Effect of age, smoking and other lifestyle factors on urinary 7-methylguanine and 8-hydroxydeoxyguanosine

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Urinary 8-hydroxydeoxyguanosine (8-OH-dG) and 7-methylguanine (m^7Gua) were measured by a column-switching high performance liquid chromatography method as markers of oxidative and methylating DNA damage, respectively. We investigated the associations between urinary 8-OH-dG or m^7Gua and various lifestyle and demographic factors, such as age and sex. The urinary 8-OH-dG excretion level was positively correlated with cigarette smoking, but inversely correlated with fruit consumption, physical activity and total energy consumed per day. A multiple regression analysis revealed that daily physical activity and healthy meal combinations decreased the urinary 8-OH-dG level, whereas alcohol consumption increased it. In terms of the urinary m^7Gua measurement, cigarette smoking, age and consumption of meat, fish, egg, soybean, etc. were positively correlated with the urinary m^7Gua level, whereas body weight, BMI, physical activity, and dietary index score, which indicates good nutritional balance, were negatively correlated with the amount of m^7Gua. Based on a multiple regression analysis, cigarette smoking and age correlated with the m^7Gua level, while high BMI and healthy meal combinations have significant reducing effects on m^7Gua level. Therefore, the urinary m^7Gua level is considered to be a useful marker of DNA methylation, not only from smoking, but also from aging and unhealthy dietary habits. (*Cancer Sci* 2009; 100: 715–721)

Oxygen radicals are formed in cells by oxygen metabolism and various environmental agents, and they damage DNA, RNA, and proteins.[1] Among the many types of oxidative DNA damage, 8-hydroxydeoxyguanosine (8-OH-dG) is a major product and is frequently analyzed as a marker of cellular oxidative stress related to carcinogenesis,[2,3] because 8-OH-dG induces mutations,[4,5] is excreted in the urine, and it has been analyzed by high performance liquid chromatography-electrochemical detection (HPLC-ECD),[6,7] liquid chromatography-tandem mass spectrometry (LC-MS),[8] gas chromatography-mass spectrometry (GC-MS),[9] and enzyme linked immunosorbent assay (ELISA).[10] However, the reproducibility and accuracy of its measurement are much higher with the HPLC-ECD and LC-MS/MS methods, as compared to the ELISA method.[11,12] We have reported that higher 8-OH-dG levels were observed in the lung DNA of smokers,[13] the liver DNA of chronic hepatitis patients,[14] and in the stomach DNA of patients infected with *Helicobacter pylori*.[15] It has also been reported that the urinary 8-OH-dG level is higher in cancer patients than in healthy people,[16] higher in smokers than in non-smokers,[17] and lower in people who exercise moderately.[17] In addition, the urinary 8-OH-dG level was higher in men than in women,[7] and it negatively correlated to body mass index (BMI).[7] As an explanation for the relationship between a lean BMI and high urinary 8-OH-dG excretion, it has been suggested that lean persons have a higher metabolic rate than obese persons,[18] and therefore have higher oxidative stress. Thus, various factors affect the 8-OH-dG levels in humans.

On the other hand, 7-methylguanine (m^7Gua) is a biomarker of DNA damage induced by methylating agents. m^7Gua may serve as a good biomarker of DNA damage caused by nitrosamines in tobacco smoke,[19] and other environmental methylating agents, such as methyl bromide.[20] It is also possible that m^7Gua is formed in cellular DNA by an endogenous methylating agent, S-adenosylmethionine.[21] m^7Gua is also a degradation product from RNA,[22,23] and is known as a metabolic rate marker. Urinary m^7Gua was measured by several researchers,[24] as a product of DNA damage. For instance, the amount of m^7Gua excreted in the urine is increased after exposure to methylating agents in laboratory animals.[25,26] Higher levels of m^7Gua excretion have been reported among patients with colon cancer,[27] although not in patients with gastric cancer.[28] In particular, the urinary excretion of m^7Gua has been shown to be higher among smokers than non-smokers.[29]

Therefore, urinary 8-OH-dG and m^7Gua seem to be useful biomarkers of DNA damage caused by oxidation and methylation, respectively. Measuring the two markers may be very meaningful, because the mechanisms of mutagenesis and carcinogenesis due to DNA oxidation and methylation are different. Therefore, it would be beneficial if the amounts of 8-OH-dG and m^7Gua in human urine could be analyzed simultaneously. Recently, we developed a new HPLC method to analyze 8-OH-dG and m^7Gua simultaneously, based on an anion exchange and reverse phase column-switching system.[30] This HPLC method was further modified to measure 8-OH-dG and m^7Gua in not only human urine samples, but also those from rat and mouse.[30] In this study, with this new HPLC method, we examined the influence of various lifestyle factors on the levels of urinary 8-OH-dG and urinary m^7Gua among a sample of 361 Japanese healthy male employees.

Materials and Methods

Urine collection and questionnaire investigation. After informed consent was obtained, urine samples were collected from 578 healthy employees in a steel-manufacturing company. At the same time, each individual's information on age, height and weight (for BMI), sex, status of cigarette smoking and alcohol drinking, status of dietary habits (for dietary score), status of rest (for rest score), and status of daily physical activity was obtained through a questionnaire. However, in the present study

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Table 1. The characteristics of categorical lifestyle factors and urinary 8-hydroxydeoxyguanosine (8-OH-dG) levels and urinary 7-methylguanine (m^7Gua) levels in 361 male subjects

Variables	Category	*n*	%	Urinary markers†			
				8-OH-dG	*P**	m^7Gua	*P**
Sleep	Deficient	10	2.8	4.37 ± 0.25	0.53	8.03 ± 0.47	0.26
	Slightly deficient	181	50.1	4.19 ± 0.12		8.98 ± 0.21	
	Sufficient	170	47.1	4.20 ± 0.11		8.60 ± 0.19	
Holiday	Little or none	2	0.6	5.41 ± 1.20	1.00	9.49 ± 0.12	0.15
	Once a week	51	14.1	3.96 ± 0.21		9.42 ± 0.40	
	Twice a week	308	85.3	4.23 ± 0.08		8.66 ± 0.15	
Fatigue	Always	18	5.0	3.66 ± 0.32	0.86	8.07 ± 0.36	0.37
	Sometimes	256	70.9	4.17 ± 0.09		8.87 ± 0.17	
	Rarely	87	24.1	4.39 ± 0.17		8.62 ± 0.25	
Rhythm	Irregular	66	18.3	4.05 ± 0.19	0.25	8.28 ± 0.27	0.24
	Mostly regular	215	59.6	4.20 ± 0.10		8.87 ± 0.19	
	Regular	80	22.2	4.30 ± 0.16		8.91 ± 0.28	
Refreshing	Difficult	13	3.6	4.40 ± 0.38	0.48	7.85 ± 0.56	0.36
	Moderate	255	70.6	4.13 ± 0.09		8.86 ± 0.17	
	Easy	93	25.8	4.33 ± 0.17		8.67 ± 0.23	
Size of a meal	Full stomach every time	24	6.6	3.87 ± 0.27	0.85	8.84 ± 0.55	0.55
	No pattern	198	54.8	4.29 ± 0.11		8.90 ± 0.20	
	Moderation every time	139	38.5	4.12 ± 0.11		8.58 ± 0.20	
Healthy	Rerely	46	12.7	4.25 ± 0.20	0.11	8.57 ± 0.37	0.72
Meal	Consider sometimes	187	51.8	4.33 ± 0.12		8.87 ± 0.20	
Combination	Consider every time	128	35.5	3.99 ± 0.11		8.70 ± 0.23	
Skipping meals	One meal every day	63	17.5	4.37 ± 0.20	0.62	9.32 ± 0.39	0.10
	2 or 3 meals a week	103	28.5	4.09 ± 0.15		8.88 ± 0.28	
	Rarely	195	54.0	4.20 ± 0.10		8.53 ± 0.16	
Light-colored	Rarely	17	4.7	4.32 ± 0.42	0.78	9.30 ± 0.94	0.35
Vegetable	Once a day	268	74.2	4.22 ± 0.09		8.83 ± 0.16	
	Each meal	76	21.1	4.09 ± 0.14		8.43 ± 0.29	
Green- and yellow-collored vegetables	Rarely	26	7.2	4.10 ± 0.32	0.75	9.93 ± 0.68	0.06
	2 or 3 times a week	244	67.6	4.21 ± 0.10		8.74 ± 0.16	
	Everyday	91	25.2	4.18 ± 0.12		8.53 ± 0.25	
Fruit	Rarely	140	38.8	4.24 ± 0.12	*0.03*	8.54 ± 0.22	0.06
	2 or 3 times a week	187	51.8	4.28 ± 0.11		9.07 ± 0.20	
	Everyday	34	9.4	3.57 ± 0.19		8.10 ± 0.36	
Meat, fish, egg, etc.	Rarely	11	3.0	4.72 ± 0.49	0.16	8.15 ± 0.55	*0.05*
	Twice a day	194	53.7	4.25 ± 0.11		9.08 ± 0.19	
	Each meal	156	43.2	4.09 ± 0.11		8.43 ± 0.21	
Milk	Rarely	118	32.7	4.32 ± 0.16	0.23	8.86 ± 0.24	0.68
	2 or 3 times a week	171	47.4	4.10 ± 0.10		8.82 ± 0.21	
	Everyday	72	19.9	4.23 ± 0.15		8.53 ± 0.29	
Oil	Rarely	9	2.5	4.41 ± 0.58	0.55	9.30 ± 1.20	0.26
	2 or 3 times a week	192	53.2	4.15 ± 0.12		8.95 ± 0.19	
	Eeveryday	160	44.3	4.24 ± 0.10		8.52 ± 0.20	
Seaweed	Rarely	58	16.1	3.99 ± 0.18	0.75	8.42 ± 0.34	0.50
	2 or 3 times a week	266	73.7	4.26 ± 0.09		8.86 ± 0.16	
	Everyday	37	10.2	4.04 ± 0.21		8.69 ± 0.49	
Physical	Light	192	53.2	4.39 ± 0.11	*0.03*	8.71 ± 0.17	0.35
activity-1	Moderate	119	33.0	4.09 ± 0.12		9.07 ± 0.28	
	Moderately heavy	12	3.3	3.85 ± 0.48		8.23 ± 0.67	
	Heavy	38	10.5	3.68 ± 0.19		8.32 ± 0.41	

*One-way analysis of variance.
†Data are mean ± SE: 8-OH-dG (μg/g creatinine), m^7Gua (mg/g creatinine).

we only selected the participants who answered all of the items in the questionnaire, to avoid bias as much as possible. Consequently, the data from 361 male subjects (aged 18–59 years, mean 36.3 ± 10.3) were analyzed.

With regard to the questionnaire, the total scores of rest and meals (rest index, dietary index) were expressed as the sum of each score. For example, the rest index score is the sum of the scores (1–3) of sleeping hours, frequency of holidays, feeling of fatigue, rhythm of daily life, and ability to refresh (Table 1). Therefore, a low rest index score means insufficient rest, whereas a high score shows sufficient rest status. Similarly, the status of the dietary habits (dietary index score) is the total score of 10 items consisting of meal size, healthy combinations of meals, frequency of skipping meals, intake of light-colored vegetables, green- and yellow-colored vegetables, fruits, milk, edible oil, seaweed, and intake of meat, fish, egg, soybean, etc. Consequently, a

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Table 2. Association of 8-hydroxydeoxyguanosine (8-OH-dG) and 7-methylguanine (m^7Gua) with continuous variables

Variables	Mean ± SE	Min	Max	Correlation coefficient			
				8-OH-dG	*P*	m^7Gua	*P*
Age	36.28 ± 0.54	18	59	–0.014	0.698	0.190	*<0.001*
Weight	67.14 ± 0.52	45.6	104	–0.033	0.343	–0.094	*0.008*
BMI	22.63 ± 0.16	15.4	34.0	–0.065	0.066	–0.078	*0.028*
Energy consumed	2487.10 ± 21.72	1892	3916	–0.069	*0.049*	–0.086	*0.015*
Physical activity-2	82.92 ± 5.90	3	1207	0.040	0.258	–0.057	0.11
Alcohol drinking	0.89 ± 0.05	0	4.3	0.065	0.073	0.047	0.191
Smoking	15.50 ± 0.57	0	40	0.088	*0.023*	0.247	*<0.001*
Brinkman index	259.79 ± 14.45	0	1520	0.082	*0.024*	0.278	*<0.001*
Rest index score	11.75 ± 0.09	6	15	0.069	0.071	–0.005	0.902
Dietary index score	21.56 ± 0.16	13	30	–0.011	0.757	–0.077	*0.036*

high dietary index score means good nutritional balance. Physical activity was calculated by two different methods (physical activity-1, -2), based on resting metabolic rate (RMR) and physical activity by commuting, working and sports, etc. Namely, 'physical activity-1' was calculated by the ratio of (physical activity by commuting, working and sports/RMR) and was categorized into four groups (scores from 1 to 4). A higher value means high physical activity. 'Physical activity-2' was calculated by physical activity due to commuting and sports, age, sex and weight and was expressed by kCal/day. Total energy consumed per day was calculated from height, age, sex and physical activity by commuting, working and sports, and was expressed by kCal/day. As continuous variables, age, weight, BMI, total energy consumed (KCal/day), physical activity-2 (kCal/day), alcohol drinking (number of glasses drunk per day: converted to Japanese sake), cigarette smoking (number of cigarettes smoked per day) and Brinkman index obtained through the questionnaire were used.

Analysis of m^7Gua, 8-OH-dG and creatinine. Urinary m^7Gua and 8-OH-dG were determined by the method previously described,[31] Briefly, a human urine sample was mixed with the same volume of a dilution solution containing the ribonucleoside marker, 8-hydroxyguanosine. A 20-μL aliquot of the diluted urine sample was injected into HPLC-1 (MCI GEL CA08F, 7 μm, 1.5 × 120 mm; elution, 2% acetonitrile in 0.3 mM sulfuric acid, 50 μL/min, 65°C), via the guard column (1.5 × 40 mm), and the chromatograms were recorded by a Gilson UV detector (UV/VIS-155 with 0.2 mm light path cell). Creatinine and m^7Gua were detected at 245 and 305 nm, respectively. The 8-OH-dG fraction was collected, depending on the relative elution position from the peak of the added marker, 8-OH-G, and was automatically injected into the HPLC-2 column. The 8-OH-dG fraction was fractionated by the HPLC-2 column (Shiseido, Capcell Pak C18, 5 μm, 4.6 × 250 mm; elution, 10 mM sodium phosphate buffer [pH 6.7] containing 5% methanol and an antiseptic Reagent MB [100 μL/L], 1 mL/min, 40°C). The 8-OH-dG was detected by a Coulochem II EC detector (ESA Inc., Chemsford, MA, USA) with a guard cell (5020) and an analytical cell (5011) (applied voltage: guard cell, 350 mV; E1, 170 mV; E2, 300 mV).

Statistics. The relationships between the urinary 8-OH-dG levels and the urinary m^7Gua levels with categorical and continuous variables were analyzed by using oneway analysis of variance (ANOVA) and Kendall's rank correlation coefficients, respectively. In addition to the ANOVA analysis, multiple comparisons between groups were conducted with Scheffe's test. Since the distributions of 8-OH-dG and m^7Gua were skewed, the log-transformed values of 8-OH-dG and m^7Gua, which showed normal distributions, were used in the multiple regression analysis. *P*-values less than 0.05 (two-tailed) were considered to indicate significant differences. All data were analyzed using the SPSS statistical package (SPSS, Chicago, IL, USA) for Windows 14.0.

Results

The mean level of urinary 8-OH-dG (μg/g creatinine) in the 361 male subjects was 4.20 ± 1.47 (SD). A 19.4-fold interindividual variation was found (0.53–10.28 μg/g creatinine). The mean level of m^7Gua normalized to creatinine (mg/g creatinine) was 8.77 ± 2.61 (SD), and a 4.80-fold interindividual variation was found (3.94–18.93 mg/g creatinine). The relationships between the 16 categorical lifestyle factors and the urinary 8-OH-dG level or the urinary m^7Gua level are shown in Table 1. The ANOVA analysis revealed that the urinary 8-OH-dG level was significantly negatively related to fruit consumption ($P = 0.03$) and physical activity-1 ($P = 0.03$). It is noteworthy that the urinary 8-OH-dG levels of the 'rarely' and 'two or three times per week' groups were significantly higher than that of the 'everyday' group ($P = 0.03$) in the fruits item. The results of the Scheffe's test also indicated that fruit consumption significantly reduced the urinary 8-OH-dG level. On the other hand, only the intake of meat, fish, egg, soybean, etc. significantly influenced the m^7Gua excretion ($P < 0.05$). Although the Scheffe's test was conducted to facilitate multiple comparisons of the urinary m^7Gua levels between the groups, no significant differences were observed in all categorical variables.

Table 2 shows the correlations of the continuous variables to the 8-OH-dG (μg/g creatinine) and m^7Gua (mg/g creatinine) levels. Significant positive correlations were observed between the urinary 8-OH-dG level and the average number of cigarettes smoked per day ($r = 0.088$, $P = 0.023$) and the Brinkman index ($r = 0.082$, $P = 0.024$), whereas there were significant inverse correlations between the urinary 8-OH-dG level and physical activity-2 ($r = -0.069$, $P = 0.049$). In contrast, more factors affect the m^7Gua levels. Namely, significant positive correlations were observed between the urinary m^7Gua level and age ($r = 0.190$, $P < 0.001$), the average number of cigarettes smoked per day ($r = 0.247$, $P < 0.001$) and the Brinkman index ($r = 0.278$, $P < 0.001$), whereas significant inverse correlations were observed between the urinary m^7Gua level and weight ($r = -0.094$, $P = 0.008$), BMI ($r = -0.078$, $P = 0.028$), total energy consumed ($r = -0.086$, $P = 0.015$), and the total score obtained from the meal index ($r = -0.077$, $P = 0.036$). In particular, the relationships of age, cigarettes smoked per day and Brinkman index with urinary m^7Gua excretion were remarkable, as shown in Figs 1, 2 and 3, respectively.

The results of the multiple regression analysis of 8-OH-dG by the stepwise method in the 361 male subjects are shown in Table 3. Due to the significant correlation between fatigue and rest score ($r = -0.728$, $P < 0.001$), the fatigue item was not used in the analysis, to avoid collinearity. Similarly, there was a significant correlation between cigarettes smoked per day and Brinkman index ($r = 0.725$, $P < 0.001$), so the Brinkman index item was not included in the analysis. Accordingly, the following

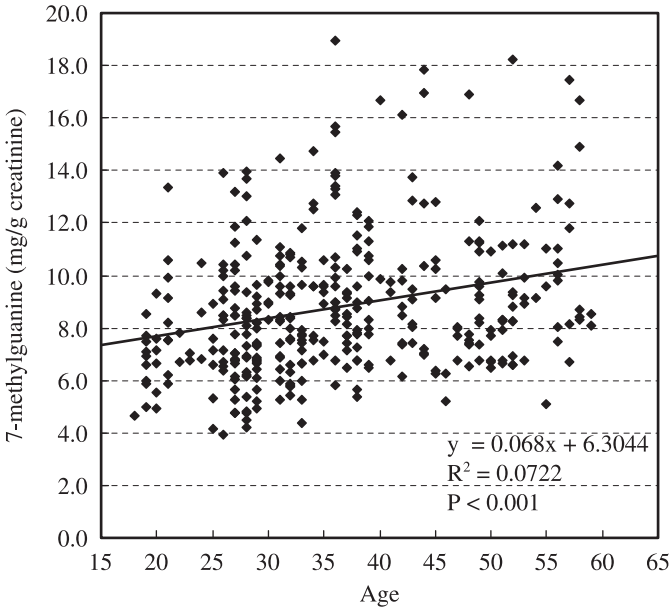

Fig. 1. Association between age and urinary 7-methylguanine level.

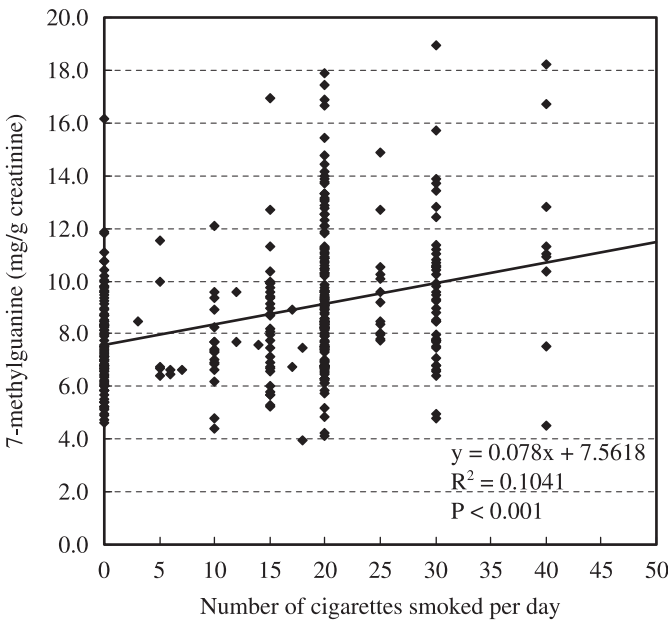

Fig. 2. Association between cigarettes smoked per day and urinary 7-methylguanine level.

24 items were used in the analysis as the independent variables for the subjects with complete data: 15 categorical variables consisting of sleep, holiday, rhythm of daily life, ability to refresh, size of a meal, healthy meal combination, frequency of skipping meals, consumption of light-colored vegetables, green- and yellow-colored vegetables, fruit, meat, milk, oil, seaweed, and physical activity-1, and nine continuous variables consisting of age, weight, BMI, energy consumption, total energy consumed, alcohol drinking, cigarette smoking, rest index score, and dietary index score. The results of the multiple regression analysis by the stepwise method indicated that physical activity-1 and healthy meal combination decreased the urinary 8-OH-dG level, whereas alcohol drinking significantly increased it. The consumption of meat, fish, egg, soybean, etc. showed a tendency to reduce the 8-OH-dG level, and the intakes of green- and yellow-colored vegetables showed a tendency of increasing it. These five independent factors obtained from the multiple regression analysis (Table 3) explain only 5.6% of the total variance. On the other hand, the total energy consumed, cigarette smoking, and BMI were not correlated with the urinary 8-OH-dG level.

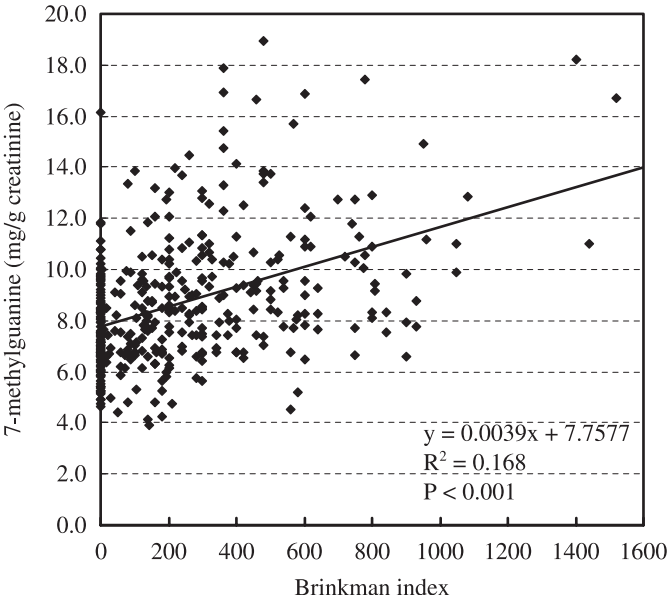

Fig. 3. Association between Brinkman index and the urinary 7-methylguanine level.

Table 4 shows the results of the multiple regression analysis using m^7Gua as the dependent variable. The 24 items as above were used in the regression analysis as the independent variables. As a result, cigarette smoking and age were significantly correlated to the urinary m^7Gua level, whereas high BMI and dietary index score (healthy meal style) were negatively correlated to it. These four independent factors obtained from the multiple regression analysis explain 19.6% of the entire variation.

Discussion

In this article, we analyzed how the urinary 8-OH-dG and m^7Gua levels are related to various lifestyle factors. In the univariate analysis of the urinary 8-OH-dG level by the lifestyle and demographic variables, we found a decrease in the urinary 8-OH-dG level with fruit consumption and daily physical activity. Many studies have shown significant relationships between the dietary consumption of fruits and vegetables and the low urinary excretion of 8-OH-dG,[7,32,33] although other studies found no associations between fruits and vegetables and 8-OH-dG.[34,35]

According to Kendall's rank correlation coefficients (Table 2), the urinary 8-OH-dG level was inversely correlated with the total energy consumed. On the other hand, factors positively related to the urinary 8-OH-dG level were cigarettes smoked per day and Brinkman index. Significant relationships between the urinary 8-OH-dG level and cigarette smoking have been observed not only in urine,[17] but also in leukocytes,[36] and lung tissue,[13] However, alcohol consumption was not significantly correlated to urinary 8-OH-dG excretion. Similarly, we did not obtain a significant association between the urinary 8-OH-dG level and the rest index, while good correlations were reported between the urinary 8-OH-dG level and the average number of working hours per day,[37] and the working conditions.[17]

In our previous work,[17,37] and the report by Loft *et al.*[7] there were significant negative correlations between 8-OH-dG and

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Table 3. Multiple regression analysis of log (8-OH-dG) against related factors in 361 male subjects

Independent variables Male subjects ($n = 361$, $R^2 = 0.056$)	Partial *r*	SE	Beta	*P*
Physical activity-1	–0.065	0.020	–0.169	*0.001*
Alcohol drinking	0.060	0.020	0.156	*0.003*
Healthy meal combination	–0.065	0.030	–0.117	*0.034*
Intakes of meat, fish, egg, soybean, etc.	–0.066	0.035	–0.100	0.064
Green- and yellow-colored vegetable consumption	0.680	0.038	0.101	0.076

8-OH-dG, 8-hydroxydeoxyguanosine. Note: Statistical analysis was conducted by a stepwise multiple regression analysis. Partial *r* indicates partial regression coefficient. Beta indicates standardized partial regression coefficient.

Table 4. Multiple regression analysis of log (m^7Gua) against related factors in 361 male subjects

Independent variables Male subjects ($n = 361$, $R^2 = 0.196$)	Partial *r*	SE	Beta	*P*
Smoking	0.070	0.001	0.281	*<0.001*
Age	0.080	0.001	0.281	*<0.001*
BMI	–0.012	0.005	–0.125	*0.010*
Dietary index score	–0.010	0.005	–0.113	*0.026*
Frequency of holiday	–0.069	0.036	–0.092	0.058

m^7Gua, 7-methylguanine. Note: Statistical analysis was conducted by a stepwise multiple regression analysis. Partial *r* indicates partial regression coefficient. Beta indicates standardized partial regression coefficient.

BMI. In the present study, the same tendency was observed in the univariate analysis ($r = -0.065$, $P = 0.066$) (Table 2), but a significant association was not observed in the multiple regression analysis. This discrepancy may be due to differences in statistical calculation methods and in other lifestyle and demographic factors between the present and previous studies. Our present results are consistent with those reported by Pilger *et al*.[38]

In the multiple regression analysis (Table 3), physical activity-1, which includes physical activity by working, showed a strong negative correlation to the urinary 8-OH-dG. This is in good agreement with our previous findings that physical exercise reduced the 8-OH-dG levels in rat organs (liver, lung and heart),[39] human urine,[17] and human leukocyte,[40] although high-intensity exercise has been shown to increase 8-OH-dG excretion.[41–43] Alcohol drinking also correlated with the 8-OH-dG level in the multiple regression analysis. Many studies have shown a significant relationship between alcohol consumption and 8-OH-dG generation in peripheral leukocytes,[44,45] esophageal tissues,[46] liver,[47] and urine.[48,49] Cigarette smoking was not related to the urinary 8-OH-dG level, whereas it was correlated with the urinary 8-OH-dG level, according to the calculation with continuous variables (Kendall's correlation coefficients, Table 2). The discrepancy between the current results and those from other investigations can be explained by variations in sample size, sample composition, methods of urinary 8-OH-dG measurement and statistical methods.

In terms of urinary m^7Gua measurement results, the categorical lifestyle item related to the elevation of urinary m^7Gua excretion was the intake of meat, fish and other protein-rich foods. It is possible that N-nitroso compounds that methylate DNA are produced by the consumption of these foods.[50,51]

In the analysis of continuous variables, age, cigarette smoking and Brinkman index were positively correlated with the urinary m^7Gua level, whereas weight, BMI, total energy consumed, and total meal index score were negatively correlated to the amount of m^7Gua. Particularly, the multiple regression analysis showed that cigarette smoking, age, BMI and meal index score were related to the urinary amount of m^7Gua. These factors explain 19.6% of the entire variation. The inverse correlation between m^7Gua and BMI can be explained by the fact that m^7Gua is a marker of the metabolic rate, and it is lower in people with a high BMI, mainly due to the lower physiological production of heat to maintain body temperature.[52]

Previous studies have shown the strong link between cigarette smoking and the urinary m^7Gua level. For instance, methylated DNA adducts were detected in animal and human tissues, as a result of exposure to tobacco smoke.[19,53] In other studies, the urinary excretion of m^7Gua was shown to be higher in smokers than in non-smokers.[29] Furthermore, the m^7Gua level in human urine decreased after smoking cessation.[54] Therefore, our results confirmed those of previous studies. Moreover, considering that tobacco-specific nitrosamines are a group of carcinogens present in tobacco smoke,[55] the urinary m^7Gua level can be analyzed to monitor DNA methylation and to assess the risk of lung cancers. The measurment of urinary m^7Gua levels would be useful not only to assess the harmful effects of smoking, but also the effects of environmental tobacco smoke.

The present analyses revealed that the urinary m^7Gua level was linked to age, food-related items, such as meat intake, weight, BMI, total energy consumed, and the meal index score. With respect to the effect of age, m^7Gua may be increased due to lower glutathione (GSH) concentration in aged people,[56] because GSH may be involved in scavenging alkylating agents. Ames and collaborators[57] reported that the m^7Gua levels in rat liver DNA were increased 2.5-fold in old rats (24 months old) as compared to the levels in young rats (6 months old). Our results are compatible with their data. It has been argued that age can affect the overall DNA repair capacity. Thus, the amount of m^7Gua in DNA could reflect a balance between methylating stress and DNA repair activity. However, urinary m^7Gua may be related to the total amount of m^7Gua released from DNA, by repair and by spontaneous depurination due to the labile glycosylic bond.

In our study, the creatinine value was used to normalize the urinary m^7Gua level. Urinary creatinine excretion is influenced by muscle mass. This may be a possible explanation for the higher levels of m^7Gua normalized to creatinine with increasing age. To clarify this point, we conducted a correlation analysis between creatinine and age. Although significant associations were obtained not only between age and m^7Gua, but also between age and creatinine, the association between age and m^7Gua ($r^2 = 0.08$) was stronger than the association between age and creatinine ($r^2 = 0.01$). Considering statistical values (r^2), we decided that the present results are not remarkably influenced by the association between age and creatinine.

We also found a significant correlation between the 8-OH-dG and m^7Gua concentrations when the Pearson's correlation coefficient was calculated ($r = 0.122$, $r^2 = 0.015$, $P = 0.02$). This may be explained by the fact that some factors, such as energy consumed, physical activity and smoking, have similar effects on the 8-OH-dG and m^7Gua levels (Table 2). However, the

coefficient of determination (r^2) explains only 1.5% of the entire variation. Therefore, 8-OH-dG and m^7Gua can be considered as independent markers affected by many factors.

The present study suggested that the amount of m^7Gua excreted in the urine is a very sensitive marker in response to aging and lifestyle, such as smoking or dietary habits. Lifestyle has a more significant effect on urinary m^7Gua than 8-OH-dG, based on all statistical analyses. The urinary excretion of m^7Gua has not been extensively investigated as a biomarker,[58] except for the influences of smoking.[29,54] In this study, urine samples from male working subjects at a specific company were analyzed. In the future, in order to prove the usefulness of urinary m^7Gua as a biomarker, we should confirm the reliability and validity of the present findings, according to appropriately designed large-scale studies.

We demonstrated that urinary m^7Gua is a useful biomarker for DNA methylation in humans, in addition to 8-OH-dG, a form of oxidative DNA damage. The urinary m^7Gua excretion value can be a useful marker not only for the assessment of lung cancer risk, but also for evaluating the aging process and various lifestyles.

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