Evaluation of urinary hydrogen peroxide as an oxidative stress biomarker in a healthy Japanese population.

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Key words

H_2O_2; 8-OHdG; lifestyle; total cholesterol; exercise

Abstract

The usefulness of urinary hydrogen peroxide (H_2O_2) as an oxidative stress biomarker was evaluated in 766 healthy Japanese people. The mean level of urinary concentrations of H_2O_2 was 5.66 ± 8.27 μmol/g creatinine, and was significantly higher in females than that in males. Significant correlations of H_2O_2 were observed with age, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), insulin, 8-hydroxy-2'-deoxyguanosine (8-OHdG), and exercise habit in females. In both sexes, H_2O_2 showed a significant correlation with 8-OHdG. By a multiple logistic regression analysis, urinary H_2O_2 was positively associated with urinary 8-OHdG and TC and was inversely associated with insulin. By stratification of sex and age, the association of urinary H_2O_2 with TC was positive in both sexes under 50 years old and was inverse in males over 50 years old, and that with insulin was inverse in males over 50 years old and in females under 50 years old. Moreover, by stratification of sex and age, a positive association of H_2O_2 with exercise and an inverse association of H_2O_2 with alcohol consumption became clear in males under 50 years old, although there were no significant odds for H_2O_2 after adjustment for covariates. In conclusion, the present results suggest that urinary H_2O_2 is a useful biomarker for oxidative stress, showing an association with 8-OHdG, TC, and insulin independently.

Introduction

Cells need oxygen for energy supply. However, they continuously generate reactive oxygen species (ROS) such as superoxide anion radicals (O_2-) and hydroxyl radicals (OH•) in the energy conversion process [1, 2]. Under physiological conditions, ROS are generally reduced by enzyme systems such as superoxide dismutase, catalase, and glutathione peroxidase or by non-enzymatic low-molecular-weight antioxidants such as ascorbate, β-carotene, α-tocopherol, urate, and bilirubin [3]. Oxidative stress is defined as a status of predominant increases in ROS generation beyond the antioxidative defense capacity, resulting in oxidative damage to lipids, DNA, and proteins [1]. Oxidative stress is involved in the initiation and progression of many diseases and even in the normal aging process. It is evaluated by measuring oxidatively modified cellular constituents in biological samples because ROS, when generated, can easily react with adjacent molecules and their life span is very short.

Hydrogen peroxide (H_2O_2), a metabolite of O_2-, is usually generated in mitochondria through a specialized enzyme to control cellular growth and death and is metabolized to water and oxygen by catalase or glutathione peroxidase. However, in the presence of iron, H_2O_2 generates OH• by the Fenton reaction. In human studies, urinary H_2O_2 was evaluated as a biomarker of ROS [4, 5] showing high values in cancer patients [6] and in persons after coffee drinking [4, 7]. However, little is known about urinary H_2O_2 in association with lifestyle and biomedical parameters of clinical examinations.

Oxidative stress biomarkers were presumed to change in the ‘pre-clinical stages of disease’ among healthy people because of the influence of unhealthy behavior related to lifestyle, such as smoking and alcohol drinking. However, few studies engaged in the assessment of these oxidative stress biomarkers for a population who have no disease [8]. Moreover, there are few data to show the critical correlation between these oxidative biomarkers in a healthy population study supporting basic biochemical reactions such as a cascade from H_2O_2 to 8-OHdG via OH•.

The present study aimed to examine the usefulness of urinary H_2O_2 as a biomarker of ROS and to investigate if the biochemical cascade from O_2- to H_2O_2 in the laboratory occurs in the human body by statistical analysis of related variables among healthy Japanese people.

Methods

Subjects

Data were obtained from a worksite lifestyle intervention study in Japanese city offices in which 847 individuals participated. For the purpose of this study, we excluded subjects who had any history of asthma, atopic dermatitis, or diabetes. A total of 766 subjects were selected. All subjects were instructed to fast overnight and not consume any beverage or food, except for plain water, before blood and urine collection. The Ethics Committee of Okayama University approved the study (No. 168) and all subjects gave informed consent.

Measurement of health assessment parameters

Blood samples were collected after overnight fasting for at least 10 h. Serum and plasma were preserved at 4°C for the measurement of red blood cells (RBC), white blood cells (WBC), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol (TC), high density lipoprotein-cholesterol (HDL-c), low density lipoprotein-cholesterol (LDL-c), triglycerides (TG), hemoglobin A1c (HbA1c), insulin, glucose, and high-sensitivity C-reactive protein (Hs-CRP). Body composition was evaluated using the following respective parameters such as body weight and body mass index (BMI). BMI was calculated by body weight (kg) / height $(m)^2$. Information on lifestyles including cigarette smoking, alcohol consumption, and exercise was obtained by self-reported questionnaires.

Analysis of oxidative stress biomarkers

Urinary H_2O_2 and 8-OHdG were determined in spot urine samples stored at -80°C before analysis. Urinary H_2O_2 was measured by the ferrous ion oxidation xylenol orange version-1 (FOX-1) method [9], with minor modification. In brief, urine specimens were centrifuged at 1500 rpm for 5 min at room temperature to remove the cellular fractions. Twenty μl of the urine samples were incubated with 20 μl of catalase solution (2,200 U/ml in 25 mM phosphate buffer, pH 7.0) or 25 mM phosphate buffer, pH 7.0. Then, the samples were reacted with 160 μl of FOX-1 reagent (100 μM xylenol orange, 100 mM sorbitol, 250 μM ammonium ferrous sulfate, and 25 mM H_2PO_4, pH adjusted to 1.7-1.8 by addition by Na_2HPO_4) at room temperature for 30min. The absorbance was measured with a microplate reader at 560 nm. The concentration of H_2O_2 was calculated from the absorbance difference (with and without

catalase) using a standard curve. The intra-assay and inter-assay coefficients of variation (CV) were 4.3% and 9.7%, respectively. Møller and Loft indicated that the correlation coefficient of 8-OHdG measurements by enzyme-linked immunosorbent assay (ELISA) between spot and 24-h urine samples was 0.87 [10]. Measurement of 8-OHdG was carried out with an ELISA kit from the Japan Institute for the Control of Aging, Fukuroi, Shizuoka, Japan [11]. The incubation with primary antibody was performed at 4 ℃ overnight [12, 13]. The intra-assay and inter-assay CV were 5.2% and 8.1%, respectively. Values for H_2O_2 and 8-OHdG were normalized by per milligram of creatinine measured in urine (Creatinine test kit, R&D Systems, Minneapolis, MN).

Statistical analysis

Data are presented as the mean ± standard deviation (SD) unless stated elsewhere. The Mann-Whitney U test was used to compare the concentrations of oxidative stress biomarkers by sex. Spearman's correlation analysis and logistic regression analysis were performed to examine the relationship between oxidative stress biomarkers and variables. Linear trends in biomedical parameters were tested according to urinary H_2O_2 quartiles. A probability value of $p<0.05$ was considered to be significant. All analyses were performed using the Statistical Package of SPSS 19 for Windows.

Results

Characteristics of subjects

The clinical characteristics of subjects are presented in Table 1. Their average age was 42.4 years. Levels of BMI, Hs-CRP, blood pressure (systolic and diastolic), RBC, WBC, AST, ALT, TC, LDL-c, TG, and glucose in males were significantly higher than those in females. Urinary H_2O_2 and 8-OHdG in females were significantly higher than those in males. The lifestyle profiles of subjects are shown in Table 2. Smokers accounted for 25.8%. The ratio of people who drank alcohol 4 times or more per week was 26.8% and those who exercised 3 times or more per week was 15.5%.

Relationship between oxidative stress biomarkers and health assessment variables

Spearman's correlation analysis between urinary H_2O_2 and health assessment data are shown in Table 3. Urinary H_2O_2 in all subjects was significantly and positively correlated with age, WBC, AST, ALT, TC, LDL-c, urinary 8-OHdG, and exercise and was negatively correlated with insulin. In males, significant correlations were shown between urinary H_2O_2 and urinary 8-OHdG. In females, significant positive correlations for urinary H_2O_2 were observed in age, AST, ALT, TC, LDL-c, urinary 8-OHdG and exercise and significant negative correlations for urinary H_2O_2 were shown in insulin. The association between the urinary hydrogen peroxide and 8-OHdG levels is presented in Figure 1.

Sex–specific mean values for several clinical profiles and oxidative stress markers according to quartiles of urinary H_2O_2

Table 4 demonstrated that mean values from the lowest to the highest quartiles of H_2O_2 were 0.34, 2.42, 4.84, and 12.70 μmol/g creatinine for males and 0.01, 1.28, 4.53, and 18.76

μmol/g creatinine for females. Tests for linear trends showed TC, LDL-c, and 8-OHdG increased as urinary H_2O_2 increased for females, while insulin decreased as urinary H_2O_2 increased. These significant associations of several factors with urinary H_2O_2 did not show a completely linear trend. In the 2nd quartile range of H_2O_2 from 0.02 to 2.59 μmol/g creatinine, age, TC, and LDL-c showed a J-shaped curve and insulin showed an inverse J-shaped curve in females. Urinary 8-OHdG showed an increasing trend as urinary H_2O_2 increased for males.

Multiple logistic regression analysis for urinary H_2O_2

The associations of H_2O_2 with 8-OHdG were evaluated by a sex-stratified multiple logistic regression analysis in Table 5. After adjustment for demographic, physical, and clinical variables such as age, BMI, Hs-CRP, WBC, ALT, TC, insulin, and exercise, the prevalence of a high H_2O_2 increased in the highest quartile of urinary 8-OHdG in a dose-dependent manner (odds ratio (OR) =2.31 (95% confidence interval (CI), 1.47-3.62) (*p* for trend <0.001)). Moreover, by stratification of sex and age, in males, a higher OR of H_2O_2 for 8-OHdG was observed in ages over 50 (OR=12.33 (95% CI, 2.07-73.40) (*p* for trend 0.001)) than that in ages under 50 (OR=2.26 (95% CI, 1.01-5.03) (*p* for trend 0.019)). In females under 50, there was a clear association of H_2O_2 with 8-OHdG in urine (OR=2.44 (95% CI, 1.19-5.01) (*p* for trend 0.021)) relative to those over 50 (OR=1.47 (95% CI, 0.53-4.10) (*p* for trend 0.441)).

In Table 6, the OR of H_2O_2 showed an inverse association with the quartiles of insulin (OR=0.50 (95% CI, 0.30-0.83) (*p* for trend 0.002)) even after adjustment of sex, age, BMI, Hs-CRP, systolic blood pressure, RBC, WBC, ALT, TC, HbA1c, 8-OHdG, smoking habit,

alcohol consumption, and exercise. However, after the stratification of sex and age and adjustment of confounding factors, the most reduced OR of urinary H_2O_2 in males over 50 was shown in the 3rd quartile of insulin (OR=0.14 (95% CI, 0.02-0.91)) in model 2. In females, the most reduced OR of H_2O_2 was shown in the 4th quartile of insulin (OR=0.35 (95% CI, 0.16-0.80)) among those under 50 after the adjustment of confounding factors.

Concerning the association of urinary H_2O_2 with TC, the highest OR was shown in the 2nd quartile of TC (OR=1.84 (95% CI, 1.20-2.81)) after the adjustment of confounding factors (Table 7). By the stratification of sex and age and by the adjustment of confounding factors, high OR of urinary H_2O_2 was shown in the 4th quartile of TC in males under 50 (OR=2.57 (95% CI, 1.14-5.82)) and in females under 50 (OR=2.42 (95% CI, 1.22-4.78)). In contrast, in males over 50, reduced OR (OR=0.11 (95% CI, 0.02-0.62)) was shown in the 3rd quartile of TC and no significant changes in OR of urinary H_2O_2 for TC was observed in females over 50.

The association of H_2O_2 with exercise was shown in Table 8. The OR of H_2O_2 for engaging exercise 3 times or more per week versus no exercise was 1.56 (95% CI, 1.02-2.39) (*p* for trend 0.042) after the adjustment of demographic variables (sex and age). Moreover, after the adjustment of biomedical parameters, markers, and lifestyle factors in addition to sex and age, the OR of H_2O_2 for engaging exercise 3 times or more per week versus no exercise was 1.55 (95% CI, 0.99-2.442) (*p* for trend 0.053). Then, we further analysed the association of urinary H_2O_2 with exercise stratified with sex and age and found that high OR of H_2O_2 was not

observed in groups of exercise 3 times or more per week, but it was observed in groups of twice or less per week in males under 50 years old (2.22 (95% CI, 1.17-4.20)).

The association of H_2O_2 with alcohol consumption was shown in Table 9. The OR of H_2O_2 for alcohol consumption was not significant; however, by the stratification of sex and age and the adjustment of the biomedical parameters, markers, and lifestyle factors, low OR of H_2O_2 (0.47 (95% CI, 0.22-0.99)) was observed in groups of drinking 3 times or less per week in males under 50 years old.

Discussion and conclusions

In this study, we evaluated urinary H_2O_2 compared with urinary 8-OHdG as oxidative stress biomarkers by analyzing the association between this biomarker and clinical examinations or lifestyles in Japanese people.

Hydrogen peroxide is generated by the dismutation of O_2- and enzymatic reactions such as monoamine oxidase, xanthine oxidase, urate oxidase, and D-amino acid oxidase [14]. A small amount of H_2O_2 is derived from superoxide-dependent autooxidation of autooxidizable molecules in urine [4]. Although urinary H_2O_2 increased in colorectal cancer patients [6], little is known for urinary H_2O_2 in healthy populations. The most important evidence in this study is a positively significant association of H_2O_2 with 8-OHdG after the adjustment of confounding factors. This implies a verification of the source of 8-OHdG from OH• by the reaction of H_2O_2 with metals.

Urinary H_2O_2 showed significantly inverse correlations with fast insulin in both sexes. However, there was no correlation between urinary H_2O_2 and serum fasting glucose. High ORs of H_2O_2 for insulin in females under 50 years old by a multiple logistic regression analysis suggests that an increase in oxidative stress is associated with a decrease in fasting serum levels of insulin. That is to say, an increase in oxidative stress is associated with low secretion of insulin from the beta-cells of pancreatic islets. Beta-cells are threatened by oxidative stress induced by excess glucose metabolism because they have a low antioxidant defense capacity [15]. On the other hand, it has been reported that insulin reduces ROS generation by nuclear transcription factors such as nuclear factor erythroid 2-related factor 2 (Nrf2) and nuclear factor-kB (NF-kB)-mediated induction of anti-oxidative enzymes such as catalase, SOD, and glutathione peroxidase (GPx) [16]. In the present study, it is not clear whether ROS reduced insulin generation or insulin reduced ROS generation.

Hypercholesterolemia induced oxidative stress by upregulation of the NADPH oxidase complex [17] and by reduction of mitochondrial antioxidants [18]. In this study, high ORs of H_2O_2 were shown between the 3rd and 4th quartile of TC in participants under 50 years old. TC concentrations of the 3rd and 4th quartile were 202-227 mg/dl and 228-417 mg/dl, respectively. Normal levels of serum total cholesterol were 130-200 mg/dl for Japanese [19]. Therefore, TC levels of the 3rd and 4th quartile in the present results were hyperlipidemic. However, in males over 50 years old, ORs of H_2O_2 for quartiles of TC tended to be suppressive. This means that the lower quartile of TC concentrations corresponds to high

H_2O_2 or a high oxidative stress state when the upper quartile of TC (quartile 3) is set as a reference. Moreover, this data may support low levels of TC in men being associated with high mortality [20], although the association of low TC and high oxidative stress in senior males is not clear.

In this study, we observed that urinary H_2O_2 was significantly associated with the habit of exercising twice or less per week in men under 50 years old. However, no association was observed between exercise habits and urinary 8-OHdG. It has been reported that exercise and cycling increases urinary 8-OHdG [21-25]. As mentioned above, from the characteristics of urinary H_2O_2 having a large interindividual variation relative to urinary 8-OHdG, the association of H_2O_2 with exercise habit may be more reliable. Therefore, ORs of H_2O_2 for exercise showing the highest concentration of urinary H_2O_2 in moderate levels of exercise group may suggest that oxidative stress was higher in a group who exercised twice or less per week than that of 3 times or more per week because anti-oxidative enzymes such as Mn-SOD, catalase, and GPx may be induced in a group who exercised 3 times or more per week and may have reduced ROS formation, although we have no data for the level of anti-oxidative enzymes among participants. Exercise induces ROS in contracting muscle. However, the precise origin of ROS in muscle during exercise is not clear because of overestimation of mitochondrial O_2- generation in recent research. However, ROS-dependent transcriptional coactivators PGC1α and PGC1 ß, and the transcriptional factor PPARγ, may be involved in the induction of anti-oxidative enzymes such as SOD2, GPx, and catalase [26-28].

After stratification of sex and age, the association of H_2O_2 with alcohol consumption became prominent in the group of alcohol consumption 3 times or less per week showing significant reduced OR (0.47 (95% CI 0.22-0.99) in males under 50 years old. This implies decreased generation of H_2O_2 due to reduced O_2- generation by the NADPH oxidase complex, reduced O_2- dismutation by superoxide dismutase (SOD), or increased consumption of H_2O_2 by catalase and glutathione peroxidase (GPx). Yeligar et al. found that alcohol induced oxidative stress by upregulation of NADPH oxidase [29]. Moreover, moderate consumption of alcohol reduced the activity of SOD and GPx [30, 31]. Therefore, it is likely that the reduced activity of SOD may be associated with this reduced OR, although we have no data for the activity of antioxidative enzymes.

Coffee intake augments urinary H_2O_2 probably due to the contaminating 1,2,4-benzenetriol [32, 33]. Therefore, in this study, as described in the Methods section, all subjects were instructed to fast overnight and not consume any beverage, food, or coffee, except for plain water, before blood and urine collection. However, the contribution of other unknown factors to the determination of urinary H_2O_2 and to inter-individual variations of urinary H_2O_2 cannot be ruled out.

Although the present results, which showed the relationship between urinary H_2O_2 and oxidative stress biomarkers, health examination data, and lifestyle habits in healthy people, are important, several limitations of this study should be noted. First, the number of cases was small. Second, causal relationships could not be determined because of the cross-sectional

study. Third, some reporting bias may have been introduced because of self-reported questionnaires. Fourth, the use of commercial ELISA kits for urinary 8-OHdG measurements has been questioned by several scientists in the literature because of its overestimation of 8-OHdG, particularly at 37℃ [13, 34, 35] although we performed an overnight incubation with the primary antibody at 4 ℃ in order to improve the 8-OHdG analysis [12, 13]. Furthermore, urinary creatinine concentration is commonly used for adjustment of analytes in urine. Individual variation in urinary creatinine excretion has been blamed for the substantial interindividual difference of urinary 8-OHdG concentration [36]. Mesaros et al. [36] and Greenblatt et al. [37] reported that factors like age, gender, and body weight can affect urinary creatinine excretion. Although urinary 8-OHdG appeared to be relatively fluctuating among the subjects in the present study, to minimize the influence of urinary creatinine on 8-OHdG level, we analyzed the relation of urinary 8-OHdG with H_2O_2 by adjustment of age, gender, BMI, smoking, alcohol consumption, and exercise.

In conclusion, this study examined the usefulness of urinary H_2O_2 as a biomarker of ROS and investigated if the biochemical cascade from O_2- to H_2O_2 in the laboratory occurs in the human body by statistical analysis of related variables among healthy Japanese people. Moreover, this study showed that H_2O_2 was associated with insulin secretion, total cholesterol, and exercise habit. In the future, further studies with an increased sample size and longitudinal examination of causal relationships are necessary to confirm such associations.

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Declaration of interest

The authors have no conflicts of interest related to this manuscript.

Abbreviations

H_2O_2	hydrogen peroxide
AST	aspartate aminotransferase
ALT	alanine aminotransferase
TC	total cholesterol
LDL-c	low-density lipoprotein cholesterol
8-OHdG	8-hydroxy-2’-deoxyguanosine
ROS	reactive oxygen species
O_2-	superoxide anion radicals
OH•	hydroxyl radicals
RBC	red blood cells
WBC	white blood cells

HDL-c high density lipoprotein-cholesterol

TG triglycerides

HbA1c hemoglobin A1c

Hs-CRP high-sensitivity C-reactive protein

BMI body mass index

FOX-1 ferrous ion oxidation xylenol orange version-1

CV coefficients of variation

ELISA enzyme-linked immunosorbent assay

SD standard deviation

OR odds ratio

CI confidence interval

Nrf2 nuclear factor erythroid 2-related factor 2

NF-kB nuclear factor-kB

MnSOD Manganese Superoxide Dismutase

GPx glutathione peroxidase

PGC1α peroxisome proliferator-activated receptor gamma, coactivator 1 alpha

PGC1ß peroxisome-proliferator- activated receptor-g co-activator 1b

PPARγ peroxisome proliferator-activated receptor gamma

NADPH nicotinamide adenine dinucleotide phosphate

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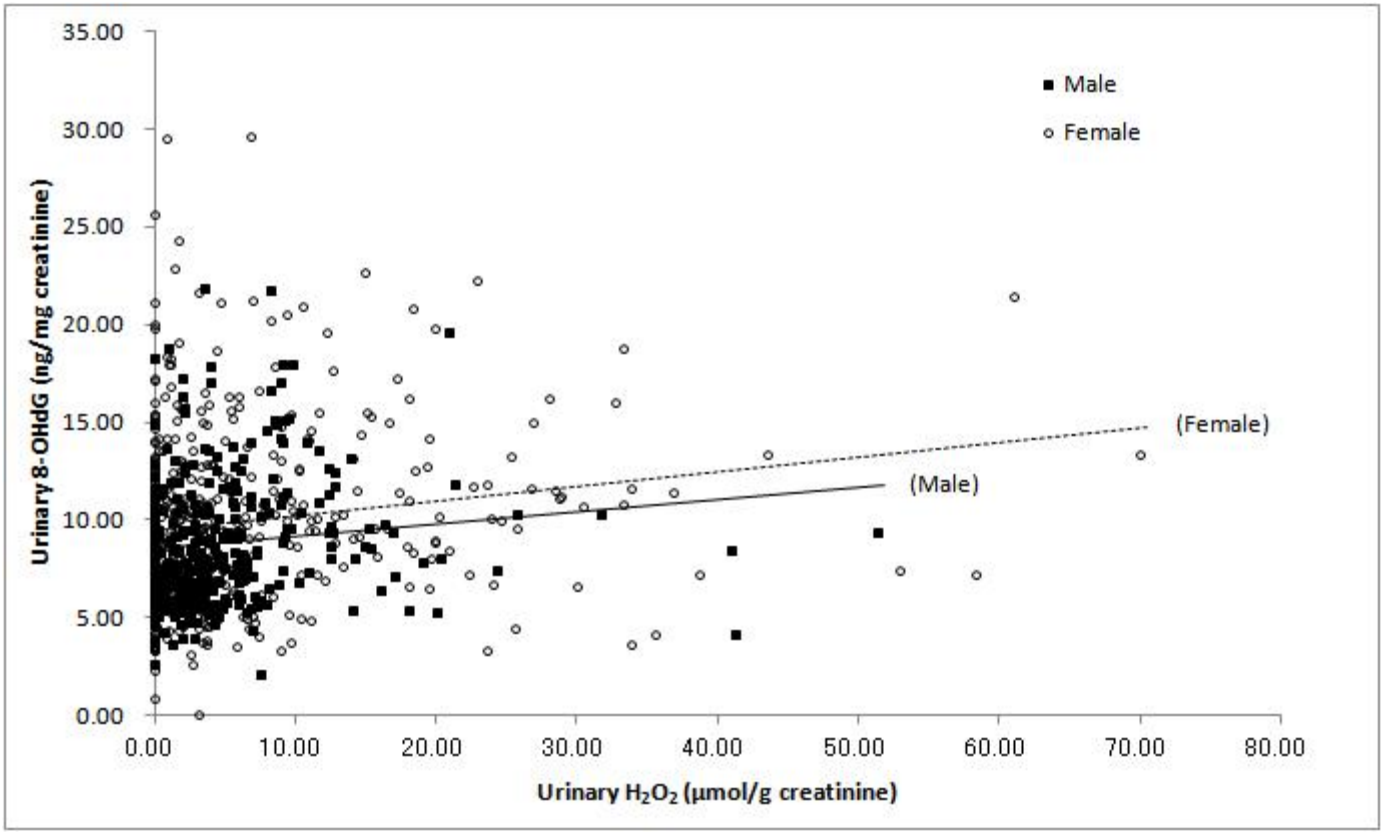
35.00
30.00
25.00
20.00
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10.00
5.00
0.00
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40.00
50.00
60.00
70.00
80.00
Urinary 8-OHdG (ng/mg creatinine)
Urinary H_2O_2 (μmol/g creatinine)
Male
Female
(Female)
(Male)

Table 1

Clinical profile of subjects

Clinical parameter	All (n=766)	Male (n=323)	Female (n=443)	p value
Age (year)	42.4 ± 10.6	42.0 ± 10.2	42.7 ± 10.9	0.344
BMI (kg/m^2)	22.7 ± 3.7	23.7 ± 3.4	21.9 ± 3.7	<0.001
Hs-CRP (mg/dl)	0.06 ± 0.10	0.07 ± 0.10	0.06 ± 0.10	<0.001
Systolic blood pressure (mmHg)	129.6 ± 21.8	133.5 ± 19.6	126.8 ± 23.0	<0.001
Diastolic blood pressure (mmHg)	78.4 ± 14.9	80.5 ± 14.4	76.8 ± 15.0	<0.001
Blood profile				
RBC (cell/μl)	465.6 ± 44.0	493.1 ± 42.8	445.6 ± 32.7	<0.001
Hb (mg/dl)	14.1 ± 1.6	15.5 ± 0.9	13.2 ± 1.3	<0.001
WBC (cell/μl)	5656.9 ± 1521.3	5931.4 ± 1580.9	5457.3 ± 1445.8	<0.001
Liver function profile				
AST (IU/l)	21.1 ± 8.1	23.7 ± 8.9	19.2 ± 6.9	<0.001
ALT (IU/l)	22.1 ± 17.7	28.6 ± 21.3	17.3 ± 12.6	<0.001
Lipid/lipoprotein profile				
TC (mg/dl)	203.9 ± 36.4	205.8 ± 33.7	202.5 ± 38.3	0.036
LDL-c (mg/dl)	124.6 ± 33.9	129.6 ± 32.4	121.0 ± 34.7	<0.001
TG (mg/dl)	97.3 ± 69.4	122.5 ± 89.1	78.9 ± 41.8	<0.001
Glucose profile				
HbA1c (%)	4.95 ± 0.37	4.95 ± 0.39	4.94 ± 0.35	0.840
Insulin (μU/ml)	5.2 ± 3.4	5.2 ± 3.4	5.2 ± 3.5	0.770
Glucose (mg/dl)	91.9 ± 10.1	93.8 ± 11.7	90.6 ± 8.6	<0.001
Oxidative stress markers				
H_2O_2 (μM/g creatinine)	5.66 ± 8.27	5.05 ± 6.18	6.10 ± 9.49	0.034
8-OHdG (ng/mg creatinine)	9.45 ± 4.09	8.85 ± 3.29	9.89 ± 4.54	0.004

BMI, body mass index; Hs-CRP, high-sensitivity C-reactive protein; RBC, red blood cells; Hb, hemoglobin; WBC, white blood cells; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol; TG, triglycerides; HbA1c, hemoglobin A1c; H_2O_2, hydrogen peroxide; 8-OHdG, 8-hydroxy-2'-deoxyguanosine.

Each value represents the mean ± SD.

Data were analyzed by the Mann-Whitney U test between males and females.

Table 2

Lifestyle profiles of subjects

	All *n* (%)	Male *n* (%)	Female *n* (%)
Total	766	323	443
Smoking			
Nonsmoker	507 (66.2)	138 (42.7)	369 (83.3)
Past smoker	61 (8.0)	47 (14.6)	14 (3.2)
Current smoker	198 (25.8)	138 (42.7)	60 (13.5)
Alcohol consumption			
No	252 (32.9)	64 (19.8)	188 (42.4)
3 times or less per week	309 (40.3)	122 (37.8)	187 (42.2)
4 times or more per week	205 (26.8)	137 (42.4)	68 (15.3)
Exercise			
No	435 (56.8)	138 (42.7)	297 (67.0)
2 times or less per week	212 (27.7)	115 (35.6)	97 (21.9)
3 times or more per week	119 (15.5)	70 (21.7)	49 (11.1)

Table 3

Spearman's correlation of urinary H_2O_2 with each parameter

Variable	All (*n* =766)		Male (*n* =323)		Female (*n* =443)	
	r	*p*	r	*p*	r	*p*
Age (year)	0.078	0.031	0.045	0.421	0.095	0.045
BMI (kg/m^2)	0.043	0.234	-0.016	0.778	0.050	0.291
Waist circumference (cm)	0.026	0.467	-0.069	0.215	0.036	0.444
Hs-CRP(mg/dl)	0.000	0.992	-0.033	0.558	-0.008	0.867
Systolic blood pressure (mmHg)	0.035	0.335	-0.024	0.667	0.036	0.447
Diastolic blood pressure (mmHg)	0.042	0.241	0.002	0.973	0.052	0.272
Blood profile						
RBC (cell/μl)	0.024	0.507	-0.017	0.757	-0.027	0.569
Hb (mg/dl)	0.050	0.170	0.017	0.760	-0.018	0.699
WBC (cell/μl)	0.071	0.049	0.041	0.467	0.077	0.104
Liver function profile						
AST (IU/l)	0.110	0.002	0.016	0.777	0.143	0.003
ALT (IU/l)	0.095	0.008	0.000	0.997	0.125	0.008
Lipid/lipoprotein profile						
TC (mg/dl)	0.126	<0.001	0.066	0.236	0.144	0.002
LDL-c (mg/dl)	0.107	0.003	0.074	0.182	0.104	0.028
TG (mg/dl)	0.021	0.559	0.002	0.973	-0.002	0.961
Glucose profile						
HbA1c (%)	0.069	0.055	0.061	0.275	0.072	0.132
Insulin (μU/ml)	-0.143	<0.001	-0.099	0.076	-0.164	0.001
Glucose (mg/dl)	-0.011	0.757	-0.075	0.178	0.004	0.933
Oxidative stress markers						
8-OHdG (ng/mg creatinine)	0.185	<0.001	0.196	<0.001	0.200	<0.001
Lifestyle						
Alcohol consumption	0.024	0.508	-0.051	0.361	0.036	0.455
Exercise	0.115	0.001	0.082	0.142	0.102	0.032

BMI, body mass index; Hs-CRP, high-sensitivity C-reactive protein; RBC, red blood cells; Hb, hemoglobin; WBC, white blood cells; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol; TG, triglycerides; HbA1c, hemoglobin A1c; 8-OHdG, 8-hydroxy-2'-deoxyguanosine.

Table 4

Mean values according to quartiles of H_2O_2 concentrations

Variable	Male (*n* =323)					Female (*n* =443)				
	Quartiles of H_2O_2 concentrations				*p* for trend	Quartiles of H_2O_2 concentrations				*p* for trend
	Q1	Q2	Q3	Q4		Q1	Q2	Q3	Q4	
Range	0.01-1.33	1.39-3.47	3.52-6.33	6.39-51.38		0.01-0.01	0.02-2.59	2.60-7.41	7.48-70.03	
Age (year)	41.6	41.9	41.1	43.4	0.373	43.3	39.9	42.0	45.3	0.077
BMI (kg/m^2)	23.9	23.7	23.7	23.6	0.684	21.7	21.8	22.0	22.1	0.337
Hs-CRP(mg/dl)	0.07	0.08	0.07	0.07	0.619	0.04	0.08	0.06	0.05	0.993
Systolic blood pressure (mmHg)	135.0	133.2	130.0	136.0	0.965	126.2	125.3	125.0	130.8	0.158
Diastolic blood pressure (mmHg)	79.6	81.8	78.1	82.5	0.474	76.1	76.2	76.2	78.8	0.213
RBC (cell/μl)	494.7	491.7	494.0	491.8	0.762	446.4	444.5	446.7	444.8	0.852
Hb (mg/dl)	15.5	15.3	15.5	15.5	0.445	13.1	13.2	13.2	13.2	0.681
WBC (cell/μl)	5570.4	5995.1	6129.6	5827.9	0.698	5172.4	5563.2	5761.3	5349.1	0.229
AST (IU/l)	25.1	22.5	22.7	24.6	0.771	18.7	18.8	19.5	20.0	0.115
ALT (IU/l)	32.1	23.9	28.0	30.6	0.971	15.9	17.7	17.5	18.2	0.204
TC (mg/dl)	201.6	206.2	206.1	209.4	0.170	198.8	195.9	202.8	212.4	0.003
LDL-c (mg/dl)	125.7	129.7	130.8	132.0	0.220	119.1	116.1	120.7	128.3	0.028
TG (mg/dl)	116.6	119.6	133.5	120.1	0.583	82.1	76.2	78.4	78.5	0.628
HbA1c (%)	4.92	5.01	4.92	4.94	0.941	4.92	4.92	4.94	4.99	0.138
Insulin (μU/mL)	5.5	5.1	5.2	5.2	0.579	5.5	5.9	4.9	4.4	0.003
Glucose (mg/dl)	94.1	95.8	92.2	92.9	0.209	91.2	89.8	90.7	90.8	0.943
Oxidative stress markers										
H_2O_2 (μM/g creatinine)	0.34	2.42	4.84	12.70	—	0.01	1.28	4.53	18.76	—
8-OHdG (ng/mg creatinine)	8.26	8.17	8.95	10.04	<0.001	8.84	9.98	9.42	11.37	<0.001

BMI indicates body mass index; Hs-CRP, high-sensitivity C-reactive protein; RBC, red blood cells; Hb, hemoglobin; WBC, white blood cells; AST, aspartate amino transferase; ALT, alanine aminotransferase; TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol; TG, triglycerides; HbA1c, hemoglobin A1c; H_2O_2, hydrogen peroxide; 8-OHdG, 8-hydroxy-2'-deoxyguanosine.

Table 5

Odds ratio of urinary H_2O_2 according to quartiles of 8-OHdG

	Quartiles of 8-OHdG concentrations				
	Q1	Q2	Q3	Q4	*p* for trend
All (*n* =766)					
Model 1[a]	1.00	1.36 (0.91-2.04)	1.64 (1.10-2.46)*	2.34 (1.55-3.53)**	<0.001
Model 2[b]	1.00	1.31 (0.87-1.97)	1.62 (1.08-2.44)*	2.33 (1.52-3.57)**	<0.001
Model 3[c]	1.00	1.30 (0.85-1.99)	1.68 (1.10-2.57)*	2.31 (1.47-3.62)**	<0.001
Male (*n* =323)					
Age < 50 (n=242)					
Model 1[a]	1.00	1.10 (0.54-2.27)	1.76 (0.86-3.62)	2.16 (1.05-4.46)*	0.018
Model 2[c]	1.00	1.15 (0.53-2.47)	2.11 (0.96-4.64)	2.26 (1.01-5.03)*	0.019
Age ≥ 50 (n=81)					
Model 1[a]	1.00	0.54 (0.14-2.07)	1.99 (0.57-6.90)	6.50 (1.59-26.51)**	0.002
Model 2[c]	1.00	0.71 (0.14-3.75)	4.37 (0.87-21.94)	12.33 (2.07-73.40)**	0.001
Female (n=443)					
Age < 50 (n=304)					
Model 1[a]	1.00	1.14 (0.61-2.16)	1.20 (0.63-2.28)	2.07 (1.08-3.96)*	0.033
Model 2[c]	1.00	1.29 (0.65-2.56)	1.29 (0.64-2.60)	2.44 (1.19-5.01)*	0.021
Age ≥ 50 (n=139)					
Model 1[a]	1.00	1.78 (0.69-4.60)	2.00 (0.77-5.18)	1.33 (0.51-3.46)	0.525
Model 2[c]	1.00	1.92 (0.68-5.42)	2.21 (0.79-6.23)	1.47 (0.53-4.10)	0.441

Data were analyzed by multiple logistic regression analysis.

Data in parentheses are 95% CI.

*p <0.05, **p <0.01

[a]Not adjusted.

[b]Adjusted for sex and age.

[c]Adjusted for BMI, Hs-CRP, systolic blood pressure, RBC, WBC, ALT, TC, HbA1c, insulin, smoking, alcohol consumption, and exercise.

Table 6

Odds ratio of urinary H_2O_2 according to quartiles of insulin

	Quartiles of insulin concentrations				
	Q1	Q2	Q3	Q4	*p* for trend
All (*n* =766)					
Model 1[a]	1.00	1.19 (0.79-1.78)	0.75 (0.51-1.13)	0.55 (0.36-0.82)**	0.001
Model 2[b]	1.00	1.25 (0.83-1.89)	0.81 (0.54-1.21)	0.57 (0.38-0.87)**	0.001
Model 3[c]	1.00	1.27 (0.83-1.94)	0.81 (0.52-1.25)	0.50 (0.30-0.83)**	0.002
Male (*n* =323)					
Age < 50 (n=242)					
Model 1[a]	1.00	1.23 (0.60-2.52)	1.00 (0.49-2.02)	0.90 (0.45-1.83)	0.653
Model 2[c]	1.00	1.04 (0.49-2.24)	0.87 (0.38-1.98)	0.76 (0.30-1.93)	0.526
Age ≥ 50 (n=81)					
Model 1[a]	1.00	0.64 (0.18-2.30)	0.14 (0.04-0.53)**	0.47 (0.13-1.69)	0.067
Model 2[c]	1.00	1.86 (0.33-10.58)	0.14 (0.02-0.91)*	2.31 (0.28-18.76)	0.979
Female (n=443)					
Age < 50 (n=304)					
Model 1[a]	1.00	1.13 (0.60-2.13)	0.95 (0.50-1.81)	0.46 (0.24-0.89)*	0.019
Model 2[c]	1.00	1.14 (0.58-2.27)	0.93 (0.46-1.89)	0.35 (0.16-0.80)*	0.012
Age ≥ 50 (n=139)					
Model 1[a]	1.00	1.19 (0.47-3.02)	1.50 (0.58-3.89)	1.06 (0.41-2.72)	0.794
Model 2[c]	1.00	1.26 (0.47-3.40)	1.35 (0.47-3.89)	0.69 (0.19-2.47)	0.614

Data were analyzed by multiple logistic regression analysis.

Data in parentheses are 95% CI.

*p <0.05, **p <0.01

[a]Not adjusted.

[b]Adjusted for sex and age.

[c]Adjusted for BMI, Hs-CRP, systolic blood pressure, RBC, WBC, ALT, TC, HbA1c, 8-OHdG, smoking, alcohol consumption, and exercise.

Table 7

Odds ratio of urinary H_2O_2 according to quartiles of TC

	Quartiles of TC concentrations				
	Q1	Q2	Q3	Q4	*p* for trend
All (*n* =766)					
Model 1[a]	1.00	2.03 (1.35-3.05)**	1.85 (1.23-2.77)**	1.97 (1.31-2.96)**	0.003
Model 2[b]	1.00	1.94 (1.28-2.92)**	1.61 (1.05-2.48)*	1.71 (1.10-2.66)*	0.049
Model 3[c]	1.00	1.84 (1.20-2.81)**	1.66 (1.06-2.59)*	1.66 (1.03-2.66)*	0.069
Male (*n* =323)					
Age < 50 (n=242)					
Model 1[a]	1.00	1.65 (0.80-3.41)	2.07 (1.01-4.23)*	2.12 (1.01-4.42)*	0.037
Model 2[c]	1.00	1.75 (0.81-3.79)	2.39 (1.09-5.25)*	2.57 (1.14-5.82)*	0.017
Age ≥ 50 (n=81)					
Model 1[a]	1.00	0.31 (0.09-1.11)	0.25 (0.07-0.91)*	0.22 (0.06-0.82)*	0.025
Model 2[c]	1.00	0.29 (0.05-1.58)	0.11 (0.02-0.62)*	0.26 (0.04-1.63)	0.078
Female (n=443)					
Age < 50 (n=304)					
Model 1[a]	1.00	1.60 (0.83-3.07)	2.52 (1.33-4.79)**	2.55 (1.33-4.86)**	0.002
Model 2[c]	1.00	1.33 (0.67-2.63)	2.34 (1.19-4.58)*	2.42 (1.22-4.78)*	0.004
Age ≥ 50 (n=139)					
Model 1[a]	1.00	0.65 (0.26-1.63)	1.17 (0.44-3.09)	0.67 (0.26-1.72)	0.675
Model 2[c]	1.00	0.72 (0.27-1.91)	1.17 (0.41-3.37)	0.60 (0.21-1.75)	0.548

Data were analyzed by multiple logistic regression analysis.

Data in parentheses are 95% CI.

*p <0.05, **p <0.01

[a]Not adjusted.

[b]Adjusted for sex and age.

[c]Adjusted for BMI, Hs-CRP, systolic blood pressure, RBC, WBC, ALT, HbA1c, insulin, 8-OHdG, smoking, alcohol consumption, and exercise.

Table 8

Odds ratio of urinary H_2O_2 according to exercise

	Exercise			
	No	2 times or less per week	3 times or more per week	*p* for trend
All (n=766)				
Model 1[a]	1.00	1.41 (1.02-1.96)*	1.77 (1.17-2.68)**	0.007
Model 2[b]	1.00	1.32 (0.94-1.85)	1.56 (1.02-2.39)*	0.042
Model 3[c]	1.00	1.29 (0.91-1.84)	1.55 (0.99-2.42)	0.053
Male (n=323)				
Age < 50 (n=242)				
Model 1[a]	1.00	1.90 (1.07-3.37)*	1.03 (0.52-2.06)	0.931
Model 2[c]	1.00	2.22 (1.17-4.20)*	1.29 (0.60-2.78)	0.521
Age ≥ 50 (n=81)				
Model 1[a]	1.00	0.62 (0.22-1.73)	0.82 (0.28-2.46)	0.728
Model 2[c]	1.00	0.63 (0.18-2.22)	0.76 (0.18-3.30)	0.718
Female (n=443)				
Age < 50 (n=304)				
Model 1[a]	1.00	1.32 (0.75-2.31)	1.39 (0.50-3.87)	0.525
Model 2[c]	1.00	1.35 (0.74-2.46)	1.36 (0.46-3.98)	0.575
Age ≥ 50 (n=139)				
Model 1[a]	1.00	1.72 (0.76-3.90)	1.55 (0.68-3.55)	0.302
Model 2[c]	1.00	1.65 (0.67-4.08)	1.37 (0.56-3.32)	0.490

Data were analyzed by multiple logistic regression analysis.

Data in parentheses are 95% CI.

*p <0.05, **p <0.01

[a]Not adjusted.

[b]Adjusted for sex and age.

[c]Adjusted for BMI, Hs-CRP, systolic blood pressure, RBC, WBC, ALT, TC, HbA1c, insulin, 8-OHdG, smoking, and alcohol consumption.

Table 9

Odds ratio of urinary H_2O_2 according to alcohol consumption

	Alcohol consumption			
	No	3 times or less per week	4 times or more per week	*p* for trend
All (n=766)				
Model 1[a]	1.00	0.95 (0.68-1.33)	1.04 (0.72-1.50)	0.835
Model 2[b]	1.00	0.96 (0.68-1.35)	0.86 (0.58-1.27)	0.440
Model 3[c]	1.00	0.92 (0.65-1.31)	0.78 (0.51-1.19)	0.245
Male (n=323)				
Age < 50 (n=242)				
Model 1[a]	1.00	0.45 (0.22-0.92)*	0.47 (0.23-0.97)*	0.042
Model 2[c]	1.00	0.47 (0.22-0.99)*	0.47 (0.21-1.05)	0.066
Age ≥ 50 (n=81)				
Model 1[a]	1.00	1.38 (0.39-4.87)	1.25 (0.41-3.79)	0.693
Model 2[c]	1.00	1.88 (0.35-10.26)	1.51 (0.31-7.26)	0.606
Female (n=443)				
Age < 50 (n=304)				
Model 1[a]	1.00	1.41 (0.87-2.28)	0.83 (0.39-1.77)	0.631
Model 2[c]	1.00	1.32 (0.79-2.21)	0.74 (0.32-1.70)	0.480
Age ≥ 50 (n=139)				
Model 1[a]	1.00	1.69 (0.76-3.75)	1.23 (0.53-2.84)	0.635
Model 2[c]	1.00	1.48 (0.63-3.49)	1.05 (0.41-2.67)	0.918

Data were analyzed by multiple logistic regression analysis.

Data in parentheses are 95% CI.

*p <0.05, **p <0.01

[a]Not adjusted.

[b]Adjusted for sex and age.

[c]Adjusted for BMI, Hs-CRP, systolic blood pressure, RBC, WBC, ALT, TC, HbA1c, insulin, 8-OHdG, smoking, and exercise.