Biomarkers of oxidative damage to DNA and repair

Steffen Loft[1], Pernille Høgh Danielsen, Lone Mikkelsen, Lotte Risom, Lykke Forchhammer and Peter Møller
Department of Environmental Health, University of Copenhagen, Øster Farimagsgade 5A, DK-1014 Copenhagen K, Denmark

Abstract

Oxidative-stress-induced damage to DNA includes a multitude of lesions, many of which are mutagenic and have multiple roles in cancer and aging. Many lesions have been characterized by MS-based methods after extraction and digestion of DNA. These preparation steps may cause spurious base oxidation, which is less likely to occur with methods such as the comet assay, which are based on nicking of the DNA strand at modified bases, but offer less specificity. The European Standards Committee on Oxidative DNA Damage has concluded that the true levels of the most widely studied lesion, 8-oxodG (8-oxo-7,8-dihydro-2′-deoxyguanosine), in cellular DNA is between 0.5 and 5 lesions per 10^6 dG bases. Base excision repair of oxidative damage to DNA can be assessed by nicking assays based on oligonucleotides with lesions or the comet assay, by mRNA expression levels or, in the case of, e.g., OGG1 (8-oxoguanine DNA glycosylase 1), responsible for repair of 8-oxodG, by genotyping. Products of repair in DNA or the nucleotide pool, such as 8-oxodG, excreted into the urine can be assessed by MS-based methods and generally reflects the rate of damage. Experimental and population-based studies indicate that many environmental factors, including particulate air pollution, cause oxidative damage to DNA, whereas diets rich in fruit and vegetables or antioxidant supplements may reduce the levels and enhance repair. Urinary excretion of 8-oxodG, genotype and expression of OGG1 have been associated with risk of cancer in cohort settings, whereas altered levels of damage, repair or urinary excretion in case-control settings may be a consequence rather than the cause of the disease.

Introduction

Multiple exogenous and endogenous sources generate ROS (reactive oxygen species) in mammalian cells. The exogenous sources include radiation, air pollution and a wide range of chemicals, whereas endogenous sources of ROS include mitochondrial respiration, inflammation, biotransformation and other metabolic processes [1]. Resulting oxidative stress can cause damage to any base or sugar moiety in DNA and SBs (strand breaks), some of which are depicted in Figure 1 and are described thoroughly elsewhere [2,3]. The importance of DNA oxidations is emphasized by their mutagenic potential, although there are multiple additional roles in aging and cancer, including, e.g., mitochondrial function, microsatellite instability and telomere shortening. To deal with oxidized DNA lesions, a multitude of repair systems have evolved, including BER (base excision repair), ligation, NER (nucleotide excision repair) and mismatch repair which have overlapping specificity and may interact or function as back-up systems [3]. The oxidatively damaged bases are preferentially repaired by enzymes of the BER pathway [4,5].

The recognition of the importance of oxidative damage to DNA has spawned a great interest in possibilities and problems related to its formation, measurement, prevention, repair and consequences in both experimental settings and human populations [6]. The use of such measurements in populations may characterize relevant environmental exposures and predict risk of diseases such as cancer [7]. The notion that fruits and vegetables or antioxidant supplements might prevent cancer by reducing the DNA damage rate or improving repair has received particularly great attention in human studies [8].

Measurement and importance of DNA oxidation lesions

Oxidative damage to DNA in cells and tissues can be measured by GLC–MS, HPLC with electrochemical or MS/MS (tandem MS) detection, immunoassays, ^{32}P-post-labelling and methods such as the single-cell gel electrophoresis (comet) assay and alkaline elution based on nicking of DNA strands at lesions [9]. Except for the nicking-based and some immunoassays, all of the chromatographic techniques require extraction of DNA before enzymatic digestion, whereas GLC–MS requires both acid hydrolysis and derivatization. The major problem found in measuring oxidized bases in DNA is the occurrence of spurious oxidation during these sample-preparation steps as outlined in Figure 1 [9]. DNA extraction is a critical issue, and the derivatization required for GLC–MS is particularly problematic, as described by the European Standards Committee on Oxidative DNA Damage (ESCODD), which organized a large inter-laboratory validation exercise with 25 member laboratories in which coded samples with defined numbers

Key words: base excision repair, biomarker, DNA damage, DNA repair, nucleotide excision repair, oxidative damage.

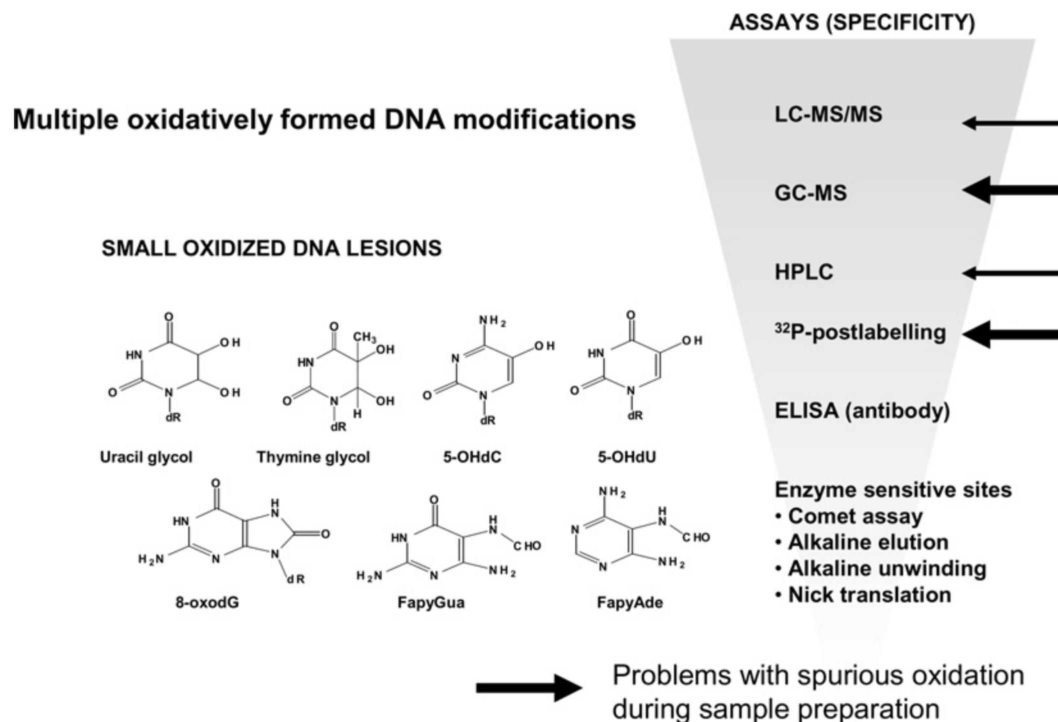
Abbreviations used: BER, base excision repair; EC, electrochemical detection; MS/MS, tandem MS; MUTYH, mutY homologue; NEIL1, nei-like 1; NER, nucleotide excision repair; NUDT1, NDP-linked moiety X-type motif 1; OGG1, 8-oxoguanine DNA glycosylase 1; hOGG1, human OGG1; 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; 8-oxoG, 8-oxoguanine; ROS, reactive oxygen species; SB, strand break.

[1]To whom correspondence should be addressed (email s.loft@pubhealth.ku.dk).

Biochem. Soc. Trans. (2008) 36, 1071–1076; doi:10.1042/BST0361071 ©The Authors Journal compilation ©2008 Biochemical Society

Figure 1 | The most well described oxidatively formed lesions in DNA and the most widely used assays with indication of specificity and extent of potential problems of spurious oxidation during sample work up and analysis

FapyAde, 4,6-diamino-5-formamidopyrimidine; FapyGua, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; 5-OHdC, 5-hydroxy-2′-deoxycytidine; 5-OHdU, 5-hydroxy-2′-deoxyuridine.



of 8-oxodG (8-oxo-7,8-dihydro-2′-deoxyguanosine) and other oxidized guanine lesions were analysed [9]. ESCODD also concluded that the true levels of 8-oxodG in cellular DNA is between 0.5 and 5 lesions per 10^6 dG bases. Among the oxidized DNA lesions, 8-oxodG has been the most studied so far because of mutagenic properties in terms of G > T transversions [5]. The advances particularly in MS-based analysis now allows inclusion and highly specific determination of more lesions, particularly by the use of heavy-isotope-labelled internal standards [10–12].

The comet assay may be the most widely used non-chromatographic method for assessment of oxidative damage to DNA [13]. It detects SBs and abasic sites, whereas base oxidations can be detected by digesting the DNA with enzymes such as FPG (formamidopyrimidine glycosylase) or endonuclease III, which nick DNA at oxidized purines and pyrimidines respectively [14]. This method is recommended for detection of these lesions with minimum risk of spurious oxidation [9]. However, it cannot determine specific base oxidations because of the insufficient specificity of the enzymes.

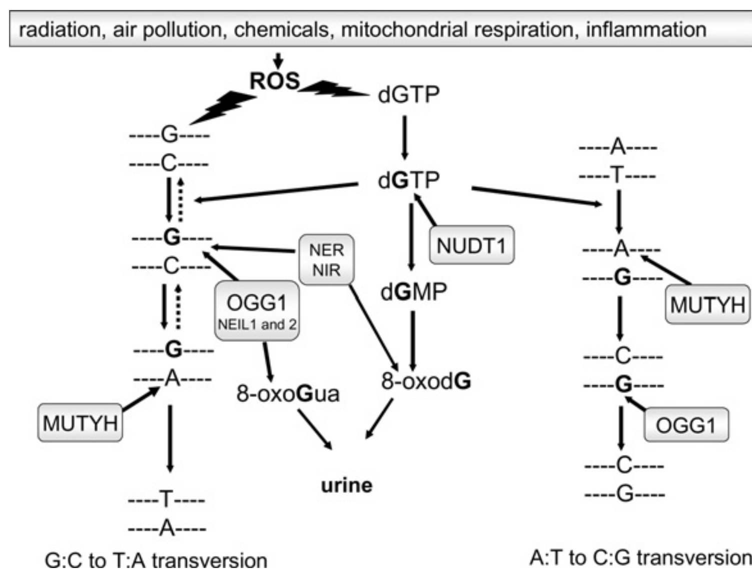
Measurement and importance of repair of DNA oxidation lesions

The complex repair pathways of oxidized bases need to be taken into account in the interpretation of the levels in DNA [5]. As shown in Figure 2, a system of repair enzymes OGG1 (8-oxoguanine DNA glycosylase 1), NEIL1 (nei-like 1) and MUTYH (mutY homologue) have been shown to be central for the prevention of mutations arising from 8-oxodG in DNA with potential supplementary roles of NER and the suggested nucleotide incision mechanisms [3]. OGG1 cleaves the 8-oxoG (8-oxoguanine) base from DNA, whereas the NEIL1 glycosylase may serve as OGG1 backup [4]. Formamidopyrimidine derivates of guanine and adenine, 5-hydroxyuracil and thymine glycol are preferred substrates of NEIL1, whereas the activity towards 8-oxoG is weak and mainly limited to excising lesions in close proximity to the 3′-end of single SBs, DNA bubbles and single-stranded structures, where OGG1 has very limited activity [4]. Additionally, NEIL1 may, like AP (apurinic/apyrimidinic) endonuclease 1, support the action of OGG1, which is bound to the abasic site after removal of 8-oxoG in DNA [5]. MUTYH is a mismatch DNA glycosylase that removes adenine mis-incorporated opposite an oxidized guanine. This allows insertion of a dC nucleotide and a new possibility for removal of 8-oxoG by OGG1, which fortunately has low affinity when this lesion is opposite adenine.

In addition to oxidation in the DNA, guanine can also be oxidized in dGTP. NUDT1 (NDP-linked moiety X-type motif 1) cleaves the pyrophosphate of 8-oxodGTP, which, upon further degradation, is excreted in the urine as 8-oxodG. The removal of 8-oxodGTP from the nucleotide pool is important because otherwise it may be incorporated into DNA during replication. Recently, a salvage pathway recycling

©The Authors Journal compilation ©2008 Biochemical Society

Figure 2 | Sources of formation and repair pathway of guanine oxidation in DNA or in the nucleotide pool
OGG1, NEIL1, NUDT1 and MUTYH have been shown to be central for the prevention of mutations arising from 8-oxodG in DNA with potential supplementary roles of NER transcription-coupled repair and the suggested nucleotide incision mechanisms (NIR).



8-oxodG by phosphorylation has been described *in vitro* [15]. However, it appears unlikely that the high substrate concentrations can reflect the *in vivo* situation and recycling of a mutagenic lesion removed with high specificity does not seem favourable from an evolutionary point of view [16].

In principle, there are two different approaches to measure the enzymatic DNA repair activity in cells [17]. Lesions can be introduced in DNA in viable cells, and the repair activity is assessed as the removal of lesions during a subsequent incubation period. The most popular type of repair activity measured by the comet assay has been the measurement of rejoining of SBs after *ex vivo* exposure to clastogens such as hydrogen peroxide or ionizing radiation [14]. Although these assays for DNA repair activity are simple, they suffer from the drawback that the genotoxic exposure may damage the repair proteins and initiate apoptosis. Moreover, SBs are generated in cultured lymphocytes as a result of exposure to atmospheric oxygen, and they can also accumulate as a consequence of poor repair activity. The validity of repair assays based on the rejoining of SBs in antioxidant intervention studies has been questioned because they cannot distinguish between rejoining of SBs and *ex vivo* scavenging effects by antioxidants [18].

These problems can be overcome by assays where extracts of cells are incubated with substrate DNA containing a defined number of lesions. Nicking assays with 8-oxodG-containing oligonucleotides have been used in large population studies using extracts from leucocytes and, to a more limited extent, tumour tissues collected in human population studies [19–21]. A novel version of the comet assay has been developed for the measurement of oxoG repair activity of cell extracts using substrate nuclei treated with the Ro19-8022 photosensitizer and white light that generates 8-oxodG and very few SBs [22]. The glycosylase enzymes incise at the sites of oxidized bases and the repair activity is measured as the number of incisions that are generated during the incubation. This approach has been used in a few studies and indicates that dietary antioxidants can alter DNA repair activity [17,23]. The repair activity of Ro19-8022-damaged DNA is believed to mainly represent OGG1 [22], whereas NEIL1 and NER enzymes probably make little contribution to the overall repair phenotype.

The activity of NUDT1 can be assessed in tissue extracts by measurement of the 8-oxodGMP formed from 8-oxodGTP by HPLC analysis [24]. The expression levels of mRNAs of DNA repair enzymes have also been used to estimate repair capacity and demonstrate up-regulation of, e.g., *OGG1* transcription from oxidative stress [25,26]. However, the correlation between the mRNA levels and the OGG1-related incision activity is not strong [20,23,27]. *NUDT1* also appears to be subject to regulation as shown by increased expression by oxidants in cell culture systems [15,28].

The *hOGG1* (human OGG1) gene has a common genetic polymorphism with a variant S326C, which appears to increase susceptibility to ROS *in vitro*; however, 8-oxodG levels and incision activity towards an 8-oxodG-containing oligonucleotide in extracts from leucocytes and target tissues generally showed no difference between the genotypes [23,24], whereas the comet-assay-based OGG1 activity measurement showed substantial difference between the genotypes [29].

Urinary excretion of DNA base damage repair products

The urinary excretion of products of damaged nucleotides from cellular pools or DNA may also be important. Of the many oxidative damage products, 8-oxodG is also the most studied in urine with assays based on HPLC–EC

©The Authors Journal compilation ©2008 Biochemical Society

Table 1 | **Studies addressing the possible protective effect on oxidative damage to DNA of diet rich in or supplements of antioxidants and of high-quality design as identified in [43]**

Of a total of 145 identified intervention studies, 45 with a total of more than 2300 participants could be included for the listing here, and they are divided into studies showing a significant effect reduction in levels of damage (pos) or showing null effects of the intervention on the biomarkers.

Type of intervention	All interventions	Antioxidant supplements	Antioxidant-rich diets
Oxidized pyrimidine by use of endonucelase III enzyme in the comet assay	5 pos/10 null	3 pos/3 null	2 pos/7 null
Oxidized purines by use of FPG enzyme in the comet assay	2 pos/5 null	1 pos/2 null	1 pos/3 null
8-OxodG measured in mononuclear blood cells by HPLC-based analysis, excluding studies with levels >10 per 10^6 dG bases	3 pos/3 null	1 pos/3 null	2 pos/0 null
8-OxodG measured in urine by HPLC-based analysis	6 pos/10 null	1 pos/6 null	5 pos/4 null
8-OxodG measured in urine by ELISA	3 pos/8 null	1 pos/5 null	2 pos/3 null
Total	16 pos/29 null	6 pos/14 null	10 pos/15 null

(electrochemical detection), CE (capillary electrophoresis)–EC, GLC–MS, HPLC–MS/MS and ELISA [30]. ELISA yielded severalfold higher concentrations, although with reasonable correlations; $r = 0.42$, 0.83 and 0.88 were obtained in three different trials, with the highest correlations being achieved when HPLC-purified fractions of 8-oxodG were used before the antibody-based detection step [9–11]. This discrepancy was substantiated further by comparison between the ELISA and a HPLC–MS/MS-based analysis [31].

In a steady state, the urinary excretion of 8-oxodG and similar products should, in principle, reflect the average rate of oxidative damage in the whole body, whereas the level of lesions in DNA from target cells or surrogate cells, such as leucocytes, should reflect the balance between damage and repair only in these cell types [12]. The relatively limited influence of the repair capacity in steady state was demonstrated by the only 26% lower 8-oxoG excretion and unchanged 8-oxodG excretion in *OGG1*-knockout mice [32]. So far, the presence of purines or oxidized DNA in the diet has not been shown to contribute to the urinary excretion of 8-oxoG and 8-oxodG [33,34]. Nevertheless, a study with more than 100 subjects showed no correlation on an individual level between the levels of 8-oxodG in leucocytes and the excretion of 8-oxodG and 8-oxoG measured by chromatographic methods [13].

Influence of environment, diet and supplements

Inhalation of direct or indirect oxidants has been associated with elevated DNA oxidation. Short-term exposure to ambient air levels of vehicle emissions, especially ultrafine particles mainly found in diesel exhaust, has been shown to increase the levels of oxidized purines in lymphocytes [27,35–37]. Animal studies indicate that prolonged exposure to diesel exhaust particles induce OGG1 expression and no increase in 8-oxodG levels [25]. Very recently, exposure to wood smoke in humans was shown to increase *hOGG1* mRNA levels and possibly excretion of 8-oxoG and 8-oxodG, although this was not statistically significant [38]. Similarly, long-term heavy exposure in bus drivers can cause increased urinary excretion of 8-oxodG [39]. Smokers have consistently elevated urinary excretion of 8-oxodG, whereas the levels in lymphocytes appears to be more variable [6]. This could be due to different regulation of the repair mechanisms among smokers and non-smokers, although such studies of repair activity have shown mixed results, and there might be interactions with nutritional status and antioxidant intake [17,21,40].

The possible protective effect on oxidative damage to DNA of diets rich in or supplements of antioxidants has been addressed in a large number of studies, as outlined in several reviews [8,41–43] and in Table 1. The biomarkers have included oxidized bases in leucocytes or urinary excretion of 8-oxodG. Of 145 intervention studies identified, 45 were of good design quality with control of confounding from other time-dependent factors. Typically parallel group or cross-over designs in random orders were used. More than a third of the intervention studies showed reduced levels of damage during intervention. This is significantly more than expected by chance, and similar size and power in studies with and without reports of significant effects indicate that this is not likely to be due to publication bias [8,43]. Although not statistically significant, there are trends that antioxidant foods are more efficient than supplements, especially with respect to urinary excretion of 8-oxodG, and that the effects are more likely to be demonstrated in subjects with oxidative stress [43]. Nevertheless, the real problem may be that very few studies have statistical power to detect effects that are of realistic size, i.e. reductions of 10% or less, and the large differences in protocols preclude use of meta-analysis.

Relationship between oxidative damage to DNA and risk of cancer

The variant allele of the *hOGG1* polymorphism has been shown to be weakly associated with risk of lung cancer in a large scale study and meta-analysis [44,45]. The relatively weak association may be related to the fact that the genotype requires interaction with environmental factors to affect risk. Thus exposure to smoky coal was a particular risk factor for adenocarcinoma of the lung among carriers of the variant *hOGG1* allele [46], whereas fruit and vegetable intake was only protective in relation to lung cancer in subjects homozygous for the variant *hOGG1* allele [47]. The importance of

©The Authors Journal compilation ©2008 Biochemical Society

8-oxodG for carcinogenesis is also underscored by observations that biallelic germline variants of the *MUTYH* gene are associated with multiple colorectal tumours [4].

Several case-control studies have indicated a relationship between high excretion rates of 8-oxodG or levels of oxidized bases in DNA from leucocytes and risk of cancers [6]. Similarly, hOGG1 activity was shown to be reduced in mononuclear blood cells from patients with cancer of the lung, head or neck as compared with healthy controls [19,21,48]. Moreover, the activity was correlated between the lung tissue and mononuclear blood cells [19]. The 8-oxodGTPase activity by NUDT1 has been shown to be higher in tumour than normal tissue from 33 patients [49]. However, these differences could well be because the cancer disease affects the levels of DNA damage and the repair capacity, and prospective cohort studies are required to address causality [6]. Such studies are extremely challenging because large numbers of subjects are needed and measurement of oxidative damage to DNA in leucocytes requires isolation of cells immediately after blood sampling and cryopreservation in specific medium. So far, only a few prospective studies have been able to address this issue by means of urinary excretion of 8-oxodG and by mRNA expression of *hOGG1*, *NUDT1* and *MUTYH* in buffy coat samples stored from a cohort for 9–13 years [7,50]. Urinary excretion of 8-oxodG and expression of *hOGG1* mRNA were positively associated with risk of lung cancer in non-smokers and subjects with variant *hOGG1* alleles indicate further that these biomarkers of oxidative stress to DNA bases are relevant for carcinogenesis.

Conclusion

Analytical validation of biomarkers of oxidative damage to DNA bases, the related repair capacity and urinary excretion of the resulting products is making good progress. Biomonitoring studies show consistent dose–response relationships with respect to relevant exposures, e.g. air pollution, and the variation is well described. Intervention studies of effects of antioxidant supplements and diets indicate decreased levels of oxidatively damaged DNA, partly due to increased repair. Levels of oxidatively damaged DNA may be high and repair capacity low in tumour tissue and leucocytes from cancer patients, although these findings could be due to reverse causality. Nevertheless, prospective study of oxidatively damaged DNA of surrogate or target cells as a predictor of risk is extremely difficult due to sampling and storage problems, whereas genetic polymorphisms in the relevant DNA repair genes are free from such concerns and support the importance of oxidative damage to DNA for cancer development. So far, urinary excretion of repair products and expression of *hOGG1* mRNA were positively associated with risk of lung cancer in non-smokers, and subjects with variant *hOGG1* alleles support further this view.

Work is supported by the Danish Research Councils and ECNIS (Environmental Cancer Risk, Nutrition and Individual Susceptibility), a network of excellence operating within the European Union 6th Framework Program, Priority 5: 'Food Quality and Safety' (contract no. 513943).

References

1 Halliwell, B. (2007) Biochemistry of oxidative stress. Biochem. Soc. Trans. **35**, 1147–1150
2 Cadet, J., Douki, T., Gasparutto, D. and Ravanat, J.L. (2003) Oxidative damage to DNA: formation, measurement and biochemical features. Mutat. Res. **531**, 5–23
3 Evans, M.D., Dizdaroglu, M. and Cooke, M.S. (2004) Oxidative DNA damage and disease: induction, repair and significance. Mutat. Res. **567**, 1–61
4 Hazra, T.K., Das, A., Das, S., Choudhury, S., Kow, Y.W. and Roy, R. (2007) Oxidative DNA damage repair in mammalian cells: a new perspective. DNA Repair **6**, 470–480
5 David, S.S., O'Shea, V.L. and Kundu, S. (2007) Base-excision repair of oxidative DNA damage. Nature **447**, 941–950
6 Loft, S. and Møller, P. (2006) Oxidative DNA damage and human cancer: need for cohort studies. Antioxid. Redox Signaling **8**, 1021–1031
7 Loft, S., Svoboda, P., Kasai, H., Tjonneland, A., Vogel, U., Møller, P., Overvad, K. and Raaschou-Nielsen, O. (2006) Prospective study of 8-oxo-7,8-dihydro-2′-deoxyguanosine excretion and the risk of lung cancer. Carcinogenesis **27**, 1245–1250
8 Møller, P. and Loft, S. (2006) Dietary antioxidants and beneficial effect on oxidatively damaged DNA. Free Radical Biol. Med. **41**, 388–415
9 European Standards Committee on Oxidative DNA Damage (ESCODD) (2003) Measurement of DNA oxidation in human cells by chromatographic and enzymic methods. Free Radical Biol. Med. **34**, 1089–1099
10 Dizdaroglu, M., Jaruga, P., Birincioglu, M. and Rodriguez, H. (2002) Free radical-induced damage to DNA: mechanisms and measurement. Free Radical Biol. Med. **32**, 1102–1115
11 Cadet, J., Douki, T., Frelon, S., Sauvaigo, S., Pouget, J.P. and Ravanat, J.L. (2002) Assessment of oxidative base damage to isolated and cellular DNA by HPLC-MS/MS measurement. Free Radical Biol. Med. **33**, 441–449
12 Weimann, A., Belling, D. and Poulsen, H.E. (2002) Quantification of 8-oxo-guanine and guanine as the nucleobase, nucleoside and deoxynucleoside forms in human urine by high-performance liquid chromatography-electrospray tandem mass spectrometry. Nucleic Acids Res. **30**, E7
13 Gedik, C.M. and Collins, A. (2005) Establishing the background level of base oxidation in human lymphocyte DNA: results of an interlaboratory validation study. FASEB J. **19**, 82–84
14 Collins, A.R. (2002) The comet assay: principles, applications, and limitations. Methods Mol. Biol. **203**, 163–177
15 Hah, S.S., Mundt, J.M., Kim, H.M., Sumbad, R.A., Turteltaub, K.W. and Henderson, P.T. (2007) Measurement of 7,8-dihydro-8-oxo-2′-deoxyguanosine metabolism in MCF-7 cells at low concentrations using accelerator mass spectrometry. Proc. Natl. Acad. Sci. U.S.A. **104**, 11203–11208
16 Cooke, M.S. and Evans, M.D. (2007) 8-Oxo-deoxyguanosine: reduce, reuse, recycle? Proc. Natl. Acad. Sci. U.S.A. **104**, 13535–13536
17 Guarnieri, S., Loft, S., Riso, P., Porrini, M., Risom, L., Poulsen, H.E., Dragsted, L.O. and Møller, P. (2008) DNA repair phenotype and dietary antioxidant supplementation. Br. J. Nutr. **99**, 1018–1024
18 Torbergsen, A.C. and Collins, A.R. (2000) Recovery of human lymphocytes from oxidative DNA damage: the apparent enhancement of DNA repair by carotenoids is probably simply an antioxidant effect. Eur. J Nutr. **39**, 80–85
19 Paz-Elizur, T., Krupsky, M., Blumenstein, S., Elinger, D., Schechtman, E. and Livneh, Z. (2003) DNA repair activity for oxidative damage and risk of lung cancer. J. Natl. Cancer Inst. **95**, 1312–1319
20 Paz-Elizur, T., Elinger, D., Leitner-Dagan, Y., Blumenstein, S., Krupsky, M., Berrebi, A., Schechtman, E. and Livneh, Z. (2007) Development of an enzymatic DNA repair assay for molecular epidemiology studies: distribution of OGG activity in healthy individuals. DNA Repair **6**, 45–60
21 Gackowski, D., Speina, E., Zielinska, M., Kowalewski, J., Rozalski, R., Siomek, A., Paciorek, T., Tudek, B. and Olinski, R. (2003) Products of oxidative DNA damage and repair as possible biomarkers of susceptibility to lung cancer. Cancer Res. **63**, 4899–4902

©The Authors Journal compilation ©2008 Biochemical Society

22 Collins, A.R., Dusinska, M., Horvathova, E., Munro, E., Savio, M. and Stetina, R. (2001) Inter-individual differences in repair of DNA base oxidation, measured *in vitro* with the comet assay. Mutagenesis **16**, 297–301
23 Collins, A.R., Harrington, V., Drew, J. and Melvin, R. (2003) Nutritional modulation of DNA repair in a human intervention study. Carcinogenesis **24**, 511–515
24 Bialkowski, K. and Kasprzak, K.S. (2004) Cellular 8-oxo-7,8-dihydro-2′-deoxyguanosine 5′-triphosphate pyrophosphohydrolase activity of human and mouse MTH1 proteins does not depend on the proliferation rate. Free Radical Biol. Med. **37**, 1534–1541
25 Risom, L., Dybdahl, M., Bornholdt, J., Vogel, U., Wallin, H., Mwøller, P. and Loft, S. (2003) Oxidative DNA damage and defence gene expression in the mouse lung after short-term exposure to diesel exhaust particles by inhalation. Carcinogenesis **24**, 1847–1852
26 Risom, L., Møller, P., Vogel, U., Kristjansen, P.E.G. and Loft, S. (2003) X-ray-induced oxidative stress: DNA damage and gene expression of *HO-1*, *ERCC1* and *OGG1* in mouse lung. Free Radical Res. **37**, 957–966
27 Brauner, E.V., Forchhammer, L., Møller, P., Simonsen, J., Glasius, M., Wahlin, P., Raaschou-Nielsen, O. and Loft, S. (2007) Exposure to ultrafine particles from ambient air and oxidative stress-induced DNA damage. Environ. Health Perspect. **115**, 1177–1182
28 Haghdoost, S., Sjolander, L., Czene, S. and Harms-Ringdahl, M. (2006) The nucleotide pool is a significant target for oxidative stress. Free Radical Biol. Med. **41**, 620–626
29 Vodicka, P., Stetina, R., Polakova, V., Tulupova, E., Naccarati, A., Vodickova, L., Kumar, R., Hanova, M., Pardini, B., Slyskova, J. et al. (2007) Association of DNA repair polymorphisms with DNA repair functional outcomes in healthy human subjects. Carcinogenesis **28**, 657–664
30 Cooke, M.S., Olinski, R. and Loft, S. (2008) Measurement and meaning of oxidatively modified DNA lesions in urine. Cancer Epidemiol. Biomarkers Prev. **17**, 3–14
31 Cooke, M.S., Singh, R., Hall, G.K., Mistry, V., Duarte, T.L., Farmer, P.B. and Evans, M.D. (2006) Evaluation of enzyme-linked immunosorbent assay and liquid chromatography-tandem mass spectrometry methodology for the analysis of 8-oxo-7,8-dihydro-2′-deoxyguanosine in saliva and urine. Free Radical Biol. Med. **41**, 1829–1836
32 Rozalski, R., Siomek, A., Gackowski, D., Foksinski, M., Gran, C., Klungland, A. and Olinski, R. (2005) Substantial decrease of urinary 8-oxo-7,8-dihydroguanine, a product of the base excision repair pathway, in DNA glycosylase defective mice. Int. J. Biochem. Cell Biol. **37**, 1331–1336
33 Cooke, M.S., Evans, M.D., Dove, R., Rozalski, R., Gackowski, D., Siomek, A., Lunec, J. and Olinski, R. (2005) DNA repair is responsible for the presence of oxidatively damaged DNA lesions in urine. Mutat. Res. **574**, 58–66
34 Rozalski, R., Siomek, A., Gackowski, D., Foksinski, M., Gran, C., Klungland, A. and Olinski, R. (2004) Diet is not responsible for the presence of several oxidatively damaged DNA lesions in mouse urine. Free Radical Res. **38**, 1201–1205
35 Vinzents, P., Møller, P., Sørensen, M., Knudsen, L.E., Hertel, O., Schibye, B. and Loft, S. (2005) Personal exposure to ultrafine particles and oxidative DNA damage. Environ. Health Perspect. **113**, 1485–1490
36 Sørensen, M., Schins, R.P., Hertel, O. and Loft, S. (2005) Transition metals in personal samples of $PM_{2.5}$ and oxidative stress in human volunteers. Cancer Epidemiol. Biomarkers Prev. **14**, 1340–1343
37 Avobge, P., Ayi-Fanou, L., Autrup, H., Loftm, S., Fayomi, B., Sanni, A., Vinzents, P. and Møller, P. (2005) Ultrafine particulate matter and high-level benzene urban air pollution in relation to oxidative DNA damage. Carcinogenesis **26**, 613–620
38 Danielsen, P.H., Brauner, E.V., Barregard, L., Sallsten, G., Wallin, M., Olinski, R., Rozalski, R., Møller, P. and Loft, S. (2008) Oxidatively damaged DNA and its repair after experimental exposure to wood smoke in healthy humans. Mutat. Res. **642**, 37–42
39 Loft, S., Poulsen, H.E., Vistisen, K. and Knudsen, L.E. (1999) Increased urinary excretion of 8-oxo-2′-deoxyguanosine, a biomarker of oxidative DNA damage, in urban bus drivers. Mutat. Res. **441**, 11–19
40 Asami, S., Hirano, T., Yamaguchi, R., Tomioka, Y., Itoh, H. and Kasai, H. (1996) Increase of a type of oxidative DNA damage, 8-hydroxyguanine, and its repair activity in human leukocytes by cigarette smoking. Cancer Res. **56**, 2546–2549
41 Møller, P. and Loft, S. (2002) Oxidative DNA damage in human white blood cells in dietary antioxidant intervention studies. Am. J. Clin. Nutr. **76**, 303–310
42 Møller, P. and Loft, S. (2004) Interventions with antioxidants and nutrients in relation to oxidative DNA damage and repair. Mutat. Res. **551**, 79–89
43 Loft, S., Møller, P., Cooke, M.S., Rozalski, R. and Olinski, R. (2008) Antioxidant vitamins and cancer risk: is oxidative damage to DNA a relevant biomarker? Eur. J Nutr. **47**, (Suppl. 2), 19–28
44 Hung, R.J., Brennan, P., Canzian, F., Szeszenia-Dabrowska, N., Zaridze, D., Lissowska, J., Rudnai, P., Fabianova, E., Mates, D., Foretova, L. et al. (2005) Large-scale investigation of base excision repair genetic polymorphisms and lung cancer risk in a multicenter study. J. Natl. Cancer Inst. **97**, 567–576
45 Hung, R.J., Hall, J., Brennan, P. and Boffetta, P. (2005) Genetic polymorphisms in the base excision repair pathway and cancer risk: a HuGE review. Am. J. Epidemiol. **162**, 925–942
46 Lan, Q., Mumford, J.L., Shen, M., DeMarini, D.M., Bonner, M.R., He, X., Yeager, M., Welch, R., Chanock, S., Tian, L. et al. (2004) Oxidative damage-related genes *AKR1C3* and *OGG1* modulate risks for lung cancer due to exposure to PAH-rich coal combustion emissions. Carcinogenesis **25**, 2177–2181
47 Sørensen, M., Raaschou-Nielsen, O., Hansen, R.D., Tjonneland, A., Overvad, K. and Vogel, U. (2006) Interactions between the OGG1 Ser326Cys polymorphism and intake of fruit and vegetables in relation to lung cancer. Free Radical Res. **40**, 885–891
48 Paz-Elizur, T., Ben-Yosef, R., Elinger, D., Vexler, A., Krupsky, M., Berrebi, A., Shani, A., Schechtman, E., Freedman, L. and Livneh, Z. (2006) Reduced repair of the oxidative 8-oxoguanine DNA damage and risk of head and neck cancer. Cancer Res. **66**, 11683–11689
49 Speina, E., Arczewska, K.D., Gackowski, D., Zielinska, M., Siomek, A., Kowalewski, J., Olinski, R., Tudek, B. and Kusmierek, J.T. (2005) Contribution of hMTH1 to the maintenance of 8-oxoguanine levels in lung DNA of non-small-cell lung cancer patients. J. Natl. Cancer Inst. **97**, 384–395
50 Hatt, L., Loft, S., Risom, L., Møller, P., Sorensen, M., Raaschou-Nielsen, O., Overvad, K., Tjonneland, A. and Vogel, U. (2008) OGG1 expression and OGG1 Ser326Cys polymorphism and risk of lung cancer in a prospective study. Mutat. Res. **639**, 45–54

Received 30 April 2008
doi:10.1042/BST0361071

©The Authors Journal compilation ©2008 Biochemical Society