those who had a history of CVD. In total population, we further adjusted history of CVD in combination with the variables in model 2. And then we performed a similar analysis in participants with a history of CVD. We used Cox proportional hazards regression with fractional polynomials to search for the best-fitting functional form of Prx4 in the model for incident CVD (model 2).

Given the number of each event, we had 80% power at a 0.05 significance level to detect a hazard ratio=1.29 for myocardial infarction, 1.25 for cerebrovascular disease, and 1.23 for CVD mortality. All statistical analyses were carried out using IBM SPSS 19.0 and R version 2.10.1 (Vienna, Austria; http://75cran.r-project.org/).

Results

Baseline Characteristics

Baseline clinical characteristics of the total population and corresponding tertiles of serum Prx4 are summarized in Table 1. Median (Q1, Q3) Prx4 levels were 0.71 (0.45 to 1.16) U/L in men and 0.66 (0.42 to 1.08) U/L in women ($P<0.001$). Across tertiles of Prx4, those who had higher Prx4 levels were older, more obese, less frequent alcohol drinkers, and more likely to have a history of CVD, hypertension, and prevalent diabetes.

Table 1. Baseline Characteristics of Study Participants for Total Population's Corresponding Tertiles of Serum Peroxiredoxin 4*

Characteristic	Total		Tertiles†	
n	8222	2730	2671	2821
Peroxiredoxin 4, U/L	0.69 (0.44 to 1.12)	0.37 (0.37 to 0.44)	0.68 (0.59 to 0.78)	1.38 (1.10 to 1.97)
Age, year	49.2±12.7	47.1±11.9	48.7±12.4	51.8±13.2
Male sex, n (%)	4107 (50.0)	1276 (46.7)	1325 (49.6)	1506 (53.4)
History of CVD, n (%)	431 (5.4)	80 (3.0)	116 (4.5)	235 (8.7)
Family history of CVD, n (%)	3817 (50.2)	1292 (50.7)	1249 (50.3)	1276 (49.7)
Current smoker, n (%)	2787 (34.0)	1071 (39.4)	897 (33.7)	819 (29.1)
Ex-smoker, n (%)	2984 (36.4)	886 (32.6)	954 (35.9)	1144 (40.7)
Alcohol drinker, n (%)	6110 (74.7)	2121 (78.0)	2021 (75.9)	1968 (70.2)
BMI, kg/m^2	26.1±4.2	25.3±3.8	26.1±4.2	26.8±4.5
Waist circumference, cm	88.5±13.0	86.0±12.1	88.3±12.8	91.2±13.8
Systolic blood pressure, mm Hg	124.5±19.6	121.0±17.5	124.0±19.3	128.3±21.1
Diastolic blood pressure, mm Hg	71.8±9.7	70.3±9.0	71.8±9.8	73.3±10.2
Antihypertensive therapy, n (%)	1282 (15.6)	294 (10.8)	396 (14.8)	592 (21.0)
Prevalent diabetes, n (%)	315 (4.0)	49 (1.9)	96 (3.7)	170 (6.2)
Fasting glucose, mg/dL	4.9±1.2	4.7±0.9	4.9±1.2	5.0±1.4
Total cholesterol, mmol/L	5.64±1.13	5.63±1.09	5.60±1.12	5.70±1.16
HDL cholesterol, mmol/L	1.32±0.40	1.37±0.40	1.32±0.39	1.27±0.40
Triglyceride, mmol/L	1.16 (0.85 to 1.68)	1.11 (0.81 to 1.56)	1.13 (0.83 to 0.164)	1.26 (0.90 to 1.88)
Metabolic syndrome, n (%)	1378 (18.2)	314 (12.3)	444 (17.9)	629 (24.5)
Fasting insulin, mmol/L	8.0 (5.6 to 12.1)	7.2 (5.2 to 10.2)	8.0 (5.5 to 11.9)	9.1 (6.2 to 14.3)
hs-CRP, mg/L	1.27 (0.55 to 2.96)	0.93 (0.42 to 2.08)	1.22 (0.55 to 2.82)	1.85 (0.79 to 4.27)
Procalcitonin, ng/mL	0.016 (0.013 to 0.20)	0.015 (0.013 to 0.018)	0.016 (0.013 to 0.019)	0.017 (0.014 to 0.021)
UAE, mg/24 h	9.45 (6.33 to 17.8)	8.76 (6.16 to 14.36)	9.11 (6.21 to 16.23)	10.87 (6.64 to 24.99)

CVD indicates cardiovascular disease; BMI, body mass index, which is the weight in kilograms divided by the square of the height in meters; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; and UAE, urine albumin excretion. Metabolic syndrome was defined according to the National Cholesterol Education Program's Adult Treatment Panel III report criteria.

*Data are mean (±SD) and median (quartiles 1 and 3) for continuous variables and percentage for categorical variables in complete baseline data set. For clinical variables, up to 1.2% was missing. For self-reported data, 0.4% to 7.8% was missing. For biomarkers, 0.2% to 6.4% was missing.

†$P<0.001$ for the comparison among all peroxiredoxin 4 tertile groups, except for total cholesterol ($P=0.005$) and family history of CVD ($P=0.77$).

Table 2. Spearman Correlation Coefficients of Serum Peroxiredoxin 4 With Baseline Variables*

Variable	Correlation Coefficient (95% CI)
Age	0.154 (0.131 to 0.177)
Systolic blood pressure	0.148 (0.117 to 0.164)
Body mass index	0.150 (0.128 to 0.170)
Waist circumference	0.167 (0.146 to 0.187)
Glucose	0.114 (0.088 to 0.135)
Total cholesterol	0.020 (0.000 to 0.044)
HDL cholesterol	−0.118 (−0.141 to −0.098)
Triglycerides	0.105 (0.080 to 0.133)
Insulin	0.181 (0.160 to 0.201)
hs-CRP	0.229 (0.205 to 0.249)
Procalcitonin	0.129 (0.107 to 0.153)
UAE	0.126 (0.104 to 0.150)

HDL indicates high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; and UAE, urine albumin excretion.
*Data were available for 7638 to 8222 participants. We used the bootstrapping method to calculate 95% confidence intervals (CIs). *P* values were <0.001 for all correlations, except for total cholesterol (*P*=0.096).

Prx4 levels were positively correlated with age, BMI, waist circumference, systolic blood pressure, glucose, triglycerides, insulin, 24-hour UAE, and inflammatory biomarkers of hs-CRP and procalcitonin and were inversely correlated with HDL cholesterol ($P<0.001$ for all correlations) (Table 2). In the backward-elimination regression model, $\log_2$ Prx4 was positively associated with age ($\beta=0.006$, $P<0.001$), history of CVD ($\beta=0.203$, $P=0.001$), triglycerides ($\beta=0.048$, $P<0.001$), $\log_2$ hs-CRP ($\beta=0.102$, $P<0.001$), $\log_2$ UAE ($\beta=0.050$, $P<0.001$), and $\log_2$ insulin ($\beta=0.102$, $P<0.001$) and was inversely associated with female sex ($\beta=-0.059$, $P=0.03$), alcohol use ($\beta=-0.083$, $P<0.001$), and total cholesterol ($\beta=-0.060$, $P<0.001$) (Table 3).

Incident CVD and All-Cause Mortality

During median (Q1, Q3) follow-up of 10.5 (9.9 to 10.8) years, 663 participants (8.1%) developed incident CVD events, 708 participants (8.7%) developed incident CVD events or CVD mortality, and 517 participants (6.3%) died (of whom 135 died of cardiovascular causes). Median (Q1, Q3) Prx4 levels were 0.88 (0.54 to 1.45) U/L and 0.85 (0.53 to 1.46) U/L in participants who developed incident CVD events or CVD

Table 3. Association of Baseline Variables With Serum Peroxiredoxin 4 as Dependent Variable*

	Unadjusted		Age- and Sex Adjusted		Multivariable Adjusted†	
	β Coefficients (SE)	*P*	β Coefficients (SE)	*P*	β Coefficients (SE)	*P*
Age, per increase of 1 year	0.011 (0.001)	<0.001	0.011 (0.001)	<0.001	0.006 (0.001)	<0.001
Sex, female vs male	−0.081 (0.022)	<0.001	−0.061 (0.022)	0.005	−0.059 (0.027)	0.03
History of CVD, yes vs no	0.406 (0.049)	<0.001	0.261 (0.050)	<0.001	0.203 (0.052)	0.001
Smoking, yes vs no	0.032 (0.013)	0.02	0.006 (0.013)	0.67	—	—
Alcohol use, yes vs no	−0.172 (0.025)	<0.001	−0.158 (0.025)	<0.001	−0.083 (0.026)	0.001
BMI, per increase of 1 kg/m^2	0.029 (0.003)	<0.001	0.021 (0.003)	<0.001	—	—
Waist circumference, per increase of 1 cm	0.010 (0.001)	<0.001	0.008 (0.001)	<0.001	−0.002 (0.001)	0.071
Systolic blood pressure, per increase of 1 mm Hg	0.007 (0.001)	<0.001	0.004 (0.001)	<0.001	0.001 (0.001)	0.074
Antihypertensive therapy, yes vs no	0.309 (0.030)	<0.001	0.194 (0.023)	<0.001	0.059 (0.035)	0.10
Prevalent diabetes, yes vs no	0.422 (0.057)	<0.001	0.291 (0.058)	<0.001	—	—
Total cholesterol, per increase of 1 mmol/L	0.027 (0.035)	0.006	−0.015 (0.010)	0.13	−0.060 (0.011)	<0.001
HDL cholesterol, per increase of 1 mmol/L	−0.227 (0.027)	<0.001	−0.204 (0.029)	<0.001	—	—
Triglycerides, per increase of 1 mmol/L	0.104 (0.011)	<0.001	0.081 (0.011)	<0.001	0.048 (0.013)	<0.001
Insulin, per increase of $\log_2$ unit	0.198 (0.013)	<0.001	0.171 (0.013)	<0.001	0.102 (0.017)	<0.001
hs-CRP, per increase of $\log_2$ unit	0.220 (0.023)	<0.001	0.114 (0.007)	<0.001	0.102 (0.008)	<0.001
Procalcitonin, per increase of $\log_2$ unit	0.127 (0.007)	<0.001	0.152 (0.024)	<0.001	0.049 (0.026)	0.10
24-hour UAE, per increase of $\log_2$ unit	0.101 (0.009)	<0.001	0.075 (0.009)	<0.001	0.050 (0.010)	<0.001

BMI indicates body mass index, which is the weight in kilograms divided by the square of the height in meters; SE, standard error; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; UAE, urine albumin excretion.
*Base-two logarithmically transformed serum level of peroxiredoxin 4 was the dependent variable.
†Backward selection was used when dropping nonsignificant variables (probability for removal was 0.10 by *F* test).

mortality and all-cause mortality, respectively. In Figure 1, the cumulative survival to incident CVD events and to incident CVD events or CVD mortality is shown based on tertile groups. The crude cumulative incidence rates (per 1000 person-years) and hazard ratios (HRs) with 95% confidence intervals (95% CIs) in crude and multivariable-adjusted models for the risk of developing incident CVD events, incident CVD events or CVD mortality, and all-cause mortality are shown in Table 3. Age- and sex-adjusted HRs (95% CIs) ranged from 1.32 (1.07 to 1.63) for incident CVD events to 1.40 (1.08 to 1.79) for all-cause mortality when comparing the highest tertile with the first tertile of Prx4 (P for trend <0.001). In a model adjusted for the Framingham risk factors, Prx4 was significantly associated with an increased risk of incident CVD and all-cause mortality. The proportional hazards assumptions were met for all models.

In further models, stepwise adjustment for alcohol use, triglycerides, hs-CRP, and 24-hour UAE minimally attenuated the associations of Prx4 with incident CVD events or CVD mortality. This was comparable to calculation of HR per doubling of Prx4 level for each outcome. We observed a 15% increased risk of incident CVD events independent of the Framingham risk factors per doubling of Prx4 level (HR 1.15, 95% CI 1.05 to 1.26). This was a 16% and a 17% increase for incident CVD events or CVD mortality (HR 1.16, 95% CI 1.06 to 1.27) and all-cause mortality (HR 1.17, 95% CI 1.06 to 1.29), respectively (Table 4). In subsequent analyses, the associations of Prx4 with the risk of either incident CVD or all-cause mortality were similar for men and women (data not shown).

To assess the incremental predictive value of Prx4 for the risk of CVD, we added Prx4 to the Framingham Risk Score as a continuous variable.[28] In our data set, the Framingham risk score had a C-statistic (95% CI) of 0.80 (0.78 to 0.82) for the 10-year risk of CVD. The addition of Prx4 modestly improved the C-statistic to 0.81 (0.79 to 0.82, P=0.02) and led to IDI of 0.003 (P<0.001) and NRI of 2.7% (95% CI 0.7% to 4.7%; P=0.01) (Table 5). In patients without incident CVD events or CVD mortality, use of Prx4 reclassified 1% and 4% of participants in lower- and higher-risk categories (<6% and >20%), respectively. In patients with incident CVD events or CVD mortality, use of Prx4 reclassified 2% and 4% of participants in lower- and higher-risk categories, respectively.

Secondary Analyses

Tables 6 and 7 show the results of secondary analyses. Separately, adjustment for BMI or waist circumference in combination with the Framingham risk factors (ie, model 2) did not materially change the association of Prx4 with risk of incident CVD events or CVD mortality. Moreover, our results adjusted for both kidney disease and family history of CVD were similar to those of model 2. In addition, further adjustment for metabolic syndrome or insulin in model 2

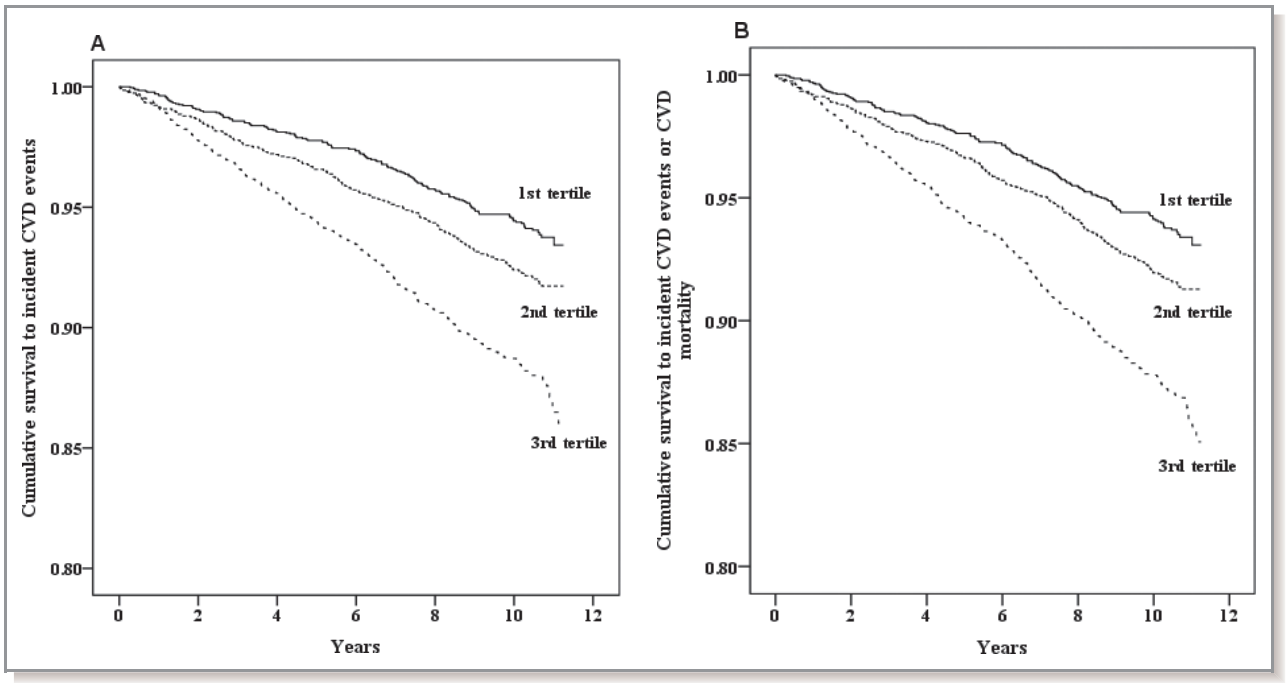

Figure 1. The cumulative probability of incident CVD events (A) and incident CVD events or CVD mortality (B) is shown by tertiles of Prx4. Data are shown for 8141 participants without CVD at baseline. CVD events were defined as a composite of nonfatal incident cardiac, cerebral, and peripheral vascular events. Overall, the log-rank tests were significant for all outcomes according to the tertiles of serum peroxiredoxin 4 (P<0.001). CVD indicates cardiovascular disease.

Table 4. Association of Serum Peroxiredoxin 4 With Incident CVD Events, Incident CVD Events or CVD Mortality, and All-Cause Mortality (n=8141)*

	HR (95% CI) or No. of Cases (Incidence Rate) According to Tertiles of Prx4				
	1	2	3	HR (95% CI) Per Log_2 Unit Increase*	*P*
Incident CVD events					
No. of cases, per 1000 person-years	151 (5.8)	204 (7.8)	308 (12.3)		
Unadjusted analysis	1.00	1.19 (0.95 to 1.48)	1.73 (1.40 to 2.13)	1.32 (1.21 to 1.44)	<0.001
Model 1	1.00	1.04 (0.84 to 1.30)	1.32 (1.07 to 1.63)	1.21 (1.10 to 1.33)	<0.001
Model 2	1.00	1.06 (0.84 to 1.32)	1.26 (1.01 to 1.57)	1.15 (1.05 to 1.26)	0.003
Model 2+alcohol use	1.00	1.06 (0.84 to 1.32)	1.25 (1.01 to 1.55)	1.14 (1.04 to 1.25)	0.004
Model 2+TG	1.00	1.06 (0.85 to 1.33)	1.28 (1.03 to 1.59)	1.16 (1.06 to 1.27)	0.001
Model 2+CRP	1.00	1.05 (0.84 to 1.31)	1.22 (0.98 to 1.52)	1.11 (1.01 to 1.22)	0.02
Model 2+UAE	1.00	1.05 (0.84 to 1.32)	1.25 (1.01 to 1.55)	1.15 (1.05 to 1.25)	0.003
Model 2+alcohol use, TG, CRP, and UAE	1.00	1.06 (0.84 to 1.32)	1.23 (1.00 to 1.53)	1.12 (1.02 to 1.23)	0.02
Incident CVD events or CVD mortality					
No. of cases, per 1000 person-years	160 (6.1)	215 (8.2)	333 (13.3)		
Unadjusted analysis	1.00	1.17 (0.94 to 1.45)	1.78 (1.46 to 2.19)	1.33 (1.23 to 1.45)	<0.001
Model 1	1.00	1.02 (0.82 to 1.27)	1.35 (1.10 to 1.66)	1.22 (1.12 to 1.33)	<0.001
Model 2	1.00	1.03 (0.83 to 1.29)	1.29 (1.05 to 1.59)	1.16 (1.06 to 1.27)	<0.001
Model 2+alcohol use	1.00	1.03 (0.83 to 1.29)	1.29 (1.04 to 1.58)	1.16 (1.06 to 1.26)	0.001
Model 2+TG	1.00	1.04 (0.84 to 1.30)	1.32 (1.07 to 1.62)	1.18 (1.08 to 1.28)	<0.001
Model 2+CRP	1.00	1.03 (0.82 to 1.28)	1.26 (1.02 to 1.56)	1.13 (1.03 to 1.23)	0.01
Model 2+UAE	1.00	1.03 (0.83 to 1.29)	1.29 (1.05 to 1.59)	1.16 (1.06 to 1.27)	0.001
Model 2+alcohol use, TG, CRP, and UAE	1.00	1.03 (0.83 to 1.29)	1.27 (1.03 to 1.57)	1.13 (1.03 to 1.24)	0.008
Incident all-cause mortality					
No. of cases, per 1000 person-years	117 (4.4)	160 (5.9)	240 (9.1)		
Unadjusted analysis	1.00	1.43 (1.10 to 1.86)	2.00 (1.56 to 2.57)	1.36 (1.23 to 1.50)	<0.001
Model 1	1.00	1.25 (0.96 to 1.62)	1.40 (1.08 to 1.79)	1.20 (1.08 to 1.33)	<0.001
Model 2	1.00	1.27 (0.98 to 1.66)	1.41 (1.09 to 1.82)	1.17 (1.06 to 1.29)	0.003
Model 2+alcohol use	1.00	1.27 (0.98 to 1.66)	1.41 (1.09 to 1.82)	1.17 (1.06 to 1.30)	0.002
Model 2+TG	1.00	1.26 (0.97 to 1.65)	1.39 (1.07 to 1.79)	1.16 (1.05 to 1.29)	0.004
Model 2+CRP	1.00	1.24 (0.95 to 1.62)	1.26 (0.97 to 1.64)	1.09 (0.98 to 1.21)	0.11
Model 2+UAE	1.00	1.27 (0.98 to 1.66)	1.40 (1.08 to 1.81)	1.15 (1.04 to 1.28)	0.006
Model 2+alcohol use, TG, CRP, and UAE	1.00	1.23 (0.94 to 1.60)	1.24 (0.96 to 1.61)	1.08 (0.97 to 1.20)	0.18

Cardiovascular disease (CVD) events were defined as a composite of incident cardiac, cerebral, and peripheral vascular events. Participants with a history of CVD were excluded. These associations did not differ by sex. Hazard ratios (HRs) with 95% confidence intervals (CIs) have been adjusted for age and sex in model 1 and for the Framingham risk factors including age, sex, smoking, systolic blood pressure, use of antihypertensive therapy, diabetes at baseline, total cholesterol, and HDL cholesterol in model 2. TG indicates triglyceride; CRP, C-reactive protein; and UAE, urine albumin excretion.

*Base-two logarithmically transformed Prx4, CRP, and 24-hour UAE were analyzed as continuous variables. Individuals with prevalent CVD were excluded.

did not affect the association. We also investigated whether Prx4 was associated with the components of incident CVD events or CVD mortality including myocardial infarction, cerebrovascular disease, and CVD mortality. After adjustment for the variables in model 2, HR (95% CI) in the highest tertile compared with the first tertile of Prx4 was 1.03 (0.71 to 1.50), 1.28 (0.85 to 1.93), and 1.22 (0.71 to 2.12) for myocardial infarction, cerebrovascular disease, and CVD mortality, respectively.

In another analysis, we examined the association of Prx4 with risk of incident CVD after adjustment for the variables in model 2 and a history of CVD in the total population. The adjusted HR (95% CI) in the highest tertile compared with the first tertile of Prx4 was 1.32 (1.11 to 1.59) for incident CVD events or CVD mortality. In

Table 5. Reclassification of Participants for the 10-Year Risk Prediction of Cardiovascular Disease Corresponding to the Framingham Risk Score and After Adding Serum Peroxiredoxin 4*

Framingham Risk Category	Framingham Risk Score With Prx4			
	Low Risk	Intermediate Risk	High Risk	Reclassification (%)
In participants without outcome				
Low risk	4647	38	0	1.0
Intermediate risk	207	1984	81	13.0
High risk	0	23	494	4.0
In participants with outcome				
Low risk	104	2	0	2.0
Intermediate risk	9	299	24	10.0
High risk	0	9	213	4.0
Total sample				
Low risk	4751	40	0	1.0
Intermediate risk	216	2283	105	12.0
High risk	0	32	707	4.0

*Corresponding to the Framingham risk score and after adding serum peroxiredoxin 4, low risk denotes <6%, intermediate risk 6% to 20%, and high risk >20% for the 10-year of cardiovascular disease. Net reclassification index was 2.7 (95% CI 0.7 to 4.7; P=0.01), and integrated discrimination improvement was 0.0032 (95% CI 0.0014 to 0.0049; P<0.001).

participants with a history of CVD, the adjusted HR (95% CI) for the variables in model 2 in the highest tertile compared with the first tertile of Prx4 was 1.07 (0.73 to 1.56) for incident CVD events or CVD mortality (n=181).

Figure 2 depicts the relationship of continuous Prx4 with incident CVD events or CVD mortality. We plotted for Framingham risk factor–adjusted (model 2) HRs and their 95% CIs as a function of Prx4. The optimal transformation was one in which the terms $(Prx4)^{1/2}$ and $(Prx4)^{1/2}\times\ln(Prx4)$ were incorporated. The solid line demonstrates that after a slight decrease in risk with levels slightly higher than the lowest ones that can be detected, the risk associated with increasing levels of Prx4 steeply increases until a plateau is reached with high levels of Prx4. Incremental value for risk prediction with the addition of $(Prx4)^{1/2}$ and $(Prx4)^{1/2}\times\ln(Prx4)$ to the model rather than $\log_2$-linear transformed Prx4 (C-statistic=0.81, 95% CI 0.79-0.82; IDI=0.004, P<0.001; NRI=3.6%, 95% CI 1.5% to 5.7%, P<0.001) was slightly higher, but very similar to that with $\log_2$-linear transformed Prx4.

Discussion

In this study, we demonstrated that serum Prx4, a circulating biomarker with antioxidant properties, was associated with the most common risk factors of CVD in a large general population cohort enriched with individuals with microalbuminuria. We found it to have an statistically significant positive association with age, history of CVD, systolic blood pressure, antihypertensive therapy, triglycerides, hs-CRP, UAE, and procalcitonin, wherea there was an inverse association with alcohol use and total cholesterol. Moreover, higher serum Prx4 levels were associated with significantly higher risk of incident CVD and all-cause mortality. For potential clinical application, we examined the incremental predictive value of Prx4 compared with the Framingham risk score. In particular, Prx4 modestly improved prediction for the 10-year CVD risk when added to the Framingham risk score in terms of discriminative ability and net reclassification.

Our findings showing the associations of serum Prx4 level with cardiovascular risk factors and events support previous clinical studies suggesting a role of oxidative stress in the pathogenesis of CVD.[1,2,4,34] In an earlier study, a higher

Table 6. Association of Serum Peroxiredoxin 4 With Incident Cardiovascular Events or CVD Mortality (n=8141)

Model	HR (95% CI) by Tertiles of Peroxiredoxin 4 (U/L)		
	1	2	3
Adjusted for model 2	1.00	1.03 (0.83 to 1.29)	1.29 (1.05 to 1.59)
Adjusted for model 2+BMI	1.00	1.03 (0.83 to 1.28)	1.29 (1.05 to 1.59)
Adjusted for model 2+waist circumference	1.00	1.03 (0.83 to 1.28)	1.31 (1.06 to 1.59)
Adjusted for model 2+family history of CVD	1.00	1.04 (0.83 to 1.29)	1.30 (1.06 to 1.60)
Adjusted for sex, age, smoking+metabolic syndrome	1.00	1.03 (0.82 to 1.28)	1.38 (1.12 to 1.76)
Adjusted for model 2+insulin	1.00	1.04 (0.83 to 1.30)	1.31 (1.06 to 1.61)
Adjusted for model 2+kidney disease	1.00	1.03 (0.83 to 1.29)	1.30 (1.05 to 1.60)

Hazard ratios (HRs) with 95% confidence intervals (95% CIs) have been adjusted for model 2, in which the Framingham risk factors age, sex, smoking, systolic blood pressure, use of antihypertensive therapy, diabetes at baseline, total cholesterol, and HDL cholesterol were included. Kidney disease was defined on the basis of a history of kidney disease requiring dialysis or estimated glomerular filtration rate (eGFR) below 60 mL/min per 1.73 m^2. We used the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation to calculate eGFR. BMI indicates body mass index; CVD, cardiovascular disease; and HDL, high-density lipoprotein.

Table 7. Association of Serum Peroxiredoxin 4 With Incident Myocardial Infarction, Cerebrovascular Events, and Cardiovascular Mortality (n=8141)

	Tertiles of Peroxiredoxin 4, U/L		
	1	2	3
Incident myocardial infarction			
No. of cases, %	51 (5.8)	63 (7.8)	94 (10.1)
Unadjusted HR (95% CI)	1.00	1.04 (0.71 to 1.52)	1.43 (1.00 to 2.06)
Multivariate-adjusted HR (95% CI)*	1.00	0.98 (0.67 to 1.43)	1.06 (0.74 to 1.53)
Incident cerebrovascular disease			
No. of cases, %	39 (6.1)	58 (8.2)	88 (13.3)
Unadjusted HR (95% CI)	1.00	1.00 (0.64 to 1.55)	1.73 (1.16 to 2.58)
Multivariate-adjusted HR (95% CI)*	1.00	0.93 (0.60 to 1.45)	1.28 (0.85 to 1.91)
Incident cardiovascular mortality			
No. of cases, %	28 (1.0)	44 (5.9)	63 (9.1)
Unadjusted HR (95% CI)	1.00	1.48 (0.84 to 2.59)	2.10 (1.23 to 3.58)
Multivariate-adjusted HR (95% CI)*	1.00	1.35 (0.77 to 2.36)	1.38 (0.80 to 2.36)

*Hazard ratios (HRs) with 95% confidence intervals (95% CIs) have been adjusted for model 2, in which the Framingham risk factors included age, sex, smoking, systolic blood pressure, use of antihypertensive therapy, diabetes at baseline, total cholesterol, and HDL cholesterol.

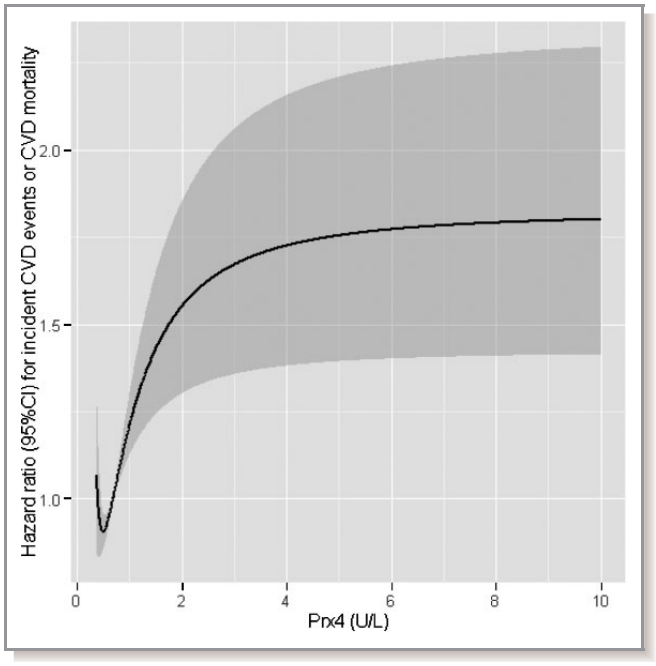

Figure 2. The relationship of peroxiredoxin 4 with incident CVD events or CVD mortality. Data are shown for 8141 participants without CVD at baseline. The plotted hazard ratio (95% CI) was adjusted for the Framingham risk factors and centered on the Prx4 median value. The optimal transformation of Prx4 was one in which the terms $(Prx4)^{1/2}$ and $(Prx4)^{1/2} \times \ln(Prx4)$ were incorporated. CVD indicates cardiovascular disease.

urinary excretion of oxidative stress indices was reported in individuals with renovascular hypertension, which was correlated with endothelium-dependent vasodilatation.[4] Another study showed the independent association of oxidized low-density lipoprotein with the incidence of metabolic syndrome.[2] In line with this, genotypes and serum activity of paraoxonase 1, an HDL-related antioxidant, has been shown to be associated with systemic oxidative stress and cardiovascular outcomes in humans.[1] We now extend accumulating information obtained from animal and human studies on Prx4 of the family of thiol-dependent antioxidants in this era. An animal model of type 1 diabetes has indicated that Prx4 may have a pivotal role in the suppression of apoptosis and the proliferation of progenitor cells to protect against oxidative stress-induced β-cell dysfunction.[12] In line with this, higher gene expression of Prx4 has been found in the islets of a high-fat diet model of β-cell dysfunction[35] and downregulated expression of Prx4 in islets in diabetic mice with chronic hyperglycemia.[36] Moreover, recent studies have suggested that Prx4 might be involved in the protection against celiac disease and cancer in the pancreas and lung with increased expression and production of Prx4 in the related human tissues.[12,37,38] Consistently, Prx1, another member of the Prx family, has also shown to be involved in a broad range of oxidative stress-related outcomes in the cardiovascular system.[39,40]

Importantly, Prx4 is the only secretable member of the family in animals and humans.[8,11] Recently, clinical data have shown that Prx4 levels were increased in sera of septic patients compared with healthy individuals.[14,15] Consistent with this, we showed the relation between Prx4 and inflammatory markers and also explored its relation with measures of adiposity, blood pressure, glycemia index, lipids, and albuminuria. All these markers are underlying in central

biological pathways of metabolic traits and CVD.[27,41] Other recent data from patients who presented to emergency departments showed that serum Prx4 level was increased in nonsurvivors and that there was improved prognostic information for survival at 30 days when it was added to a clinical risk score.[15] One main explanation for these findings may be that concomitant production and secretion of Prx4 can be augmented to protect against the increased rate of oxidative stress in relation to adverse cardiovascular and metabolic conditions.[42] Interestingly, in secondary analyses with fractional polynomials, we found support for the notion that very low levels of Prx4 compared with somewhat higher levels are associated with a slightly increased risk. Possibly this makes up a subgroup of subjects with constitutively low levels of Prx4, which predisposes to CVD. The high risk with more elevated levels of Prx4 is consistent with a compensatory increase in Prx4 in response to existing oxidative stress. At the cellular level, Prx4 may promote antioxidant activity via several pathways, such as nuclear factor kB,[43] p53,[44] thromboxane A2 receptor,[45] and NF-E2-related factor 2 (Nrf2).[46] Despite equivocal findings of supplementing antioxidant vitamins to prevent cardiovascular and metabolic diseases,[1,47] a recent trial[48] showed an effective intervention of a specific antioxidant, that is, an Nrf2 antagonist that is related to Prx4 pathway, against decline of renal function in patients with chronic kidney disease and type 2 diabetes. Therefore, this might be an opening line for novel therapeutic targets and monitoring tools for metabolic traits and CVD.

Next, we assessed the clinical application of Prx4 for the risk prediction of CVD. To do this, we used information from a general 10-year risk of CVD based on the Framingham risk score[28] and examined whether Prx4 might have incremental predictive value above this risk score. We did not develop new models, but chose an appropriate approach in prediction research by using the information retained in the existing prediction model.[19,30,31,49] Of note, there are usually missing values for baseline data in an observational study. We had <10% of data missing, and we used a single imputation and predictive mean matching. Imputation for missing values will increase the power and precision of a study and minimize the risk of biased findings.[50] Finally, we used reclassification measures, which are more sensitive and more clinically relevant than C-statistic alone.[18,19,49] For CVD, the addition of Prx4 to the Framingham risk score marginally improved prediction in terms of discrimination and reclassification. Overall, the addition of Prx4 correctly reclassified 2.7% of participants for risk of CVD obtained by the Framingham risk score. Further studies are warranted to confirm our findings for this oxidative stress biomarker in different settings[17] and among individuals with other comorbidities such as diabetes.

There are some limitations to this study that should be addressed. Our cohort predominantly comprised white adults, and it is therefore unknown whether our findings can be generalized to nonwhites. The PREVEND cohort was enriched for microalbuminuria at baseline. However, a weighted method was performed to compensate for this, which did not affect the results. Moreover, we investigated the association of only baseline and not serial circulating levels of Prx4 with the future risk of outcomes of interest. The extent of within-individual variation over time might affect the statistical power of a study.[51,52] For example, higher variation can lead to less power, and then the estimated risk is attenuated using a single Prx4 measurement. Although we accounted for confounding of traditional cardiovascular risk factors, a potential for uncontrolled or residual confounding would be plausible. Finally, we had no data on other oxidative stress markers such as oxidized low-density lipoprotein and homocysteine to compare their predictive values with that of Prx4.

In conclusion, our results suggest that higher serum Prx4 levels are associated with most cardiovascular risk factors including albuminuria and hs-CRP in a general-population cohort study. Moreover, higher serum Prx4 levels were associated with a significantly higher risk of incident CVD events or CVD mortality and all-cause mortality after adjustment for traditional cardiovascular risk factors. Prx4 marginally improves the 10-year risk prediction for CVD when added to the Framingham risk score.

Acknowledgments

We thank L. T. W. de Jong-van den Berg and S. T. Visser from the Department of Social Pharmacy, Pharmacoepidemiology and Pharmacotherapy, Groningen University Institute for Drug Exploration, University of Groningen, University Medical Center Groningen, for providing the data on pharmacy-registered use of antidiabetic medication.

Sources of Funding

This work was supported by the Netherlands Heart Foundation, Dutch Diabetes Research Foundation, and Dutch Kidney Foundation. This research was performed within the framework of the Center for Translational Molecular Medicine (http://www.ctmm.nl) and project PREDICCt (grant 01C-104-07). None of the study sponsors had a role in the study design; in data collection, analysis, or interpretation; in writing the report; or in the decision to submit for publication.

Disclosures

Drs Struck and Schulte hold patent rights to the peroxiredoxin 4 assay and are employees of BRAHMS GmbH, the manufacturer of the peroxiredoxin 4 assay. The present study was not financed by BRAHMS GmbH. No other author has any potential conflicts of interest relevant to this article.

References

1. Bhattacharyya T, Nicholls SJ, Topol EJ, Zhang R, Yang X, Schmitt D, Fu X, Shao M, Brennan DM, Ellis SG, Brennan ML, Allayee H, Lusis AJ, Hazen SL. Relationship of paraoxonase 1 (PON1) gene polymorphisms and functional activity with systemic oxidative stress and cardiovascular risk. *JAMA.* 2008;299:1265–1276.
2. Holvoet P, Lee DH, Steffes M, Gross M, Jacobs DR Jr. Association between circulating oxidized low-density lipoprotein and incidence of the metabolic syndrome. *JAMA.* 2008;299:2287–2293.
3. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. *Nature.* 2000;408:239–247.
4. Higashi Y, Sasaki S, Nakagawa K, Matsuura H, Oshima T, Chayama K. Endothelial function and oxidative stress in renovascular hypertension. *N Engl J Med.* 2002;346:1954–1962.
5. Giustarini D, Dalle-Donne I, Tsikas D, Rossi R. Oxidative stress and human diseases: origin, link, measurement, mechanisms, and biomarkers. *Crit Rev Clin Lab Sci.* 2009;46:241–281.
6. Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of oxidative damage in human disease. *Clin Chem.* 2006;52:601–623.
7. Strobel NA, Fassett RG, Marsh SA, Coombes JS. Oxidative stress biomarkers as predictors of cardiovascular disease. *Int J Cardiol.* 2011;147:191–201.
8. Okado-Matsumoto A, Matsumoto A, Fujii J, Taniguchi N. Peroxiredoxin IV is a secretable protein with heparin-binding properties under reduced conditions. *J Biochem.* 2000;127:493–501.
9. Schulte J, Struck J, Bergmann A, Köhrle J. Immunoluminometric assay for quantification of peroxiredoxin 4 in human serum. *Clin Chim Acta.* 2010;411:1258–1263.
10. Neumann CA, Krause DS, Carman CV, Das S, Dubey DP, Abraham JL, Bronson RT, Fujiwara Y, Orkin SH, Van Etten RA. Essential role for the peroxiredoxin Prdx1 in erythrocyte antioxidant defence and tumour suppression. *Nature.* 2003;424:561–565.
11. Wood ZA, Schröder E, Robin Harris J, Poole LB. Structure, mechanism and regulation of peroxiredoxins. *Trends Biochem Sci.* 2003;28:32–40.
12. Ding Y, Yamada S, Wang KY, Shimajiri S, Guo X, Tanimoto A, Murata Y, Kitajima S, Watanabe T, Izumi H, Kohno K, Sasaguri Y. Overexpression of peroxiredoxin 4 protects against high-dose streptozotocin-induced diabetes by suppressing oxidative stress and cytokines in transgenic mice. *Antioxid Redox Signal.* 2010;13:1477–1490.
13. Wei Q, Jiang H, Xiao Z, Baker A, Young MR, Veenstra TD, Colburn NH. Sulfiredoxin–peroxiredoxin IV axis promotes human lung cancer progression through modulation of specific phosphokinase signaling. *Proc Natl Acad Sci USA.* 2011;108:7004–7009.
14. Schulte J, Struck J, Köhrle J, Müller B. Circulating levels of peroxiredoxin 4 as a novel biomarker of oxidative stress in patients with sepsis. *Shock.* 2011;35:460–465.
15. Nickel CH, Ruedinger J, Misch F, Blume K, Maile S, Schulte J, Köhrle J, Hartmann O, Giersdorf S, Bingisser R. Copeptin and peroxiredoxin-4 independently predict mortality in patients with nonspecific complaints presenting to the emergency department. *Acad Emerg Med.* 2011;18:851–859.
16. Shah SH, de Lemos JA. Biomarkers and cardiovascular disease: determining causality and quantifying contribution to risk assessment. *JAMA.* 2009;302:92–93.
17. Hlatky MA, Greenland P, Arnett DK, Ballantyne CM, Criqui MH, Elkind MS, Go AS, Harrell FE Jr, Hong Y, Howard BV, Howard VJ, Hsue PY, Kramer CM, McConnell JP, Normand SL, O'Donnell CJ, Smith SC Jr, Wilson PW; American Heart Association Expert Panel on Subclinical Atherosclerotic Diseases and Emerging Risk Factors and the Stroke Council. Criteria for evaluation of novel markers of cardiovascular risk: a scientific statement from the American Heart Association. *Circulation.* 2009;119:2408–2416.
18. Pencina MJ, D'Agostino RB Sr, Steyerberg EW. Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers. *Stat Med.* 2011;30:11–21.
19. Cook NR, Ridker PM. Advances in measuring the effect of individual predictors of cardiovascular risk: the role of reclassification measures. *Ann Intern Med.* 2009;150:795–802.
20. Lambers Heerspink HJ, Brantsma AH, de Zeeuw D, Bakker SJ, de Jong PE, Gansevoort RT; PREVEND Study Group. Albuminuria assessed from first-morning-void urine samples versus 24-hour urine collections as a predictor of cardiovascular morbidity and mortality. *Am J Epidemiol.* 2008;168:897–905.
21. Mahmoodi BK, Gansevoort RT, Veeger NJ, Matthews AG, Navis G, Hillege HL; Prevention of Renal and Vascular End-stage Disease (PREVEND) Study Group. Microalbuminuria and risk of venous thromboembolism. *JAMA.* 2009;301:1790–1797.
22. Monster TB, Janssen WM, de Jong PE, de Jong-van den Berg LT; PREVEND Study Group Prevention of REnal and Vascular ENT Stage Disease. Pharmacy data in epidemiological studies: an easy to obtain and reliable tool. *Pharmacoepidemiol Drug Saf.* 2002;11:379–384.
23. Abbasi A, Corpeleijn E, Postmus D, Gansevoort RT, de Jong PE, Gans RO, Struck J, Hillege HL, Stolk RP, Navis G, Bakker SJ. Plasma procalcitonin and risk of type 2 diabetes in the general population. *Diabetologia.* 2011;54:2463–2465.
24. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF III, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. *Ann Intern Med.* 2009;150:604–612.
25. Abbasi A, Corpeleijn E, Postmus D, Gansevoort RT, de Jong PE, Gans RO, Struck J, Hillege HL, Stolk RP, Navis G, Bakker SJ. Plasma procalcitonin is associated with obesity, insulin resistance, and the metabolic syndrome. *J Clin Endocrinol Metab.* 2010;95:E26–E31.
26. Stricker BH, Herings RM. Plea for the retention of the Dutch National Medical Registration (LMR) to provide reliable information regarding public health and healthcare. *Ned Tijdschr Geneeskd.* 2006;150:1916–1917.
27. Hillege HL, Fidler V, Diercks GF, van Gilst WH, de Zeeuw D, van Veldhuisen DJ, Gans RO, Janssen WM, Grobbee DE; Prevention of Renal and Vascular End Stage Disease (PREVEND) Study Group. Urinary albumin excretion predicts cardiovascular and noncardiovascular mortality in general population. *Circulation.* 2002;106:1777–1782.
28. D'Agostino RB Sr, Vasan RS, Pencina MJ, Wolf PA, Cobain M, Massaro JM, Kannel WB. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. *Circulation.* 2008;117:743–753.
29. Steyerberg EW, Vickers AJ, Cook NR, Gerds T, Gonen M, Obuchowski N, Pencina MJ, Kattan MW. Assessing the performance of prediction models: a framework for traditional and novel measures. *Epidemiology.* 2010;21:128–138.
30. Janssen KJ, Moons KG, Kalkman CJ, Grobbee DE, Vergouwe Y. Updating methods improved the performance of a clinical prediction model in new patients. *J Clin Epidemiol.* 2008;61:76–86.
31. Polak JF, Pencina MJ, Pencina KM, O'Donnell CJ, Wolf PA, D'Agostino RB Sr. Carotid-wall intima-media thickness and cardiovascular events. *N Engl J Med.* 2011;365:213–221.
32. Marshall A, Altman DG, Holder RL. Comparison of imputation methods for handling missing covariate data when fitting a Cox proportional hazards model: a resampling study. *BMC Med Res Methodol.* 2010;10:112.
33. van Buuren S, Boshuizen HC, Knook DL. Multiple imputation of missing blood pressure covariates in survival analysis. *Stat Med.* 1999;18:681–694.
34. Horstkotte J, Perisic T, Schneider M, Lange P, Schroeder M, Kiermayer C, Hinkel R, Ziegler T, Mandal PK, David R, Schulz S, Schmitt S, Widder J, Sinowatz F, Becker BF, Bauersachs J, Naebauer M, Franz WM, Jeremias I, Brielmeier M, Zischka H, Conrad M, Kupatt C. Mitochondrial thioredoxin reductase is essential for early postischemic myocardial protection. *Circulation.* 2011;124:2892–2902.
35. Dreja T, Jovanovic Z, Rasche A, Kluge R, Herwig R, Tung YC, Joost HG, Yeo GS, Al-Hasani H. Diet-induced gene expression of isolated pancreatic islets from a polygenic mouse model of the metabolic syndrome. *Diabetologia.* 2010;53:309–320.
36. Xie X, Li S, Liu S, Lu Y, Shen P, Ji J. Proteomic analysis of mouse islets after multiple low-dose streptozotocin injection. *Biochim Biophys Acta.* 2008;1784:276–284.
37. Simula MP, Cannizzaro R, Canzonieri V, Pavan A, Maiero S, Toffoli G, De Re V. PPAR signaling pathway and cancer-related proteins are involved in celiac disease-associated tissue damage. *Mol Med.* 2010;16:199–209.
38. Chung JC, Oh MJ, Choi SH, Bae CD. Proteomic analysis to identify biomarker proteins in pancreatic ductal adenocarcinoma. *ANZ J Surg.* 2008;78:245–251.
39. Kisucka J, Chauhan AK, Patten IS, Yesilaltay A, Neumann C, Van Etten RA, Krieger M, Wagner DD. Peroxiredoxin1 prevents excessive endothelial activation and early atherosclerosis. *Circ Res.* 2008;103:598–605.
40. Martinez-Pinna R, Ramos-Mozo P, Madrigal-Matute J, Blanco-Colio LM, Lopez JA, Calvo E, Camafeita E, Lindholt JS, Meilhac O, Delbosc S, Michel JB, de Ceniga MV, Egido J, Martin-Ventura JL. Identification of peroxiredoxin-1 as a novel biomarker of abdominal aortic aneurysm. *Arterioscler Thromb Vasc Biol.* 2011;31:935–943.
41. Emerging Risk Factors Collaboration, Wormser D, Kaptoge S, Di Angelantonio E, Wood AM, Pennells L, Thompson A, Sarwar N, Kizer JR, Lawlor DA, Nordestgaard BG, Ridker P, Salomaa V, Stevens J, Woodward M, Sattar N, Collins R, Thompson SG, Whitlock G, Danesh J. Separate and combined associations of body-mass index and abdominal adiposity with cardiovascular disease: collaborative analysis of 58 prospective studies. *Lancet.* 2011;377:1085–1095.
42. Schulte J. Peroxiredoxin 4: a multifunctional biomarker worthy of further exploration. *BMC Med.* 2011;9:137.
43. Jin DY, Chae HZ, Rhee SG, Jeang KT. Regulatory role for a novel human thioredoxin peroxidase in NF-kappaB activation. *J Biol Chem.* 1997;272:30952–30961.

44. Wong CM, Chun AC, Kok KH, Zhou Y, Fung PC, Kung HF, Jeang KT, Jin DY. Characterization of human and mouse peroxiredoxin IV: evidence for inhibition by Prx-IV of epidermal growth factor- and p53-induced reactive oxygen species. *Antioxid Redox Signal.* 2000;2:507–518.

45. Giguère P, Turcotte ME, Hamelin E, Parent A, Brisson J, Laroche G, Labrecque P, Dupuis G, Parent JL. Peroxiredoxin-4 interacts with and regulates the thromboxane A(2) receptor. *FEBS Lett.* 2007;581:3863–3868.

46. Bertolotti M, Yim SH, Garcia-Manteiga JM, Masciarelli S, Kim YJ, Kang MH, Iuchi Y, Fujii J, Vené R, Rubartelli A, Rhee SG, Sitia R. B- to plasma-cell terminal differentiation entails oxidative stress and profound reshaping of the antioxidant responses. *Antioxid Redox Signal.* 2010;13:1133–1144.

47. Münzel T, Gori T, Bruno RM, Taddei S. Is oxidative stress a therapeutic target in cardiovascular disease? *Eur Heart J.* 2010;31:2741–2748.

48. Pergola PE, Raskin P, Toto RD, Meyer CJ, Huff JW, Grossman EB, Krauth M, Ruiz S, Audhya P, Christ-Schmidt H, Wittes J; BEAM Study Investigators. Bardoxolone methyl and kidney function in CKD with type 2 diabetes. *N Engl J Med.* 2011;365:327–336.

49. McGeechan K, Macaskill P, Irwig L, Liew G, Wong TY. Assessing new biomarkers and predictive models for use in clinical practice: a clinician's guide. *Arch Intern Med.* 2008;168:2304–2310.

50. van der Heijden GJ, Donders AR, Stijnen T, Moons KG. Imputation of missing values is superior to complete case analysis and the missing-indicator method in multivariable diagnostic research: a clinical example. *J Clin Epidemiol.* 2006;59:1102–1109.

51. Koenig W, Sund M, Fröhlich M, Löwel H, Hutchinson WL, Pepys MB. Refinement of the association of serum C-reactive protein concentration and coronary heart disease risk by correction for within-subject variation over time: the MONICA Augsburg studies, 1984 and 1987. *Am J Epidemiol.* 2003;158:357–364.

52. Danesh J, Wheeler JG, Hirschfield GM, Eda S, Eiriksdottir G, Rumley A, Lowe GD, Pepys MB, Gudnason V. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. *N Engl J Med.* 2004;350:1387–1397.