Oxidative DNA damage correlates with oxygen consumption in humans

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ABSTRACT: Generation of reactive oxygen species from mitochondrial respiration has been proposed as an important determinant of longevity and cumulative cancer risk. However, studies correlating mitochondrial respiration with aging-restriction studies of metabolic rate and oxidative DNA damage support this notion. In the present study we have demonstrated a close association between oxidative DNA damage as assessed by the urinary excretion of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) and oxygen consumption in 33 healthy premenopausal women (n = 64; p = 0.0007). In the 12 women who smoked, 8-oxodG excretion was increased by 35%, although oxygen consumption increased only 10% compared with the 21 non-smoking women. Apparently, the rate of oxidative DNA damage relates to mitochondrial respiration in humans and is aggravated by smoking. — Loft, S., Astrup, A., Buemann, B., Poulsem, H. E. Oxidative DNA damage correlates with oxygen consumption in humans. PNAS 91: 534–537; 1994.

Key Words: mitochondrial respiration • 8-oxodG excretion • smoking

Mitochondrial respiration as a source of reactive oxygen species (1) (ROS) with resulting damage to DNA has been proposed as an important determinant of longevity and cumulative cancer risk (2–4). Indeed, oxidative modifications are abundant in mammalian DNA, amounting to 1-25 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) per 10^9 g in normal tissue; higher levels are found with advanced age in malignant tumors, and after treatment with ionizing radiation or chemical carcinogens (2, 3, 5). Besides 8-oxodG, a whole series of oxidative DNA modifications are found in human tissue, in particular from cancers (5, 6). The oxidative hit rate has been estimated to 10^4 to 10^5 DNA bases per cell per day from the urinary excretion of 8-oxodG and similar biomarkers (8–10). The formation of such urinary or tissue biomarkers of oxidative DNA damage correlates with metabolic rate across species (3, 8, 11) and is reduced by calorie restriction (11, 12), supporting a relationship with mitochondrial respiration. However, for the individual variation among humans the relevance of such an association remains to be established. Recently, the urinary excretion of 8-oxodG in humans was shown to be increased in smokers and correlated with body mass index (BMI) and gender, suggesting an association with the metabolic rate (13). In the present study we have examined the relationship between urinary excretion of 8-oxodG and oxygen consumption in 12 smoking and 31 non-smoking, healthy premenopausal women.

MATERIAL AND METHODS

After informed consent and approval from the local ethics committee, 33 healthy premenopausal women (age 35 ± 10 years; BMI 20-25 kg m^-2) were investigated in the follicular phase of the menstrual cycle. Twenty-four-hour oxygen consumption was measured by means of two open-circuit respiratory chambers as previously published along with data on plasma hormones and energy expenditure in some of the subjects (13). The within-subject coefficient of variation for repeated measurements was 2.5%. During the measurements the subjects were kept under continuous surveillance and 24-h urine was collected under metabolic ward conditions. Subjects were allowed to smoke no more than six cigarettes while in the respiratory chamber so as not to contaminate the airflow. For at least 4 days before the experiments the subjects were instructed to be in a weight maintenance, conventional diet supplying 55% of the energy as carbohydrates, 30% as fat, and 15% as protein. At bed times during the measurement the subjects were fed a similar diet with an energy content corresponding to an estimated 24-h energy expenditure computed from lean body mass (LBM) measured by the bioimpedance method using an Anitome (HTS Engineering Inc., Odense, Denmark). The excretion of 8-oxodG in 24 h urine was measured by an automated three-dimensional HPLC method with isocratic separation and electrochemical detection (10). The urine and interday coefficients of variation for the analysis were 8% and 10%, respectively. All samples were analyzed independently on at least two different days and the average value was used for calculation. The concentration of 8-oxodG was constant in urine samples stored at –20°C for as least 25 years. The DNA excretion of 8-oxodG was calculated from its concentration and the volume of urine and expressed per subject, body weight (BW), LBM, and oxygen consumption.

Biostatistical analysis of the effect of smoking on 8-oxodG excretion and oxygen consumption was done by means of the t test. The total effect of the two factors on 8-oxodG excretion was studied by multivariate analysis of variance with oxygen consumption as covariate. Linear regression was performed by the method of least squares. Probability values of less than 0.05 were considered significant.

RESULTS

The excretion of 8-oxodG was closely associated with oxygen consumption whether the former was expressed per subject, body weight, or LBM (Fig. 1; Table 1). Linear regression of 8-oxodG excretion on BW or LBM was 35% higher than in non-smokers. Although smoking increased oxygen consumption by only 10% (P < 0.05), its effect on 8-oxodG excretion lost statistical significance (P > 0.5) when oxygen consumption was included as covariate. Corrected for consumed oxygen, the smoking-related difference in 8-oxodG excretion was 85% and just failed to reach statistical significance (P = 0.08).

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2Abbreviations: 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; ROS, reactive oxygen species; BW, body mass index; LBM, lean body mass; BW, body weight; Fpg, fecamidopirimidin-DNA glycosylase.
In the present study 8-oxodG excretion was closely associated with oxygen consumption whether the former was expressed per subject, body weight, or LBM. Indeed, the slope of linear regression of 8-oxodG excretion on oxygen consumption was compatible with inter species comparisons of metabolic rate and body weight of oxidative DNA damage including 8-oxodG (3, 12), whereas another interspecies correlation showed a much less steep slope (5).

Although the present data excluded elaborate testing of alternatives to a linear model, a negative intercept of 8-oxodG excretion is without biological meaning. Rather, the relationship with oxygen consumption may be more like a hockey stick, reflecting limited capacity of antioxidant defenses (3) and a near linear production of ROS with increasing metabolic rate. Thus, the 1-5% fraction of consumed oxygen undergoing single electron transfer to generate ROS during mitochondrial respiration (1) may differ between species and between human individuals. In fact, both the hydrogen peroxide formation per nulligrannul of mitochondrial protein (4) and the assayed mitochondrial surface area (14) have been shown to correlate with the metabolic rate, suggesting that total ROS production from leakage in the respiratory chain correlates with a power, e.g., the square, of the oxygen consumption. Moreover, the inverse relationship between longevity and metabolic rate appears to have different coefficients within various groups of mammals, e.g., humans and some primates, other primates, and non-primates; hypothetically related to differences in antioxidant defense systems (5).

In the smokers, 8-oxodG excretion per BW or LBM was 35% higher than in the abstainers. Although smoking increased oxygen consumption by only 10% its effect on 8-oxodG excretion lost statistical significance when oxygen consumption was included as a covariate. Apparently, the effect of smoking on 8-oxodG excretion was not exclusively related to an increase in mitochondrial respiration and a proportional increase in ROS generation. Thus, smoking may increase ROS formation by a partial decoupling of the respiratory chain as shown in heart muscle mitochondria from rabbits exposed to tobacco smoke (35). In addition, tobacco smoke contains large amounts of oxidants and induces oxidative DNA damage in vitro (46). Indeed, smokers notoriously have low plasma antioxidant levels due to increased consumption of vitamin C.

From a previously studied of women aged 40–94 years (90) the average excretion of 8-oxodG per BW was 35% lower than

**TABLE 1. Urinary 8-oxodG excretion in relation to smoking and oxygen consumption in 33 healthy premenopausal women**

<table>
<thead>
<tr>
<th></th>
<th>All,  n = 33</th>
<th>Smokers,  n = 42</th>
<th>Non-smokers,  n = 27</th>
<th>Coefficient of correlation; 8-oxodG consumption (r) 8-oxodG</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>O2 consumption (lit 24 h)</strong></td>
<td>396 ± 53</td>
<td>420 ± 35*</td>
<td>382 ± 32</td>
<td>0.16 (r = 0.207)</td>
<td>0.16</td>
</tr>
<tr>
<td><strong>8-oxodG excreted per subject (mol 24 h)</strong></td>
<td>21.5 ± 10.0</td>
<td>26.7 ± 11*</td>
<td>18 ± 7.8</td>
<td>3.14 (r = 0.157)</td>
<td>3.14</td>
</tr>
<tr>
<td><strong>BW (pound 24 h)</strong></td>
<td>359 ± 191</td>
<td>431 ± 168*</td>
<td>318 ± 130</td>
<td>0.16 (r = 0.198)</td>
<td>0.16</td>
</tr>
<tr>
<td><strong>LBM (pound 24 h)</strong></td>
<td>469 ± 194</td>
<td>562 ± 203*</td>
<td>416 ± 168</td>
<td>0.16 (r = 0.198)</td>
<td>0.16</td>
</tr>
</tbody>
</table>

*P < 0.05 vs. non-smoker value in r univariate analysis of variance. **P > 0.1 (P < 0.5) for effect of smoking with inclusion of O2 consumption as covariate in multivariate analysis of variance.

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REFERENCES

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27. Ma, J. Y., Mak, H., and Sekiguchi, M. (1992) Hydrolytic elimination of a mutagenic nucleotide, F-8-oxoGTP, by human 8-oxoguanine pro-


associated oxygen damage and mutations in mitochondrial DNA in hu-


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