

# Urinary 8-Hydroxy-2'-Deoxyguanosine Levels and Cardiovascular Disease Incidence in Japan

Masanori Nagao<sup>1,2,3</sup>, Gen Kobashi<sup>1</sup>, Mitsumasa Umesawa<sup>1</sup>, Renzhe Cui<sup>4</sup>, Kazumasa Yamagishi<sup>5,6</sup>, Hironori Imano<sup>4</sup>, Takeo Okada<sup>7</sup>, Masahiko Kiyama<sup>7</sup>, Akihiko Kitamura<sup>8</sup>, Toshimi Sairenchi<sup>1</sup>, Yasuo Haruyama<sup>1</sup>, Tetsuya Ohira<sup>2,3</sup> and Hiroyasu Iso<sup>4</sup> for the CIRCS Investigators

<sup>1</sup>Department of Public Health, School of Medicine, Dokkyo Medical University, Tochigi, Japan

<sup>2</sup>Office of Epidemiology, Radiation Medical Science Center for the Fukushima Health Management Survey, Fukushima Medical University, Fukushima, Japan

<sup>3</sup>Department of Epidemiology, School of Medicine, Fukushima Medical University, Fukushima, Japan

<sup>4</sup>Public Health, Department of Social Medicine, Osaka University Graduate School of Medicine, Osaka, Japan

<sup>5</sup>Department of Public Health Medicine, Faculty of Medicine, and Health Services Research and Development Center, University of Tsukuba, Tsukuba, Japan

<sup>6</sup>Ibaraki Western Medical Center, Ibaraki, Japan

<sup>7</sup>Osaka Center for Cancer and Cardiovascular Diseases Prevention, Osaka, Japan

<sup>8</sup>Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan

**Aim:** The association between urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG), an oxidative stress marker, and the incidence of cardiovascular disease (CVD) has not been confirmed because no previous studies evaluated 24-hour 8-OHdG excretion levels in the general population. We aimed to confirm the association between 24-hour urinary 8-OHdG levels and CVD risk among Japanese men and women.

**Methods:** A nested case-control study was performed based on a 24-hour urine collection in a community-based cohort study performed from 1996 to 2005. Seventy-six cases (55 men and 21 women) who experienced their first CVD incidence during the follow-up period (median: 5.9 years) were recruited. The controls were frequency-matched 1:2, with each case for sex, age, area of residence, and baseline year. The 8-OHdG level was measured by enzyme-linked immunosorbent assay. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using conditional logistic regression models adjusted for body mass index, ethanol intake, smoking status, and estimated glomerular filtration rate.

**Results:** The geometric mean and geometric standard deviation (SD) of 8-OHdG levels (nmol/day) for cases and controls were 35.5 (1.55) and 35.5 (1.54) for men and 32.1 (1.35) and 25.0 (1.39) for women, respectively. The multivariable OR (95% CI) of CVD incidence according to the 1-SD increment of the log-transformed 8-OHdG level was 2.08 (0.99–4.37) for women. The multivariable ORs (95% CIs) for the 1st (lowest) and 4th versus 2nd quartile according to 8-OHdG for men were 3.29 (1.02–10.61) and 2.77 (0.96–7.96), respectively.

**Conclusion:** A high 8-OHdG level tended to be associated with CVD incidence among women.

**Key words:** 8-OHdG, Oxidative stress, Cardiovascular diseases, Incidence, Population- based

## Introduction

Cardiovascular diseases (CVDs) are major health threats worldwide. Various researchers have described

CVD risk factors. Although many studies have reported known risk factors, these cannot explain all causes of cardiovascular events<sup>1)</sup>.

Oxidative stress appears to be a risk factor for

Address for correspondence: Masanori Nagao, Office of Epidemiology, Radiation Medical Science Center for the Fukushima Health Management Survey, Fukushima Medical University, Fukushima, Japan, 960-1295 E-mail: [nagao-masanori-hm@alumni.osaka-u.ac.jp](mailto:nagao-masanori-hm@alumni.osaka-u.ac.jp)

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CVD<sup>1-9</sup>). Oxidative stress is constantly generated in the body because of aerobic energy production, while antioxidant activity occurs simultaneously to resist this oxidative stress and maintain redox balance. If the redox balance is disrupted, oxidative stress increases to harmful levels<sup>2</sup>). Excessive oxidative stress results in oxidation and degeneration of various molecules (proteins, lipids, DNA, and others)<sup>3</sup>), and it induces key components of atherogenesis, including the upregulation of cytokines<sup>2, 4</sup>). Indeed, large epidemiological studies have reported that oxidative stress or antioxidants are associated with CVD events<sup>5-8</sup>), and the Japan Atherosclerosis Society Guidelines for Prevention of Atherosclerotic Cardiovascular Diseases 2017 stated that measuring the level of malondialdehyde-modified low-density lipoprotein (LDL), which is oxidized LDL, is useful for the prognostic prediction of the incidence of coronary artery disease (CAD) in diabetic patients who have a history of CAD (evidence level: E-1b)<sup>9</sup>).

Although direct measurement of oxidative stress is difficult, it is easy to measure oxidized metabolites. Therefore, various targets have been established as biomarkers for oxidative stress. Among them, 8-hydroxy-2'-deoxyguanosine (8-OHdG), a metabolite of DNA oxidation, has been used for this purpose in many epidemiological studies<sup>10-15</sup>) because its chemical stability and urinary excretion make it easy to collect and manipulate specimens<sup>16, 17</sup>). To evaluate the excretion level of 8-OHdG, it is necessary to prove the validity of the measurement of the biomarker using spot urine specimens or to measure the absolute amount by 24-hour urine collection. However, no studies have proved the validity of measuring the 8-OHdG excretion level using spot urine specimens. In fact, it has been reported that 8-OHdG has a circadian rhythm<sup>18, 19</sup>). It cannot be denied that this circadian rhythm may change, depending on the prevalence of sex, age, or disease.

To our best knowledge, only two population-based studies<sup>10, 11</sup>) have evaluated the association between 8-OHdG levels and the risk of CVD based on data from a prospective study. However, these studies evaluated the 8-OHdG levels using spot urine specimens.

## Aim

As previous studies have not evaluated 24-hour 8-OHdG excretion levels in the general population, the purpose of the present study was to confirm an association between 24-hour urinary 8-OHdG excretion levels and the risk of CVD among Japanese men and women by utilizing a nested case-control study

based on a population-based cohort. We hypothesized that individuals with CVD would have higher levels of 8-OHdG than those without CVD.

## Methods

### Ethical Approval

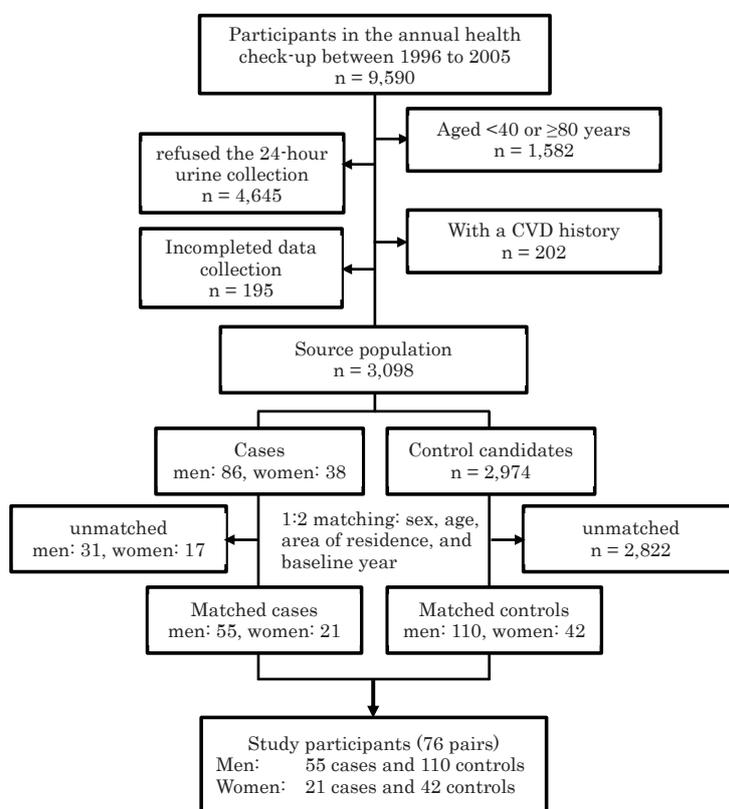
Informed consent was obtained from all participants. This study protocol was approved by the ethics committees of the Dokkyo Medical University, University of Tsukuba, Osaka Center for Cancer and Cardiovascular Disease Prevention, and Osaka University and conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

### Study Participants

We conducted a nested case-control study as part of the Circulatory Risk in Communities Study (CIRCS) cohort. The study protocol of the CIRCS study has been reported previously<sup>20</sup>). In brief, CIRCS was a dynamic cohort study that included annual health check-ups (cardiovascular risk survey) and surveillance for the incidence of stroke and CADs among the general population in five communities across Japan. From 9,590 individuals who participated in the annual health check-up between 1996 to 2005 in two communities (Kyowa in the Ibaraki prefecture and Ikawa in the Akita prefecture, Japan), we excluded participants who were not aged 40–79 years ( $n=1,582$ ), refused the 24-hour urine collection in annual health check-ups ( $n=4,645$ ), had a history of CVD ( $n=202$ ), and had incomplete data collection ( $n=195$ ). As a result, the population of the present study included 3,098 men and women. When comparing the urine collection between non-participants and participants, the differences of the characteristics between the two were as follows: the proportion of men was 41.0% versus 43.2%, average age was 59.7 years versus 55.9 years, the proportion of smokers was 17% versus 18%, alcohol consumption was 11.7 g/day versus 11.1 g/day, the proportion with a history of CVD was 2.8% versus 2.1%, and the proportion of new-onset CVD during the study period was 8.7% versus 5.9%, respectively. Finally, we analyzed 228 participants by matching cases and controls according to the procedure described as follows (Fig. 1).

### Cases and Selection of Controls

During a median follow-up of 5.9 years, we identified 124 cases (86 men and 38 women) with CVD among 3,098 participants. Two controls per case were selected by matching sex, age, area of residence, and year of urine sample collection (defined as baseline year). As a result, we analyzed 228 individuals (76



**Fig. 1.** Flow diagram of enrollment and selection for the present study  
CVD, cardiovascular disease

cases and 152 controls; 165 men and 63 women) who were successfully matched.

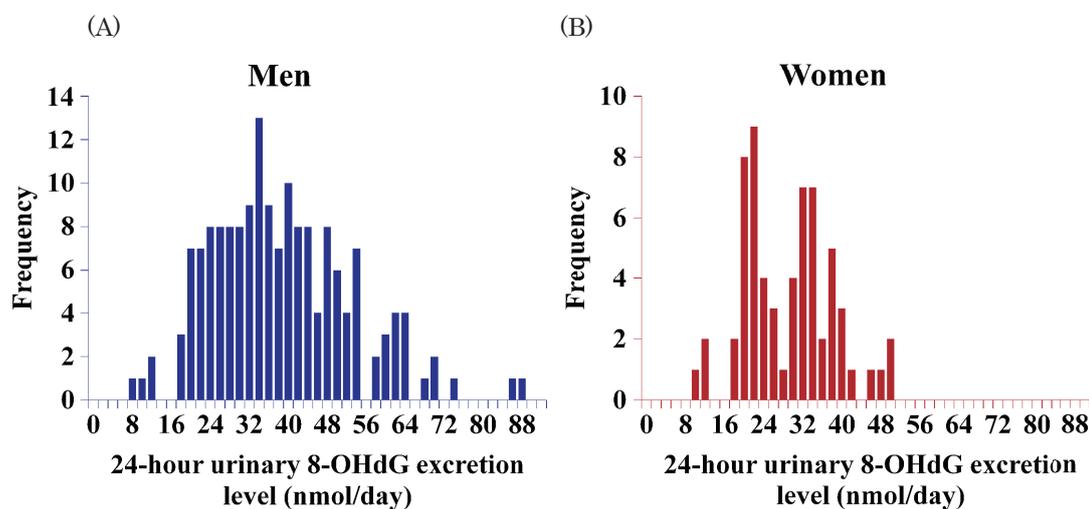
### Follow-Up and Ascertainment of CVD

Participants were followed to confirm outcomes (incidence of CVDs: stroke, angina of effort, myocardial infarction [MI], or sudden cardiac death within 1 hour of symptom onset) until the end of 2011 in Kyowa or 2009 in Ikawa.

Since 1981, we have obtained information on the incidence of CVD from one or more of the following: national health insurance claims, ambulance records, reports by local physicians or public health nurses and volunteers, questionnaires completed during an annual health check-up, and death certificates. Subsequently, to confirm the diagnosis of CVD, the participants or their families were interviewed to obtain information concerning the symptoms and time course of onset by telephone or in-person interview. Several physician-epidemiologists independently determined whether each case was a definite or suspected case of stroke or CAD based on their review of the medical records from local clinics and hospitals;

the data from the annual health check-ups were blinded.

The diagnostic criteria of CVD have been described elsewhere<sup>21</sup>. The diagnosis of MI was based on the modified World Health Organization criteria for CAD. Individuals were diagnosed with definite MI if they met the following criteria: (1) typical severe chest pain (lasting  $\geq 30$  min) and (2) new abnormal Q or QS waves on electrocardiography or consistent changes in cardiac enzyme levels. Probable MI was defined as meeting criterion (1) but not meeting criterion (2). Angina of effort was defined as repeated episodes of chest pain during effort, especially when walking, that usually disappeared rapidly after the cessation of effort or with sublingual nitroglycerin<sup>22</sup>. Other than MI or angina of effort, death that occurred within 1 hour of onset was regarded as sudden cardiac death<sup>23</sup>. Stroke was diagnosed if individuals had focal neurological symptoms with rapid onset, persisting for at least 24 hours or until death, and confirmed primarily based on computed tomography or magnetic resonance imaging, which was available in approximately 90% of cases<sup>24</sup>.



**Fig. 2.** Frequencies of 24-hour urinary 8-hydroxy-2'-deoxyguanosine excretion levels among men (A) and women (B)

The average periods for urine collection are 1439 min among men and 1,433 min among women. 8-OHdG, 8-hydroxy-2'-deoxyguanosine.

### Measurement of Biological Values

Urinary specimens were part of a 24-hour urine collection performed during an annual health check-up from 1996 to 2005 in Kyowa or from 1996 to 2001 in Ikawa. The collected urine samples were stored at  $-80^{\circ}\text{C}$  until just before measurement because 8-OHdG is stable for 800 days at a minimum temperature of  $-80^{\circ}\text{C}$ <sup>25</sup>. The concentration of urinary 8-OHdG was measured between 2016 and 2017 using a commercial enzyme-linked immunosorbent assay kit (Japan Institute for the Control of Aging, Shizuoka, Japan) according to the manufacturer's instructions. We calculated the 24-hour urinary excretion level of 8-OHdG according to the following formula: 24-hour urinary 8-OHdG excretion level (nmol) = concentration of 8-OHdG in the specimen (nmol/mL)  $\times$  collecting volume (mL)  $\times$  1,440 (min) / collection period (min).

Serum cholesterol (total cholesterol, high-density cholesterol [HDL]) and triglyceride levels were measured using standardized methods in the laboratory of the Osaka Medical Center for Health Science and Promotion, an international member of the Cholesterol Reference Method Laboratory Network<sup>26-28</sup>. Blood pressure was measured by trained physicians using standard mercury sphygmomanometers and standardized epidemiological methods.

### Statistical Analysis

To compare the baseline characteristics between cases and controls, we used the unpaired *t*-test for the mean values and the chi-square test for the proportion

values. For quartile analyses, cases and controls were categorized according to quartiles of 24-hour urinary 8-OHdG excretion levels among the sex-specific control group. We calculated sex-specific odds ratios (ORs) and 95% confidence intervals (95% CIs) for CVD incidence using conditional logistic regression models according to sex-specific quartiles and 1-standard deviation (SD) increments of the 24-hour urinary 8-OHdG excretion levels. We analyzed the data using three models: model 1 was not adjusted for covariates (matched only for sex, age, area of residence, and baseline year); model 2 was adjusted for body mass index (BMI: kg/m<sup>2</sup>, continuous), ethanol intake (g/day, continuous), and smoking status (current, former, never); and model 3 was additionally adjusted for the estimated glomerular filtration rate (eGFR) calculated by the following formula<sup>29, 30</sup>:  $\text{eGFR (mL/min)} = 194 \times [\text{serum creatinine level (mg/dL)}]^{-1.094} \times [\text{age (years)}]^{-0.287} \times (0.739: \text{only women}) \times [\text{body weight (kg)}]^{0.425} \times [\text{body height (cm)}]^{0.725} \times 0.007184 / 1.73 \text{ (m}^2\text{)}$ . The 8-OHdG value was logarithmically transformed because it was not normally distributed (Fig. 2). We excluded individuals who had a history of CVD at baseline. The geometric mean and geometric SD were calculated by the exponential converted value of the average or SD of the logarithmic value of the 8-OHdG, respectively<sup>31</sup>.

Statistical data were analyzed using Statistical Analysis Software (SAS) version 9.4 (SAS Institute, Cary, North Carolina). All probability values for statistical tests were two-tailed, and  $p < 0.05$  was considered statistically significant.

**Table 1.** Sex-specific mean values with standard deviations and the prevalence of cardiovascular risk factors in cases and controls

	Men			Women		
	Case	Control <sup>†</sup>	<i>p</i> value	Case	Control <sup>†</sup>	<i>p</i> value
No. of participants, <i>n</i>	55	110		21	42	
Age, years	64.9 ± 5.4	64.9 ± 5.4	1.00	61.9 ± 7.9	61.9 ± 7.8	1.00
BMI, kg/m <sup>2</sup>	24.0 ± 3.2	23.6 ± 2.8	0.42	25.7 ± 3.4	24.0 ± 3.5	0.07
Ethanol intake, g/day	24.2 ± 28.1	20.5 ± 19.9	0.38	0.2 ± 1.1	0.2 ± 1.5	1.00
Total cholesterol, mmol/L	5.25 ± 0.78	5.14 ± 0.80	0.42	5.33 ± 0.72	5.41 ± 0.78	0.70
HDL cholesterol, mmol/L	1.40 ± 0.49	1.38 ± 0.35	0.77	1.43 ± 0.25	1.56 ± 0.35	0.15
Triglyceride, mmol/l	1.78 ± 1.09	1.39 ± 0.81	<b>0.02</b>	1.33 ± 0.57	1.31 ± 0.86	0.90
Serum creatinine, mg/dL	1.1 ± 0.2	1.0 ± 0.1	0.09	0.8 ± 0.1	0.9 ± 0.1	0.12
eGFR, mL/min	1.71 ± 0.25	1.61 ± 0.29	0.55	1.92 ± 0.40	1.96 ± 0.33	0.18
Urinary 8-OHdG level <sup>‡</sup> , nmol/day	35.5 (1.55)	35.5 (1.54)	0.98	32.1 (1.35)	25.0 (1.39)	<b>0.005</b>
Current smoker, %	40.0	35.5	0.57	4.8	2.4	0.61

<sup>†</sup> Controls were matched with cases for sex, age, area of residence, and year of urine sample collection (defined as baseline year), with 2 controls per case.

<sup>‡</sup> For urinary 8-OHdG level, the geometric mean and geometric standard deviation (in parenthesis) are shown. BMI, body mass index; eGFR, estimated glomerular filtration rate; 8-OHdG, 8-hydroxy-2'-deoxyguanosine

## Results

**Fig. 2** shows sex-specific frequencies of the 24-hour urinary 8-OHdG excretion levels. The distributions were not normal. The average periods for urine collection were 1,439 min among men and 1,433 min among women.

The baseline characteristics of cases and case-matched controls indicated by mean ( $\pm$ SD) or prevalence values are shown in **Table 1**. In men, there were no differences in BMI; the amount of ethanol intake; levels of serum total cholesterol, HDL cholesterol, and serum creatinine; eGFR; the prevalence of current smokers. However, the triglyceride level was higher among cases than among controls. Urinary 8-OHdG levels were not different between the groups. In women, there were no differences in any of these variables except for the urinary 8-OHdG levels. The *p* values of the log-transformed 8-OHdG values, according to the t-test, were 0.98 for men and 0.005 for women.

**Table 2** shows model 1 (sex, age, area of residence, and baseline year matched) conditional ORs (95% CIs) for the incidence of CVD among men and women. In men, 8-OHdG excretion levels were not associated with CVD incidence in model 1. In women, there was a statistically significant association between 8-OHdG excretion levels and CVD incidence. The model 1 OR (95% CI) for CVD incidence according to the 1-SD increment in log-transformed 8-OHdG levels among women (SD=0.34) was 2.38 (1.19–4.77). No statistically significant association was found in the quartile analysis.

Multivariable adjustment for CVD risk factors did not change the tendency of the results, but it changed the statistical significance between men and women (**Table 2**). In men, the U-shaped tendency was found according to the quartile analysis in model 2 and model 3. The conditional ORs (95% CIs) of the 1st, 3rd, and 4th quartiles versus the 2nd quartile were 3.36 (1.05–10.77), 1.60 (0.50–5.08), and 2.72 (0.95–7.80) in model 2, and 3.29 (1.02–10.61), 1.68 (0.52–5.40), and 2.77 (0.96–7.96) in model 3, respectively. In women, the model 2 and model 3 ORs (95% CIs) for the 1-SD increment in 8-OHdG excretion level were 2.29 (1.08–4.81) and 2.08 (0.99–4.37), respectively.

We performed the same analyses stratified by age groups (younger than 65 years of age or not) among men (**Table 3**). This analysis was not possible among women because there were few participants older than 65 years of age. In the quartile analysis among men, the ORs were slightly attenuated in the 1st quartile and increased in the 4th quartile among the < 65-year-old group compared with all men. The ORs (95% CI) of the 1st, 3rd, and 4th quartiles versus the 2nd quartile in model 3 were 2.62 (0.36–19.28), 1.92 (0.27–13.75), and 4.57 (0.75–27.75), respectively, in the < 65-year-old group. However, in the  $\geq$  65-year-old group, the ORs were increased in the 1st quartile and attenuated in the 4th quartile. The ORs (95% CI) of the 1st, 3rd, and 4th quartiles versus the 2nd quartile in model 3 were 4.11 (0.73–23.18), 1.56 (0.28–8.77), and 1.80 (0.42–7.68), respectively.

**Table 2.** Sex-specific conditional odds ratios and 95% confidence intervals for the incidence of cardiovascular disease according to quartiles and 1-standard deviation increment of 24-hour urinary 8-hydroxy-2'-deoxyguanosine excretion

	24-hour urinary 8-OHdG excretion level				
	Q1 (low) OR 95% CI	Q2 Reference	Q3 OR 95% CI	Q4 (high) OR 95% CI	1-SD increment <sup>‡</sup>
<b>Men</b>					
24-hour urinary 8-OHdG excretion <sup>†</sup>					
Geometric mean <sup>§</sup> , nmol/day	20.8	31.9	40.4	57.8	
Range, nmol/day	(6.6-26.8)	(26.8-35.9)	(36.1-45.7)	(46.2-128.7)	
No. of cases	18	8	10	19	
No. of controls	27	28	28	27	
character-matched OR (model 1)	2.68 (0.91-7.91)	1.00	1.39 (0.46-4.22)	2.70 (0.95-7.65)	1.01 (0.73-1.38)
Multivariable OR (model 2)	3.36 (1.05-10.77)	1.00	1.60 (0.50-5.08)	2.72 (0.95-7.80)	0.94 (0.67-1.32)
Multivariable OR (model 3)	3.29 (1.02-10.61)	1.00	1.68 (0.52-5.40)	2.77 (0.96-7.96)	0.96 (0.69-1.35)
<b>Women</b>					
24-hour urinary 8-OHdG excretion <sup>†</sup>					
Geometric mean <sup>§</sup> , nmol/day	17.1	21.7	29.3	38.4	
Range, nmol/day	(9.0-19.9)	(20.4-23.3)	(24.5-33.0)	(33.2-52.3)	
No. of cases	2	3	4	12	
No. of controls	10	11	11	10	
character-matched OR (model 1)	0.79 (0.10-6.38)	1.00	1.59 (0.31-8.16)	3.93 (0.83-18.66)	2.38 (1.19-4.77)
Multivariable OR (model 2)	0.99 (0.11-8.49)	1.00	1.62 (0.24-11.04)	3.94 (0.73-21.20)	2.29 (1.08-4.81)
Multivariable OR (model 3)	0.77 (0.08-7.16)	1.00	1.19 (0.14-9.92)	2.91 (0.46-18.57)	2.08 (0.99-4.37)

We divided all participants into four groups by sex-specific quartile of the control group.

Model 1: Matched for sex, age, area of residence, and year of urine sample collection (defined as baseline year).

Model 2: Model 1 plus body mass index (continuous), ethanol intake (continuous), smoking status (current, former, never).

Model 3: Model 2 plus estimated glomerular filtration rate (continuous).

<sup>†</sup>Urinary 8-OHdG value was log-transformed.

<sup>‡</sup>The standard deviation of log-transformed 8-OHdG were 0.43 among men and 0.34 among women.

<sup>§</sup>Geometric mean value was calculated by exponential converted of the average of the logarithmic value of 8-OHdG.

8-OHdG, 8-hydroxy-2'-deoxyguanosine; SD, standard deviation; OR, odds ratio; CI, confidence interval

## Discussion

In this nested case-control study of a population-based cohort, although a significant difference could not be detected, we found that high levels of 24-hour urinary 8-OHdG tended to be associated with CVD incidence among women; the results in women support our hypothesis, but the results in men do not. There was a U-shaped tendency between the 8-OHdG level and CVD incidence among men, which does not support our hypothesis. To our knowledge, this is the first analysis using 24-hour urine specimens from a population-based cohort to evaluate the association between 8-OHdG levels and CVD incidence.

Some patient-based studies have suggested an association between 8-OHdG and CVD. In heart failure patients, the 8-OHdG level positively correlated with the severity of the New York Heart Association class<sup>12)</sup> and with hazard ratios of re-hospitalization and death<sup>13)</sup>. In acute MI patients, 8-OHdG levels before and after reperfusion therapy were higher in patients

who had cardiac events within 2 weeks of admission for reperfusion therapy than in patients without cardiac events<sup>14)</sup>. In patients with cerebral hemorrhage, high levels of 8-OHdG were associated with 30-day functional outcomes<sup>15)</sup>. Since these studies were patient-based, the associations between 8-OHdG and new-onset CVD were not examined. However, they suggested that 8-OHdG would be associated with the severity of CVD. Several studies reported the potential molecular mechanisms of oxidative stress involved in the pathogenesis of CVD based on the pathway of atheroma formation and vessel wall fragility, including induced uncoupling of endothelial NO synthase, proliferation of smooth muscle cells, LDL oxidization, and upregulation of cytokines (e.g., interleukin-6, tumor necrosis factor- $\alpha$ , and monocyte chemoattractant protein-1), adhesion molecules, or matrix metalloproteinases<sup>2, 4)</sup>. It appeared that 8-OHdG was also involved in these components. However, it remained unclear whether the involvement of these molecular mechanisms could be implicated in the general popu-

**Table 3.** Age-stratified conditional odds ratios and 95% confidence intervals for the incidence of cardiovascular disease according to quartiles and 1-standard deviation increment of 24-hour urinary 8-hydroxy-2'-deoxyguanosine excretion among men

	24-hour urinary 8-OHdG excretion level				
	Q1 (low) OR 95% CI	Q2 Reference	Q3 OR 95% CI	Q4 (high) OR 95% CI	1-SD increment <sup>‡</sup>
<b>&lt; 65 years</b>					
24-hour urinary 8-OHdG excretion <sup>†</sup>					
Geometric mean <sup>§</sup> , nmol/day	21.4	32.4	40.8	56.6	
Range, nmol/day	(8.1-26.8)	(27.6-35.7)	(36.1-45.7)	(47.0-87.1)	
No. of cases	5	3	5	11	
No. of controls	11	13	13	11	
character-matched OR (model 1)	2.57 (0.41-16.00)	1.00	1.60 (0.29-8.91)	4.66 (0.88-24.79)	1.27 (0.75-2.14)
Multivariable OR (model 2)	2.78 (0.42-18.60)	1.00	1.41 (0.23-8.56)	4.38 (0.79-24.33)	1.21 (0.67-2.19)
Multivariable OR (model 3)	2.62 (0.36-19.28)	1.00	1.92 (0.27-13.75)	4.57 (0.75-27.75)	1.24 (0.69-2.25)
<b>≥ 65 years</b>					
24-hour urinary 8-OHdG excretion <sup>†</sup>					
Geometric mean <sup>§</sup> , nmol/day	20.5	31.4	40.0	58.9	
Range, nmol/day	(6.6-26.8)	(26.8-35.9)	(36.4-45.4)	(46.2-128.7)	
No. of cases	13	5	5	8	
No. of controls	16	15	15	16	
Character-matched OR (model 1)	2.87 (0.70-11.73)	1.00	1.17 (0.26-5.22)	1.58 (0.39-6.43)	0.85 (0.55-1.30)
Multivariable OR (model 2)	4.35 (0.77-24.50)	1.00	1.68 (0.31-9.21)	1.82 (0.42-7.85)	0.81 (0.51-1.27)
Multivariable OR (model 3)	4.11 (0.73-23.18)	1.00	1.56 (0.28-8.77)	1.80 (0.42-7.68)	0.82 (0.52-1.30)

We divided all participants into four groups by age group-specific quartile of the control group.

Model 1: Matched for sex, age, area of residence, and year of urine sample collection (defined as baseline year).

Model 2: Model 1 plus body mass index (continuous), ethanol intake (continuous), smoking status (current, former, never).

Model 3: Model 2 plus estimated glomerular filtration rate (continuous).

<sup>†</sup>Urinary 8-OHdG value was log-transformed.

<sup>‡</sup>The standard deviation of log-transformed 8-OHdG were 0.40 among < 65 years group and 0.46 among ≥ 65 years group.

<sup>§</sup>Geometric mean value was calculated by exponential converted of the average of the logarithmic value of 8-OHdG.

8-OHdG, 8-hydroxy-2'-deoxyguanosine; SD, standard deviation; OR, odds ratio; CI, confidence interval

lation because only a few epidemiological studies have been reported on this topic<sup>32</sup>. A cross-sectional study that<sup>32</sup> included 51 male cases and the same number of male controls reported that urinary 8-OHdG was associated with an increased prevalence of high carotid intima media thickness, an indicator of arteriosclerosis. The ORs (95% CI) of 8-OHdG according to the lowest tertile versus the middle and highest tertiles were 0.77 (0.22–2.64) and 4.45 (1.27–15.56), respectively<sup>32</sup>. In this study, although the OR was somewhat decreased in the middle tertile, it was not significant, and because it was a cross-sectional study, the temporal relationship is unknown.

Only two studies have evaluated the association between 8-OHdG or DNA oxidation marker and CVD onset using data from a population-based cohort study, and the results were inconsistent<sup>10, 11</sup>. A Taiwanese nested case-control study consisting of 131 cases (57 women) and the same number of controls reported that the OR (95% CI) of the 8-OHdG level according to the lowest versus the highest quartile for

stroke incidence was 0.97 (0.45–2.08) and that over the course of four quartiles, a significant association was not observed<sup>10</sup>. Another study was based on 9949 older adults from Germany with 14 years of follow up. The study reported a significant association between urinary oxidized guanine/guanosine levels (including 8-OHdG) and CVD mortality. The hazard ratio (95% CI) of the highest versus lowest tertile was 1.32 (1.06–1.64)<sup>11</sup>. However, both studies analyzed only sex-combined data and used spot urine specimens corrected for creatinine. They did not discuss associations between the sex difference and CVD incidence.

In the present study, a U-shaped association was observed among men, especially ORs in the 4th quartile that were higher than those in the 1st quartile in the younger age group, and the opposite association was observed in the elderly group. We propose the following possible reason for this result. Genomic guanine with oxidative damage (8-OHdG or its tautomer 8-oxo-7,8-dihydro-2'-deoxyguanosine) is excised from

the genome by 8-oxoguanine DNA glycosylase (OGG1) and excreted in urine<sup>33</sup>). The activity of OGG1 is enhanced by apurinic/aprimidinic endonuclease (APE1)<sup>34</sup>. A study in rats showed that APE1 gene expression and liver 8-OHdG levels were inversely correlated, and APE1 messenger RNA levels may decrease with aging<sup>35</sup>. A study in humans reported that the older participant group ( $63.4 \pm 4.7$  years) had higher levels of 8-oxo-7,8-dihydroguanine in skeletal muscle and lower levels of active OGG1 (acetylated OGG1) than the younger group<sup>36</sup>. These studies indicate that the repair and excretion of 8-OHdG may decrease with aging even though the 8-OHdG level in the tissue increases. These reports may explain the increased risk of CVD incidence in the group with low levels of urinary 8-OHdG excretion among older men in the present study. In women, a U-shaped association was not observed as in men; however, it is thought that the risk of CVD in women appears later than in men<sup>37</sup>, probably because of female hormones. Moreover, the proportion of those older than 65 years of age was small among women participants. Therefore, the increase of OR in the 1st quartile observed among older men may not have been observed in the female group. Another possible reason is that older men have lower OGG1 activity than younger men, although this association was not observed among women<sup>38</sup>. We speculate that women may have shown a linear association in this study because of the simple association between 8-OHdG levels in tissues and urinary excretion regardless of age. Other possible reasons might involve reverse causation and residual confounding; that is, case subjects with CVD risk factors unrelated to or that lowered the 8-OHdG level, such as uric acid<sup>39</sup>, could be categorized in the lowest quartile group. Further, large epidemiological studies are needed to evaluate these hypotheses.

### Strengths and Limitations

The present study has some strengths. First, since this investigation was a nested case-control study based on a prospective cohort study, all exposure information and specimens were collected before the onset of CVD. Second, the present study was based on the general population; therefore, bias due to the presence of disease could be smaller than the bias in studies based on the clinical population. Third, we evaluated the 24-hour 8-OHdG excretion levels using 24-hour urine collection specimens, reflecting 8-OHdG excretion levels more accurately than spot urine specimen measurements. To our knowledge, no study has reported a valid method for estimating the 24-hour 8-OHdG excretion level using spot urine

specimens. The correlation coefficient between 8-OHdG excretion in 24-hour urine specimens and morning spot urine specimens corrected for creatinine was 0.5 ( $p < 0.05$ )<sup>40</sup>. Spot urine has an advantage that it can be used for large-scale epidemiological studies because collecting spot urine is simple. On the other hand, collecting 24-hour urine samples from the general population is difficult but represents the ideal method to evaluate the 8-OHdG excretion level more accurately by eliminating the influence of circadian variation. Fourth, since we conducted a systematic follow-up of CVD incidence, it was possible to evaluate CVD incidence as the outcome measure, rather than mortality, and to assess the association between 8-OHdG and the development of CVD more directly.

The present study also has limitations. First, there was a limit in our ability to control confounding factors and stratification analysis because of the small sample size, although we did attempt to control these factors as much as possible by matching and multivariable adjustments. Second, since 8-OHdG was measured using urine specimens stored at  $-80^{\circ}\text{C}$  for a maximum of 22 years in this study, the possibility that urinary 8-OHdG was degraded during storage cannot be denied. However, 8-OHdG was reported to be stable for at least 800 days at  $-80^{\circ}\text{C}$ <sup>25</sup>). In addition, cases and controls were matched by the urine collecting year. Therefore, if 8-OHdG is degraded, then the difference in 8-OHdG levels between the case group and the control group tends to be small, but the tendency of the associations was not disturbed. Third, the evaluation of urinary 8-OHdG levels was performed only once at baseline throughout the study period. Therefore, we were not able to evaluate fluctuations in 8-OHdG levels during the follow-up period. Fourth, because 24-hour urine collection always involves participants carrying a urine storage bottle, which is inconvenient, it was difficult to recruit individuals willing to participate in this research study. Therefore, selection bias in participation may have existed. When comparing the urine collection of non-participants and participants, the proportion of new-onset CVD during the study period among non-participants was more frequent (8.7% versus 5.9%). However, considering other characteristics, there were few differences between non-participants and participants (described in method section). Fifth, because participants collected urine at home, we could not confirm whether specimens were collected accurately. However, urine collection is a simple procedure; individuals urinate in a cup and then transfer it to a bottle. Furthermore, we interviewed these individuals regarding urine collection when we received their specimens. Therefore, it is assumed that the sampling accuracy is not low. Finally,

because all participants in the present study were Japanese, the results should be carefully generalized to other races. Further large-scale cohort studies are needed to determine the association between 8-OHdG levels and CVD incidence, especially among men.

## Conclusions

Although a significant difference could not be detected, this nested case-control study demonstrates that 24-hour urinary 8-OHdG excretion levels tended to be associated with the risk of CVD incidence among women. The reason for this sex difference remains unclear. Further large, prospective studies are warranted.

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The CIRCS Investigators are: Takeo Okada, Yuji Shimizu, Yasuhiko Kubota, Shinichi Sato, Mina Hayama-Terada and Masahiko Kiyama, Osaka Center for Cancer and Cardiovascular Disease Prevention; Hironori Imano, Renzhe Cui, Isao Muraki, Akihiko Kitamura, Hiroshige Jinnouchi, Mizuki Sata and Hiroyasu Iso, Osaka University; Kazumasa Yamagishi, Mitsumasa Umesawa and Tomoko Sankai, University of Tsukuba; Koutatsu Maruyama, Ehime University; Ai Ikeda and Takeshi Tanigawa, Juntendo University, and Masanori Nagao and Tetsuya Ohira, Fukushima Medical University.

## Conflicts of Interest

M Nagao, G Kobashi, M Umesawa, R Cui, K Yamagishi, H Imano, T Okada, M Kiyama, A Kitamura, T Sairenchi, Y Haruyama, T Ohira, and H Iso have no conflicts of interest.

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