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Meta- and Pooled Analyses

Antioxidant Enzyme Activity and Coronary Heart Disease: Meta-analyses of Observational Studies

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Controversial data exist concerning the relation between the activities of scavenger antioxidant enzymes and coronary heart disease (CHD) risk. The authors report updated meta-analyses of studies assessing the activities of 3 antioxidant enzymes—glutathione peroxidase, superoxide dismutase, and catalase—and CHD risk. Computer-based and manual searches of the relevant literature from January 1966 to January 2008 were performed. Studies assessing glutathione peroxidase, superoxide dismutase, and catalase activities in cells or biologic fluids and clinical CHD outcomes were selected. Pooled odds ratios for CHD were calculated by using an inverse-variance-weighted random-effects model. Forty-two case-control studies and 3 prospective studies were included. The pooled odds ratios for CHD associated with a 1-standard-deviation increase in glutathione peroxidase, superoxide dismutase, and catalase activity levels were 0.51 (95% confidence interval: 0.35, 0.75), 0.48 (95% confidence interval: 0.32, 0.72), and 0.32 (95% confidence interval: 0.16, 0.61), respectively, with substantial between-study heterogeneity ($I^2 > 90\%$ for the 3 enzymes). These findings were remarkably robust in the sensitivity analysis. The authors' meta-analyses support an inverse association between circulating levels of superoxide dismutase, glutathione peroxidase, and catalase activities with CHD and emphasize the need for additional high-quality prospective studies.

cardiovascular diseases; catalase; coronary disease; glutathione peroxidase; meta-analysis; superoxide dismutase

Abbreviations: CHD, coronary heart disease; GSH-Px, glutathione peroxidase; SOD, superoxide dismutase.

Cardiovascular disease is the main cause of mortality and a major cause of morbidity and disability worldwide (1). Atherosclerosis, the most common pathologic process underlying cardiovascular disease, represents a state of heightened oxidative stress characterized by lipid and protein oxidation in the vascular wall (2, 3). Oxidation of low density lipoproteins is considered a key initial step in atherosclerosis and coronary heart disease (CHD) development and progression (4). Oxidative stress is also considered a central factor in endothelial dysfunction and plaque disruption (2).

Among antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase constitute a first line of defense against oxidative stress by removing key reactive oxygen species (3). SOD, which operates primarily within cells and in extracellular matrices, catalyzes the dismutation of the superoxide anion $\left(O_2^*\right)$ into hydrogen peroxide (H_2O_2). Catalase and GSH-Px remove hydrogen peroxide, and GSH-Px can also convert lipid peroxyl radicals to nontoxic alcohols (3).

It has been hypothesized that low activity levels of antioxidant enzymes are associated with an increased risk of CHD (5). The literature on this topic is scattered, however, and, to our knowledge, no systematic review or meta-analysis is available on the relation of GSH-Px, SOD, or catalase activity levels with CHD. The objective of the present meta-analyses was to systematically review the issue.

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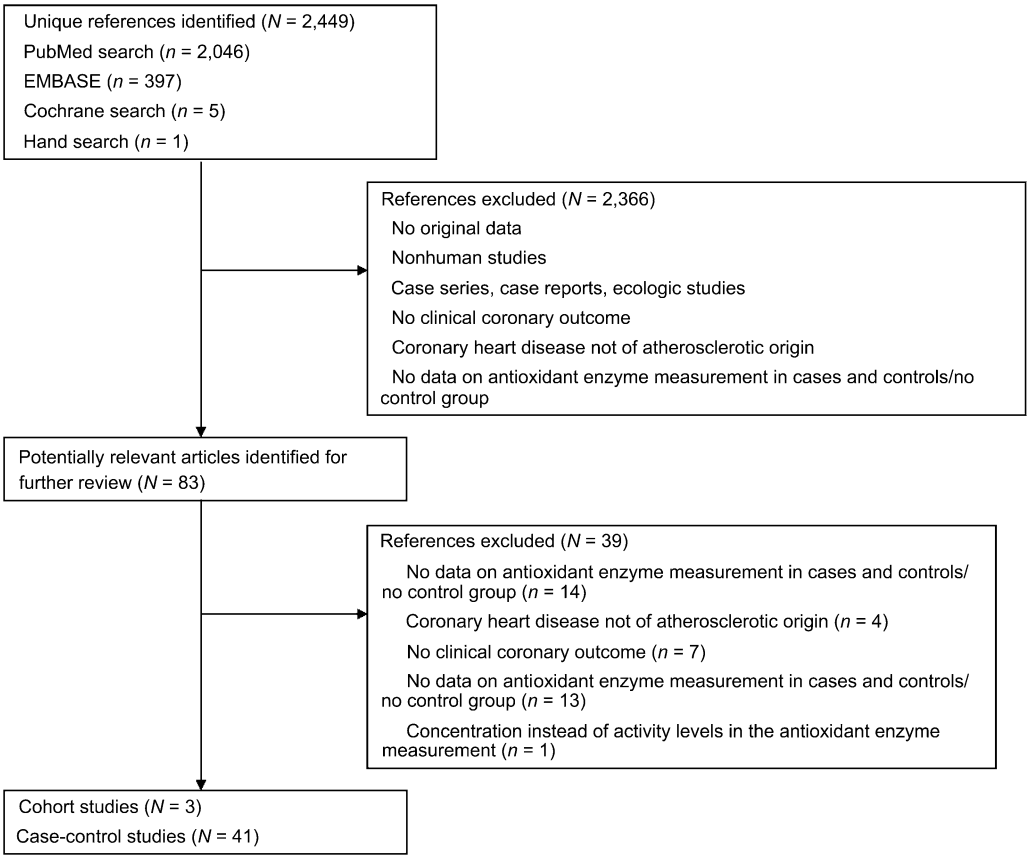

Figure 1. Flow diagram of the study selection process.

MATERIALS AND METHODS

Search strategy and study selection

We searched the MEDLINE database (National Library of Medicine, Bethesda, Maryland) and EMBASE (The Excerpta Medica Database; Elsevier, the Netherlands) for epidemiologic studies investigating the relation of antioxidant enzyme activities with CHD by using the following free-text and medical subject heading (MeSH) terms: *glutathione peroxidase, superoxide dismutase, catalase, selenium, antioxidant enzyme, oxidative stress, atherosclerosis, cardiovascular disease, myocardial infarction, coronary heart disease, angina pectoris, stroke, peripheral arterial disease, atherosclerosis, meta-analysis, review, mortality,* and *morbidity*. The search period was from January 1966 to January 2008. There were no language restrictions. We also searched the Cochrane Central Register of Controlled Trials (The Cochrane Library; Wiley InterScience, Malden, Massachusetts) and reviewed the reference lists of relevant original papers and review articles.

We aimed to identify all studies assessing GSH-Px, SOD, and catalase activities in cells (erythrocytes, platelets) or biologic fluids (plasma, serum, or whole blood) and clinical CHD outcomes (including acute myocardial infarction or angina). Our exclusion criteria were 1) no original research (reviews, editorials, and nonresearch letters); 2) nonhuman studies (experimental studies); 3) case reports and case series (no control group); 4) lack of a clinical CHD outcome (e.g., subclinical atherosclerosis); 5) no data on antioxidant enzyme activity; and 6) concentration instead of activity levels in the antioxidant enzyme measurements. When time-course changes for an enzymatic activity after an acute event were measured, the first measurement was selected. In addition, when several articles using the same population were published, the publication with the longest follow-up was selected. Figure 1 summarizes the study selection process.

Data abstraction

Three investigators (G. F.-M., R. E., and P. C.-S.) independently abstracted the articles that met the selection criteria. They resolved discrepancies by consensus. Data abstracted were author, country, age, percentage of men, design, outcomes, number of cases and noncases (controls), biomarker used, and method used to measure enzymatic activity.

The primary endpoint selected a priori was CHD. This endpoint was defined as any combination of fatal CHD and nonfatal acute myocardial infarction or angina with or without coronary revascularization.

Case-control studies were further classified as acute-event studies, when antioxidant enzyme activities were determined during the acute phase of myocardial infarction or unstable angina, and as chronic/stable–event studies, when enzyme activities were determined during a more stable phase of the disease. To assess study quality, we adapted the criteria used by Longnecker et al. (6) for observational studies.

Statistical analyses

We abstracted the hazard ratios and their associated 95% confidence intervals from 3 studies (5, 7, 8). The rest of the studies (9–49) did not report standard measures of association. We thus abstracted the mean and standard-deviation levels of antioxidant enzyme activity in cases and noncases, and we used a linear discriminant function method to calculate the odds ratios for CHD associated with a 1-standard-deviation increase in antioxidant enzyme activity (50). Pooled odds ratios were estimated by using an inverse-variance-weighted random-effects model. Heterogeneity was quantified with the I^2 statistic (51), which describes the proportion of the total between-study variability due to heterogeneity. We used meta-regression and subgroup analyses to evaluate whether results were different according to the 1) matrix in which the antioxidant enzyme was measured (serum vs. other matrices); 2) outcome (acute vs. chronic or stable events); 3) country (West-European populations vs. others); and 4) degree of adjustment for traditional CHD risk factors ($\geq$3 vs. $<$3).

We assessed publication bias by using funnel plots. In sensitivity analyses, we assessed the relative influence of each study on the pooled estimate by omitting one study at a time. Statistical analyses were conducted by using Stata, version 9.0 software (Stata Corporation, College Station, Texas).

RESULTS

Literature search

The search strategy retrieved 2,449 unique citations (Figure 1). Of these citations, 2,366 were excluded after screening on the basis of title and abstract and 39 after full-text review, leaving 3 cohort and 41 case-control studies for final inclusion in the meta-analyses (Table 1). The AtheroGene cohort contributed 2 papers (5, 7). We used GSH-Px activity data from the longest follow-up report (7), but, because this report did not include SOD activity, we used the SOD activity data from the earlier publication in this cohort (5).

Cohort studies fulfilled most prespecified quality criteria. Case-control studies varied widely in their degree of fulfilling these criteria (Table 2), although most studies had limitations in terms of lack of adjustment for potential confounders and description of the control selection process.

GSH-Px activity

Thirty-two case-control studies (9–15, 18–28, 30, 32–42, 46, 49) and 2 prospective cohort studies (5, 8) evaluated the association between GSH-Px and CHD (Table 1). Twenty-five studies were from Europe and 9 from Asia. The number of CHD cases ranged from 9 (22) to 200 (18). The Paglia and Valentine method, which measures all GSH-Px isoforms, was the most commonly used (52). Only 6 case-control studies (11, 12, 19, 23, 32, 35), as well as a cohort study (7), controlled for 3 or more potential confounders.

There was substantial heterogeneity in the direction and magnitude of the association between GSH-Px activity and CHD outcomes across studies (Web Figure 1; this is the first of 3 supplementary figures, each of which is referred to as ''Web Figure'' in the text and is posted on the *Journal*'s website (http://aje.oupjournals.org/)), although a majority of studies found an inverse association with CHD. The pooled odds ratio for CHD associated with a 1-standard-deviation increase in GSH-Px activity was 0.51 (95% confidence interval: 0.35, 0.75; P for heterogeneity <0.001; $I^2 = 96.2\%$) (Table 3). In sensitivity analysis, exclusion of individual studies did not substantially modify the estimates, with pooled odds ratios ranging from 0.55 to 0.63.

SOD activity

Twenty-six case-control studies (14–18, 21, 22, 25, 26, 29–32, 34–36, 38, 39, 41–45, 47–49) and one cohort study (5) evaluated the association between SOD activity and CHD. The number of cases ranged from 9 (26) to 200 (18). Seventeen studies were performed in Europe and 10 in Asia. Among all SOD isoenzymes, copper-zinc-SOD was the isozyme most commonly assessed (5, 14–18, 21, 22, 25, 26, 30, 31, 35, 36, 38, 39, 41–45, 47–49). Only 8 case-control studies (14, 16, 21, 25, 29, 38, 45, 48), as well as a cohort study (5), controlled for potential confounders.

Most studies found inverse associations between SOD activity and CHD outcome, although they were not always statistically significant (Web Figure 2). The pooled odds ratio for CHD associated with a 1-standard-deviation increase in SOD activity was 0.48 (95% confidence interval: 0.32, 0.72; P for heterogeneity <0.001; $I^2 = 95.7\%$) (Table 3). The findings were similar after we excluded studies that measured extracellular SOD activity (29, 32) (pooled odds ratio = 0.44, 95% confidence interval: 0.29, 0.66; P for heterogeneity <0.001; $I^2 = 95.5\%$) or after conducting sensitivity analyses that excluded one study at a time (pooled odds ratios = 0.47–0.57).

Catalase activity

Ten case-control studies evaluated the association between catalase activity and CHD (15, 17, 18, 21, 26, 34, 43, 45–47). The number of cases ranged from 10 (15) to 200 (18). Six studies were from Europe and 4 from Asia. None of the studies controlled for potential confounders.

All studies except one found an inverse association between catalase activity and CHD (Web Figure 3) (34). The pooled odds ratio for CHD associated with a 1-standard-deviation increase in catalase activity was 0.32 (95% confidence interval: 0.16, 0.61; P for heterogeneity <0.001; $I^2 = 94.4\%$) (Table 3). The findings were consistent in the

Table 1. Characteristics of Observational Studies of Glutathione Peroxidase, Superoxide Dismutase, and Catalase and Coronary Heart Disease

First Author, Year (Reference No.)	Country	% of Men Among Noncases	Age of Noncases, Years	Type of Controls	Source of Cases	Outcomes	No. of Cases/ Noncases	Enzyme/Matrix/ Method	OR	95% CI	Adjusted for
Guidi, 1986 (9)	Italy	100	Mean, 46	NR	NR	CHD, chronic/ stable	25/25	GSH-Px/platelets/Lowry	0.34	0.20, 0.60	Sex
Akesson, 1987 (10)	Sweden	100	Mean, 68	General population	Men born in 1914	Angor, chronic/ stable	64/417	GSH-Px/serum/Günzler	0.93	0.72, 1.22	Age, sex
Salonen, 1988 (11)	Finland	100	Mean, 54	Kuopio Ischemic Heart Disease Study	Kuopio Ischemic Heart Disease Study	CHD, chronic/ stable	175/449	GSH-Px/whole blood/ Levander	0.82	0.69, 0.98	Sex, smoking, BMI, lipids, HT, DM
Kok, 1989 (12)	The Netherlands	70	Mean, 59	General population	Rotterdam Hospital	AMI, acute	84/84	GSH-Px/erythrocytes/ Paglia and Valentine	1.49	1.10, 2.02	Age, sex, smoking, BMI, lipids, HT, DM
Gromadzinska, 1990 (13)	Poland	42	Range, 17–90	NR	NR	AMI, acute	14/96	GSH-Px/erythrocytes/ Paglia and Valentine	0.56	0.32, 0.98	Crude
Loeper, 1991 (14)	France	NR	Mean, 67	Nursing staff or consulting patients	Hospital	CHD, acute	17/35	GSH-Px/erythrocytes/ Paglia and Valentine	1.11	0.68, 1.77	Age
								SOD/erythrocytes/Crapo, McCord, and Fridovich	0.36	0.22, 0.58	
László, 1991 (15)	Hungary	NR	NR	NR	NR	AMI, acute	10/11	GSH-Px/erythrocytes/ Little and O'Brien	0.55	0.23, 1.31	Crude
								SOD/erythrocytes/Misra and Fridovich	0.14	0.06, 0.33	
								Catalase/erythrocytes/ Beers and Sizer	0.16	0.07, 0.37	
Ciuffetti, 1992 (16)	Italy	66	Mean, 58	Outpatient clinic	Outpatient clinic	Angina, chronic/ stable	18/15	SOD/whole blood/McCord and Fridovich	0.36	0.18, 0.73	Age, sex, smoking, BMI, lipids, HT, DM
Kopff, 1993 (17)	Poland	62	Range, 29–65	NR	NR	Angina, chronic/ stable	26/86	SOD/erythrocytes/NR	0.58	0.37, 0.90	Crude
Jayakumari, 1992 (18)	India	100	NR	Hospital staff	Patients scheduled for coronary angiography	AMI, chronic/ stable	200/100	GSH-Px/erythrocytes/ Hopkins	1.12	0.88, 1.43	Sex
								SOD/erythrocytes/Elstner	0.44	0.35, 0.56	
								Catalase/erythrocytes/ Aebi	0.99	0.78, 1.26	
Porter, 1992 (19)	United Kingdom	NR	Range, 30–65	Noncardiac disease inpatients	Hospital	CHD, acute	18/18	GSH-Px/serum/Paglia and Valentine	0.38	0.20, 0.74	Age, sex, smoking
								GSH-Px/erythrocytes/ Paglia and Valentine	0.93	0.48, 1.79	
								GSH-Px/platelets/Paglia and Valentine	0.31	0.16, 0.59	
Akyol, 1993 (20)	Turkey	NR	NR	NR	Hospital	AMI, acute	15/10	GSH-Px/serum/Paglia and Valentine	0.81	0.36, 1.81	Crude
Buczynski, 1993 (21)	Poland	100	Range, 35–57	NR	Hospital	CHD, acute	41/41	GSH-Px/platelets/Little and O'Brien	0.33	0.21, 0.51	Crude
								SOD/platelets/Misra and Firdovich	0.15	0.10, 0.23	

Dubois-Rande, 1994 (22)	France	NR	NR	Hospital staff	Patients scheduled for coronary angioplasty	AMI, acute	9/23	GSH-Px/serum/Paglia and Valentine	0.76	0.36, 1.60	Age
								GSH-Px/erythrocytes/ Paglia and Valentine	1.07	0.37, 3.08	
								SOD/erythrocytes/Flohé and Oting	1.24	0.59, 2.59	
Duthie, 1994 (23)	United Kingdom	NR	Mean, 53	NR	NR	Angina, chronic/ stable	21/200	GSH-Px/serum/NR	1.29	0.85, 1.95	Age, sex, smoking, BMI, HT, DM
								GSH-Px/erythrocytes/NR	1.14	0.75, 1.73	
Blann, 1995 (24)	United Kingdom	59	Mean, 50	Hospital staff and patients without vascular disease	Lipid clinic	CHD, chronic/ stable	48/71	GSH-Px/serum/Paglia and Valentine	0.02	0.01, 0.03	Age
Akkus, 1996 (25)	Turkey	60	Mean, 56	NR	NR	CHD, chronic/ stable	42/35	GSH-Px/erythrocytes/ Paglia and Valentine	0.17	0.10, 0.30	Age, sex
								SOD/erythrocytes/Randox	0.48	0.31, 0.76	
Dusinovic, 1998 (26)	Yugoslavia	NR	Range, 33–74	NR	NR	AMI, acute	9/30	GSH-Px/whote blood/ Paglia and Valentine	2.69	1.28, 5.66	Crude
								SOD/serum/Misra and Fridovich	0.76	0.36, 1.60	
								Catalase/erythrocytes/ Beutler	0.03	0.01, 0.07	
Kaur, 1999 (27)	India	NR	NR	NR	NR	AMI, acute	10/10	GSH-Px/platelets/Lowry	0.26	0.11, 0.63	Age, sex
Bor, 1999 (28)	Turkey	83	Mean, 51	NR	Emergency room	AMI, acute	27/24	GSH-Px/serum/Paglia and Valentine	1.11	0.61, 2.01	Crude
								GSH-Px/erythrocytes/ Paglia and Valentine	3.73	2.06, 6.76	
Jain, 2000 (29)	India	88	Mean, 55	Patients without vascular disease	Hospital	AMI, acute	60/30	SOD/serum/Minami	3.73	2.40, 5.78	Age, sex
Pandey, 2000 (30)	India	NR	NR	NR	NR	Angina, acute	20/20	GSH-Px/platelets/Pinto and Bartley	0.40	0.20, 0.78	Crude
								SOD/serum/Misra and Fridovich	0.07	0.04, 0.14	
Gürlek, 2000 (31)	Turkey	NR	Mean, 60	NR	NR	CHD, acute	51/20	SOD/erythrocytes/ Winterbourn	9.89	5.89, 16.6	Age
Jang, 2001 (32)	Korea	100	Mean, 53	Volunteers from a nutrition study	Hospital	CHD, chronic/ stable	30/64	GSH-Px/serum/Paglia and Valentine	1.00	0.65, 1.54	Age
								SOD/serum/Marklund and Marklund	0.69	0.45,1.06	
Zachara, 2001 (33)	Poland	62	Mean, 57	NR	Coronary unit	AMI, chronic/ stable	49/58	GSH-Px/serum/Paglia and Valentine	0.94	0.64, 1.37	Crude
								GSH-Px/erythrocytes/ Paglia and Valentine	1.35	0.92, 1.98	
Kesavulu, 2001 (34)	India	NR	Mean, 51	NR	NR	CHD, chronic/ stable	57/69	GSH-Px/erythrocytes/ Wendel	0.74	0.33, 1.69	Sex, BMI
								SOD/erythrocytes/Misra and Fridovich	0.85	0.39, 1.85	
								Catalase/erythrocytes/ Aebi	2.57	1.62, 4.08	

Table continues

Table 1. Continued

First Author, Year (Reference No.)	Country	% of Men Among Noncases	Age of Noncases, Years	Type of Controls	Source of Cases	Outcomes	No. of Cases/ Noncases	Enzyme/Matrix/ Method	OR	95% CI	Adjusted for
Muzakova, 2001 (35)	Czech Republic	81	Mean, 55	Blood donors	Hospital	AMI, acute	48/21	GSH-Px/whole blood/ Paglia and Valentine	0.77	0.46, 1.28	Age, sex, smoking, BMI, HT, DM
								SOD/erythrocytes/Misra and Fridovich	1.75	1.05, 2.93	
Krstevska, 2001 (36)	Republic of Macedonia	0	Range, 18–55	NR	Cardiology clinic	CHD, chronic/ stable	41/51	GSH-Px/whole blood/ Paglia and Valentine	0.49	0.33, 0.74	Sex
								SOD/whole blood/ Goldstein	0.66	0.43, 1.01	
Domínguez-Rodriguez, 2002 (37)	Spain	68	Mean, 53	Hospital staff	Hospital	AMI, acute	25/25	GSH-Px/serum/ Calbichem	0.84	0.48, 1.46	Age, sex
Simic, 2003 (38)	Serbia	67	Mean, 58	NR	Hospital	AMI, acute	31/24	GSH-Px/erythrocytes/ Güntzler	0.12	0.07, 0.20	Sex
								SOD/erythrocytes/Randox	0.09	0.05, 0.18	
Weinbrenner, 2003 (39)	Spain	100	Mean, 57	Spanish Olive Oil Study	Spanish Olive Oil Study	CHD, chronic/ stable	32/32	GSH-Px/whole blood/ Paglia and Valentine	6.30	3.90, 10.2	Sex
								SOD/whole blood/Misra and Fridovich	2.15	1.32, 3.51	
Tosukhowong, 2003 (40)	Thailand	66	Mean, 58	Cardiovascular unit	NR	CHD, chronic/ stable	61/32	GSH-Px/erythrocytes/ Paglia and Valentine	0.03	0.02, 0.04	Sex
Rajasekhar, 2004 (41)	India	64	Mean, 46	General population	Cardiology clinic	CHD, chronic/ stable	139/59	GSH-Px/serum/Misra and Fridovich	0.86	0.63, 1.16	HT, DM
								GSH-Px/erythrocytes/ Misra and Fridovich	1.18	0.87, 1.60	
								SOD/erythrocytes/Misra and Fridovich	1.11	0.82, 1.50	
Colak, 2005 (42)	Serbia	52	Mean, 58	Hospital staff	Institute of Endocrinology, Diabetes and Metabolic Disorders	CHD, chronic/ stable	69/44	GSH-Px/whole blood/ Paglia and Valentine	0.48	0.33, 0.69	Crude
								SOD/whole blood/ Goldstein	0.21	0.15, 0.30	
Saha, 2005 (43)	India	58	Mean, 53	Volunteers	Hospital	CHD, chronic/ stable	33/38	SOD/erythrocytes/Paoletti	0.92	0.47, 1.82	Crude
								Catalase/erythrocytes/ Aebi			
Saxena, 2005 (44)	India	30	Mean, 51	NR	Diabetes clinic	CVD, chronic/ stable	13/13	SOD/erythrocytes/ Marklund and Marklund	0.19	0.09, 0.42	Crude
Kratnov, 2005 (45)	Russia	NR	NR	NR	NR	CHD, chronic/ stable	59/20	SOD/whole blood/ Nishikimi	0.64	0.38, 1.06	Age, sex
								Catalase/whole blood/ Mamontova and Beloborodova	0.45	0.27, 0.75	
Serdar, 2006 (46)	Turkey	54	Mean, 56	Normal coronary angiograms	Pathologic coronary angiograms	CHD, chronic/ stable	53/54	GSH-Px/erythrocytes/ Randox	0.53	0.36, 0.78	Crude
								Catalase/erythrocytes/ Randox	0.16	0.10, 0.23	

Dwivedi, 2006 (47)	India	100	Range, 45–70	NR	NR	AMI, acute	37/25	SOD/whole blood/Misra and Fridovich	0.11	0.05, 0.24	Age
								Catalase/whole blood/Lück	0.21	0.15, 0.30	
Kotur-Stevuljevic, 2007 (48)	Serbia	57	Mean, 56	NR	Institute for Cardiovascular Diseases at a clinical center	CHD, chronic/stable	188/197	SOD/erythrocytes/Misra and Fridovich	0.64	0.53, 0.78	Age, sex
Patil, 2007 (49)	India	84	Mean, 43	Intensive cardiac care unit	Annual checkup program	AMI, acute	48/50	GSH-Px/erythrocytes/Paglia and Valentine	0.02	0.01, 0.03	Age
								SOD/erythrocytes/Winterbourn	0.02	0.01, 0.03	
Hallgren, 2001 (8)	Sweden	79	Mean, 55	MONICA study participants	MONICA incidence registry	AMI, acute	78/156	GSH-Px/erythrocytes/Güntzler	0.80	0.31, 2.06	Age, sex
Blankenberg, 2003 (5); Schnabel, 2005 (7)	Germany and France	72	Mean, 62	Patients with angina pectoris	Hospital and general practitioner registry	CVD, mortality	112/527	GSH-Px/erythrocytes/Paglia and Valentine	0.41	0.22, 0.74	Age, sex, smoking, BMI, HT, DM
								SOD/erythrocytes/Misra and Fridovich	0.29	0.14, 0.60	

Abbreviations: AMI, acute myocardial infarction; BMI, body mass index; CHD, coronary heart disease; CI, confidence interval; CVD, cardiovascular disease; DM, diabetes mellitus; GSH-Px, glutathione peroxidase; HT, hypertension; MONICA, Monitoring of Trends and Determinants in Cardiovascular Disease; NR, not reported; OR, odds ratio; SOD, superoxide dismutase.

sensitivity analysis omitting one study at a time, with pooled odds ratios ranging from 0.25 to 0.40.

Stratified analyses

Subgroup analyses of studies with acute versus chronic or stable CHD cases showed similar associations for GSH-Px and SOD activities. For catalase activity, a stronger association was observed for acute compared with chronic or stable cases (Table 4). There was no significant difference in the pooled odds ratio for CHD associated with a 1-standard-deviation increase in GHS-Px ($P = 0.35$) and SOD activities ($P = 0.32$) when the analyses were restricted to studies adjusting for 3 or more potential confounders, although these associations were attenuated. Concerning catalase, none of the studies adjusted for any potential confounder. Even after stratified analysis, substantial residual heterogeneity existed in all subgroups evaluated (all $I^2 > 76\%$). Funnel plots did not suggest the presence of publication or related biases (refer to Appendix Figures 1–3).

DISCUSSION

In the present meta-analyses, we identified strong and statistically significant inverse associations of GSH-Px, SOD, and catalase activities with CHD outcomes. The quality of the study base was limited, however, and, for GSH-Px and SOD, the associations were attenuated when the analyses were restricted to studies that adjusted for at least 3 potential confounders. Etiologic inferences about the role of oxidative stress on CHD are thus complicated by limitations in the design of the original studies and by the complexity of the relation between oxidative stress and antioxidant enzyme activity. While our analyses provide evidence that CHD cases have reduced antioxidant enzyme activity levels, lower enzyme activity could be a consequence of the increased oxidative stress induced by the coronary events or by subclinical disease rather than a causal mechanism in CHD etiology.

Oxidative stress is induced by reactive oxygen and nitrogen species. Scavenger antioxidant enzymes, such as SOD, GSH-Px, or catalase, are inactivated when counteracting these reactive species. In turn, reactive oxygen and nitrogen species induce expression of the antioxidant enzymes (53). Thus, low levels of antioxidant enzyme activity could reflect either high oxidative stress or low levels of defense against it. In CHD patients, even in stable cases, a high oxidative stress status has been reported (54, 55). For instance, circulating oxidized low density lipoprotein levels are positively associated with severity of acute coronary syndromes (55) and with subclinical CHD (56). As a consequence, the association between low antioxidant enzyme activity and CHD in our data may reflect increased oxidative stress in CHD cases. Indeed, low levels of GSH-Px and intracellular SOD have been reported not only in CHD (57) but also in other oxidative-stress-associated diseases such as diabetes mellitus (58), neoplasia (59, 60), kidney diseases (61), and degenerative brain disorders (62). Conversely, high levels of GSH-Px and SOD activities may confer protection against

Table 2. Quality Criteria for Evaluating the Design and Data Analysis of Studies on the Association of Glutathione Peroxidase, Superoxide Dismutase, and Catalase With Coronary Heart Disease[a]

	Reference Number(s)																																											
	9	10	11	12	13	14	15	16	17	18	19	20	21	77	22	23	24	25	27	28	29	30	31	32	33	34	35	36	37	38	78	39	40	41	42	43	44	45	46	47	48	79	8	5; 7
All epidemiologic studies																																												
Antioxidant activity was assessed at the individual level.	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Outcomes were based on objective tests or standard criteria for ≥90% of study participants.	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
The authors presented internal comparisons within study participants.	■	■	■	■	□	■	■	■	■	■	■	■	■	■	■	■	■	■	□	■	□	□	□	■	■	■	■	■	■	■	■	■	□	■	■	■	■	■	■	■	■	■	■	■
The authors controlled for potential confounding risk factors in addition to age and sex.	□	□	■	■	□	□	□	■	□	□	■	□	□	□	■	■	□	■	□	□	□	□	□	■	□	■	■	□	□	□	□	□	□	■	□	□	□	□	□	□	□	□	□	□
Prospective cohort studies																																												
Loss of follow-up was independent of exposure.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	■	■
The intensity of the search for disease was independent of exposure status.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	■	■
Case-control studies																																												
Data were collected in a similar manner for all participants.	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	□	–	–
The same exclusion criteria were applied to all participants.	□	■	■	■	□	■	□	■	□	□	□	□	□	□	□	■	■	□	■	□	■	□	■	□	□	■	□	■	■	□	□	■	■	□	□	□	□	■	■	□	□	□	–	–
The selection process for noncases was described.	□	□	■	■	□	■	□	□	□	■	■	□	□	□	■	■	■	□	□	□	■	□	□	■	□	■	■	■	■	□	□	□	□	□	■	■	■	□	■	■	□	□	–	–
Samples were collected <48 hours from the onset of symptoms for all cases.	□	□	□	□	■	■	■	□	■	□	□	□	□	□	■	□	□	□	□	■	■	□	□	□	■	□	■	□	■	■	□	□	□	□	□	□	□	■	□	■	□	■	–	–
The study was based on incident cases of disease.	□	□	□	■	■	■	■	□	■	□	■	□	□	■	■	□	□	□	■	■	■	■	□	□	■	□	■	□	■	■	□	□	□	□	□	□	□	■	□	■	□	■	–	–
Noncases were people who would have been excluded if they had developed coronary heart disease.	■	■	■	■	■	■	□	■	■	■	■	■	■	■	■	■	■	■	■	□	■	■	■	■	□	■	■	■	□	□	■	■	□	■	■	□	□	□	□	■	■	■	–	–

[a] ■, yes; □, no ; – not applicable.

Table 3. Odds Ratio Estimates and 95% Confidence Intervals for Coronary Heart Disease per a 1-Standard-Deviation Increase in Glutathione Peroxidase, Superoxide Dismutase, and Catalase Activity

	Glutathione Peroxidase (n = 34)			Superoxide Dismutase (n = 26)			Catalase (n = 10)		
	OR	95% CI	I^2, %	OR	95% CI	I^2, %	OR	95% CI	I^2, %
Case-control studies									
Overall serum	0.61	0.31, 1.10	94.6	1.60	0.31, 8.38	96.5			
Overall erythrocytes	0.80	0.55, 1.17	90.3	0.63	0.42, 0.95	92.9	0.55	0.24, 1.26	96.1
Overall whole blood	0.44	0.13, 1.44	98.7	0.58	0.43, 0.79	97.1	0.27	0.09, 0.79	82.6
Overall platelets	0.33	0.25, 0.43	0	0.14	0.10, 0.20	0	0.28	0.18, 0.48	0
Cohort studies									
Overall erythrocytes	0.52	0.28, 0.96	26.5	0.29	0.14, 0.60	0			
Total studies	0.51	0.35, 0.75	96.2	0.48	0.32, 0.72	95.7	0.32	0.16, 0.61	94.4

Abbreviations: CI, confidence interval; OR, odds ratio.

reactive species production. Overexpression of GSH-Px (63) and intracellular SOD (57) in transgenic animal models of reperfusion injury prevents postischemic free radical injury and inhibits low density lipoprotein oxidation by endothelial cells.

Although our analysis is compatible with a higher level of oxidative stress in CHD patients, population studies of oxidative stress and CHD have been hampered by the lack of a widely accepted biomarker of oxidative stress. We restricted our meta-analyses to studies that measured antioxidant enzyme activity rather than enzyme concentrations. Methods that measure enzymatic activity assess the functional capacity of an enzyme to act on its substrate. Enzymatic concentration assays, however, often rely on polyclonal antibodies binding to enzyme epitopes, which may potentially be preserved in various states of the enzyme degradation (64).

Mendelian-randomization-based studies have also provided insight into the role of antioxidant enzymes and CHD risk. Substitution of arginine-213 by glycine (R213G), a common variant of the extracellular (*EC*)-*SOD* gene (65) described in 4%–6% of the population (66), has been associated with a 10-fold increase in SOD plasma concentration (66). The -262C>T polymorphism in the promoter region of the catalase gene has been associated with a 1.5-fold increase in catalase activity (67). However, to our knowledge, no association between these genetic variants and CHD risk has been reported (66, 67). Further studies are necessary to elucidate the role of antioxidant enzyme genetic variants in CHD risk.

Interpretation of the inverse association between antioxidant enzyme levels and CHD observed in our meta-analysis is further complicated by methodological limitations of the studies. Several studies in the meta-analysis measured

Table 4. Stratified Pooled Odds Ratios for Coronary Heart Disease for a 1-Standard-Deviation Increase in Antioxidant Enzyme Activity

	Glutathione Peroxidase					Superoxide Dismutase					Catalase				
	No.	OR	95% CI	I^2, %	P for Interaction	No.	OR	95% CI	I^2, %	P for Interaction	No.	OR	95% CI	I^2, %	P for Interaction
Acute coronary heart disease as the primary outcome															
Yes	21	0.57	0.36, 0.89	93.3		14	0.40	0.16, 1.01	97.2		6	0.12	0.05, 0.30	84.4	
No[a]	13	0.43	0.22, 0.84	97.9	0.77	13	0.58	0.43, 0.79	87.7	0.74	4	0.57	0.27, 1.19	95.0	0.02
Adjusted for confounding factors															
≥3 factors	7	0.84	0.61, 1.16	76.2		3	0.58	0.77, 1.92	89.6						
<3 factors	27	0.47	0.29, 0.77	96.4	0.35	24	0.46	0.30, 0.72	95.8	0.32					
Country															
West-European	9	0.55	0.29, 1.03	95.6		3	0.50	0.22, 1.86	78.8						
Others	25	0.50	0.30, 0.82	96.2	0.63	24	0.47	0.31, 0.74	95.9	0.40					

Abbreviations: CI, confidence interval; OR, odds ratio.

[a] Chronic or stable angina.

antioxidant enzyme activity during the acute phase of coronary events. Antioxidant enzyme activity changes rapidly during an acute coronary event, with increased production of reactive oxygen species and depressed antioxidant reserves in the first 6–12 hours (68), up-regulation of enzyme activity 1–2 days after onset of the acute event, and a gradual return to the basal range over the next days (69). The magnitude and the time course of these changes are affected by the severity of the acute event, by the development of complications, and by some therapeutic interventions such as reperfusion (33, 69, 70). Variability in the timing of assessment of antioxidant enzyme activity with respect to onset of the acute event across studies may result in increased within-study variability and between-study heterogeneity. Even in studies that measured antioxidant enzyme activity in chronic or stable CHD cases, enzyme activity may have been modified by postinfarction changes in medication (e.g., statin use) or lifestyle (e.g., smoking cessation, physical activity (71–73), or diet (74)).

In addition, most studies in this meta-analysis were small case-control studies. This design can be affected by survival bias because fatal CHD episodes are not included in the case group, by biases in the selection of the control group, and by differences between cases and controls in traditional and nontraditional risk factors that were not adjusted for in the analysis (e.g., many studies did not adjust for factors such as age, smoking, diet, and physical activity). The attenuation in the odds ratios when the analyses were restricted to studies that adjusted for 3 or more potential confounders opens the possibility that more rigorous adjustment for other cardiovascular risk factors may completely remove the association between antioxidant enzyme activity and CHD.

Although recent meta-analyses have been performed on the relation between antioxidant enzyme activities, or polymorphic gene variations, in several diseases (e.g., bipolar disorders or tardive dyskinesia), the relation between antioxidant enzyme activities and CHD risk has not been previously evaluated to our knowledge (75, 76). From our meta-analyses, large, prospective cohort studies with adequate control for standard cardiovascular risk factors are needed to determine the impact of antioxidant enzyme activity on CHD risk. Surprisingly, none of the large population-based cohorts of cardiovascular risk factors have evaluated these exposures. The AtheroGene Study (5, 7) was the largest prospective study to assess the effect of SOD and GSH-Px activities on CHD outcomes. Participants in this study had already developed cardiovascular disease at baseline, however. As a consequence, this study provides information on the role of antioxidant enzyme activity levels in CHD recurrence only. In this study (5, 7), GSH-Px activity was inversely associated with CHD events after adjustment for traditional cardiovascular risk factors, but no association was found for SOD.

In summary, our meta-analyses identified a strong, inverse association of GSH-Px, SOD, and catalase activity levels with CHD. These analyses provide one of the strongest pieces of evidence to date supporting the fact that CHD patients experience a state of increased oxidative stress. Unfortunately, this association is difficult to interpret in mechanistic or etiologic terms because of methodological limitations of the available studies. High-quality prospective studies evaluating the association between low antioxidant enzyme activity levels and cardiovascular endpoints are warranted.

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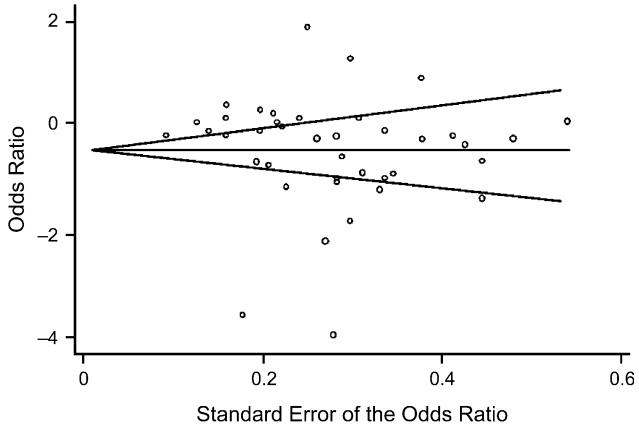
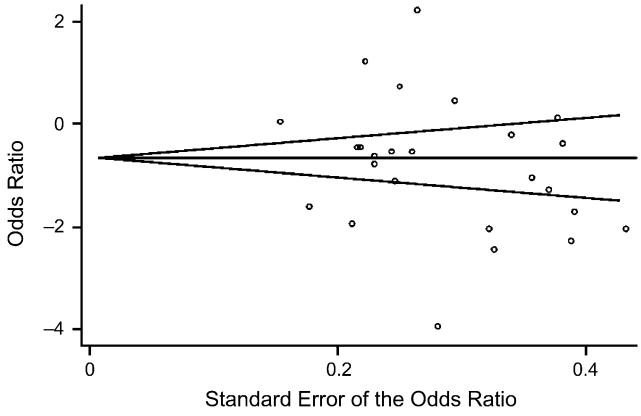
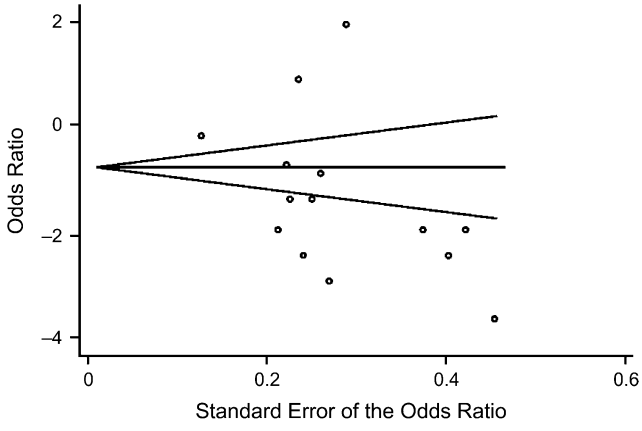


Appendix Figure 1. Begg's funnel plot, with pseudo–95% confidence limits, of the studies on glutathione peroxidase activity and coronary heart disease.



Appendix Figure 2. Funnel plot, with pseudo–95% confidence limits, of the studies on superoxide dismutase activity and coronary heart disease.



Appendix Figure 3. Funnel plot, with pseudo–95% confidence limits, of the studies on catalase activity and coronary heart disease.