# Why and how should we measure oxidative DNA damage in nutritional studies? How far have we come?[1,2]

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**ABSTRACT** Free radicals and other reactive species are constantly generated in vivo and cause oxidative damage to DNA at a rate that is probably a significant contributor to the age-related development of cancer. Agents that decrease oxidative DNA damage should thus decrease the risk of cancer development. That is, oxidative DNA damage is a "biomarker" for identifying persons at risk (for dietary or genetic reasons, or both) of developing cancer and for suggesting how the diets of these persons could be modified to decrease that risk. This biomarker concept presupposes that we can measure oxidative damage accurately in DNA from relevant tissues. Little information is available on whether oxidative DNA damage in blood cells mirrors such damage in tissues at risk of cancer development. Measurement of 8-hydroxylated guanine (eg, as 8-hydroxy-2′-deoxyguanosine; 8OHdG) is the commonest method of assessing DNA damage, but there is no consensus on what the true levels are in human DNA. If the lowest levels reported are correct, 8OHdG may be only a minor product of oxidative DNA damage. Indeed, 8OHdG may be difficult to measure because of the ease with which it is formed artifactually during isolation, hydrolysis, and analysis of DNA. Mass spectrometry can accurately measure a wide spectrum of DNA base damage products, but the development of liquid chromatography–mass spectrometry techniques and improved DNA hydrolysis procedures is urgently required. The available evidence suggests that in Western populations, intake of certain fruit and vegetables can decrease oxidative DNA damage, whereas ascorbate, vitamin E, and β-carotene cannot. *Am J Clin Nutr* 2000;72:1082–7.

## WHY MEASURE OXIDATIVE DNA DAMAGE?

Free radicals and other reactive species are constantly generated in vivo and cause oxidative damage to biomolecules, a process held in check only by the existence of multiple antioxidant and repair systems as well as the replacement of damaged lipids and proteins (1, 2). DNA is probably the most biologically significant target of oxidative attack, and it is widely thought that continuous oxidative damage to DNA is a significant contributor to the age-related development of the major cancers, such as those of the colon, breast, rectum, and prostate (2–4). Consistent with this view, estimates suggest an average of at least a few hundred "oxidative hits" per day on the DNA of each of the $\approx 5 \times 10^{13}$ cells in the human body (4, 5). Damage to DNA by reactive oxygen, chlorine, and nitrogen species generates a multiplicity of different base oxidation and other base modification products (6, 7), which are repaired by a complex system of enzymes (8–10). There is no general agreement even on the order of magnitude of the purine and pyrimidine oxidation products that escape repair and persist in DNA. For example, values for levels of 8-hydroxylated guanine in cellular DNA in some studies are = $0.1/10^5$ guanines and in others = $100/10^5$ guanines (4, 6, 7, 9–15). There is a multiplicity of other oxidative DNA base damage products (6, 7), none of which has yet been subject to the methodologic comparisons that are just beginning (11–15) for 8-hydroxyguanine (8OHG) and its nucleoside 8-hydroxy-2′-deoxyguanosine (8OHdG).

For the sake of argument, I conservatively guess that the sum of all base damage products is $1/10^5$ unmodified DNA bases. The concentrations of benzpyrene-DNA adducts in DNA from malignant tumors taken from smokers have been shown to be 0.65–5.33/$10^6$ DNA bases (16, 17). In rat liver, the calculated carcinogen-DNA adduct concentration associated with a 50% incidence of liver cancer ranged from 53 to 2083 adducts/$10^8$ nucleotides for a range of carcinogens including aflatoxin and dimethylnitrosamine (18). In mouse liver the respective figures were 812–5543 adducts/$10^8$ nucleotides for ethylene oxide, dimethylnitrosamine, 4-aminobiphenyl, and 2-acetylaminofluorene (18). If we assume that DNA base oxidation products such as 8OHdG are only one-tenth as mutagenic, it still seems reasonable to propose that the steady state oxidative damage that can be measured in DNA represents a threat to the cell. The mutagenicity of 8OHdG is well established (9). Several other DNA base oxidation products are mutagenic (19–21), eg, 2-hydroxyadenine, 5-hydroxycytosine, formyluracil, and 5-hydroxyuracil. In addition, the biological consequences of many of the other base oxidation products found in cellular DNA (6, 7) have not been studied in detail.

Thus, current data on the levels of oxidative DNA damage products in cellular DNA are consistent with the concept that oxidative DNA base damage is a major contributor to the risk of cancer development. Reactive oxygen species could, of course, promote cancer development by additional mechanisms, including

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effects on cellular proliferation (22), prevention of apoptosis (23), damage to DNA repair enzymes (24), damage to DNA polymerases leading to decreased fidelity of replication (25), and the binding of end products of lipid peroxidation to DNA bases to create mutagenic lesions (26, 27). The mutagenic effects of products generated by attack of reactive species on deoxyribose residues in DNA (28) may also be worthy of consideration.

If we accept that direct damage to DNA bases by reactive species contributes significantly to the development of cancer, then agents that decrease the amount of such damage should decrease the risk of cancer development. In other words, the steady state oxidative DNA damage in human cells is a "surrogate marker" (or biomarker) predictive (to some extent) of cancer development later in life. It is well known that chronic inflammation, cigarette smoking, and diets rich in fat and poor in fruit and vegetables are associated with increased cancer incidence. Indeed, chronic inflammation elevates levels of several oxidative DNA damage products in human cells (29, 30). Cigarette smoke, many other carcinogens, and a high-fat diet appear to accelerate the formation of 8OHdG in animals (31–34). Consumption of Brussels sprouts in humans and rats and of tomatoes or vegetable juices in healthy human volunteers decreased oxidative DNA damage (35–38). By contrast, β-carotene supplementation did not decrease oxidative DNA damage in humans (39, 40), consistent with the fact that it does not exert an anticancer effect (41).

Thus, the limited data currently available on how oxidative DNA damage is affected by conditions known to influence cancer development seem to justify the use of oxidative DNA damage as a biomarker. Can this biomarker be used to answer key nutritional questions, eg, to identify which fruit and vegetables and which of their constituents are the most protective against oxidative DNA damage (and that therefore should be the most effective in delaying cancer development)? Are the protective agents ascorbate, folate, flavonoids, tocopherols, tocotrienols, carotenoids (other than β-carotene); some or all of these in combination; or something not yet identified? Recent data suggest that, in well-nourished Western subjects, increased intakes of ascorbate, tocopherols, or quercetin do not decrease oxidative DNA damage (40, 42–45). The degree of oxidative DNA damage varies widely between individuals, no matter how it is measured (6, 9, 37, 43, 46–48). It seems likely that both dietary and genetic influences [eg, on rates of free radical generation and rates of repair of oxidative DNA damage (49)] are responsible. Should subjects with oxidative DNA damage greater than the mean be selected for antioxidant therapy to decrease their risk of cancer development (47)? If so, which antioxidants should we give them and how much? Of course, any conclusions drawn from previous and future experiments are valid only if the methods used to measure oxidative DNA damage are valid. Is this the case?

## HOW SHOULD OXIDATIVE DNA DAMAGE BE MEASURED?

### The problem of artifactual oxidation

To measure oxidative DNA damage by most of the currently available methods, one must first isolate DNA. The isolated DNA is then hydrolyzed and the hydrolysate prepared for analysis of oxidized bases. The analytic methods usually involve HPLC or gas chromatography–mass spectrometry (GC-MS). DNA is susceptible to chemical oxidation, the most sensitive base being guanine (6). During isolation and preparation for analysis, DNA and its bases are exposed to ambient oxygen concentrations (hyperoxia compared with nuclear oxygen concentrations) and to transition metal ions. Such metals are potent catalysts of free radical damage and can be present as contaminants in laboratory reagents (2) and equipment, eg, dialysis membranes (50). Metal ions are liberated from intracellular sites of sequestration (eg, lysosomes) during homogenization of tissues before DNA extraction (2). In some procedures, DNA is exposed to elevated temperatures (eg, in acidic hydrolysis and in derivatization for GC-MS) and prooxidant chemicals such as phenol. Isolation, hydrolysis, and analysis all therefore have the potential to cause further artifactual oxidation of DNA (especially of guanine residues), raising the apparent level of base oxidation products and invalidating the measurement. If the steady state level of oxidized bases in cellular DNA is $\leq 1/10^5$ bases, it is easy to see how oxidation of $<0.01\%$ of unmolested DNA bases can invalidate the measurement. When mitochondria are isolated, they continue to generate reactive oxygen species and may even do so at an accelerated rate because of the exposure to 21% oxygen. Beckman and Ames (51) stated recently that for this and other reasons "it is impossible to conclude that mitochondrial DNA suffers greater oxidation than nuclear DNA," contradicting much previous work done in this field.

Many laboratories are intensively investigating better methods of isolating, hydrolyzing, and analyzing cellular DNA (5, 6, 9, 11–15, 52–57). The unspoken criterion by which the results of such endeavors are judged seems to be that the lower the level of oxidized bases in cellular DNA that is obtained, the more likely it is to be correct. This is perhaps logical because one would expect organisms to "perceive" oxidative DNA damage as a threat to the integrity of the genome, and so to minimize steady state concentrations of oxidized DNA bases using their plethora of DNA repair systems. However, there are many surprises in biology, including the realization that exposure of human tissues to potentially DNA-damaging reactive oxygen species may be much greater than is commonly supposed (58, 59); therefore, one must be wary. For example, levels of 8OHG in acid-hydrolyzed calf thymus DNA by GC-MS techniques are often, but not always, higher than those measured (as 8OHdG) by HPLC after enzymic DNA hydrolysis (11, 13). The discrepancy is usually attributed (with justification—*see* below) to artifactual oxidation of guanine during preparation of DNA for GC-MS analysis. However, studies with synthetic oligonucleotides containing known amounts of 8OHdG (12) showed that enzymic digestion by the usual protocols does not result in full release of 8OHdG from double-stranded DNA, ie, HPLC analysis of 8OHdG after enzymatic hydrolysis of DNA may produce underestimates. Protocols involving sodium iodide instead of phenol for DNA extraction were found to decrease measured levels of 8OHdG in isolated DNA (15), but it has been claimed that sodium iodide can destroy 8OHdG in DNA (60).

Perhaps the best approach would be to bypass the above problems by measuring oxidative DNA damage in the intact cell. Antibody methods have been developed (61) and are useful for visualization of damage, but they seem likely to be semiquantitative. The comet assay (50) can be applied directly to cells and measures DNA strand breaks. If a digestion step with DNA repair enzymes is included in the protocol, the increased numbers of DNA strand breaks can be used to estimate the level of oxidized DNA bases in the cell. The values so obtained are generally lower than

those generated by HPLC analysis of isolated enzymically digested DNA from the same cells (50). It may be that the comet assay seriously underestimates the number of base lesions (which I believe is likely, because the enzymes may be unable to reach oxidized bases deep in chromatin and 2 adjacent base lesions may be recorded as a single strand break), or it may be that HPLC analysis overestimates damage. Again, we must not make a priori assumptions about which (if either) technique is correct. The comet assay is fast and can be applied to small numbers of cells, making it useful for human studies, especially large epidemiologic studies, including intervention trials with putative antioxidants. Before use in this way, the comet assay needs to be validated, which can be done only by comparison with rigorous quality-controlled chemical methods applied to pure DNA isolated from the same cells. Hence we cannot as yet escape the problem of getting the "right answer" for concentrations of base oxidation products in isolated DNA.

**Where should we obtain DNA?**

One obvious problem in studying oxidative DNA damage is the limited availability of human tissues from which to obtain DNA. Most studies are performed on DNA isolated from lymphocytes (or sometimes total white cells) from human blood. Sperm, buccal cells, placenta, and biopsies of muscle, skin, colon, and other tissues are other potential sources of DNA, although biopsy samples often yield too little DNA for HPLC- or MS-based methods. Are we justified in assuming that changes in the amount of oxidative DNA damage in white blood cells caused, for example, by antioxidant supplementation, are reflected in the tissues in which cancer is most likely to develop later (eg, breast, prostate, rectum, and colon)? Are the basal amounts of oxidative damage in all body tissues the same? More work needs to be done to answer such questions. For example, studies in dogs showed that endurance exercise decreased concentrations of 8OHdG in lymphocytes and in colon, but not in other tissues (62). Phagocytic white cells such as neutrophils can easily activate during isolation, exposing themselves and adjacent lymphocytes to reactive species during the cell separation process. Thus, in recent studies we preferred to isolate DNA rapidly from whole blood (53).

**Which oxidized DNA bases should we measure?**

The most frequently measured product is 8OHdG, usually assayed by HPLC linked to electrochemical detection, after enzymic hydrolysis of isolated DNA (9, 63). The availability of this sensitive assay was a major reason for 8OHdG being adopted in many laboratories as a biomarker of oxidative DNA damage. Other factors supporting 8OHdG measurement include

*1*) its formation in DNA by several reactive species, such as singlet oxygen and hydroxyl radical (9);
*2*) its established mutagenicity in inducing GC→TA transversions (9); and
*3*) the multiple mechanisms that have evolved to remove 8OHdG from DNA, or to prevent its incorporation into cellular DNA (9, 64, 65), which suggests that the cell "perceives" 8OHdG to be a threatening lesion that has to be removed rapidly (9, 64–66).

Levels of 8OHdG are not a quantitative marker of damage to DNA by all reactive species, because 8OHdG is only a minor product of attack on DNA by reactive nitrogen (eg, peroxynitrite and nitrous acid) or chlorine (eg, hypochlorous acid) species (6). Attack of reactive oxygen species on guanine residues in DNA generates not only 8OHdG but also products such as Fapy-guanine (67). The ratio of 8OHdG to Fapy-guanine is affected by the redox state of the cell (eg, it is decreased at low oxygen concentrations) and by the presence of transition metal ions (67, 68). Hence, it is possible for changes in the level of 8OHdG in DNA to result from changes in redox state and transition metal ion availability, rather than from changes in the rate of oxidative attack on DNA. In other words, the same amount of free radical attack on DNA could give different levels of 8OHdG, depending on the environment around the DNA.

Another drawback to the analysis of 8OHdG relates to the problems described above: 8OHdG is easily formed artifactually by oxidation of guanine during DNA isolation, hydrolysis, and analysis. In addition, 8OHdG is much more oxidizable than is guanine, which allows its sensitive electrochemical detection at low voltage (63), but as a result 8OHdG can easily be destroyed in DNA by reactive species such as peroxynitrite, nitrous acid, and hypochlorous acid (69). Several of the other DNA base oxidation products seem less susceptible to artifactual generation (7, 54, 55). If levels of 8OHdG in cellular DNA really are very low, it may be that some of these other DNA base damage products, less readily formed artifactually, are quantitatively more important in contributing to the overall effect of oxidative DNA damage. In other words, 8OHdG may be only a very minor contributor to the total "mutagenic load" of oxidized DNA bases, in which case more attention to the biological consequences of the other oxidized bases, some of which are already known to be mutagenic (19–21), is required. Of course, interlaboratory comparisons based on quality-controlled materials have been undertaken only for 8OHdG, and equal or greater problems could emerge when concentrations of other oxidized bases are examined in the same way. An example of this would result from a comparison of levels of thymine glycol in the studies by Jenner et al (55) and Le et al (70).

Measuring multiple DNA base oxidation products accurately requires rigorous chemical identification. The best technique available for this is MS, which has been shown to be capable of identifying a wide range of different products of damage to DNA caused by reactive chlorine, oxygen, and nitrogen species (6, 7, 67, 68). MS methods coupled with GC-MS are currently used in several laboratories for analysis of oxidative DNA damage (6, 7, 66, 67), although liquid chromatography (LC)-MS techniques are being developed. A major problem with GC-MS has been that both DNA bases and their oxidation products can be modified artifactually by the acidic DNA hydrolysis procedures usually used and by the derivatization methods used to render products volatile for GC. In particular, significant artifactual hydroxylation of guanine can occur during high-temperature derivatization procedures, if oxygen is inadequately excluded and perhaps for other technical reasons that vary between laboratories (6, 7, 54, 55, 57, 67, 71, 72). Although techniques that appear to minimize these problems now exist (55–57, 72), there is an urgent need for interlaboratory comparisons of them. The problems lie with hydrolysis and derivatization rather than with MS itself, so that the development of LC-MS methods coupled with improved DNA hydrolysis and isolation techniques requires urgent attention.

**Should we measure steady state oxidative DNA damage or rate of oxidative DNA damage?**

Levels of 8OHdG and other modified bases, as measured in DNA isolated from cells, represent a dynamic equilibrium

between rates of oxidative DNA damage and rates of repair of that damage. It follows that levels of oxidized bases can change not only because of changes in the rate of oxidative DNA damage, but also because of alterations in the rate of repair. Fortunately, 8OHdG is excreted in human urine and its measurement has been used to assess whole-body DNA damage (32, 73). This excretion rate can be combined with measurements of 8OHdG in cellular DNA to study the question of rates of repair versus rates of damage. For example, administration of 2-nitropropane to rats led to elevated cellular levels of 8OHdG. Levels subsequently decreased, accompanied by an increase in urinary 8OHdG excretion (32). Cigarette smoking was shown in some studies (31, 34) to raise 8OHdG levels in human cells as well as 8OHdG excretion rates (39). Hence, both nitropropane and constituents of cigarette smoke appear to increase the rate of oxidative DNA damage in vivo, and the rate of repair (at least as indicated by 8OHdG excretion) also increases, although often not to an extent that prevents a rise in the steady state level of 8OHdG.

Measurements of only urinary excretion rates of 8OHdG should be interpreted with caution. For example, an agent that increases 8OHdG excretion rates might be interpreted as "bad" (seemingly increasing DNA damage) but might in fact be "good" (if it stimulated repair and therefore decreased steady state 8OHdG concentrations in DNA). The concentration of 8OHdG in urine is thought to be unaffected by diet and 8OHdG is thought to not be metabolized in humans (32, 73), although more detailed studies on both these points are probably required. In addition, some or all of the 8OHdG excreted in human urine may arise not from DNA, but from oxidation of deoxyguanosine triphosphate (dGTP) in the DNA precursor pool (64, 73). If so, it follows that 8OHdG excretion rates are not a quantitative index of oxidative damage to guanine residues in DNA. As has been the case for measurements of steady state oxidative DNA damage in cellular DNA, the measurement of additional products in urine might indicate unsuspected events. For example, in patients treated with adriamycin, 8OHdG excretion did not change, but there was a significant rise in 5-(hydroxymethyl)uracil in urine (74). Several other DNA base damage products have been identified in human urine (75, 76), although it will be necessary to rule out a confounding effect of diet (absorption and reexcretion of oxidized DNA bases from foods) before these other products can be used as biomarkers. Indeed, more data on this point are needed for 8OHdG. Methodologic questions have also been raised about many of the reported analyses of 8OHdG in urine, especially those based on HPLC, for which interfering peaks can easily co-elute (AR Collins et al, personal communication, 2000; 77–79). MS would, in principle, provide more rigorous identification. Nevertheless, the creatinine-standardized concentrations of 8OHdG measured seem broadly similar among different laboratories (72, 78, 79).

The ideal in human studies would be to measure steady state DNA damage as well as total damage (by urinary excretion rates). If only one set of measurements can be made, the steady state measurement might be preferable because miscoding induced by oxidized bases is presumably what determines the risk of mutation and in turn the risk of cancer development. The repair process itself is not error free, however, and can introduce mutations, so it could be argued that a greater "throughput" of DNA base oxidation is deleterious even if it does not result in significant rises in the steady state concentrations of DNA base damage products. The question remains unanswered.

## CONCLUSIONS

A pessimist might conclude from the above that we should not bother to even attempt to use oxidative DNA damage as a biomarker of cancer risk until we have better methods. Indeed, it could be provocatively argued that 8OHdG is the worst base to measure in cellular DNA, because it is so prone to artifactual formation, and that more attention should be given to the other base oxidation products.

We should continue aggressively the development of MS-based methods (especially LC-MS) for the measurement of DNA base oxidation products and pay less attention than is currently paid to HPLC-based analysis of 8OHdG. We urgently need to establish the biological significance (eg, mutagenicity and repair rates) of the multiple oxidized bases other than 8OHdG that can be found in cellular DNA. Finally, we need to validate assays applicable to whole cells (comet and antibody-based assays) against chemically rigorous methods.

On the basis of available data, can we conclude anything of relevance to nutrition? On the basis of incomplete (but growing) evidence, I propose that *1*) the well-established protective effects of fruit and vegetables against cancer development in Western populations are due, in part or in whole, to their ability to decrease oxidative DNA damage (35–38); *2*) in well-nourished persons, the antioxidants responsible for this action are not ascorbate, vitamin E, or β-carotene (39–42, 80) and possibly not the flavonoids (43). We need to use biomarkers of oxidative DNA damage to determine what the protective agents really are, what their optimal intake is, and whether subjects with elevated rates of DNA base oxidation would benefit from a greater intake of these agents.

## REFERENCES


1. Grune T, Davies KJ. Breakdown of oxidized proteins as a part of secondary antioxidant defenses in mammalian cells. Biofactors 1997;6:165–72.
2. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. 3rd ed. Oxford, United Kingdom: Oxford University Press, 1999.
3. Totter JR. Spontaneous cancer and its possible relationship to oxygen metabolism. Proc Natl Acad Sci U S A 1980;77:1763–7.
4. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants and the degenerative diseases of aging. Proc Natl Acad Sci U S A 1993;90:7915–22.
5. Loft S, Poulsen HE. Markers of oxidative damage to DNA: antioxidants and molecular damage. Methods Enzymol 1999;300:166–84.
6. Halliwell B. Can oxidative DNA damage be used as a biomarker of cancer risk in humans? Free Radic Res 1998;29:469–86.
7. Senturker S, Dizdaroglu M. The effects of experimental conditions on the levels of oxidatively modified bases in DNA as measured by gas chromatography-mass spectrometry. Free Radic Biol Med 1999;27:370–80.
8. Demple B, Harrison L. Repair of oxidative damage to DNA: enzymology and biology. Annu Rev Biochem 1994;63:915–48.
9. Kasai H. Analysis of a form of oxidative DNA damage, 8-hydroxy-2′-deoxyguanosine, as a marker of cellular oxidative stress during carcinogenesis. Mutat Res 1997;387:146–63.
10. Dizdaroglu M, Karakaya AE, eds. Advances in DNA damage and repair. Vol 302. New York: Kluwer Academic/Plenum Publishers, 1999. (NATO ASI series A.)
11. Lunec J. ESCODD: European Standards Committee on Oxidative DNA Damage. Free Radic Res 1998;29:601–8.
12. Lunec J, Herbert KE, Jones GDD, et al. Development of a quality control material for the measurement of 8-oxo-7,8-dihydro-2′-deoxyguanosine, an in vivo marker of oxidative stress, and a comparison of results from different laboratories. Free Radic Res (in press).

13. ESCODD. Comparison of different methods of measuring 8-oxoguanine as a marker of oxidative DNA damage. Free Radic Res 2000;32:333–41.
14. Helbock HJ, Beckman KB, Ames BN. 8-Hydroxydeoxyguanosine and 8-hydroxyguanine as biomarkers of oxidative DNA damage. Methods Enzymol 1999;300:156–66.
15. Helbock HJ, Beckman KB, Shigenaga MK, et al. DNA oxidation matters: the HPLC-electrochemical detection assay of 8-oxo-deoxyguanosine and 8-oxo-guanine. Proc Natl Acad Sci U S A 1998;95:288–93.
16. Bartsch H. DNA adducts in human carcinogenesis: etiological relevance and structure-activity relationship. Mutat Res 1996;340:67–9.
17. Poirier MC, Weston A. Human DNA adduct measurements: state of the art. Environ Health Perspect 1996;104(suppl):883–93.
18. Ottender M, Lutz WK. Correlation of DNA adduct levels with tumor incidence: carcinogenic potency of DNA adducts. Mutat Res 1999;424:237–47.
19. Wang D, Kreutzer DA, Essigmann JM. Mutagenicity and repair of oxidative DNA damage: insights from studies using defined lesions. Mutat Res 1998;400:99–115.
20. Arashidani K, Iwamoto-Tanaka N, Muraoka M, Kasai H. Genotoxicity of ribo- and deoxyribonucleosides of 8-hydroxyguanine, 5-hydroxycytosine, and 2-hydroxyadenine; induction of SCE in human lymphocytes and mutagenicity in *Salmonella typhimurium* TA100. Mutat Res 1998;403:223–7.
21. Fujikawa K, Kamiya H, Kasai H. The mutations induced by oxidatively damaged nucleotides, 5-formyl-dUTP and 5-hydroxy-dCTP, in *Escherichia coli.* Nucleic Acids Res 1998;26:4582–7.
22. Burdon RH. Superoxide and hydrogen peroxide in relation to mammalian cell proliferation. Free Radic Biol Med 1995;18:775–94.
23. Clement MV, Pervaiz S. Reactive oxygen intermediates regulate cellular response to apoptotic stimuli: an hypothesis. Free Radic Res 1999;30:247–52.
24. Lynn S, Yew FH, Chen KS, Jan KY. Reactive oxygen species are involved in nickel inhibition of DNA repair. Environ Mol Mutagen 1997;29:208–16.
25. Feig DI, Loeb LA. Mechanisms of mutation by oxidative DNA damage: reduced fidelity of mammalian DNA polymerase β. Biochemistry 1993;32:4466–73.
26. Chaudhary AK, Nokubo M, Reddy GR, et al. Detection of endogenous malondialdehyde-deoxyguanosine adducts in human liver. Science 1994;265:1580–2.
27. Barbin A. Etheno-adduct-forming chemicals: from mutagenicity testing to tumor mutation spectra. Mutat Res 2000;462:55–69.
28. von Sonntag C. The chemical basis of radiation biology. London: Taylor and Francis, 1987.
29. Wiseman H, Halliwell B. Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. Biochem J 1996;313:17–29.
30. Ohshima H, Bartsch H. Chronic infections and inflammatory processes as cancer risk factors: possible role of nitric oxide in carcinogenesis. Mutat Res 1994;305:253–64.
31. Kiyosawa H, Suko M, Okudaira H, et al. Cigarette smoking induces formation of 8-hydroxydeoxyguanosine, one of the oxidative DNA damages, in human peripheral leukocytes. Free Radic Res Commun 1990;11:23–7.
32. Loft S, Deng XS, Tuo J, et al. Experimental study of oxidative DNA damage. Free Radic Res 1998;29:525–39.
33. Loft S, Thorling EB, Poulsen HE. High-fat-diet-induced oxidative DNA damage estimated by 8-oxo-7,8-dihydro-2′-deoxyguanosine excretion in rats. Free Radic Res 1998;29:595–600.
34. Asami S, Manabe H, Miyake J, et al. Cigarette smoking induces an increase in oxidative DNA damage, 8-hydroxydeoxyguanosine, in a central site of the human lung. Carcinogenesis 1997;18:1763–6.
35. Verhagen V, Poulsen HE, Loft S, et al. Reduction of oxidative DNA damage in humans by Brussels sprouts. Carcinogenesis 1995;16:969–70.
36. Deng XS, Tuo J, Poulsen HE, Loft S. Prevention of oxidative DNA damage in rats by Brussels sprouts. Free Radic Res 1998;28:323–33.
37. Rehman A, Bourne LC, Halliwell B, Rice-Evans C. Tomato consumption modulates oxidative DNA damage in humans. Biochem Biophys Res Commun 1999;262:828–31.
38. Pool-Zobel BL, Bub A, Muller H, Wollowski I, Rechkemmer G. Consumption of vegetables reduces genetic damage in humans: first results of a human intervention trial with carotenoid-rich foods. Carcinogenesis 1997;18:1847–50.
39. von Poppel G, Poulsen HE, Loft S, Verhagen V. No influence of beta carotene on oxidative DNA damage in male smokers. J Natl Cancer Inst 1995;87:310–1.
40. Beatty ER, England TG, Geissler CA, Aruoma OI, Halliwell B. Effect of antioxidant vitamin supplementation on markers of DNA damage and plasma antioxidants. Proc Nutr Soc 1999;58:44A (abstr).
41. Rowe PM. Beta-carotene takes a collective beating. Lancet 1996;347:249 (editorial).
42. Priemé H, Loft S, Nyyssönen K, Salonen JT, Poulsen HE. No effect of supplementation with vitamin E, ascorbic acid, or coenzyme Q10 on oxidative DNA damage estimated by 8-oxo-7,8-dihydro-2′-deoxyguanosine excretion in smokers. Am J Clin Nutr 1997;65:503–7.
43. England TG, Beatty E, Rehman A, et al. The steady-state levels of oxidative DNA damage and of lipid peroxidation ($F_2$-isoprostanes) are not correlated in healthy human subjects. Free Radic Res 2000;33:115–27.
44. Podmore ID, Griffiths HR, Herbert TE, et al. Vitamin C exhibits prooxidant properties. Nature 1998;392:559 (letter).
45. McCall MR, Frei B. Can antioxidant vitamins materially reduce oxidative damage in humans? Free Radic Biol Med 1999;26:1034–53.
46. van Zeeland AA, de Groot AJ, Hall J, Donato F. 8-Hydroxydeoxyguanosine in DNA from leukocytes of healthy adults: relationship with cigarette smoking, environmental tobacco smoke, alcohol and coffee consumption. Mutat Res 1999;439:249–57.
47. Halliwell B. Antioxidant defence mechanisms: from the beginning to the end (of the beginning). Free Radic Res 1999;31:261–72.
48. Bianchini F, Elmståhl S, Martinez-Garciá C, et al. Oxidative DNA damage in human lymphocytes: correlations with plasma levels of β-tocopherol and carotenoids. Carcinogenesis 2000;21:321–4.
49. Benhamou S, Sarasin A. Variability in nucleotide excision repair and cancer risk: a review. Mutat Res 2000;462:149–58.
50. Gedik CM, Wood SG, Collins AR. Measuring oxidative damage to DNA: HPLC and the comet assay compared. Free Radic Res 1998;29:609–15.
51. Beckman KB, Ames BN. Endogenous oxidative damage of mtDNA. Mutat Res 1999;424:51–8.
52. Beckman KB, Saljovghi S, Mashiyama S, Ames BN. A simpler more robust method for the analysis of 8-oxyguanine in DNA. Free Radic Biol Med (in press).
53. Rehman A, Jenner A, Halliwell B. Gas chromatography-mass spectrometry analysis of DNA: optimisation of protocols for isolation and analysis of DNA from human blood. Methods Enzymol 2000;319:401–17.
54. England TG, Jenner A, Aruoma OI, Halliwell B. Determination of oxidative DNA base damage by gas chromatography-mass spectrometry. Effect of derivatization conditions on artifactual formation of certain base oxidation products. Free Radic Res 1998;29:321–30.
55. Jenner A, England TG, Aruoma OI, Halliwell B. Measurement of oxidative DNA damage by gas chromatography-mass spectrometry: ethanethiol prevents artifactual generation of oxidized DNA bases. Biochem J 1998;331:365–9.
56. Douki T, Delatour T, Bianchini F, Cadet J. Observation and prevention of an artifactual formation of oxidized DNA bases and nucleosides in the GC-EIMS method. Carcinogenesis 1996;17:347–53.
57. Hong J, Oh CH, Johnson F, Iden CR. Suppression of adventitious formation of 8-oxoguanine $(TMS)_4$ from guanine during trimethylsilylation. Anal Biochem 1998;261:57–63.
58. Long LH, Evans PJ, Halliwell B. Hydrogen peroxide in human urine: implications for antioxidant defense and redox regulation. Biochem Biophys Res Commun 1999;262:605–9.

59. Long LH, Halliwell B. Coffee drinking increases levels of urinary $H_2O_2$ detected in healthy human volunteers. Free Radic Res 2000; 32:463–67.
60. Lenton KJ, Therriault H, Fulop T, Payette H, Wagner JR. Glutathione and ascorbate are negatively correlated with oxidative DNA damage in human lymphocytes. Carcinogenesis 1999;20: 607–13.
61. Toyokuni S, Iwasa Y, Kondo S, Tanaka T, Ochi H, Hiai H. Intranuclear distribution of 8-hydroxy-2′-deoxyguanosine. An immunocytochemical study. J Histochem Cytochem 1999;47:833–6.
62. Kamura K, Doi T, Sakurai M, et al. Effect of endurance exercise on the tissue 8-hydroxy-deoxyguanosine content in dogs. Free Radic Res 1997;26:523–8.
63. Floyd RA, Watson JJ, Wong PK, et al. Hydroxy free radical adduct of deoxyguanosine: sensitive detection and mechanisms of formation. Free Radic Res Commun 1986;1:163–72.
64. Mo JY, Maki H, Sekiguchi M. Hydrolytic elimination of a mutagenic nucleotide, 8-oxodGTP, by human 18-kiloDalton protein: sanitization of nucleotide pool. Proc Natl Acad Sci U S A 1992;89: 11021–5.
65. Klungland A, Rosewell I, Hollenbach S, et al. Accumulation of premutagenic DNA lesions in mice defective in removal of oxidative base damage. Proc Natl Acad Sci U S A 1999;96:13300–5.
66. Spencer JPE, Jenner A, Aruoma OI, et al. Oxidative DNA damage in human respiratory tract epithelial cells. Time course in relation to DNA strand breakage. Biochem Biophys Res Commun 1996;224: 17–22.
67. Dizdaroglu M. Facts about the artifacts in the measurement of oxidative DNA base damage by gas chromatography-mass spectrometry. Free Radic Res 1998;29:551–63.
68. Fuciarelli AF, Wegher BJ, Blakely WF, Dizdaroglu M. Yields of radiation-induced base products in DNA–effects of DNA conformation and gassing conditions. Int J Radiat Biol 1990;58: 397–415.
69. Zhao K, Whiteman M, Spencer JPE, Halliwell B. DNA damage by nitrite and peroxynitrite: protection by dietary phenols. Methods Enzymol (in press).
70. Le XC, Xing JZ, Lee J, Leadon SA, Weinfeld M. Inducible repair of thymine glycol detected by an ultrasensitive assay for DNA damage. Science 1998;280:1066–9.
71. Cadet J, d'Ham C, Douki T, et al. Facts and artifacts in the measurement of oxidative base damage to DNA. Free Radic Res 1998;29:541–50.
72. Rodriguez H, Jurado J, Laval J, Dizdaroglu M. Comparison of the levels of 8-hydroxyguanine in DNA measured by GC-MS following hydrolysis of DNA by *E. coli* Fpg protein or formic acid. Nucleic Acids Res (in press).
73. Cooke MS, Evans MD, Herbert KE, Lunec J. Urinary 8-oxo-2′-deoxyguanosine—source, significance and supplements. Free Radic Res 2000;32:381–97.
74. Faure H, Mousseau M, Cadet J, et al. Urine 8-oxo-7,8-dihydro-2′-deoxyguanosine versus 5-(hydroxy-methyl)uracil as DNA oxidation marker in adriamycin-treated patients. Free Radic Res 1998;28: 377–82.
75. Stillwell WG, Xu HX, Adkins JA, Wishnock JS, Tannenbaum SR. Analysis of methylated and oxidized purines in urine by capillary gas chromatography-mass spectrometry. Chem Res Toxicol 1989;2:94–9.
76. Ravanat JL, Guicherd P, Tuce Z, Cadet J. Simultaneous determination of five oxidative DNA lesions in human urine. Chem Res Toxicol 1999;12:802–8.
77. Drury JA, Jeffers G, Cooke RW. Urinary 8OHdG in infants and children. Free Radic Res 1998;28:423–8.
78. Bogdanov MB, Beal MF, McCabe DR, Griffin RM, Matson WR. A carbon column-based liquid chromatography electrochemical approach to routine 8OHdG measurements in urine and other biologic matrices: a one-year evaluation of methods. Free Radic Biol Med 1999; 24:647–66.
79. Renner T, Fechner T, Scherer G. Fast quantification of the urinary marker of oxidative stress 8OHdG using solid-phase extraction and HPLC with triple-stage quadrupole mass detection. J Chromatogr B 2000;738:311–7.
80. Huang H-Y, Helzlsover KJ, Appel LJ. The effect of vitamin C and vitamin E on oxidative DNA damage: results from a randomized controlled trial. Cancer Epidemiol Biomark Prevent 2000;9:647–52.