# 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. Interspecies correlations and animal calorie 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 ($r$ = 0.64; $p$ = 0.00007). In the 12 women who smoked, 8-oxodG excretion was increased by 35%, although oxygen consumption increased only 10% compared with the 21 nonsmoking 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., Poulsen, H. E. Oxidative DNA damage correlates with oxygen consumption in humans. *FASEB J.* 8: 534–537; 1994.**

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

MITOCHONDRIAL RESPIRATION AS A SOURCE of reactive oxygen species (1) (ROS)[2] with resulting damage to DNA has been proposed as an important determinant of longevity and cumulated 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 $10^5$ dG in normal tissue; higher levels are found with advanced age, in malignant tumors, and after treatment with ionizing radiation or chemical carcinogens (2, 5–7). 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 (10). In the present study we have examined the relationship between urinary excretion of 8-oxodG and oxygen consumption in 12 smoking and 21 nonsmoking, healthy premenopausal women.

## MATERIAL AND METHODS

After informed consent and approval from the local ethics committee, 33 healthy premenopausal women (age 33 ± 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 measurement the subjects were kept under continuous surveillance and 24-h urine was collected under metabolic ward conditions. Smokers were allowed to smoke no more than six cigarettes while in the respiratory chamber so as not to contaminate the air filters. A fixed physical activity program included three 10-min bicycling (75 W) sessions. For at least 4 days before the experiment the subjects were instructed by a dietician in a weight maintenance, conventional diet supplying 55% of the energy as carbohydrate, 30% as fat, and 15% as protein. At fixed 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 Animeter (HTS-Engineering Inc, Odense, Denmark) (13). The concentration of 8-oxodG in 24-h urine was measured by an automated three-dimensional HPLC method with isocratic separation and electrochemical detection (10). The intra- and interday coefficients of variation for the analysis were 8% and 10%, respectively. All samples were analyzed repeatedly 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 at least 2.5 years.

The 24-h 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.

Bivariate analysis of the effect of smoking on 8-oxodG excretion and oxygen consumption was done by means of the $t$ test. The combined effect of the two factors on 8-oxodG excretion was studied by multifactorial 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 oxygen consumption showed a slope of 0.17 nmol per liter of oxygen ($P$ = 0.00007; $r$ = 0.64).

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% ($P < 0.05$), 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 29% and just failed to reach statistical significance ($P$ = 0.08).

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[2]Abbreviations: 8-oxodG, 8-oxo-7,8-dihydro-2'-deoxyguanosine; ROS, reactive oxygen species; BMI, body mass index; LBM, lean body mass; BW, body weight; Fpg, formamidopyrimidine-DNA glycosylase.



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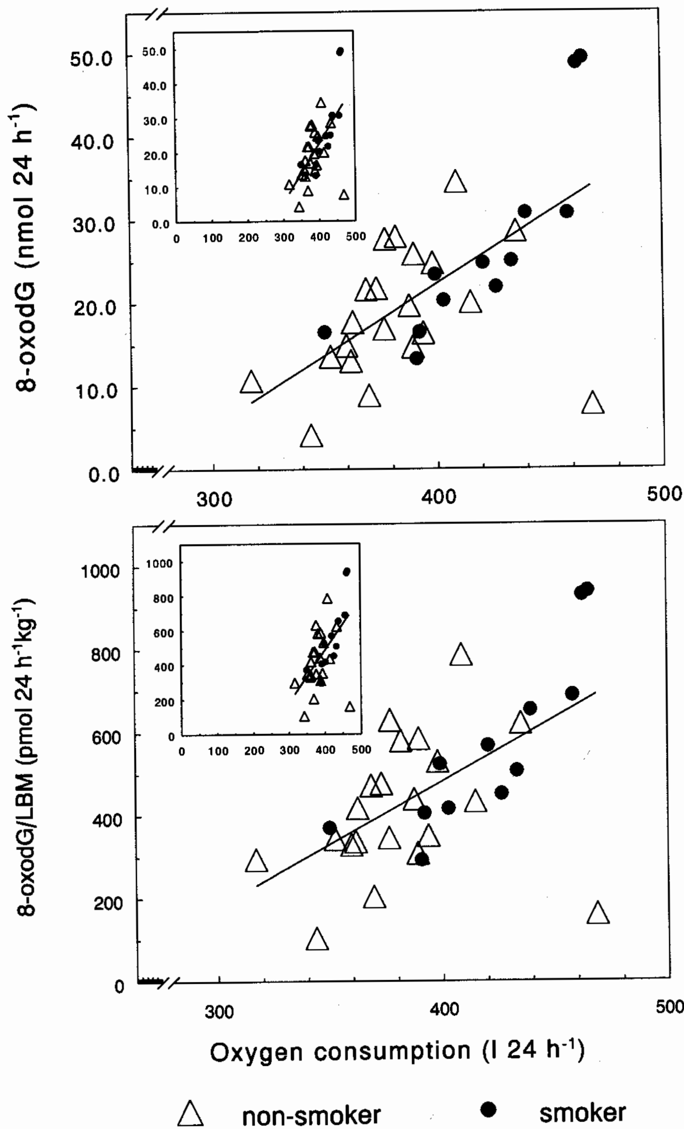


**Figure 1.** Relationship between oxygen consumption and oxidative DNA damage as assessed by the urinary excretion of 8-oxodG in 33 healthy premenopausal women. 8-OxodG excretion was expressed per subject (top) and per lean body mass (LBM; bottom). The lines of regressions are shown. Inserts show the relationships with full abscissa.

## DISCUSSION

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 interspecies comparisons of metabolic rate and biomarkers of oxidative DNA damage, including 8-oxodG (3, 11), whereas another interspecies correlation showed a much less steep slope (8).

Although the present data precluded 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 shaped like a hockey stick, reflecting limited capacity of antioxidant defences (3) and/or nonlinear production of ROS with increasing metabolic rate. Thus, the 1–5% fraction of consumed oxygen undergoing single electron transfers to generate ROS during mitochondrial respiration (1) may differ between species and between human individuals. In fact, both the hydrogen peroxide formation per milligram of mitochondrial protein (4) and the summed 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 nonprimates, hypothetically related to differences in antioxidant defence systems (3).

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 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 (15). In addition, tobacco smoke contains large amounts of oxidants and induces oxidative DNA damage in vitro (16). Indeed, smokers notoriously have low plasma antioxidant levels due to increased utilization and decreased intake (17).

From a previous study of women aged 40–64 years (10) the average excretion of 8-oxodG per BW was 33% lower than

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

| | All, $n = 33$ | Smokers, $n = 12$ | Nonsmokers, $n = 21$ | Coefficient of covariation $O_2$ consumption ($l\ 24\ h^{-1}$) |
|---|---|---|---|---|
| $O_2$ consumption ($l\ 24\ h^{-1}$) | 396 ± 33 | 420 ± 35[a] | 382 ± 32 | |
| 8-oxodG excretion per | | | | |
| subject (nmol 24 $h^{-1}$) | 21.5 ± 10.0 | 26.7 ± 11.7[a,b] | 18.6 ± 7.8 | 0.16 ($F$ = 20.7) |
| BW (pmol 24 $h^{-1} \cdot kg^{-1}$) | 359 ± 191 | 431 ± 168[a,b] | 318 ± 130 | 2.14 ($F$ = 15.2) |
| LBM (pmol 24 $h^{-1} \cdot kg^{-1}$) | 469 ± 194 | 562 ± 207[a,b] | 416 ± 168 | 2.75 ($F$ = 15.8) |
| $O_2$ consumption (pmol $l^{-1}$) | 53 ± 20 | 62 ± 23 | 48 ± 19 | |

[a] $P < 0.05$ vs. nonsmoker value in $t$ test. [b] $P > 0.5$ ($F < 0.5$) for effect of smoking with inclusion of $O_2$ consumption as covariate in multifactorial analysis of variance.

in the younger women studied more recently. This difference could be related to the well-known age-related decrease in energy expenditure (18). Indeed, 8-oxodG excretion has been reported to decrease with age in rats along with the decreasing rate of metabolism (2). Accordingly, a study of oxidative DNA damage estimated by, for example, 8-oxodG excretion in a homogeneous population with a broad age range is warranted.

In vivo 8-oxodG in DNA is extensively repaired but the relative importance of the two possible products, 8-oxodG and the corresponding base (8-oxoguanine), is yet undetermined (2, 7, 9, 19). In *Escherichia coli* the formamidopyrimidine-DNA glycosylase (Fpg) enzyme repair 8-oxodG in DNA by initial liberation of the base (20), which is also found in larger quantities than 8-oxodG in the spent medium (19). However, studies of repair-deficient *E. coli* mutants suggest that nucleotide excision by the UvrABC complex may also be a repair pathway with 8-oxodG as the putative end product (21). In mammalian cells an enzyme complex similar to the *E. coli* Fpg has been shown to repair by excision at a different site, i.e., the proximal and distal phosphodiester bond at the 3' and 5' side of lesion, respectively, although the end product is yet unknown (22). Indeed, studies with repair-deficient cell lines suggest that nucleotide excision is an important pathway for 8-oxodG in humans (23). In addition, digestion of damaged DNA from cell renewal and mitochondrial turnover will liberate 8-oxodG. Mitochondrial DNA contains relatively more 8-oxodG, presumably due to its proximity to the respiratory chain, lack of histones, and poor repair, but the total amounts are small compared with nuclear DNA (24). The cellular pools of nucleosides and nucleotides may be oxidized and lead to mutations if incorporated into DNA (25, 26). In fact, a human enzyme corresponding to the *E. coli* MutT protein hydrolyzes the phosphates of 8-oxodGTP with high affinity (27). Injected 8-oxodG is readily excreted unchanged into the urine whereas 8-oxodG in the diet or oxidation of dG during excretion does not contribute (9, 19). Thus, although the exact importance as a DNA repair product remains to be determined, the urinary excretion of 8-oxodG reflects the general average risk of a promutagenic oxidative adduct in DNA of all tissues and organs.

In replicating DNA, 8-oxodG leads to G-T A-C transversions as well as other mutations and codon 12 activation of c-Ha-ras or K-ras oncogenes in mammalian systems (25, 26, 28, 29). In human tumors G-T transversions are among the most frequent hot spot mutations in the p53 suppressor gene (30). Reports of exponential accumulation of 8-oxodG and a correlation with deletions in mitochondrial DNA from human heart muscle indicate a role of oxidative DNA damage from the respiratory chain in the muscle weakness associated with aging (31). Accordingly, the presently demonstrated close association between oxygen consumption and excretion of 8-oxodG in humans further supports the suggested relationship between mitochondrial respiration and oxidative DNA damage and, by inference, the risk of cancer and rate of aging (2-4). Moreover, this association is aggravated by smoking. FJ

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REFERENCES


1. Chance, B., Sies, H., and Boveris, A. (1979) Hydroperoxide metabolism in mammalian organs. *Physiol. Rev.* **59**, 527-605
2. Fraga, C., Shigenaga, M. K., Park, J.-W., Degan, P., and Ames, B. N. (1990) Oxidative damage to DNA during aging: 8-hydroxy-2'-deoxyguanosine in rat organ DNA and urine. *Proc. Natl. Acad. Sci. USA* **87**, 4533-4537
3. Cutler, R. G. (1991) Human longevity and aging: possible role of reactive oxygen species. *Ann. New. York. Acad. Sci.* **621**, 1-28
4. Sohal, R. S., and Orr, W. C. (1992) Relationship between antioxidants, prooxidants, and the aging process. *Ann. New. York. Acad. Sci.* **663**, 74-84
5. Olinski, R., Zastawny, T., Budzbon, J., Skokowski, J., Zegarski, W., and Dizdaroglu, M. (1992) DNA base modification in chromatin of human cancerous tissues. *FEBS. Lett.* **309**, 193-198
6. Malins, D. C., and Haimanot, R. (1991) Major alterations in the nucleotide structure of DNA in cancer of the female breast. *Cancer Res.* **51**, 5430-5432
7. Kasai, H., Crain, P. F., Kuchino, Y., Nishimura, S., Ootsuyama, A., and Tanooka, H. (1986) Formation of 8-hydroxyguanine moiety in cellular DNA by agents producing oxygen radicals and evidence for its repair. *Carcinogenesis* **7**, 1849-1851
8. Adelman, R., Saul, R. L., and Ames, B. N. (1988) Oxidative damage to DNA: relation to species, metabolic rate and life span. *Proc. Natl. Acad. Sci. USA* **85**, 2706-2708
9. Shigenaga, M. K., Gimeno, C. J., and Ames, B. N. (1989) Urinary 8-hydroxy-2'-deoxyguanosine as a biological marker of in vivo oxidative DNA damage. *Proc. Natl. Acad. Sci. USA* **86**, 9697-9701
10. Loft, S., Vistisen, K., Ewertz, M., Tjønneland, A., Overvad, K., and Poulsen, H. E. (1992) Oxidative DNA-damage estimated by 8-hydroxydeoxyguanosine excretion in man: influence of smoking, gender and body mass index. *Carcinogenesis* **13**, 1561-1568
11. Simic, M. G., and Bergtold, D. S. (1991) Dietary modulation of DNA damage in humans. *Mutat. Res.* **250**, 17-24
12. Chung, M. H., Kasai, H., Nishimura, S., and Yu, B. P. (1992) Protection of DNA damage by dietary restriction. *Free Rad. Biol. Med.* **12**, 523-525
13. Astrup, A., Buemann, B., Christensen, N. J., Madsen, J., Gluud, C., Bennet, P., and Svenstrup, B. (1992) The contribution of body composition, substrates and hormones to the variability in energy expenditure and substrate utilization in premenopausal women. *J. Clin. Endocrinol. Metab.* **74**, 279-286
14. Else, P. L., and Hulbert, A. J. (1985) Mammals: an allometric study of metabolism at tissue and mitochondrial leve. *Am. J. Physiol.* **248**, R415-R421
15. Gvozdjakova, A., Kucharska, J., and Gvorzdjak, J. (1992) Effect of smoking on the oxidative processes of cardiomyocytes. *Cardiology* **81**, 81-84
16. Leanderson, P., and Tagesson, C. (1992) Cigarette smoke-induced DNA damage in cultured human lung cells: role of hydroxyl radicals and endonuclease activation. *Chem.-Biol. Interact.* **810**, 197-208
17. Dorgan, J. F., and Schatzkin, A. (1991) Antioxidant micronutrients in cancer prevention. *Hematol. Oncol. Clin. North. Am.* **5**, 43-68
18. Vaughan, L., Zurlo, F., and Ravussin, E. (1991) Aging and energy expenditure. *Am. J. Clin. Nutr.* **53**, 821-825
19. Park, E.-M., Shigenaga, M. K., Degan, P., Korn, T., Kitzler, J. W., Wehr, C. M., Kolachana, P., and Ames, B. N. (1992) Assay of excised oxidative DNA lesions: isolation of 8-oxoguanine and its nucleoside derivatives from biological fluids with a monoclonal antibody column. *Proc. Natl. Acad. Sci. USA* **89**, 3375-3379
20. Boiteux, S., Gajewski, E., Laval, J., and Dizdaroglu, M. (1992) Substrate specificity of the *Escherischia coli* Fpg protein (formamidopyrimidine-DNA glycosylase): excision of purine lesions in DNA produced by ionizing radiation or photosensitization. *Biochemistry* **31**, 106-110
21. Czeczot, H., Tudek, B., Lambeert, B., Laval, J., and Boiteux, S. (1991) *Escherischia coli* Fpg protein and UvrABC endonuclease repair DNA damage induced by methylene blue plus visible light in vivo and in vitro. *J. Bacteriol.* **173**, 3419-3424
22. Yamamoto, F., Kasai, H., Bessho, T., Chung, M.-H., Inoue, H., Hori, T., and Nishimura, S. (1992) Ubiquitous presence in mammalian cells of enzymatic activity specifically cleaving 8-hydroxyguanine-containing DNA. *Jpn. J. Cancer Res.* **83**, 351-357
23. Klein, J. C., Bleeker, M. J., Saris, C. P., Roelen, H. C. P. F., Brugghe, H. F., van den Elst, H., van der Marel, G. A., van Boom, J. H., Westra, J. G., Kriek, E., and Berns, A. J. M. (1992) Repair and replication of

plasmids with site-specific 8-oxodG and 8-AAFdG residues in normal and repair-deficient human cells. *Nucleic Acids Res.* **20,** 4437–4443
24. Richter, C., Park, J.-W., and Ames, B. N. (1988) Normal oxidative damage to mitochondrial and nuclear DNA is extensive. *Proc. Natl. Acad. Sci. USA* **85,** 6465–6467
25. Shibutani, S., Takeshita, M., and Grollman, A. P. (1991) Insertion of specific bases during DNA synthesis past the oxidation damaged base 8-oxodG. *Nature (London)* **349,** 431–434
26. Kamiya, H., Miura, K., Ishikawa, H., Nishimura, S., and Ohtsuka, E. (1992) c-Ha-ras containing 8-hydroxyguanine at codon 12 induces point mutations at the modified and adjacent positions. *Cancer Res.* **52,** 3483–3485
27. Mo, J. Y., Maki, H., and Sekiguchi, M. (1992) Hydrolytic elimination of a mutagenic nucleotide, 8-oxodGTP, by human 18-kilodalton protein: sanitization of nucleotide pool. *Proc. Natl. Acad. Sci. USA* **89,** 11021-11025
28. Cheng, K. C., Cahill, D. S., Kasai, H., Nishimura, S., and Loeb, L. A. (1992) 8-Hydroxyguanine, an abundant form of oxidative DNA damage, causes G–T and A–C substitutions. *J. Biol. Chem.* **267,** 166–172
29. Higinbotham, K. G., Rice, J. M., Divan, B. A., Kasprzak, K. S., Reed, C. D., and Perantoni, A. O. (1992) GGT to GTT transversion in codon 12 of the K-ras oncogene in rat renal sarcomas induced with nickel subsulfide or nickel subsulfiede/iron are consistent with oxidative damage to DNA. *Cancer Res.* **52,** 4747–4751
30. Hollstein, M., Sidransky, D., Vogelstein, B., and Harris, C. C. (1991) p53 mutations in human cancers. *Science* **253,** 49–52
31. Hayakawa, M., Hattori, K., Sugiyama, S., and Ozawa, T. (1992) Age-associated oxygen damage and mutations in mitochondrial DNA in human hearts. *Biochem. Biophys. Res. Commun.* **189,** 979–985

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