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Original Research

Association of oxidative stress with mortality: the Beaver Dam Eye Study

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Abstract
Epidemiological studies have shown that oxidative stress is associated with cardiovascular disease (CVD) and diabetes. However, the association of oxidative stress marker with non-CVD and CVD mortality has not been extensively evaluated. The association of baseline serum 8-isoprostane (8-ISO) with all-cause, non-CVD, and CVD mortality was examined in a random subset (n = 1,753) of a population-based study of 4,926 adults (99% non-Hispanic whites; 56% women) aged 43-86 years from the Beaver Dam Eye Study. Cause of death was ascertained by death certificate between 1987 and 2002. 8-ISO was measured by immunoassay. Cox proportional hazards regression analysis was used to estimate mortality hazard ratios (HRs) and 95% confidence intervals (CIs) by one 8-ISO standard deviation. During a median follow-up of 13.1 years, 590 (33.7%) participants died (290 CVD deaths). After adjusting for socio-demographics and CVD risk factors, 8-ISO was significantly associated with all-cause (HR = 1.1, 95% CI 1.01-1.2) and non-CVD (HR = 1.14, 95% CI 1.01-1.28) mortality but not with CVD mortality (HR = 1.06, 95% CI 0.93-1.2). When limited to participants with BMI < 25 kg/m², individuals in the highest 8-ISO quartile had approximately 34 to 36% increased risk of all-cause, non-CVD, and CVD death compared to those at the lowest quartile. In contrast, 8-ISO was not significantly associated with mortality among those with BMI ≥ 25 kg/m². These findings suggest that baseline serum 8-ISO, a marker of oxidative stress, is an independent risk factor of all-cause, non-CVD, and CVD mortality among a cohort of middle-aged adults with normal BMI.

INTRODUCTION

Oxidative stress, the inability of the body's antioxidant defense mechanism to neutralize the effects caused by the excessive production of oxidative compounds (*e.g.*, reactive oxygen species [ROS]), has been suggested to play an important role in the development of chronic diseases [1, 2] such as diabetes [3, 4], cardiovascular disease (CVD) [5], and cancer [6-8]. Under normal physiological conditions, ROS (highly reactive oxygen molecules with one or more impaired electrons) are the by-products of normal cellular metabolism that, in excessive concentrations, may cause DNA damage, protein, and lipid oxidation [2, 6-8]. Animal studies have further shown that higher levels of ROS are associated with shorter life expectancy (ROS levels increase two to three-fold with age) and aging (free-radical theory of aging) [9-11]. Given the suggested role of oxidative stress in atherosclerosis, diabetes, and cancer, examining the association of oxidative stress markers with mortality may provide a better understanding of the pathophysiology of the most prevalent age-related chronic diseases.

Epidemiological studies have shown increased levels of 8-isoprostane (8-ISO), a prostaglandin-like compound formed from the oxidation of arachidonic acid [12] and a reliable marker of oxidative stress [13] in patients with coronary artery calcification [14], ischemic stroke [15], diabetes [16], history of smoking [17], and obesity [18]. However, the association of oxidative stress markers such as 8-ISO with non-CVD and CVD

mortality has not been extensively evaluated in population-based studies.

The Beaver Dam Eye Study (BDES) provides a unique opportunity to examine the relationship of 8-ISO with mortality in a population-based study with a long follow-up (maximum of 15 years).

MATERIAL AND METHODS

Study population

The BDES is an ongoing population-based longitudinal study designed to evaluate the prevalence and long-term cumulative effects of age-related ocular disorders and systemic diseases in residents living in the city or township of Beaver Dam, Wisconsin. A total of 4,926 (83.1%) of the 5,924 eligible residents identified by a private census of the population of Beaver Dam (99% non-Hispanic whites) between the ages of 43-86 years participated in the baseline examination between March 1, 1988 and September 14, 1990. Differences in baseline characteristics between participants and non-participants have been previously described [19].

8-ISO was assessed in a random sample of 1,793 of the original 4,926 individuals who participated in the baseline examination. Two percent (n = 40) of the participants were excluded from the analysis due to missing values of serum 8-ISO. Therefore, this analysis included 1,753 participants with a mean age of 62 ± 11.2 years (56% women) at baseline.

All participants provided written informed consent, and the study protocol conformed to the tenets of the Declaration of Helsinki and was approved by the Human Subjects Committee of the University of Wisconsin School of Medicine and Public Health.

Measurements

Standardized questionnaires were used to obtain self-reported demographics (*i.e.*, age, gender, race/ethnicity, and years of education), medical history, medication use, and history of smoking (*i.e.*, never smoker defined as history of smoking < 100 cigarettes in participant's lifetime; former smoker as history of smoking ≥ 100 cigarettes in participant's lifetime and current smoker if participant smoked at the time of the examination). Weight and height were assessed to the nearest quarter pound and quarter inch with participants wearing light clothing and no shoes using the Health-o-Meter scale (Continental Scale Corporation, Bridgeview, IL, USA). Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Two seated right arm blood pressure measurements were obtained after a 5 min rest by trained technicians using a random-zero sphygmomanometer as recommended by the Hypertension Detection and Follow-up Program Protocol [20]. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were determined using the average of the first and second readings. Hypertension was defined as a mean SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg [20], or the use of antihypertensive medication. Biomarkers were measured on the Roche Modular P Chemistry Analyzer (Roche Diagnostics Corporation, Indianapolis, IN, USA) as follows: serum creatinine was measured using the Roche enzymatic CERA plus method and glucose was assayed with the Roche hexokinase method; total cholesterol was measured using Roche cholesterol oxidase method; high density lipoprotein cholesterol (HDL-C) was measured by the cholesterol oxidase method after precipitation of non-HDL cholesterol with magnesium/dextran; a Roche bromcresol purple method was used to measure albumin, and a Roche latex particle-enhanced immunoturbidimetric assay kit was used to measure high sensitivity C reactive protein (hsCRP). Total glycosylated hemoglobin (tHgA) was determined using affinity chromatography (Isolab, Inc., Akron, OH, USA). Diabetes was defined as tHgA ≥ 8% (which is approximately equal to a glycosylated hemoglobin A_{1c} value of ≥ 6.4%) or the use of medication for diabetes. Serum 8-ISO was measured using the competitive immunoassay technique of the Correlate Enzyme Immunoassay (Assay Designs, Inc., Ann Arbor, MI, USA) from serum stored at –80°C for up to 17 years until the samples were shipped on dry ice to the University of Minnesota Laboratory. The laboratory coefficient of variability was 20.8%.

Deaths were ascertained by matching participants' names with the National Death Index. Cause of death was ascertained by using the underlying cause of death listed in the death certificate between March 1, 1988 (baseline examination) and December 31, 2002 according to the International Classification of Diseases, 9th Revision (ICD-9, for deaths before December 31, 1998) or 10th Revision (ICD-10, for death thereafter). CVD mortality was defined according to the ICD-9 codes 391.0-398.9, 402.0-402.9, 404.0-429.9 and ICD-10 codes I01.0-I99.0. Non-CVD mortality was defined as deaths other than CVD deaths.

Statistical analysis

Differences in baseline characteristics among those who were alive during the study period (alive group) compared to those who died from any cause (all-cause mortality group), non-CVD (non-CVD mortality group) and CVD (CVD mortality group) were assessed using the t-test and Wilcoxon rank-sum test for continuous variables and chi-square test for categorical variables using the alive group as the reference group. Mortality rates were calculated per 1,000 person-years for all causes of death by 8-ISO quartiles, and P values for trend were determined by fitting a Poisson regression model. Since 8-ISO was negatively skewed, a

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logarithmic transformation was used to normalize these variables. A Cox proportional hazards model was used to estimate the hazard ratios (HR) and 95% confidence intervals (CI) of the association of baseline serum 8-ISO with all-cause, non-CVD, and CVD mortality. All models were adjusted by age, gender, education, BMI, current and former smoking, SBP, serum total cholesterol/HDL-C ratio, hsCRP, diabetes, and history of CVD. Interaction terms were included in models to further explore for any effect modification of age (younger/older than the median age of the cohort), gender, BMI < 25 kg/m² or ≥ 25 kg/m², smoking status, hsCRP ≤ 3 or > 3 mg/l, medication use for hypertension and high cholesterol, presence of diabetes, hypertension, and history of CVD on the association of 8-ISO with mortality. HRs were expressed either by 8-ISO quartiles using the 1st quartile as the reference group or one standard deviation of baseline 8-ISO. The proportional hazards assumption was assessed by using log minus log survival plots and the correlation between the Schoenfeld residuals for each covariate and the ranking of the individual death times [21].

Analyses were performed using SAS version 9.1 for Windows (SAS Institute, Cary, NC, USA); a two-sided P value < 0.05 was used to assess statistical significance.

RESULTS

During a median follow-up of 13.1 years (range 0.1-14.8), 590 (33.7%) participants died (51.8% women). Of these deaths, 290 (49.2%) were due to CVD (51.7% ischemic heart disease, 14.4% stroke, 5.9% heart failure, and 28% other causes) and 300 (50.8%) were non-CVD related (50.7% cancer, 16% diseases of the respiratory system, 8.7% diseases of the nervous system, and 24.6% other causes). Baseline characteristics are shown in Table 1. Compared to those who remained alive, participants who died from all-cause, non-CVD, and CVD were significantly older, with more education, higher levels of serum glucose, tHgA, SBP, hsCRP, proportion of individuals with diabetes, CVD, and hypertension (Table 1). Similar median levels of baseline 8-ISO were observed among those who died of any cause compared to those who remained alive. Median levels of 8-ISO did not change after adjusting for age, gender, and the presence of CVD or hypertension.

Table 1. Baseline characteristics of Beaver Dam Eye Study participants

Characteristic	ALIVE (n = 1163)	All-Cause Mortality (n = 590)	Non-CVD Mortality (n = 300)	CVD Mortality (n = 290)
Age, years[a]	58 (9.6)	70 (10)[b]	68 (10)[b]	69 (10)[b]
Women, % (n)	58 (674)	58.9 (306)	51 (153)	52.7 (153)
Men, % (n)	42 (488)	48.1 (284)	49 (147)	47.2 (137)
Education, High School or Less, % (n)	68 (790)	81.5 (481)[b]	80.7 (242)[b]	82.4 (239)[b]
Never Smoker, % (n)	47.2 (548)	41.7 (246)	38.3 (115)	45.3 (131)
Former Smoker, % (n)	33.2 (385)	37.5 (221)	37.7 (113)	37.4 (108)
Current Smoker, % (n)	19.6 (227)	20.7 (122)	24 (22)	17.3 (50)
Body Mass Index, kg/m² [a]	28.8 (5.4)	28.7 (6.1)	28.7 (6.2)	29 (6.1)
Systolic Blood Pressure, mmHg[a]	129.3 (18.5)	136.3 (21.6)[b]	133.4 (20.8)	139.3 (22)[b]
Diastolic Blood Pressure, mmHg[a]	78.7 (10.5)	74.2 (11.9)[b]	74.3 (11.2)[b]	77.8 (10.8)[b]
Creatinine, mg/dl[c]	0.9 (0.2)	0.9 (0.3)[b]	0.9 (0.3)[b]	0.9 (0.4)[b]
Random Glucose, mg/dl[c]	97 (14)	100 (20)[b]	99 (17.5)[b]	100.5 (22)[b]
Total Glycosylated Hemoglobin, %[c]	5.7 (0.8)	6 (1.2)[b]	6 (1.2)[b]	6.1 (1.3)[b]
hsCRP, mg/l[c]	1.8 (2.7)	3 (5.7)[b]	2.7 (5.4)[b]	3.7 (6)[b]
8-Isoprostane, pg/ml[c]	121.9 (66.2)	126.8 (70.8)	129 (70.9)	124.4 (68.6)
Total Cholesterol, mg/100 ml[a]	234 (42.5)	232.5 (48.78)	225.5 (44.8)[b]	239.7 (47.7)[b]
HDL-C, mg/100 ml[a]	53.2 (18.1)	49.8 (18.1)[b]	50.9 (18.2)	48.7 (18.1)[b]
Total Cholesterol/HDL-C Ratio[c]	4.6 (2.5)	4.8 (2.5)[b]	4.6 (2.4)	5.1 (2.5)[b]
Prevalent Diabetes, % (n)	4.4 (51)	13.6 (80)[b]	12 (36)[b]	15.2 (44)[b]
History of CVD, % (n)	7.9 (91)	29 (167)[b]	19.7 (58)[b]	38.6 (109)[b]
Prevalent Hypertension, % (n)	48 (552)	71.3 (410)[b]	65.2 (191)[b]	77.7 (219)[b]
History of Myocardial Infarction, % (n)	2.9 (34)	14.2 (83)[b]	8.7 (28)	19.9 (57)[b]
History of Stroke, % (n)	1.5 (17)	8.3 (49)[b]	6.3 (19)[b]	10.4 (30)[b]
History of Cancer, % (n)	8.5 (99)	16.3 (96)[b]	18 (54)[b]	14.5 (42)
Medications for Hypertension, % (n)	29.5 (339)	52.1 (300)[b]	44.7 (131)[b]	59.7 (169)[b]
Medications for Diabetes, % (n)	2.7 (31)	10.3 (61)[b]	9.3 (28)[b]	11.4 (33)[b]
Medications for High Cholesterol, % (n)	4.6 (54)	5 (29)	4.7 (14)	5.3 (15)

[a]Value reported as mean (standard deviation)
[b]P < 0.05 (all-cause mortality *vs* alive, non-CVD mortality *vs* alive, and CVD mortality *vs* alive)
[c]Value reported as median (interquartile range)
Abbreviations: CVD, cardiovascular disease; HDL-C, high density lipoprotein cholesterol; hsCRP, high sensitivity C-reactive protein

Table 2. Relationship of 8-Isoprostane with all-cause, non-cardiovascular, and cardiovascular mortality

	All Cause Mortality	Non-CVD Mortality	CVD Mortality
No. of Deaths	590	300	290
Person-Years	20,121	20,121	20,121
Mortality Rate (1,000 person-years)	29.3	14.9	14.4
Model 1[a] Hazard Ratio (95% CI)	1.11 (1.02, 1.2)	1.16 (1.04, 1.3)	1.05 (0.93, 1.18)
Model 2[b] Hazard Ratio (95% CI)	1.1 (1.01, 1.2)	1.14 (1.01, 1.28)	1.06 (0.93, 1.2)

[a]Adjusted for age, gender, and education
[b]Adjusted for age, gender, education, BMI, current and former smoking, systolic blood pressure, total cholesterol/high density lipoprotein cholesterol ratio, diabetes, high sensitivity C reactive protein, history of CVD, and medication use for hypertension
Abbreviations: CI, confidence interval; CVD, cardiovascular disease

Table 3. Relationship of 8-isoprostane quartiles with all-cause, non-cardiovascular, and cardiovascular mortality

	8-Isoprostane (pg/ml)				P value for trend
	< 97.0	97.0 –123.9	124.0–163.9	≥ 164.0	
All-Cause Mortality					
No. of Deaths	147	133	154	156	
Person-Years	5183	5158	4897	4884	
Mortality Rate (1,000 person-years)	28.4	25.8	31.4	31.9	0.13
Model 1[a] Hazard Ratio (95% CI)	1	0.96 (0.75, 1.22)	1.17 (0.93, 1.47)	1.28 (1.01, 1.62)	0.01
Model 2[b] Hazard Ratio (95% CI)	1	0.95 (0.75, 1.22)	1.19 (0.94, 1.50)	1.24 (0.97, 1.57)	0.03
Non-CVD Mortality					
No. of Deaths	68	68	78	86	
Person-Years	5183	5158	4897	4884	
Mortality Rate (1,000 person-years)	131	13.2	15.9	17.6	0.04
Model 1[a] Hazard Ratio (95% CI)	1	1.03 (0.73, 1.44)	1.25 (0.9, 1.74)	1.53 (1.11, 2.11)	0.01
Model 2[b] Hazard Ratio (95% CI)	1	1.07 (0.78, 1.52)	1.4 (1, 1.96)	1.44 (1.03, 2.02)	0.01
CVD Mortality					
No. of Deaths	79	65	76	70	
Person-Years	5183	5158	4897	4884	
Mortality Rate (1,000 person-years)	15.2	12.6	15.5	14.3	0.99
Model 1[a] Hazard Ratio (95% CI)	1	0.89 (0.64, 1.25)	1.09 (0.79, 1.5)	1.04 (0.74, 1.47)	0.57
Model 2[b] Hazard Ratio (95% CI)	1	0.86 (0.61, 1.21)	1.01 (0.73, 1.41)	1.06 (0.75, 1.5)	0.56

[a]Adjusted for age, gender, and education
[b]Adjusted for age, gender, education, BMI, current and former smoking, systolic blood pressure, total cholesterol/high density lipoprotein cholesterol ratio, diabetes, high sensitivity C reactive protein, history of CVD, and medication use for hypertension.
Abbreviations: CI, confidence interval; CVD, cardiovascular disease

After adjusting for age, gender, education, BMI, smoking status, SBP, serum total cholesterol/HDL-C ratio, hsCRP, medication use for hypertension, diabetes, and history of CVD, 8-ISO was significantly associated with all-cause and non-CVD mortality but not with CVD mortality (Table 2). Individuals in the highest baseline 8-ISO quartile had a 1.24 and 1.44-fold increase in all-cause and non-CVD mortality, respectively, compared to those in the lowest quartile (Table 3). The pattern of association between 8-ISO and mortality remained the same after excluding those participants who had died by the first (n = 25), second (n = 57), or fifth (n = 188) year, or those with a BMI < 18.5 kg/m² (n = 35) (data not shown). The association of 8-ISO with mortality did not vary by age, gender, smoking status, prevalent diabetes, history of CVD, levels of hsCRP, or medication use for hypertension or high cholesterol (P > 0.05 for all interactions). However, individuals with BMI < 25 kg/m² had approximately 34 to 36% increased risk of all-cause, non-CVD, and CVD death per one standard deviation (0.45 pg/ml) of the log-transformed baseline 8-ISO (Fig.1) while 8-ISO was not significantly associated with any cause of mortality among those with BMI ≥ 25 kg/m². The prevalence of those with BMI ≥ 25 kg/m² in the BDES cohort was 76.2% (n = 1,736; by cause of death: all-cause: 75.3%; non-CVD: 72.6%; CVD: 78.1%). 8-ISO was not significantly correlated with BMI (r = 0.02; P = 0.345). Studies have shown that smokers have significantly higher levels of 8-ISO [18] and lower BMI [22]. A significant 8-ISO by smoking interaction (P = 0.033) was found among those with BMI < 25 kg/m². The independent association of 8-ISO with mortality was further explored by smoking status and BMI category. The association of 8-ISO with all-cause and non-CVD

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mortality remained significant among nonsmokers with BMI < 25 kg/m², but not with CVD mortality (Fig.2). In contrast, 8-ISO was not significantly associated with any cause of mortality among smokers and nonsmokers with BMI ≥ 25 kg/m² (Fig.2).

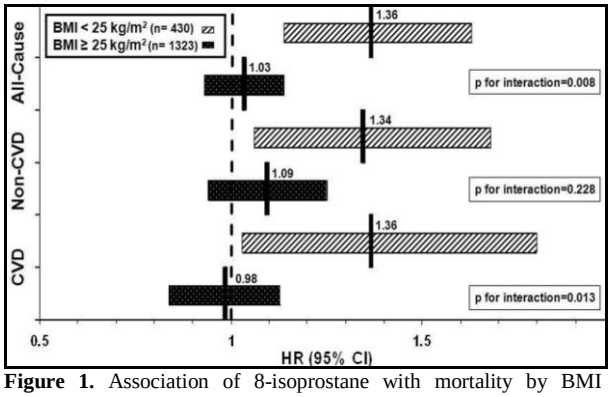

Figure 1. Association of 8-isoprostane with mortality by BMI category. Abbreviations: BMI, body mass index; CI, 95% confidence interval; CVD, cardiovascular disease; HR, hazard ratio. Adjusted for age, gender, education, current and former smoking, SBP, total cholesterol/HDL-C ratio, diabetes, hsCRP, history of CVD, and medication use for hypertension.

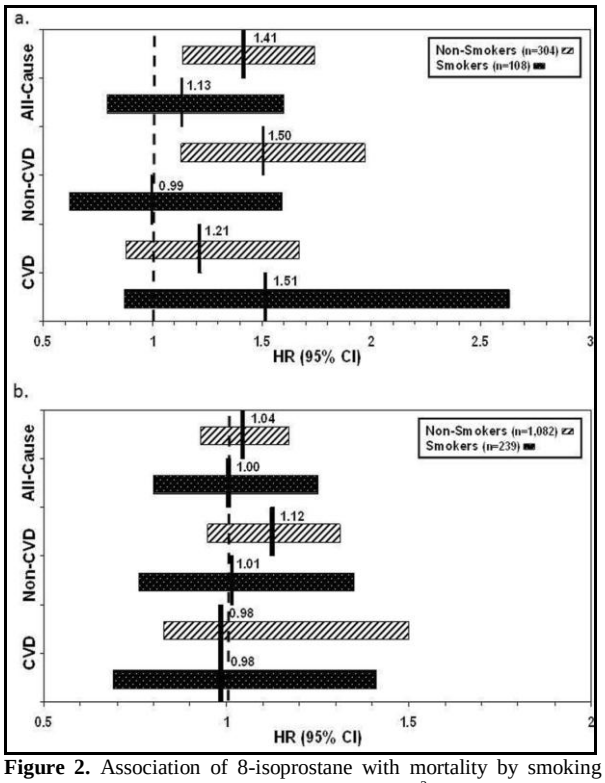

Figure 2. Association of 8-isoprostane with mortality by smoking status in individuals with BMI **(a)** < 25 kg/m² (n = 413); and **(b)** ≥ 25 kg/m² (n = 1,323). Abbreviations: BMI, body mass index; CI, 95% confidence interval; CVD, cardiovascular disease; HR, hazard ratio. Adjusted for age, gender, education, SBP, total cholesterol/HDL-C ratio, diabetes, hsCRP, history of CVD, and medication use for hypertension.

DISCUSSION

After a median follow-up of 13.1 years, serum 8-ISO was significantly associated with all-cause and non-CVD mortality but not with CVD mortality after adjusting for age, gender, education, CVD risk factors, prevalent CVD, and a marker of inflammation among a cohort of middle-aged adults. Individuals in the highest 8-ISO quartile had approximately 24-44% increased risk of all-cause and non-CVD death compared to those in the lowest quartile. However, after stratifying by BMI category, 8-ISO was significantly associated with all-cause, non-CVD, and CVD mortality among individuals with BMI < 25 kg/m², but not among those with BMI ≥ 25 kg/m². Further stratification by smoking status showed a significant association of 8-ISO with all-cause and non-CVD mortality but not with CVD mortality among nonsmokers with BMI < 25 kg/m². 8-ISO was not significantly associated with mortality among individuals with BMI ≥ 25 kg/m² irrespective of smoking status.

Studies examining the association of oxidative markers with mortality are limited. To our knowledge, the association of 8-ISO with mortality has not been examined in a population-based study. In general, the association of other markers of oxidative stress and mortality are consistent with our findings. During a follow-up of 5 years, malondialdehyde, another biomarker of lipid peroxidation, was significantly associated with all-cause mortality in a cohort of 154 ambulatory nursing home residents (mean age 73.2 years) in Spain [23]. In contrast, malondialdehyde was not significantly associated with CVD and non-CVD mortality in a random sample of 344 adults aged 65 years and older living in Finland during a follow-up of 13 years [24]. On the other hand, serum protein carbonyl, a biomarker of protein oxidation, was significantly associated with all-cause mortality in a cohort of 746 moderately to severely disabled women aged 65 years and older participating in the Women's Health and Aging Study during a follow-up period of 5 years (25).

Oxidative stress has been suggested to play an important role in the development of chronic diseases [1, 2] such as diabetes [3, 4], CVD [5], and cancer [6-8]. The free-radical and the mitochondrial theories of aging proposed by Harman in the 1950s and 1970s [26, 27], respectively, suggested that aging ("the phenomenon of growth, decline, and death") and "degenerative diseases" are products of the damaging effects of excessive accumulation of ROS in the mitochondria. Animal studies have further shown that lower levels of ROS are significantly associated with longer life expectancy in transgenic mice, worms, and flies [9, 11, 28, 29]. Oxidized low-density lipoprotein, protein modifications, and DNA mutations are a few of

the suggested effects of oxidative stress in the pathogenesis of CVD, diabetes, and cancer [5, 8]. High concentrations of 8-ISO, a reliable marker of oxidative stress [13] and lipid peroxidation, have been reported in patients with evidence of CVD, *e.g.,* coronary artery calcification [14] and ischemic stroke [15], and diabetes [16] and related risk factors, *e.g.*, smoking [17] and obesity [18]. The BDES data provide further information on the strong association of 8-ISO with mortality, especially among those with BMI < 25 kg/m². It is unclear why 8-ISO is not associated with mortality among individuals with BMI ≥ 25 kg/m². It is widely known that obesity is significantly associated with CVD, diabetes, and markers of inflammation. Interestingly, what the BDES findings may be suggesting is that 8-ISO does not add any additional information to the risk or prediction of mortality among overweight or obese participants after the effects of hypertension, diabetes, smoking, lipids, and markers of inflammation are taken into consideration. It is possible that 8-ISO may only be involved in the development or early stages of chronic diseases. Once the individual has any of the risk factors, 8-ISO does not contribute to the prediction of mortality. Further stratification by smoking status showed that 8-ISO remained significantly associated with all-cause and non-CVD mortality but not with CVD mortality among nonsmokers with BMI < 25 kg/m². However, the number of nonsmokers and smokers dying from CVD is too small to reach any significant conclusion.

Several limitations should be acknowledged. Cause of death was ascertained by using the underlying cause of death listed on the death certificate, which may be subject to misclassification. Serum 8-ISO may be subjected to auto-oxidation. However, all the samples from the BDES cohort were treated the same way, and to our knowledge there is no differential auto-oxidation in individuals who died from CVD compared to those who remained alive or died from other causes. Due to the small number of cause-specific deaths in our study, we were unable to examine the independent relationship of 8-ISO with cause-specific mortality. Several potential confounders were included in the analysis; however, the possibility of residual confounding cannot be entirely excluded. Ninety nine percent of the BDES cohort is non-Hispanic white; therefore, our findings may not be generalizable to other racial/ethnic groups. On the other hand, there are several strengths to our study. This is a large population-based study with high participation rate, long follow-up, and standardized protocol.

In conclusion, serum 8-ISO, a marker of oxidative stress, is an independent risk factor of all-cause, non-CVD, and CVD mortality among a cohort of middle-aged adults with normal BMI. Further population-based studies are needed to examine the longitudinal association of 8-ISO and BMI with mortality in a diverse population.

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Role of the Sponsors

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COMPETING INTERESTS

The authors declare that they have no conflict of interest.

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REFERENCES

1. Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of oxidative damage in human disease. Clin Chem 2006; 52:601-23.
2. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature 2000; 408:239-47.
3. Njajou OT, Kanaya AM, Holvoet P, Connelly S, Strotmeyer ES, Harris TB, Cummings SR, Hsueh WC. Association between oxidized LDL, obesity and type 2 diabetes in a population-based cohort, the Health, Aging and Body Composition Study. Diabetes Metab Res Rev 2009; 25:733-9.
4. Meigs JB, Larson MG, Fox CS, Keaney JF Jr, Vasan RS, Benjamin EJ. Association of oxidative stress, insulin resistance, and diabetes risk phenotypes: the Framingham Offspring Study. Diabetes Care 2007; 30:2529-35.
5. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. N Engl J Med 1989; 320:915-24.
6. Hussain SP, Hofseth LJ, Harris CC. Radical causes of cancer. Nat Rev Cancer 2003; 3:276-85.
7. Loft S, Poulsen HE. Cancer risk and oxidative DNA damage in man. J Mol Med 1996; 74:297-312.
8. Hollstein M, Sidransky D, Vogelstein B, Harris CC. p53 mutations in human cancers. Science 1991; 253:49-53.
9. Sohal RS, Weindruch R. Oxidative stress, caloric restriction, and aging. Science 1996; 273:59-63.
10. Sohal RS, Mockett RJ, Orr WC. Mechanisms of aging: an appraisal of the oxidative stress hypothesis. Free Radic Biol Med 2002; 33:575-86.
11. Balaban RS, Nemoto S, Finkel T. Mitochondria, oxidants, and aging. Cell 2005; 120:483-95.
12. Morrow JD, Hill KE, Burk RF, Nammour TM, Badr KF, Roberts LJ. A series of prostaglandin F2-like compounds are produced in vivo in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. Proc Natl Acad Sci USA 1990; 87:9383-7.
13. Kadiiska MB, Gladen BC, Baird DD, Germolec D, Graham LB, Parker CE, Nyska A, Wachsman JT, Ames BN, Basu S, Brot N, Fitzgerald GA, Floyd RA, George M, Heinecke JW, Hatch GE, Hensley K, Lawson JA, Marnett LJ, Morrow JD, Murray DM, Plastaras J, Roberts LJ, Rokach J, Shigenaga MK, Sohal RS, Sun J, Tice RR, Van Thiel DH, Wellner D, Walter PB, Tomer KB, Mason RP, Barrett JC. Biomarkers of oxidative stress study II: are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning? Free Radic Biol Med 2005; 38:698-710.
14. Gross M, Steffes M, Jacobs DR Jr, Yu X, Lewis L, Lewis CE, Loria CM. Plasma F2-isoprostanes and coronary artery calcification: the CARDIA Study. Clin Chem 2005; 51:125-31.
15. Kelly PJ, Morrow JD, Ning M, Koroshetz W, Lo EH, Terry E, Milne GL, Hubbard J, Lee H, Stevenson E, Lederer M, Furie KL. Oxidative stress and matrix metalloproteinase-9 in acute ischemic stroke: the Biomarker Evaluation for Antioxidant Therapies in Stroke (BEAT-Stroke) study. Stroke 2008; 39:100-4.
16. Davi G, Ciabattoni G, Consoli A, Mezzetti A, Falco A, Santarone S, Pennese E, Vitacolonna E, Bucciarelli T, Costantini F, Capani F, Patrono C. In vivo formation of 8-iso-prostaglandin f2alpha and platelet activation in diabetes mellitus: effects of improved metabolic control and vitamin E supplementation. Circulation 1999; 99:224-9.
17. Morrow JD, Frei B, Longmire AW, Gaziano JM, Lynch SM, Shyr Y, Strauss WE, Oates JA, Roberts LJ. Increase in circulating products of lipid peroxidation (F2-isoprostanes) in smokers. Smoking as a cause of oxidative damage. N Engl J Med 1995; 332:1198-203.
18. Keaney JF, Jr., Larson MG, Vasan RS, Wilson PW, Lipinska I, Corey D, Massaro JM, Sutherland P, Vita JA, Benjamin EJ. Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham Study. Arterioscler Thromb Vasc Biol 2003; 23:434-9.
19. Klein R, Klein BE, Linton KL, De Mets DL. The Beaver Dam Eye Study: visual acuity. Ophthalmology 1991; 98:1310-5.
20. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, Jones DW, Materson BJ, Oparil S, Wright JT Jr, Roccella EJ. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Hypertension 2003; 42:1206-52.
21. Hosmer DW, Lemeshow S. Applied Survival Analysis. 1st edition, John Wiley & Sons, New York, NY, USA, 1999.
22. Molarius A, Seidell JC, Kuulasmaa K, Dobson AJ, Sans S. Smoking and relative body weight: an international perspective from the WHO MONICA Project. J Epidemiol Community Health 1997; 51:252-60.
23. Huerta JM, Gonzalez S, Fernandez S, Patterson AM, Lasheras C. Lipid peroxidation, antioxidant status and survival in institutionalised elderly: a five-year longitudinal study. Free Radic Res 2006; 40:571-8.
24. Marniemi J, Jarvisalo J, Toikka T, Raiha I, Ahotupa M, Sourander L. Blood vitamins, mineral elements and inflammation markers as risk factors of vascular and non-vascular disease mortality in an elderly population. Int J Epidemiol 1998; 27:799-807.
25. Semba RD, Ferrucci L, Sun K, Walston J, Varadhan R, Guralnik JM, Fried LP. Oxidative stress is associated with greater mortality in older women living in the community. J Am Geriatr Soc 2007; 55:1421-5.
26. Harman D. Aging: a theory based on free radical and radiation chemistry. J Gerontol 1956; 11:298-300.
27. Harman D. The biologic clock: the mitochondria? J Am Geriatr Soc 1972; 20:145-7.
28. Schriner SE, Linford NJ, Martin GM, Treuting P, Ogburn CE, Emond M, Coskun PE, Ladiges W, Wolf N, Van Remmen H, Wallace DC, Rabinovitch PS. Extension of murine life span by overexpression of catalase targeted to mitochondria. Science 2005; 308:1909-11.
29. Migliaccio E, Giorgio M, Mele S, Pelicci G, Reboldi P, Pandolfi PP, Lanfrancone L, Pelicci PG. The p66shc adaptor protein controls oxidative stress response and life span in mammals. Nature 1999; 402:309-13.