Obesity has been admitted as a risk factor of cancer (1); however, there is a paucity of biological evidence supporting this relation. Subjects analyzed were 177 healthy Japanese workers who participated in a lifestyle intervention study. The levels of urinary 8-hydroxydeoxyguanosine (8-OHdG), a marker of oxidative DNA damage, were measured using an automated high-pressure liquid chromatography and urinary creatinine levels were adjusted for before statistical analysis. A clear inverse association was found between body mass index (BMI) and 8-OHdG levels among smokers [Pearson correlation coefficient ($r = -0.48$), and the association did not materially change after adjustment for potential confounding factors. In contrast, no apparent relation was observed between BMI and 8-OHdG levels among nonsmokers ($r = -0.12$), although lean nonsmokers had a slightly higher mean of 8-OHdG levels compared with nonlean nonsmokers. The interaction of smoking and BMI reached statistical significance ($P = 0.04$). Leanness may enhance oxidative DNA damage induced by smoking and thus serve as a marker of host susceptibility to smoking-related cancers. (Cancer Epidemiol Biomarkers Prev 2006;15(3):582–5)

**Leanness, Smoking, and Enhanced Oxidative DNA Damage**

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**Abstract**

An increased risk of some forms of cancer, including lung cancer, among lean individuals has been consistent; however, there is a paucity of biological evidence supporting this relation. Subjects analyzed were 177 healthy Japanese workers who participated in a lifestyle intervention study. The levels of urinary 8-hydroxydeoxyguanosine (8-OHdG), a marker of oxidative DNA damage, were measured using an automated high-pressure liquid chromatography and urinary creatinine levels were adjusted for before statistical analysis. A clear inverse association was found between body mass index (BMI) and 8-OHdG levels among smokers [Pearson correlation coefficient ($r = -0.48$), and the association did not materially change after adjustment for potential confounding factors. In contrast, no apparent relation was observed between BMI and 8-OHdG levels among nonsmokers ($r = -0.12$), although lean nonsmokers had a slightly higher mean of 8-OHdG levels compared with nonlean nonsmokers. The interaction of smoking and BMI reached statistical significance ($P = 0.04$). Leanness may enhance oxidative DNA damage induced by smoking and thus serve as a marker of host susceptibility to smoking-related cancers. (Cancer Epidemiol Biomarkers Prev 2006;15(3):582–5)

**Introduction**

Obesity has been admitted as a risk factor of cancer (1); however, little attention has been paid to the role of leanness in carcinogenesis. Epidemiologic studies have shown an inverse association between body mass index (BMI) and total cancer risk (2) or the risk of several cancer forms, including cancer of the lungs (3) and esophagus (4). Several studies (2-4) reported a stronger association among smokers than among nonsmokers. Leanness is thus hypothesized to represent a host susceptibility to smoking-related cancers. However, controversy continues regarding causal role of leanness in carcinogenesis due to a potential bias in epidemiologic studies (5). For instance, the effect of preclinical cancer on weight loss cannot be completely ruled out. Moreover, because smoking is related to lower BMI levels (6), the inverse association between BMI and cancer risk may merely reflect smoking-induced weight loss.

Oxidative DNA stress is thought to play a major role in carcinogenesis (7), and increased levels of 8-hydroxydeoxyguanosine (8-OHdG), a reliable marker of oxidative DNA damage, have been detected in urine of smokers (8, 9) or in lung cancer tissue (10). However, the association between BMI and urinary 8-OHdG levels has been inconsistent; two studies (8, 9) reported an inverse association, whereas others (11, 12) failed to detect such association. Moreover, there is limited evidence suggesting a modifying effect of smoking on BMI and 8-OHdG levels (8). We therefore investigated whether leanness modulates the relation of smoking to oxidative DNA damage among healthy working employees, using an automated high-pressure liquid chromatography (HPLC; ref. 13).

**Materials and Methods**

Data were obtained from the baseline survey of a worksite lifestyle intervention study, in which 179 volunteers ages 28 to 57 years of a Japanese city office participated. A written informed consent was obtained. The study protocol has been approved by the ethics committee of Kyushu University.

Health-related lifestyles were ascertained using a detailed questionnaire. Ever smokers were defined as those who smoked 100 cigarettes or more in their lifetime. Current smokers consuming cigarettes on a daily basis were asked about cigarette consumption a day, whereas current smokers consuming less than daily basis were defined as occasional smokers. Regular alcohol drinkers were defined as those who consumed alcohol beverage on a weekly basis over the recent 1-month period, and they were asked about the frequency and quantity per occasion of consumption for each type of five alcohol beverages—shochu, beer, sake, wine, and whisky/liquor. Those who engaged in any leisure-time physical activities during the past month were asked about the names, frequencies, and minutes or hours engaged per occasion of each activity.

Casual urine samples, collected mostly between 5 to 6 p.m. before super, were kept in tubes stored in a cooler box overnight and then frozen at −80°C until analysis. Urinary samples were analyzed for 8-OHdG using an automated HPLC system composed of two columns and an electrochemical detector (13). In short, the urinary 8-OHdG level was determined using an apparatus in which the pump 1 (Shiseido Nanospace SI-2), the sampling injector (Gilson 231XL), the guard column for the HPLC-1 (valve 1, pump 3), the HPLC-1 column, the UV detector (Toso UV-8020, microcell), the HPLC-2 column (valve 2, loop, pump 2), and the EC detector (ESA Coulochem 2) were connected. Urine samples were defrosted and 50 μL of each was mixed with the same volume of a dilution solution containing the ribonucleoside marker 8-hydroxyguanosine (120 μg/mL) and 4% acetonitrile in a solution of 130 mmol/L sodium acetate (pH 4.5) and 0.6 mmol/L H2SO4. The urine solutions were centrifuged at 13,000 rpm for 5 minutes. A 20 μL aliquot of each supernatant was injected into the first HPLC (MCI...
GEL CA08F, 7 μm, 1.5 × 150 mm, 2% acetonitrile in 0.3 mmol/L sulfuric acid, 37 μL/min from the sampling injector, via the guard column, and the chromatogram was recorded by the UV detector (254 nm). A aliquot of the fraction containing 8-OHdG was automatically injected into the second HPLC column [Shiseido, Capcell Park C18, 5 μm, 4.6 × 250 mm, 10 mmol/L sodium phosphate buffer (pH 6.7), 5% methanol, plus an antiseptic Reagent MB (100 μL/L), 1 mL/min]. Finally, the 8-OHdG was detected by an EC detector with a guard cell (5020) and an analytic cell (5011). The accuracy of the measurement, estimated from the recovery of an added 8-OHdG standard, was 90% to 98%. When the same urine sample was analyzed thrice, the variation of the data was within 7%. Urinary creatinine levels of the same urine sample were simultaneously measured by using anion exchange chromatography in the HPLC-1 step.

8-OHdG levels were adjusted for urinary creatinine levels and then log-transformed before analysis. Subjects were divided into either nonsmokers, including past smokers, or current smokers, including occasional smokers. Weight was measured in a light cloth and information about height was obtained from record of the latest checkup. BMI was calculated as body weight in kilograms divided by the square of height in meters. Ethanol consumption was estimated by multiplying the frequency of consumption and amount consumed per occasion for each of five alcohol beverages and summed. Intensity of each leisure-time physical activity was determined in terms of metabolic equivalent (MET) according to the literature (14). MET hours per week for a specific activity was calculated by multiplying weekly hours spent in that activity and the corresponding intensity, and weekly MET hours of total activity was estimated by summing the MET hours for each type of activity. The association between BMI and 8-OHdG levels was assessed by Pearson correlation coefficient (r). Multiple regression and analysis of covariance were used to estimate regression coefficients and means, respectively, while adjusting for sex, age (continuous), alcohol consumption (<3.0, 3.0-22.9, or ≥23.0 g/d), and physical activities (<1.0, 1.0-9.9, or ≥10.0 MET-h/wk). Effect modification was tested by adding a cross-product term of smoking status and BMI (continuous) in the model. Preliminary analysis indicated that data for two smokers who had extremely high BMI (over the mean value plus 3 SD) were outliers, and thus these were excluded. All statistical tests were two-tailed and were considered to be statistically significant at the 0.05 level. All analyses were done with SAS (15).

Results

Characteristics of the study subjects were shown in Table 1. Of 177 subjects, 38 (21%) were female and 49 (28%) were current smokers. Only three women were current smokers, and they were all occasional smokers. Among men, means of BMI were 24.7 and 24.3 kg/m² for smokers and nonsmokers, respectively. BMI was not significantly associated with the number of cigarettes smoked among daily smokers (r = 0.11). The levels of 8-OHdG ranged from 1.2 to 11.4 μg/g creatinine, with median of 3.9 μg/g creatinine. The geometric means of 8-OHdG levels were 3.70, 4.50, and 4.52 μg/g creatinine for nonsmokers, occasional smokers, and daily smokers, respectively. A weak positive association was observed among daily smokers between the number of cigarettes smoked a day and 8-OHdG levels (r = 0.21). Among nonsmokers, geometric means of 8-OHdG levels were 3.68 and 3.74 μg/g creatinine for men and women, respectively.

As shown in Fig. 1, a clear inverse association emerged among 49 current smokers (r = −0.48; P = 0.0004). Adjustment for daily cigarette consumption, with 0.5 assigned to occasional smokers, slightly strengthened the association (partial r = −0.51), whereas analysis excluding occasional smokers somewhat attenuated the association (r = −0.41). Regression coefficient of log-transformed 8-OHdG on BMI (−0.069 μg/g creatinine per unit BMI) did not materially change after adjustment for age, sex, alcohol consumption, and physical activities (−0.070 μg/g creatinine per unit BMI). In contrast, 8-OHdG levels did not apparently correlate with BMI among 128 nonsmokers (r = −0.12; P = 0.18), although a marginally significant inverse correlation was observed among nonsmoking men (r = −0.19; P = 0.06).

Subjects were divided into six groups by smoking status and BMI category (tertiles of BMI among smokers: <23.1, 23.1-25.1, and ≥25.2 kg/m²). Multivariate adjusted mean of 8-OHdG levels was statistically significantly higher among smokers than among nonsmokers in the lowest tertile of BMI (geometric mean: 5.22 μg/g creatinine for smokers versus 4.03 μg/g creatinine for nonsmokers; P = 0.03), whereas 8-OHdG levels did not materially differ according to smoking status in the highest tertile of BMI (geometric mean: 3.56 μg/g creatinine for smokers versus 3.56 for nonsmokers μg/g creatinine; P = 0.90). Among nonsmokers, mean of 8-OHdG levels in the lowest tertile of BMI was slightly higher than those in upper categories of BMI (geometric mean: 4.03, 3.47, and 3.50 μg/g creatinine for the lowest, medium, and highest tertile, respectively). The interaction of smoking and BMI (continuous) reached statistical significance (Pinteraction = 0.04).

Discussion

The source of urinary 8-OHdG may be the hydrolysis of 8-OHdGTP by the nucleotide sanitization enzyme MTH1, the nucleotide excision repair of DNA, and the apoptosis of oxidatively damaged cells (16, 17). Urinary excretion of 8-OHdG is a useful biomarker reflecting general average risk of a promutagenic oxidative adduct in DNA, and thus carcinogenesis of all tissues and organs (16). Using an automated HPLC method, we investigated the association of smoking, BMI, and levels of urinary 8-OHdG among a healthy working population and found a clear inverse association between BMI and urinary 8-OHdG levels among smokers.

Most HPLC methods developed thus far have not been suitable for the analysis of 8-OHdG in epidemiologic studies because of complicated manual procedures involved (reviewed in ref. 13). ELISA method is simple and cost-efficient, but it produced two to four times higher values compared with those obtained using HPLC, probably due to cross-reactions to substances having similar structure to 8-OHdG (18). The method we used is able to analyze large samples with reasonable reproducibility (13). In addition, the urinary 8-OHdG level was unchanged, even when urine samples were kept at room temperature for 24 hours (9).

Table 1. Characteristics of study subjects by gender

<table>
<thead>
<tr>
<th></th>
<th>Men (n = 139)</th>
<th>Women (n = 38)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>42 (7)</td>
<td>39 (8)</td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>33</td>
<td>8</td>
</tr>
<tr>
<td>Occasional smokers</td>
<td>13</td>
<td>100</td>
</tr>
<tr>
<td>No. cigarettes smoked</td>
<td>19 (10)</td>
<td>—</td>
</tr>
<tr>
<td>A day among daily smokers</td>
<td>40</td>
<td>3</td>
</tr>
<tr>
<td>Past smokers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsmokers (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>171 (5)</td>
<td>159 (5)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>71 (9)</td>
<td>81 (7)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.4 (2.8)</td>
<td>21.5 (2.4)</td>
</tr>
</tbody>
</table>

NOTE: Values were mean and standard deviation (in parenthesis) unless otherwise stated.
Of two studies showing an inverse association between BMI and urinary 8-OHdG levels (8, 9), Loft et al. (8) found that the association among smokers was stronger than that among nonsmokers, a finding consistent with ours. The finding suggests that leanness is related to increased oxidative DNA damage, especially among smokers. Furthermore, a pilot investigation among smokers (19) showed that leanness was associated with high DNA adducts levels, an indication for the net outcome of carcinogen exposure, bioactivation, and DNA repair. Findings from these biomarker studies indicate that leanness-cancer association found in numerous epidemiologic studies (2-4) is biologically plausible, not a mere confounding effect.

The mechanism by which leanness enhances smoking-related oxidative DNA stress is not clear. Lean individuals may have low levels of antioxidants due to insufficient nutritional status. However, increased levels of 8-OHdG were not confined to subjects with very low BMI, but 8-OHdG levels constantly decreased as BMI increased up to BMI of 28 kg/m² among smokers (Fig. 1). Therefore, nutritional deficiency is not a plausible explanation. The mechanism of oxidative stress from tobacco smoking may involve not only the presence of ROS and ROS-generating compound in the smoke but also an increased metabolic rate with an increase in mitochondrial production of ROC in the cells (16). Thus, leanness may be related to either or both of these ROC-producing pathways, leading to an enhancement of smoking-induced oxidative stress. A lack of an apparent association between BMI and 8-OHdG levels among nonsmokers suggests that leanness itself may not have significant effect on oxidative DNA damage in the absence of carcinogens. However, because 8-OHdG levels among lean nonsmokers were slightly elevated compared with those among nonlean nonsmokers, we do not deny the possibility that leanness enhances oxidative DNA damage among nonsmokers.

We should discuss methodologic issues. First, the sample size was not large and the majority of smokers were male. However, a study including greater number of smoking women (8) exhibited result similar to ours, indicating the consistency of our finding. Second, there is a concern about the use of creatinine-adjusted 8-OHdG levels in assessing their relation to BMI, because creatinine levels reflect muscle mass and may vary according to sex and age. However, the present study did not include elderly persons and analysis including only men showed similar results. We thus believe that the effect of bias associated with the creatinine adjustment is minimal. Third, because smoking influences body weight (6), whether the magnitude of weight change after smoking initiation or cessation, irrespective of the initial BMI, determines 8-OHdG levels needs to be clarified in a longitudinal study.

In conclusion, the present result of increased 8-OHdG levels among lean smokers provides mechanistic insight into epidemiologic finding of an increased risk of smoking-related cancers associated with low BMI. Leanness may represent decreased biological functions against oxidative DNA stress induced by smoking and thus could be used as a marker of host susceptibility to smoking-related cancer. Because smoking cessation leads to a substantial decline of 8-OHdG levels (20), it is expected that lean smokers may have large health benefit from smoking cessation. It remains uncertain, however, whether leanness itself or factor related to leanness modulates the carcinogenic effect of smoking, and this point deserves further investigations, including a search for genetic profiles regarding metabolism of tobacco smoke or repair of DNA damage.

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References
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