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Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass inde[*]

NB The Department has a new address

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Oxidative DNA damage may be implicated in ageing, carcinogenesis and other degenerative diseases. Oxidative DNA damage can be assessed in humans *in vivo* from the urinary excretion of the DNA-repair product 8-hydroxydeoxyguanosine (8OHdG). We investigated factors influencing the excretion of 8OHdG in 24 h urine from 83 randomly selected healthy subjects (52 women) aged 40–64 years. For 2 weeks prior to urine collection the subjects kept a weighed diet record. 8OHdG was quantified by an automatic three-dimensional HPLC analysis with electrochemical detection. The 8OHdG excretion was 252 ± 103 (mean ± SD) pmol kg body weight/24 h with a range from 78 to 527. Multiple regression analysis identified three factors, smoking, body mass index (BMI) and gender, as significant predictors of the 8OHdG excretion. In 30 smokers the 8OHdG excretion was 320 ± 99 pmol/kg/24 h opposed to 213 ± 84 pmol/kg/24 h in 53 non-smokers. According to multiple regression analysis smokers excreted 50% (31–69%; 95% confidence interval) more 8OHdG than non-smokers. In 52 women the 8OHdG excretion was 240 ± 106 pmol/kg/24 h opposed to 271 ± 96 pmol/kg/24 h in 31 men. According to the multiple regression analysis men excreted 29% (10–48%) more 8OHdG than women. According to multiple regression analysis the 8OHdG excretion decreased with 4% (2–6%) per increment in BMI measured in kg/m^2. The dietary distribution of energy demonstrated no important predictive value with respect to 8OHdG excretion. The intake of the antioxidant vitamins C and E and of vitamin A equivalents, including β-carotene, was not associated with 8OHdG excretion. The results suggest that smoking increases oxidative DNA damage by ~50%. This effect implies potential serious health effects adding to the other well-known health hazards of smoking. The higher 8OHdG excretion in men and lean subjects may be related to a higher rate of metabolism with increased availability of reactive oxygen species. The apparent 7-fold individual variation in oxidative DNA damage carries implications regarding the rate of ageing and the risk of cancer and other degenerative diseases. The excretion of 8OHdG into urine offers a valuable tool for testing such hypotheses in humans.

Introduction

Cellular damage caused by reactive oxygen species (ROS*) has been proposed to be an important factor in ageing as well as in a number of age-related degenerative diseases, including cancer, heart disease, arthritis and cataract formation (1–6). ROS are generated from cellular respiration, co-oxidation during the metabolism of xenobiotics and arachidonic acid, and from the respiratory burst in phagocytic cells and may be incompletely quenched by the various defence mechanisms.

In ageing and cancer DNA is probably the most important target for ROS. A frequent oxidative DNA damage is the 8-hydroxylation of the guanine base. The occurrence of 8-hydroxyguanine in DNA is a well-established measure of oxidative damage *in vitro* (7–9). Damaged DNA is repaired *in vivo* by exonucleases and the resulting free water-soluble 8-hydroxydeoxyguanosine (8OHdG) is excreted without further metabolism into the urine (3,10,11). Exogenous DNA, e.g. from the diet, does not contribute to this excretion (11). Thus, the urinary excretion of this hydroxylated nucleoside will reflect the current oxidative DNA damage and repair.

The available data regarding the urinary excretion of 8OHdG suggest that oxidative damage to DNA is extensive in humans, i.e. ~10^4 base oxidations per cell per day (10,11). Similar data regarding other urinary biomarkers of oxidative DNA damage, thymine and thymidine glycol, support this notion (12,13). Moreover, interspecies comparisons suggest that oxidative DNA damage correlates with specific oxidative metabolism and cancer risk and inversely with expected life-span (13,14). In the healthy organism cellular respiration is probably the quantitatively dominant source of ROS and resulting oxidative DNA damage. Whether this is changed under pathological conditions is unknown. In ageing rats 8OHdG excretion declines along with accumulation of damaged bases in cellular DNA, suggesting failing repair mechanisms (3).

In humans limited data are available regarding 8OHdG formation and excretion, except for reports of the basal levels in a few subjects and, e.g. of the increasing excretion in two patients treated with ionizing radiation (11,15,16). Currently, the analytical methods employed have been very cumbersome, i.e. complicated extraction procedures requiring radiolabelled 8OHdG as internal standard, before HPLC gradient separation and electrochemical detection or GC–MS (11,16,17).

In the present study we developed an automated triple column switch HPLC method with isocratic separation and electrochemical detection for the analysis of 8OHdG. This method was used for the study of factors influencing urinary excretion of 8OHdG in 94 healthy 40–64 year old subjects, randomly selected from the Danish population.

Material and methods

Protocol

The protocol was approved by the local ethics committee. An age-stratified sample of 300 men and 300 women, aged 40–64 years, was selected at random from the National Population Register among people living in the Østerbro area of

*Abbreviations: ROS, reactive oxygen species; 8OHdG, 8-hydroxydeoxyguanosine; BMI, body mass index.

Table I. Descriptive data of 83 subjects (82 subjects with respect to diet data) randomly selected from the Danish population aged 40–64 years

	All	Women	Men
Number of subjects	83	52	31
Age (years)	51 ± 7	51 ± 6	53 ± 7
Body weight (kg)	75 ± 14	70 ± 12	84 ± 14
BMI (kg/m^2)	26 ± 5	26 ± 4	27 ± 5
Lean body fraction (%)	73 ± 6	70 ± 7	77 ± 6
Exercise (h/week)	6.3 ± 5.3	6.5 ± 5.5	6.0 ± 5.0
Smoking habits			
No. of non-smokers/smokers	53/30	31/21	23/8
Smokers: cigarettes/day	16 ± 8	16 ± 9	15 ± 4
Caffeine consumption (cups)[a]	6.6 ± 3.9	7.1 ± 4.0	5.8 ± 3.5
Dietary factors			
Energy intake (MJ/day)	8.4 ± 2.4	7.1 ± 3.8	10.4 ± 2.2
Energy intake (kJ/kg^{-1}/day)	114 ± 36	105 ± 32	127 ± 38
Energy from fat (%)	38 ± 5	38 ± 5	38 ± 6
Energy from carbohydrate (%)	38 ± 6	39 ± 6	37 ± 6
Energy from protein (%)	14.8 ± 2.7	15.5 ± 2.6	13.6 ± 2.5
Energy from alcohol (%)	8.5 ± 7.1	6.9 ± 5.5	11.2 ± 8.7
Vitamin A equivalents (mg/day)	1.1 ± 0.6	1.1 ± 0.6	1.1 ± 0.5
Vitamin C (mg/day)	72 ± 43	69 ± 34	75 ± 55
Vitamin E equivalents (mg/day)	5.9 ± 2.7	5.2 ± 1.6	7.2 ± 3.6

[a]No of cups of coffee + 0.6 no. of cups of tea; values are mean ± SD.

Copenhagen, and invited to participate in the study. Of the 320 people who visited the study centre, 202 were selected at random for a validation study of a semi-quantitative food-frequency questionnaire (18,19). Of the 144 people who completed the questionnaire 94 (59 women) collected urine for 24 h and were included in the present study. Two subjects were excluded due to incomplete urine collection and nine subjects due to analytical problems (see below).

The subjects kept a weighed diet record for 2 weeks (for details see refs 18 and 19). One subject did not complete the diet record and was excluded from the part of the data analysis related to dietary factors. The average intake of the antioxidants, vitamin C and vitamin E, as well as vitamin A equivalents, including β-carotene, was calculated from the diet record data and questionnaire information regarding the use of supplements. The computerized tables used for the calculation of micronutrient intake did not include β-carotene alone. The subjects filled in questionnaires regarding the consumption of coffee, tea, cigarettes and the use of medicine. The body mass index (BMI) was calculated as the measured body weight in kg divided by the square of the measured height in metres. The ideal BMI is considered to be from 20 to 25 kg/m^2. The lean and fat body mass were measured by means of body impedance using an Akern BIA 109 analyser (20). The characteristics of the 83 subjects (82 subjects with respect to diet data) with successful analysis and complete urine collection are summarized in Table I.

Urine was collected for 24 h and aliquots were stored at −20°C. Completeness of urine collection was monitored by the excretion of para-aminobenzoic acid (80 mg three times a day). The collection of urine was incomplete in two subjects, who were thus excluded from the study.

Analysis

The urinary concentration of 8OHdG was measured by HPLC with electrochemical detection. To 5 ml of urine 100 μl of 2 N HCl was added. After freezing, thawing and centrifugation the acid content of a 1 ml aliquot of the supernatant was neutralized with 20 μl of 2 N sodium hydroxide. To three aliquots of 190 μl of urine sample 10 μl of water containing 0.800 and 8000 nM genuine 8OHdG was added. After further addition of 200 μl of 1 M Tris–HCl buffer pH 7.9 to each, an aliquot of 25–50 μl was injected on a Spherisorb ODS2 5 μ 15 cm column eluted with acetonitrile (2.5%, v/v) and methanol (1.5%, v/v) in 10 mM borate buffer pH 7.9 at 1 ml/min. The retention time of 8OHdG was determined from injection of samples of genuine 8OHdG dissolved in the Tris–HCl buffer and monitored by UV absorbance. Immediately before the elution of 8OHdG a Valco automatic six-port valve switched the effluent from the Spherisorb column to a 2 cm Hamilton cation-exchange column. Sixty seconds later a switch of the automatic valve brought the fraction of the effluent retained in the ion exchange column on a Nucleosil ODS 3 μ 25 cm column. The flow path of the 25 cm column was equipped with an SSI LP-21 pulse dampener and an electrochemical guard cell set at 300 mV. This column was isocratically eluted with acetonitrile (2.5%, v/v) methanol (1%, v/v) in 100 mM phosphate buffer pH 2.2. The effluent was monitored by an ESA Coluchem II electrochemical detector in the oxidation mode and equipped with a high sensitivity analytical cell 5011 set at 120 mV (electrode 1) and 300 mV (electrode 2) and 50 nA as full range deflection. The

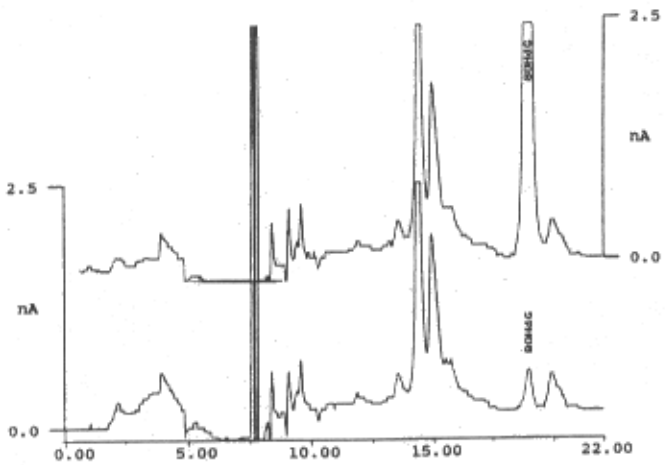

Fig. 1. Electrochemical tracing from a triple column HPLC analysis of 50 μl injections from a urine sample with (**top**) and without (**bottom**) addition of genuine 8OHdG. The sample contained 12.0 nM 8OHdG.

chromatographic system was controlled and the data were processed by means of Merch-Hitachi D-6000 chromatographic software.

With the triple column switch HPLC method 8OHdG could be automatically analysed with a run time of ~20 min. A representative chromatogram is presented in Figure 1.

Quantification of 8OHdG was done by sample addition. For each sample calibration curves were constructed from the peak heights after injections of aliquots with and without addition of 40 and 400 nM of genuine 8OHdG. The calibration curves were linear in the relevant range (Figure 2). The samples were usually analysed in duplicate and the mean value used for data analysis. The genuine 8OHdG was synthesized as described by Kasai *et al.* (21) and calibrated against solutions of pure compound kindly supplied by Dr Dawid W.Potter, Rohm and Haas Co., Spring House, PA, and Dr Per Leanderson, Dept of Occupational Medicine, Lindköbing Hospital, Sweden.

Statistics

The distribution of the 24 h urinary excretion of 8OHdG was investigated by means of probit analysis. The fit to the normal distribution before and after log transformation of the data was tested by the Kolmogorov–Smirnoff and χ^2 tests. The relationship between the recorded host factors and the excretion was investigated in bivariate analysis, i.e. *t*-tests were used for the effect of binominal variables and according to the distribution, Pearson product moment or Spearman rank correlations coefficients were calculated for continuous variables. The effect

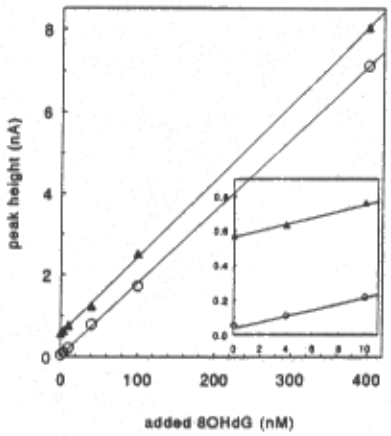

Fig. 2. Representative calibration curves for urine samples containing different concentrations 8OHdG. The insert shows an enlargement of the lower left corner of the graph. Sample 1 (○) contained 3.16 nM and sample 2 (▲) contained 33.3 nM.

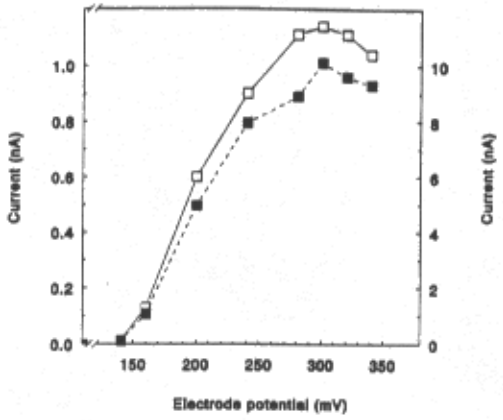

Fig. 3. Dynamic voltamograms of electrochemical substance eluting as 8OHdG in chromatograms of a urine sample from a smoker with (■—■; right ordinate) and without (□——□; left ordinate) addition of genuine 8OHdG (400 nM).

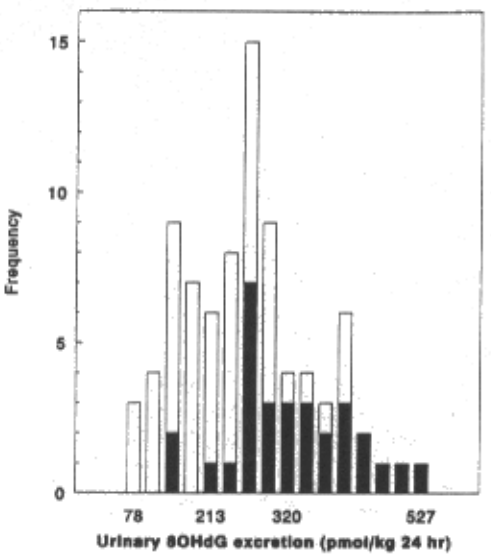

Fig. 4. Histogram of the urinary excretion of 8OHdG in 83 healthy subjects randomly selected from the Danish population aged 40–64 years. Black bars represent smokers.

of all recorded host factors was investigated by stepwise multiple linear regression analysis with forward variable selection as well as backward variable exclusion. The multiple regression analysis was repeated after stratification of the subjects according to smoking habits and gender. Probability levels <0.05 were considered statistically significant.

Results

The recovery of 8OHdG after the column extraction procedure was 65–70% depending on the wear of the extraction column. By varying the time of valve switching it could be demonstrated that the extraction of the 8OHdG peak was identical with or without addition of genuine substance. Moreover, dynamic voltamograms of the 8OHdG peak, e.g. of a sample from a smoker with a high concentration, was identical before and after addition of genuine substance (Figure 3).

Estimated from 30 samples reanalysed once and six samples reanalysed six times on different days the interday coefficient of variation for the analysis was 10%. The intraday coefficient of variation was 8%. The limit of detection was 0.2 nM. The concentration of 8OHdG was constant in urine samples stored at −20°C for at least 1 year.

The 8OHdG concentration could be measured in the samples from 85 subjects, two of whom had collected urine incompletely. In nine subjects interfering chromatographic peaks precluded analysis. In at least five subjects the interfering peaks were related to recent intake of paracetamol which interferes with the analysis (data not shown).

The excretion of 8OHdG ranged from 78 to 527 pmol/kg body weight/24 h and fitted a normal distribution (Table II; Figure 4). In bivariate analyses the following host factors, body weight, BMI and smoking were significantly associated with the 8OHdG excretion (Table II; Figure 4). Thus, the 30 smokers had a 50% (31–69%; 95% confidence interval) higher level of 8OHdG excretion than non-smokers. The excretion of 8OHdG was similar in nine subjects smoking <10 cigarettes/day and in 21 subjects smoking ≥ 10 cigarettes/day, i.e. 328 ± 34 and 317 ± 22 pmol/kg/24 h. This does not support a dose-related effect, but the smoking habits were too uniform in this material to allow a firm conclusion.

Multivariate regression analysis identified gender, BMI and smoking, as independent predictors of the 8OHdG excretion (Table III; Figure 5). A regression model including these three variables offered an explanation of 40% ($= r^2$) of the variation in excretion in the present population sample. In addition to the enhancing effect of smoking shown in the bivariate analysis, men excreted 29% (10–48%) more than women, whereas lean subjects had a higher excretion than the overweight subjects; according to the regression coefficients the 8OHdG excretion decreased 4% (2–6%) per unit BMI. As seen in Figure 5 and Tables IV and V the effects of these three independent variables on the 8OHdG excretion were not entirely additive. In non-smokers, gender was the most important determinant of 8OHdG excretion, whereas BMI was the only significant predictor in smokers. In women, both smoking and BMI were strong predictors of the 8OHdG excretion, whereas in men only smoking was a marginally significant predictor. As extremes, the 18 normal weight (BMI <25) smokers of either sex had an average 8OHdG excretion 2.2 times (2.1–2.4 times) the excretion of the 14 obese (BMI >25) female non-smokers and there was no overlap between these two groups.

There were no significant interactions between the three predicting factors. However, the BMI of smokers was 25.2 ± 4.3 kg/m^2 opposed to 26.8 ± 4.6 kg/m^2 of non-smokers ($P = 0.14$). The values of men and women are shown in Table

I. Moreover, a relatively large number of women were smokers ($P = 0.0009$).

The association between the body weight and the 8OHdG excretion in the bivariate analysis did not prove important in the multivariate analysis, suggesting that the association could be explained by BMI and smoking.

If the lean fraction of the body mass estimated by means of body impedance (20) was introduced in the regression analysis, it substituted BMI and gender as predictors with forward variable selection, having an F value of 13.7. However, with backward variable exclusion BMI and gender were still significant predictors whereas the lean body fraction was excluded. Thus, either of these characteristics may be used. Moreover, in a multiple regression analysis with the total 8OHdG excretion, i.e. not divided by body weight, as dependent variable, smoking and the lean body mass were the important predictors, having F values of 33.7 and 26.9 respectively.

The factors describing the distribution of consumed energy also produced some information in the multivariate regression analyses. However, these factors were related to the total energy intake, the body weight and the BMI. Accordingly, the dietary composition was not an important independent predictor of the 8OHdG excretion in the present setting. The intake of the antioxidant vitamins C and E and of vitamin A equivalents, including β-carotene, was not associated with the 8OHdG excretion.

Table II. Bivariate analysis of relationship between host factors and excretion of 8OHdG in 83 subjects (82 subjects with respect to diet data) randomly selected from the Danish population aged 40–64 years

	8OHdG excretion pmol/kg/24 h	Correlation coefficient	
		Pearson	Spearman
All $n = 83$	252 ± 103		
Gender			
Women ($n = 52$)	240 ± 106		
Men ($n = 31$)	271 ± 96		
Age		−0.17	
Body weight		−0.23*	
BMI		−0.36*	
Exercise			−0.04
Smoking habits			0.50*
Non-smokers ($n = 53$)	213 ± 84		
Smokers ($n = 30$)	320 ± 99*		
Coffee + tea consumption			0.19
Dietary factors			
Energy intake		0.17	
Energy from fat		−0.09	
Energy from carbohydrate		0.12	
Energy from protein		0.08	
Energy from alcohol		−0.07	
Vitamin A		0.15	
Vitamin C		0.01	
Vitamin E		−0.12	

*$P < 0.05$.

Discussion

In the present investigation the urinary excretion of 8OHdG was used to study factors influencing oxidative DNA damage in randomly selected healthy subjects aged 40–64 years. The excretion was normally distributed with a 7-fold range and the levels were in accordance with other reports of an average excretion of 2–300 pmol/kg/24 h (10,11). The most important determining factor was smoking followed by the BMI and gender. Thus, lean smokers of either sex excreted more than twice as much 8OHdG as obese non-smoking women, indicating at least a doubling of the oxidative DNA damage.

In vitro tobacco smoke and several of its constituents, e.g. hydroquinone and cathecol, have been shown to generate ROS and to induce oxidative damage to isolated DNA as well as to produce 8OHdG in cell cultures (22,23). The volatile phase of tobacco smoke induced lipid peroxidation and lipoprotein oxidation in human plasma *in vitro* (24). In the plasma of smokers low concentrations of the important antioxidants, ascorbic acid and β-carotene, have been found, suggesting increased consumption and reduced antioxidant capacity (24–26). Moreover, in smokers leukocyte DNA has been found to contain ~50% more 8OHdG than DNA of leukocytes from non-smokers (27). Our finding of increased urinary 8OHdG excretion in smokers is the first verification of these suggestions *in vivo* in humans.

Studies across species have shown a significant correlation

Table III. Stepwise multiple regression analysis of the relationship between host factors and the excretion of 8OHdG in 83 subjects randomly selected from the Danish population aged 40–64 years

	F values for		Multiple regression coefficients (95% confidence intervals)
	Forward entry	Backward removal	
Constant			201 (173–228)
Gender (value 0 or 1)	12.9	14.7	59 (21–97)
Age	0.6	0.6	
Body weight	1.0	0.9	
BMI (−25)	16.7	15.3	−7.4 (−11.4 to −3.3)
Exercise	0.2	0.3	
Smoking habits (value 0 or 1)	30.5	30.1	106 (68–143)
Coffee + tea consumption	1.9	2.0	
Dietary factors			
Energy intake	2.1	1.9	
Energy from fat	0.6	6.3	
Energy from carbohydrate	2.3	4.2	
Energy from protein	5.6	2.2	
Energy from alcohol	0.5	7.1	
Vitamin A	1.4	1.7	
Vitamin C	0.4	0.0	
Vitamin E	0.8	1.8	

between specific metabolic rate and measures of oxidative DNA damage, including 8OHdG excretion (11,13). The high mitochondrial oxygen metabolism in small animals, such as rodents, allows generation of more ROS as the most important source of oxidative DNA damage. Thus, the apparent increasing effect of tobacco smoking on 8OHdG excretion may be related to its well-documented enhancing effect on the basic metabolic rate (28). Moreover, from the present data an inverse relationship between relative 8OHdG excretion and BMI emerged. This may relate to the fact that lean persons have a higher metabolic rate than obese (29). By the same token, men have a higher basic metabolic rate than women (30) which may provide an explanation of the higher 8OHdG excretion in men in the present material. In accordance, we found a slightly lower BMI and lean body fraction in smokers than non-smokers and in men as opposed to women. Furthermore, in two human subjects the urinary excretion of 8OHdG and thymidine glycol was found to decrease after restriction of the calorie intake to −60%, which would be expected to decrease the metabolic rate (31).

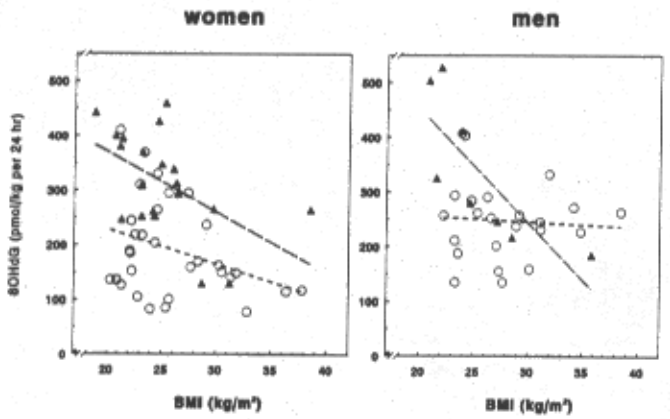

Fig. 5. The excretion of 8OHdG according to gender, smoking status and BMI in 83 healthy subjects randomly selected from the Danish population aged 40−64 years. The lines of regression for the excretion versus BMI are given for each group as short dashed lines for non-smokers (○) and long dashed lines for smokers (▲).

An alternative explanation of the association between 8OHdG excretion and BMI and gender may be that oxidative DNA damage mainly occurs in the lean body fraction. Accordingly, concurrent measurements of 8OHdG excretion and metabolic rate in subjects with different body compositions are warranted. Nevertheless, the data suggest that, ideally, the 8OHdG excretion should be corrected for lean body mass for comparison between individuals.

The 50% increased excretion in smokers may be caused by a much larger increase in oxidative DNA base damage in a limited number of cells in contact with ROS generated from tobacco smoke. Alternatively, tobacco smoking causes a general increase in base damage in all cells of the body. Probably, tobacco smoking acts by several simultaneous mechanisms, i.e. generally by increasing the metabolic rate with enhanced generation of ROS from cellular respiration and by consuming protective antioxidants, as well as directly through ROS or ROS-generating constituents in the smoke and by activating leukocytes to produce ROS.

The excretion of 8OHdG as well as other DNA repair products suggests that each cell in the human body may suffer 10^4 oxidative damages to the DNA bases (11−13). The excretion of 14 nmol 8OHdG in an average non-smoker represents 140 oxidatively damaged and repaired guanine bases for each of the

Table IV. Stepwise multiple regression analysis of the relationship between gender, BMI and the excretion of 8OHdG in non-smokers and smokers among 83 subjects randomly selected from the Danish population aged 40−64 years

	F values for		Multiple regression coefficients (95% confidence intervals)
	Forward entry	Backward removal	
Non-smokers			
Constant			194 (164−223)
Gender (value 0 or 1)	12.0	12.0	79 (33−124)
BMI (−25)	5.2	5.2	−5.6 (−10.6 to −0.7)
Smokers			
Constant			324 (295−353)
Gender (value 0 or 1)	0.1	0.1	8.1 (−61 to 77)
BMI (−25)	17.7	17.7	−13.9 (−20.7 to −7.1)

Table V. Stepwise multiple regression analysis of the relationship between smoking, BMI and the excretion of 8OHdG in men and women among 83 subjects randomly selected from the Danish population aged 40−64 years

	F values for		Multiple regression coefficients (95% confidence intervals)
	Forward entry	Backward removal	
Women			
Constant			195 (165−224)
Smoker (value 0 or 1)	31.0	31.0	124 (80−170)
BMI (−25)	11.1	11.1	−8.3 (−10.6 to −0.7)
Men			
Constant			248 (209−285)
Smoker (value 0 or 1)	5.1	5.1	79 (7.4−150)
BMI (−25)	2.9	2.9	−6.1 (−13.3 to 1.1)

6×10^{13} cells of the body per day. In animal experiments and cell cultures strong inducers of ROS, such as radiation and redox cycling chemicals, may double the 8OHdG content in DNA (8,21,23). However, the base damage and repair processes are dynamic and a marginally increased steady-state level may reflect a substantial increase in the rate of damage. Moreover, in two patients receiving 1.8 Gray of radiation therapy for breast or colon cancer, i.e. a limited radiation field, the excretion of 8OHdG and thymine glycol, another repair product of oxidative DNA damage, was 4-fold increased (16).

Age was not a significant predictor of 8OHdG excretion in the present population sample which had a rather narrow age range. In animal studies 8OHdG accumulates in DNA and the excretion decreases with age, partly due to failing repair (3). Moreover, 8OHdG appears to accumulate with age in the mitochondrial DNA of human diaphragm muscle cells (32). Nevertheless, considering the abundant daily oxidation of bases in the cellular DNA, even a minimal decrease in the repair will soon lead to massive accumulation of damaged bases. Thus, the excretion of 8OHdG is more a correlate of damage than of repair.

The intake of vitamins A, C and E was not correlated with 8OHdG excretion, although as antioxidants they could have been expected to have a protective effect. Thus, a high intake of vitamin C has been reported to protect against 8OHdG formation in human seminal DNA (33). In a single subject a high intake of fruit and vegetables was found to decrease the urinary excretion of thymidine glycol (31). However, in the present material the generally modest intake, which may not be accurately recorded from the diet records, may have little or no effect on 8OHdG formation. In fact, vitamin C may also exhibit pro-oxidant effects if ROS are generated by transition metals in Fenton-like reactions (34,35).

In women at high risk of breast cancer a low-fat diet decreased oxidative DNA damage in nucleated peripheral blood cells as measured by the levels of oxidized thymine (36). In the present study, however, the relationship between dietary fat and oxidative DNA damage was negative, if any.

The possible health implications of the variation in 8OHdG excretion and the inferred oxidative DNA damage are undetermined. *In vitro* it has been shown that oxidized guanine bases in replicating DNA will lead to G→T and A→C substitution mutations (37,38). The extent of oxidative DNA damage across species is correlated with a cumulative cancer risk and inversely correlated to life-span (2,13). Ionizing radiation and some cancer chemotherapeutics that cause oxidative DNA damage are known to cause cancer in humans (39). In this context, the effect of smoking is equivalent to a radiation dose of 0.25 Gray with respect to 8OHdG excretion, assuming linearity from the data on the two patients mentioned above treated with 1.8 Gray for cancer (16). Many other important diseases, such as atherosclerosis, are thought to involve ROS-related cellular damage in their pathogenesis (40,41). The use of simple non-invasive measures of oxidative DNA damage, such as urinary excretion of 8OHdG, gives unique opportunities for investigation of the related health effects in prospective studies or in nested case-control studies with urine from bioligocal banks. Thus, 8OHdG appears to be stable in urine stored at −20°C for at least 1 year. Unfortunately, in some cases, i.e. ~10% of our samples, urine cannot be analysed by our method due to interfering peaks, probably due to recent intake of paracetamol (unpublished data).

In conclusion, the present study demonstrates that smoking is associated with a 50% increased excretion of a repair product from oxidative DNA damage, 8OHdG. This effect adds to the other well-known harmful effects of tobacco smoke and may be important in carcinogenesis and other diseases as well as ageing. Two other factors, being lean and male, were also associated with an increased excretion of 8OHdG, possibly due to a higher metabolic rate related to these factors. With the urinary excretion of 8OHdG as a tool, the numerous hypotheses of the importance of ROS in ageing, degenerative diseases and carcinogenesis can be challenged. The postulated beneficial effects of antioxidant supplementation and other dietary intervention also represent unchallenged hypotheses to be tested.

Acknowledgements

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