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REVIEW

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Cancer risk and oxidative DNA damage in man

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Abstract In living cells reactive oxygen species (ROS) are formed continuously as a consequence of metabolic and other biochemical reactions as well as external factors. Some ROS have important physiological functions. Thus, antioxidant defense systems cannot provide complete protection from noxious effects of ROS. These include oxidative damage to DNA, which experimental studies in animals and in vitro have suggested are an important factor in carcinogenesis. Despite extensive repair oxidatively modified DNA is abundant in human tissues, in particular in tumors, i.e., in terms of 1–200 modified nucleosides per 10^5 intact nucleosides. The damaged nucleosides accumulate with age in both nuclear and mitochondrial DNA. The products of repair of these lesions are excreted into the urine in amounts corresponding to a damage rate of up to 10^4 modifications in each cell every day. The most abundant of these lesions, 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), is also the most mutagenic, resulting in GT transversions which are frequently found in tumor relevant genes. A series of other oxidative modifications of base and sugar residues occur frequently in DNA, but they are less well studied and their biological significance less apparent. The biomarkers for study of oxidative DNA damage in humans include urinary excretion of oxidized nucleosides and bases as repair products and modifications in DNA isolated from target tissue or surrogate cells, such as lymphocytes. These biomarkers reflect the rate of damage and the balance between the damage and repair rate, respectively. By means of biomarkers a number of important factors have been studied in humans. Ionizing radiation, a carcinogenic and pure source of ROS, induced both urinary and leukocyte biomarkers of oxidative DNA damage. Tobacco smoking, another carcinogenic source of ROS, increased the oxidative DNA damage rate by 35–50% estimated from the urinary excretion of 8-oxodG, and the level of 8-oxodG in leukocytes by 20–50%. The main endogenous source of ROS, the oxygen consumption, showed a close correlation with the 8-oxodG excretion rate although moderate exercise appeared to have no immediate effect. So far, cross-sectional study of diet composition and intervention studies, including energy restriction and antioxidant supplements, have generally failed to show an influence on the oxidative DNA modification. However, a diet rich of Brussels sprouts reduced the oxidative DNA damage rate, estimated by the urinary excretion of 8-oxodG, and the intake of vitamin C was a determinant for the level of 8-oxodG in sperm DNA. A low-fat diet reduced another marker of oxidative DNA damage in leukocytes. In patients with diseases associated with a mechanistically based increased risk of cancer, including Fanconi anemia, chronic hepatitis, cystic fibrosis, and various autoimmune diseases, the biomarker studies indicate an increased rate of oxidative DNA damage or in some instances deficient repair. Human studies support the experimentally based notion of oxidative DNA damage as an important mutagenic and apparently carcinogenic factor. However, the proof of a causal relationship in humans is still lacking. This could possibly be supported by demonstration of the rate of oxidative DNA damage as an independent risk factor for cancer in a prospective study of biobank material using a nested case control design. In addition, oxidative damage may be important for the aging process, particularly with respect to mitochondrial DNA and the pathogenesis of inflammatory diseases.

Key words Oxidative DNA damage · 8-Oxo-7,8-dihydro-2′-deoxyguanosine · Biomarker · Cancer risk · Mutation

Abbreviations *EC* Electrochemical detection · *ELISA* Enzyme linked immunosorbent assay · *GC*/MS Gas chromatography – mass spectrometry · *HPLC* High-performance liquid chromatography · *8-oxodG* 8-Oxo-7,8-dihydro-2′-deoxyguanosine · *RA* Rheumatoid arthritis · *ROS* Reactive oxygen species · *SIM* Selective ion monitoring · *SLE* Systemic lupus erythematosus · *dTg* Thymidine glycol · *Tg* Thymine glycol

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Reactive oxygen species and cancer

Knowledge about the pathogenesis of cancer is a prerequisite for both prevention and treatment of cancer. In living cells reactive oxygen species (ROS) are formed continuously as a consequence of both biochemical reactions and external factors (Fig. 1). Antioxidant defense systems are frequently inadequate, and damage from ROS has been proposed to be involved in carcinogenesis and age-related degenerative diseases [1–9]. Indeed, ROS may act at several steps in multistage carcinogenesis (Fig. 1) [8–10]. ROS can damage DNA, and division of cells with unrepaired or misrepaired damage leads to mutations. If these relate to critical genes such as oncogenes or tumor suppressor genes, initiation and/or progression may result. ROS can interfere directly with cell signaling and growth [10, 11], and cellular damage inflicted by ROS can induce mitosis, increasing the risk that damaged DNA leads to mutations and increasing the exposure of DNA to mutagens, including ROS [12]. Mutations may also be induced by recombination, gene conversion, and nondisjunction during mitosis [12]. Moreover, mitogenesis increases the possibility of clonal expansion of cells transformed by oncogene activation and/or tumor suppressor inactivation.

Epidemiological evidence from cross-cultural, longitudinal, and case-control studies points almost unanimously to reduced risks for cancer, particularly in the upper gastrointestinal tract and airways, associated with a diet rich in antioxidants and/or a high content of antioxidants in plasma samples [8, 13, 14]. Table 1 summarizes a review of studies on the apparent relationship between cancer risk and the intake of fruit and vegetables. However, so far the potentially active principals of the fruit and vegetables have yet to be identified. Several extremely costly large-scale clinical intervention studies of individual antioxidants have not been successful with respect to prevention of lung cancer or colon adenoma [15–18] although a combination of vitamin E, β-carotene, and selenium reduced the risk of gastric cancer in a micronutrient-deficient, high-risk population [19]. Accordingly, the role of oxidative DNA damage in carcinogenesis needs to be defined. Moreover, this may provide possibilities of using intermediary end-points of cancer risk allowing improved focus of intervention strategies before large-scale trials.

The present review focuses on estimation and risk factors of oxidative DNA damage in man, in particular related to the C-8 oxidation of guanine, the most abundant and best studied lesion.

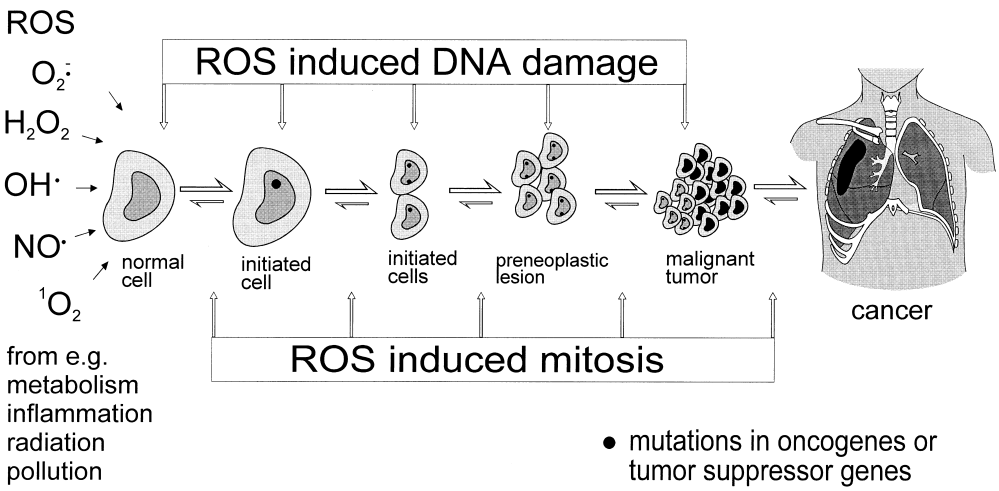

Fig. 1 Possible roles of ROS in multistage carcinogenesis

Table 1 Studies of cancer risk in relation to intake of fruit and vegetables. (Modified from a review by Block et al. [13])

Cancer	No. of studies	Protective (P<0.05)	Harmful (P<0.05)	Relative risk: low vs. high intake (95% CI)
All sites	170	132	6	2.2 (1.2–7.0)
Lung	25	24	0	2.3 (2.1–2.8)
Larynx	4	4	0	2.0 (1.7–2.5)
Oral, pharynx	9	9	0	2.5 (0.5–5.8)
Esophagus	16	15	0	1.9 (0.7–4.8)
Stomach	19	17	1	2.5 (0.5–5.8)
Colorectal	27	20	3	1.9 (0.3–3.3)
Bladder	5	3	0	2.1 (1.6–2.1)
Pancreas	11	9	0	2.8 (1.4–6.4)
Cervix	8	7	0	2.0 (1.2–6.4)
Ovary	4	3	0	1.8 (1.1–2.3)
Breast	14	8	0	1.3 (1.1–2.8)
Prostate	14	4	2	1.3 (0.6–3.5)

Sources of ROS

There are many sources of ROS in the human body (Fig. 2) [20, 21]. During mitochondrial respiration 1–5% of the oxygen undergoes single electron transfer, generating the superoxide anion radical (superoxide, $O_2^{-\bullet}$) in amounts corresponding to 2 kg per year for a human being [22]. Superoxide shows limited reactivity but is converted to hydrogen peroxide (H_2O_2) by superoxide dismutase [20, 21]. Reduction of hydrogen peroxide to water is secured by catalase and glutathione peroxidase. However, in the presence of transition metal such as iron and copper hydrogen peroxide is reduced to hydroxyl radicals ($OH^{\bullet}$), the most deleterious ROS. Inhaled smoke and polluted air as well as ingested food contain ROS and compounds generating ROS by cooxidation/metabolism [23, 24]. A variety of carcinogens, including benzene, aflatoxin, and benzo[a]pyrene, may exert their effect partly through generation of ROS during their metabolism [25–27]. Reperfusion of ischemic tissue leads to generation of ROS through a variety of mechanisms [28, 29]. The metabolism of arachidonic acid gives rise to ROS and the respiratory burst from leukocytes includes superoxide and hypochlorous acid, a ROS known from household bleach [20]. Nitric oxide is also a physiological free radical produced by phagocytes in addition to being a vascular relaxant and neurotransmitter [30]. Although neither nitric oxide nor superoxide is highly reactive, the product of their mutual reaction, peroxynitrite, may generate hydroxyl radicals oxidizing DNA or lead to nitrosation of proteins or deamination of DNA [31]. Low wavelength ionizing radiation (e.g., gamma and X-rays) generates ROS by cleaving water to hydroxyl radicals, superoxide, and hydrogen radicals [32]. UV light is also an important source of ROS, including singlet oxygen [33].

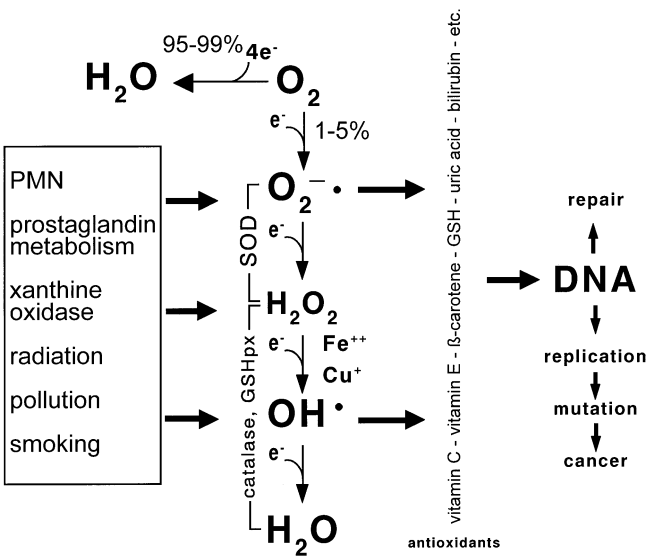

Fig. 2 ROS generated from one electron transfer in the mitochondrial respiration (1–5% of the consumed oxygen [22]) and other endogenous and exogenous sources, the antioxidant defense systems, and potential damage to DNA with possible consequences. *PMN*, Polymorphonuclear granulocytes; *SOD*, superoxide dismutase; *GSHpx*, glutathione peroxidase

Oxidative DNA damage products

ROS can attack any cellular structure or molecule. However, with respect to cancer DNA is considered the most important target (Figs. 1, 2) [8, 9, 34]. Oxidative damage to DNA includes a range of specifically oxidized purines and pyrimidines as well as alkali labile sites and strand breaks, formed directly or by the repair processes [35–37]. The apparently most abundant and certainly the most studied oxidative modification of DNA bases involves the C-8 hydroxylation of guanine (Fig. 3), frequently estimated as the oxidized deoxynucleoside, 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) [5, 35, 38–40]. Oxidation of guanine can also lead to the ring opened product 2,6-diamino-4-hydroxy-5-formamidopyrimidine [35]. Other abundant oxidatively modified purines and pyrimidines include, 8-oxoadenine, 2-hydroxy adenine, FapyAdenine, 5-hydroxymethyluracil, 5-hydroxycytosine, cytosine glycol and thymine glycol (Tg). In addition, a large number of other modifications of bases and sugars have been identified [35, 36]. All these modifications are present in DNA, and the levels can be increased *in vivo* or *in vitro* by systems generating ROS. The yield of the individual DNA modifications is highly dependent on which reactive oxygen species are involved. Thus, whereas singlet oxygen induces preferentially 8-oxodG [33], superoxide has too low reactivity to induce this modification at all [41], and hydroxyl radicals can cause almost any modification [35]. In addition, the target molecule conditions, such as oxygen tension, chelation of the transition metals, and the presence of reductants, may influence the yield and pattern of oxidative DNA modifications [35, 36].

Repair of oxidative DNA damage

In vivo oxidative DNA damage is repaired continuously by a variety of enzymes. Strand breaks are annealed, and modified bases are excised as such or as nucleotides (Fig. 3) [42, 43]. DNA glycosylases excise bases and subsequently phosphodiester bonds on each site of the abasic site are incised by endonucleases, allowing insertion of an intact nucleotide. Some enzymes such as endonuclease III possess both glycosylase and endonuclease activities for repair of oxidized pyrimidines. The repair products of this excision, including thymidine, Tg, and hydroxyuracil, are excreted into the urine. The formamidopyrimidine-DNA glycosylase enzyme (Fpg; mutM) in *Echerichia coli* repairs the oxidized purines 8-oxoguanine and 2,6-diamino-4-hydroxy-5-formamidopyrimidine and to a lesser extent the corresponding adenine derivatives by base excision [44]. The formamidopyrimidine-DNA glycosylase protein appears to repair 8-oxodG

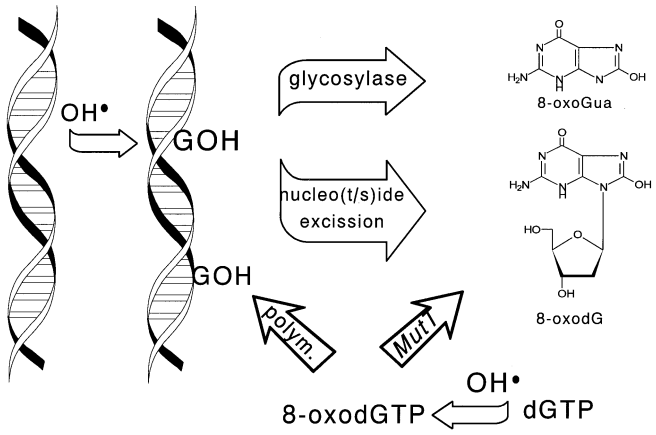

Fig. 3 Oxidative damage to guanine residues (*G*) in DNA or in the nucleotide pool and the relevant repair pathways. *OH*, Hydroxyl radical; *8-oxodG*, 8-oxo-7,8-dihydro-2′-deoxyguanosine; *8-oxoGua*, 8-oxoguanine; *polym*, DNA polymerase; *MutT*, enzyme system that cleaves the phosphates of 8-oxodGTP excretion [50, 111]

in noncoding and actively transcribed mammalian DNA sequences with equal efficiency [45]. After liberation of the damaged base exonucleases incise the apurinic site for insertion of a new nucleotide. In humans similar two-step pathways yielding 8-oxoguanine have been identified, in addition, to repair of 8-oxodG by single-step excision of the complete nucleotide [46]. The UvrABC complex in *E. coli* repair some oxidative DNA lesions by excision of 11–13 nucleotides, including a damaged base such as Tg, 8-oxoguanine, and 2,6-diamino-4-hydroxy-5-formamidopyrimidine as well as abasic sites [47].

Oxidized nucleosides and nucleotides from the cellular pools may be incorporated into DNA and lead to AT to CG base substitution mutations in case of 8-oxoGTP (Fig. 2) [48, 49]. However, the so-called mutT enzyme sanitizes the nucleotide pool by cleaving the phosphates of 8-oxodGTP with high affinity [50]. In humans a homologue to this enzyme has been identified and the genomic structure and chromosome allocation described [51, 52]. The expression of this enzyme appears to be partly tissue and cell type specific [53]. In *E. coli* the mutM and mutT repair enzymes cooperate functionally with mutY, an enzyme which removes adenine misinserted opposite 8-oxoguanine in DNA [54].

A number of other mechanisms and postreplication repairs are functional as further lines of defense [42]. Bacteria lacking one or more of the DNA repair mechanisms have increased mutation frequencies. In humans there are several disease categories with functionally defect DNA repair systems [55, 56]. Some of these have been shown to have increased cancer risk and increased levels of oxidative DNA damage.

Consequences of oxidative DNA damage

Oxidative modifications of DNA bases can lead to mutations if left unrepaired or repaired with errors before replication. The mutational spectra induced by specific ROS have been studied in bacteriophage and plasmid DNA [57]. From these studies it appears that although radicals generated by ionizing radiation damage all four bases, mutations are usually related to modifications of GC pairs whereas modifications of AT pairs rarely lead to mutations ([57] and references therein). The mutations are clustered in hot spots and are mainly basepair substitutions, whereas base deletions, large deletions, and insertions are less frequent. Hydroxyl radicals from ionizing radiation induce mainly GC to CG or AT basepair substitutions, depending on the DNA expression system, whereas hydrogen radicals preferentially induce GC to AT substitutions [57]. Singlet oxygen and 1,2-dioxetanes preferentially induce 8-oxodG and GC to AT basepair substitutions [33, 57–59]. In agreement, modified guanine such as 8-oxoguanine and apurinic sites are mispaired mainly with adenine, at a frequency dependent on the adjacent base sequences and the involved polymerase [48, 60, 61]. In contrast, modified adenine, for example, 8-oxoadenine, does not seem to mispair or lead to mutations [62]. Accordingly, the majority of mutations induced by ROS appear to involve modification of guanine, in particular 8-oxoguanine causing G to T transversions. Indeed, 8-oxodG induces codon 12 activation of c-Ha-*ras* or K-*ras* oncogenes in mammalian systems [48, 49, 63, 64]. In human tumors G to T transversions are among the most frequent hot spot mutations in the p53 suppressor gene [65, 66]. Moreover, in human fibroblasts radicals generated from iron and hydrogen peroxide induce GT and CA transversions at the important codon 249 hot spot in the p53 suppressor gene [67]. In addition to the mutations related to mispairing to oxidized bases, ROS may also cause reduced fidelity of DNA polymerase β [68] and alter the methylation of cytosine and thus gene control [69].

A role for ROS in the development of cancer in humans is further supported by the abundant presence of oxidative DNA modifications in cancer tissue. Thus, the lungs from cancer patients contain 25–75 8-oxodG per 10^5 deoxyguanosine in the apparently normal tissue and two- to threefold higher values in the tumor, in addition to a whole series of other oxidative DNA modifications (Table 1) [70]. For comparison, polyaromatic adducts, the hitherto suspected tobacco-related carcinogens, determined by ^{32}P postlabeling are 3–4 orders of magnitude less frequent than the oxidative modifications in lung tissues of smokers and nonsmokers [71]. The urinary excretion of 8-oxodG and related biomarkers suggests that the rate of oxidative DNA modifications corresponds to up to 10^4 affected bases per cell per day [38, 72–74].

In experimental carcinogenesis involving oxidants, 8-oxodG and other oxidative DNA modifications accumulate in tumor tissue [75, 76]. Moreover, in rats the accumulation of 8-oxodG in liver tissue and the later development of preneoplastic lesions and in turn hepatocellular cancer induced by etinyl estradiol have been reduced considerably by administration of the antioxidants, vita-

min C and E, and/or β-carotene [76]. Furthermore, rat renal sarcomas induced by oxidizing nickel compounds contained GGT to GTT transversion, consistent with 8-oxodG related mutations, in codon 12 of the K-*ras* oncogene [64].

Reports of exponential accumulation of 8-oxodG and a correlation with deletions in mitochondrial DNA from human heart muscle also indicate a role of oxidative DNA modification from the respiratory chain in the muscle weakness associated with aging [77]. Similarly, 8-oxodG accumulates with age in both the mitochondrial and nuclear DNA in the brain [78].

Biomarkers of DNA damage

Cellular DNA

The presence of oxidative damage in genomic and mitochondrial DNA from tissues and isolated cells may be studied by a variety of methods. Gas chromatography/mass spectroscopy with selective ion monitoring (GC/MS-SIM) after acid hydrolysis of chromatin or isolated DNA and derivatization of the bases has been developed and used extensively for determination of at least 20 different oxidized base and sugar products [35, 79]. For quantitative analysis the use of isotopes as internal standards is recommended. However, the most widely used methods involve enzymatic digestion of the DNA isolated by various procedures to nucleotides. Usually the nucleotides are further digested by alkaline phosphatase to nucleosides which are subjected to high-performance liquid chromatographic (HPLC) separation and detection by UV and electrochemistry (8-oxodG) [39]. Conveniently, this allows determination of a ratio between the modified and intact nucleoside in one chromatographic run. Electrochemical detection (EC) may be used for some other oxidized nucleosides such as 8-oxodeoxyadenosine and the corresponding bases whereas thermospray mass spectrometry and radiometric detection of tritiated nucleosides can be applied to the liquid chromatography methods [80, 81]. Alternatively, nucleotides can be subjected to ^{32}P postlabeling and chromatographic separation and radiometric detection [82, 83]. In humans these methods have been applied to accessible cells, such as leukocytes, with or without separation of lymphocytes, and sperm cells as well as surgically or postmortem removed tissues from cancers and various nonmalignant diseases (Table 2). Immunological methods have recently been introduced with slot blot based estimation of 8-oxodG in intact isolated DNA by means of polyclonal antibodies or enzyme-linked immunoassay (ELISA) based estimation of 8-oxodG in DNA digested to nucleosides and purified by an immunoaffinity column [84, 85]. The results of the latter assay were highly correlated with the conventional HPLC-EC assay, although when applied to human tissue the values were six times higher [85].

One problem in relation to the analytical methods is the frequently 10- to 100-fold higher values obtained by GC/MS-SIM than those obtained by the other methods for oxidized base residues in DNA (Table 2) [86]. This difference relates particularly to 8-oxodG although one group has analyzed samples of DNA from plasma immune complexes and of urine with both HPLC-EC and GC/MS, finding identical results [87]. One early explantation of the apparent discrepancies between the methods involves a suggested breakdown of 8-oxodG to 8-oxoguanine and deoxyribose during the preparations for HPLC [86]. Recently, however, values similar to HPLC results have been obtained by GC/MS analysis with new DNA handling procedures [88–90]. These recent studies also show that guanine and other base residues are extensively oxidized during the derivatization procedures usually required for GC/MS analysis [89, 90]. Other problems relate to too high values from the HPLC methods due to artifactual oxidation during the preparation of the nucleosides. To minimize this it is recommended either to avoid phenol or use a special pure grade for DNA isolation and minimize the treatment with alkaline phosphatase [54, 91]. The use of antioxidants such as BHT has also been recommended [92].

In groups of healthy subjects the reported mean levels of 8-oxodG in leukocyte DNA are 0.33–7.1 per 10^5 deoxyguanosine, with interindividual coefficients of variations between 20% and 64%. Regarding variation within subjects the 8-oxodG levels in leukocytes showed no significant correlation (r=0.28) in 19 subjects studied twice [93] whereas another study reported an intraindividual variation below 25% in an unknown number of repeated samples [94].

Indirect methods of estimating oxidative DNA damage include determination of strand breaks by alkaline elution of DNA isolated from cells or tissue or by single cell gel electrophoresis [95–97]. In the latter assay cells, typically lymphocytes, are embedded in a gel, their membranes are lysed and subjected to electrophoresis where the DNA migrates to a comet shape according to the strand breaks. Both these assays can be refined by treating the DNA with the specific repair enzymes which introduce strand breaks where oxidized bases are present [95, 98, 99].

Urine

The repair products from oxidative DNA damage, i.e., oxidized bases and nucleosides, are poor substrates for the enzymes involved in nucleotide synthesis and are fairly water soluble. Thus they are generally excreted into the urine without further metabolism [38, 100]. Injected 8-oxodG is completely excreted into the urine within 4 h in pigs [100]. Among the possible repair products from oxidative DNA modifications 8-oxodG, 8-oxoguanine, Tg, dTg, and 5-hydroxymethyluracil have so far been identified in urine [38, 72, 88, 101–103] (Table 3). Of these 8-oxodG and the thymine derivatives are the most intensively studied.

The assays for the urinary DNA repair products include HPLC with EC for 8-oxodG and 8-oxoguanine and

Table 2 Published values regarding oxidative modifications in nuclear DNA from humans. (*FapyGua* 2,6-diamino-4-hydroxy-5-formamidopyrimidine, *5-OHmU* 5-hydroxymethyluracil, *^{32}P-TLC* ^{32}P postlabeling–thin-layer chromatography, *PMN* polymorphonuclear granulocytes, *MN* mononuclear leukocytes)

Experimental protocol	Tissue/cellular source of DNA	Lesion	Assay	Mean±SD or (range) per 10^5	Reference intact nucleoside/base
10 healthy subjects before vs. after smoking	Leukocytes	8-oxodG	HPLC-EC	0.33±0.08 vs. 0.51±0.25*	[117]
180 nonsmokers vs. 73 smokers	Leukocytes	8-oxodG	HPLC-EC	5.9±3.8 vs. 7.1±4.3*	[118]
15 Fanconi anemia patients and 24 of their parents	Leukocytes	8-oxodG	HPLC-EC	33±26 and 32±46	[118]
19 healthy subjects	MN vs. PMN	8-oxodG	HPLC-EC	1.2±0.4 vs. 1.1±0.4	[93]
79 healthy factory workers	Leukocytes	8-oxodG	HPLC-EC	1.1±0.2**	[93]
7 asbestosis patients vs. 6 control patients	Leukocytes	8-oxodG	HPLC-EC	1.0±0.2 vs. 1.0±0.2	[147]
1 unexposed subject vs. 5 irradiated patients	Leukocytes	8-oxodG	TLC-^{32}P+HPLC-EC	8 vs. 16–112	[119]
9 healthy subjects before vs. immediately after swimming	Lymphocytes	8-oxodG	HPLC-EC	3.5±1.8 vs. 1.6±0.2*	[128]
9 healthy subjects before vs. immediately after running	Lymphocytes	8-oxodG	HPLC-EC	2.2±1.2 vs. 2.3±1.1	[128]
43 control subjects vs. 85 patients with autoimmune disease patients	Lymphocytes	8-oxodG	HPLC-EC	7.0±2.0 vs. 8.1–13.70*;***	[79]
47 controls subjects vs. 93 patients with autoimmune disease patients	PMN	8-oxodG	HPLC-EC	11.8±7.1 vs. 9.6–14.9***	[79]
12 women on normal diet vs. 9 women on low fat diet (n = 12 vs. 9 women)	Leukocytes	5-OHmU	GC/MS	93±19 vs. 30±6*	[141]
50 healthy semen donors	Sperm cells	8-oxodG	HPLC-EC	2.1±3.2	[150]
5 patients with lung cancer	Normal vs. cancer tissue	8-oxoG	GC/MS-SIM	25–75 vs. (50–200)	[156]
5 patients with lung cancer	Tissue	FapyGua	GC/MS-SIM	25–33 vs. (50–120)	[156]
5 patients with lung cancer	Tissue	5-OHmU	GC/MS-SIM	(4–15) vs. (5–19)	[156]
31 patients with renal cancer	Normal vs. cancer tissue	8-oxodG	HPLC-EC	3.6±1.1 vs. 5.6±2.3*	[159]
22 patients with breast cancer	Normal vs. cancer tissue	8-oxodG	HPLC-EC	5.1±3.3 vs. 3.6±1.9	[157]
10 organ donors vs. -	Liver tissue	8-oxodG	HPLC-EC	7.4 (4.5–13.4) vs	[162]
8 Wilson's disease patients vs. 6 hemochromatosis patients	Cirrhotic liver	8-oxodG	HPLC-EC	4.4 (3.2–6.0) vs. 7.2 (4.0–11.6)	[162]
11 livers with metastasis vs. 15 chronic hepatitis patients -	Normal vs. inflamed liver	8-oxodG	HPLC-EC	1.6±0.7 vs. 3.2±2.1*	[158]
vs. 13 liver cirrhosis patients vs. 18 liver cancer patients	Cirrhotic vs. cancer tissue	8-oxodG	HPLC-EC	2.3±1.4 vs. 2.3±1.6	[158]
10 autopsies	Brain tissue	8-oxodG	HPLC-EC	(1.3–2.7)	[78]
23 healthy mothers	Placenta	8-oxodG	HPLC-EC vs. ELISA	(0.1–1.0) vs. (1.5–7.8)	[85]
15 patients with prostatic hyperplasia	Normal vs. hyperplastic tissue	8-oxodG	GC/MS-SIM	(10–100) vs. (30–180)	[161]

* $p<0.05$; ** no correlation with age or smoking status; *** mean values of groups with SLE, RA, vasculitis, scleroderma and Behçet's disease; similarly increased values were obtained for other modifications and in cancers of brain, colon, stomach , ovary [70] and breast [154] by GC/MS.

Table 3 Published values regarding urinary biomarkers of oxidative DNA damage in humans (*8-oxoG* 8-oxoguanosine)

Experimental protocol	Age (years)	Lesion	Assay	Mean±SD or (range)	Reference
9 healthy subjects	Unknown	dTg and Tg	HPLC	390±360 and 100±50 pmol/kg 24h	[72]
10 healthy subjects vs. 20 cancer patients	Unknown	dTg	HPLC	435±120 vs. 347±156 pmol/kg 24h	[160]
10 healthy subjects vs. 20 cancer patients	Unknown	Tg	HPLC	174±54 vs. 125±45 pmol/kg 24 h	[160]
2 cancer patients before vs. after radiotherapy	57–59	dTg	GC/MS	8–10 vs. 20–37 nmol/24 h	[114]
100% vs. 60% energy in diet (n = 1)	50	dTg	GC/MS	250 vs. 106 pmol/kg 24 h	[103]
5 healthy subjects	Unknown	8-oxodG	HPLC	323±23 pmol/kg 24 h	[38]
63 healthy subjects	Unknown	8-oxodG	HPLC	172±79 pmol/kg 24 h	[105]
23 healthy subjects	Unknown	8-oxodG	GC/MS	300±100 pmol/kg 24 h	[103]
53 healthy nonsmokers vs. 30 healthy smokers	40–64	8-oxodG	HPLC	213±84 vs. 320±99 pmol/kg 24 h*	[74]
21 healthy nonsmokers vs. 12 healthy smokers	33±10	8-oxodG	HPLC	318±130 vs. 431±168 pmol/kg 24 h*	[120]
24 healthy nonsmokers vs. 10 healthy smokers	Unknown	8-oxodG	HPLC	1.0±0.4 vs. 1.3±0.4 nmol/mmol creatinine*	[104]
Smokers on placebo (n = 57) vs. β-carotene 20 mg/day (n = 65)	39±10	8-oxodG	HPLC	2.8±1.2 vs. 3.0±1.1 nmol/mmol creatinine	[149]
300 g vegetable diet vs. 300 g Brussels sprouts diet (n = 5)	Young	8-oxodG	HPLC	300–630 vs. 210–490 pmol/kg 24 h*	[151]
100% vs. 80% energy in diet (n = 16)	35–50	8-oxodG	HPLC	629±218 +78±189 pmol/kg 24 pmol/kg*	[113]
100% vs. 60% energy in diet (n = 1)	50	8-oxodG	GC/MS	345 vs. 110 pmol/kg 24 h	[103]
9 swimmers studied before vs. immediately after swimming	20±1	8-oxodG	HPLC	2.3±0.5 vs. 2.5±0.4 nmol/mmol creatinine	[128]
9 runners studied before vs. immediately after running	21±2	8-oxodG	HPLC	1.4±0.5 vs. 1.6±0.5 nmol/mmol creatinine	[128]
10 rowers studied before vs. immediately after a rowing bout	23 (19–30)	8-oxodG	HPLC	(1.6–3.7) vs. (1.4–14.7) nmol/mmol creatinine	[129]
7 noncancer patients vs. 10 cancer patients	Unknown	8-oxodG	HPLC	1.1±0.6 vs. 1.8 to 3.4 nmol/mmol creatinine*	[104, 107]
10 vs. 11 healthy subjects vs	20–47 vs. 62–82	8-oxodG	HPLC	(1.0–5.2) vs. (0.8–3.9) nmol/mmol creatinine	[87]
10 RA patients vs. 33 SLE patients	57–80 vs. 20–64	8-oxodG	HPLC	(25–32) vs. (<0.1) nmol/mmol creatinine*	[87]
10 healthy subjects vs. 11 cystic fibrosis patients	Mean 9 vs. 5–16	8-oxodG	HPLC	1.5±0.4 vs. 2.8±1.2 nmol/mmol creatinine*	[166]
2 cancer patients before and after radiotherapy	57–59	8-oxodG	GC/MS	8–14 vs. 31–40 nmol/24 h	[114]
6 healthy nonsmokers vs. 7 healthy smokers vs	22–25 vs. 23–60	8-oxoguanine	HPLC	3.8±1.9 vs. 7.41±1.5 ng/mg creatinine*	[102]
3 healthy subjects exposed to polluted air	22–25	8-oxoguanine	HPLC	max. 12–18 ng/mg creatinine*	[102]
11 healthy subjects before vs. after exercise vs	18–35	8-oxoG	HPLC	405±85 vs. 310±85 pmol/kg 24 h	[112]
after vitamin C and E and β-carotene supplementation	18-35	8-oxoG	HPLC	390±85 pmol/kg 24 h	[112]
53 healthy subjects	Unknown	8-oxoG	HPLC	335±125 pmol/kg 24 h	[105]
Unknown	Unknown	8-oxoG	HPLC	950 pmol/kg 24 h	[167]

* P<0.05

UV for dTg and Tg, whereas all the repair products can be measured by GC/MS-SIM [88, 103]. The HPLC methods require extensive clean-up procedures such as multiple solid-phase extractions, HPLC column switching techniques, or immunoaffinity columns [38, 72, 74, 104–107]. In both the GC/MS-SIM and HPLC methods recovery may be a problem requiring labeled internal standards, and the complicated procedures limit the analytical capacity. An ELISA assay based on monoclonal antibodies has been developed for estimation of 8-oxodG in urine samples [108]. However, the values obtained in rat urine are three to five times higher than other published values. Similarly, in four smokers studied before and after smoking cessation the urinary 8-oxodG excretion values estimated by the ELISA method were eight times higher than and showed only a weak correlation (r=0.42) with the values obtained by HPLC [109].

The repair pathway of 8-oxoguanine in DNA has been debated in relation to the use of the base or the nucleoside as urinary biomarkers [105]. Two different DNA repair enzymes, one with glycosylase activity and one excising single 8-oxodG as a nucleotide, have been isolated from nuclear extracts of a human cell line [46]. In excision repair deficient human cell lines plasmids containing 8-oxodG were replicated at a rate of only 25% of the rate of proficient cell lines and with a three to fivefold increased frequency of G-T transversion mutations typical for 8-oxodG [110]. This suggests that nucleotide excision is the most important pathway for 8-oxodG in humans. Moreover, the 8-oxodG phosphatase and 8-oxodGMP nucleotidase selectively and rapidly convert the liberated oxidized nucleotide to a nucleoside ready for excretion [50, 111]. These enzymes also sanitize oxidized dGTP from the cellular pool and allow its excretion as 8-oxodG. In addition, digestion of damaged DNA from cell renewal and mitochondrial turnover liberates 8-oxodG. Animal experiments have shown that injected 8-oxodG is readily excreted unchanged into the urine whereas 8-oxodG in the diet or oxidation of deoxyguanosine during excretion does not contribute [38, 100, 105]. At least in rats dietary purines are an important determinant of the excretion of 8-oxoguanine, which is far larger than the excretion of 8-oxodG [105]. However, in humans the excretion of 8-oxoguanine and 8-oxodG are in the same range and both are increased by smoking [74, 102]. Accordingly, although the exact relative importance of the repair pathways remains to be determined, the urinary excretion of 8-oxodG reflects the general average risk of a promutagenic oxidative adduct in DNA of all tissues and organs. It is possible that 8-oxoguanine in the urine will become a valuable addition allowing a complete account of the repair of 8-oxodG in DNA. The RNA oxidation product 8-oxoguanosine has also been used a urinary biomarker [112]. The excretion is three to four times higher than of 8-oxodG.

The 24-h urinary excretion of 8-oxodG shows a sevenfold range within the studied populations and an intersubject coefficient of variation of 30–40% (Table 3). The intraindividual coefficient of variation was 22% in 8 subjects examined twice on a controlled diet for 10 weeks [113], and in 20 subjects studied twice separated by 130 days the measured 8-oxodG excretion rates were significantly correlated (r=0.73) [105]. In urine stored at –20°C 8-oxodG is stable for at least 4 years ([113]; unpublished observations). For Tg and dTg excretion the interindividual coefficients of variation range from 28% to 92% (Table 3).

The use of the urinary excretion of repair products as biomarkers of oxidative DNA assumes extensive repair. After ionizing radiation the increase in urinary excretion of Tg and 8-oxodG occurred within 24-h in humans whereas excess 8-oxodG was removed from mouse liver DNA after approximately 90 min [114–116]. In a study of 8-oxodG in human brain the accumulation rate in the nuclear DNA corresponded to two lesions per cell per day [78]. The reported values of the urinary excretion of the repair product, 8-oxodG, are in the range 200–600 pmol/kg 24 h, which correspond to an average of 168–504 lesions per day for each of the assumed 5×10^{13} cells in the body per day [74, 113]. In addition, the alternative repair product 8-oxoguanine appears to be excreted in similar amounts [102] Accordingly, the calculated repair efficiency under these assumptions ranges from 99.4% to 99.8%.

Due to the extensive and rapid repair the urinary excretion of the repair products reflects the average rate of oxidative DNA damage in all the cells in the body. In contrast, the levels of oxidized bases in DNA lymphocytes or other accessible cells reflects the steady-state levels, i.e., the balance between damage and repair, albeit only in a surrogate for target tissues. Accordingly, the two groups of biomarkers are supplementary.

Determinants of oxidative DNA damage

The extent of oxidative modifications in DNA in material from humans has been studied in relation to a large number of factors and conditions (Table 2 and 3). In the majority of studies 8-oxodG has been the choice of measurement. Whereas there is good agreement between different laboratories regarding the values of urinary excretion of the repair products, the values obtained from DNA isolated from tissues or cells differ by several orders of magnitude, some of which may be related to the choice of analytical method. For example GS/MS usually reports 10- to 100-fold higher values than HPLC-EC, as discussed above. However, even using HPLC-EC the average values of for example, 8-oxodG in leukocyte DNA range from 0.3 per 10^5 deoxyguanosine in a group of Japanese subjects to 7 per 10^5 deoxyguanosine in a group of Italian subjects (Table 2) [117, 118]. Nevertheless, the relative difference between groups within each laboratory appears to be of the same magnitude. Moreover, even the lowest reported number of 8-oxodG equals 0.1 per 10^5 intact deoxyguanosine, i.e., two orders of magnitude higher than adducts of classical aromatic carcinogens, such as benzo[a]pyrene in lymphocyte or lung

tissue DNA [71]. The combined urinary excretion rates of 8-oxodG and 8-oxoguanine in healthy humans correspond to an average oxidation rate of approximately to 300–1000 guanine bases per cell every day as previously described. In addition, a plethora of other possible oxidative modifications of DNA take place.

Ionizing radiation, a classical and pure source of ROS, particularly hydroxyl radicals, has in a few subjects been shown to increase 8-oxodG levels in circulating leukocytes (Table 2) [119] and to increase the urinary excretion rate of 8-oxodG and dTg (Table 3) [107, 114]. This indicates that oxidative DNA modification in vivo can be detected in humans by means of the biomarkers.

Age

So far no published study has systematically addressed age as a determinant of the markers of oxidative modification of DNA in humans. From the studies involving different age groups it appears that the urinary excretion rate of the repair products decreases with age (Table 3). However, the 8-oxodG levels in leukocyte DNA appeared to increase linearly with age corresponding to 0.09 ± 0.01 per 10^5 deoxyguanosine per year [118]. Similarly, mitochondrial DNA from muscles and both nuclear and mitochondrial DNA from brain accumulate 8-oxodG with age [77, 78]. The accumulation of 8-oxodG in nuclear DNA from brain corresponds to approximately 0.04 modifications per 10^5 deoxyguanosine per year, or two modifications per cell per day [78]. Similar results regarding tissue DNA and urinary excretion of 8-oxodG have been obtained in aging rats [3]. These data thus suggest that the rate of damage decreases with age, possibly along with the decreasing rate of metabolism, whereas the steady-state levels increases due to failing repair.

Metabolism, exercise, and diet

A close relationship between the urinary excretion rate of 8-oxodG and 24-h oxygen consumption or the change in resting metabolic rate after energy restriction has been shown in humans [113, 120]. Similar correlations, including dTg excretion, have been reported across species [73, 103, 121, 122]. This apparent relationship between oxidative DNA damage and oxygen consumption is thought to be due to the 1–5% undergoing single electron transfers to generate ROS during mitochondrial respiration [22]. Hydrogen peroxide formation per milligram of mitochondrial protein [123] and the summed mitochondrial surface area [124] have been shown to be correlated with the metabolic rate across species. In accordance, 8-oxodG is 10–15 times more abundant in mitochondrial DNA than in nuclear DNA [78, 125, 126]

Exercise would be expected to increase the metabolic rate and might thus increase the rate of oxidative DNA damage in parallel with the signs of lipid and protein oxidation related to exercise that has been well described [127]. However, this notion is only partially supported by the limited available data from athletes. Immediately after running or swimming, involving energy expenditure of approximately 3000–4000 kJ, the creatinine-corrected concentration of 8-oxodG in urine was unchanged whereas the levels of 8-oxodG in lymphocyte DNA were unchanged and reduced, respectively (Tables 2, 3) [128]. An unchanged 8-oxodG to creatinine ratio was also reported after a 6-min all-out rowing bout [129]. After 90-min exercise bouts at 65% of maximal oxygen uptake there was no significant change in the 24-h excretion of a product of oxidative RNA modification, 8-oxoguanosine [112]. However, these data should be interpreted with caution since the extent of exercise was limited, the urinary collection periods for 8-oxodG were very short, and immediately after exercise and the creatinine concentration used for correction could be affected by increased production during exercise, as supported by increased plasma concentrations of creatinine after running [128]. Similarly, acute changes in the lymphocyte DNA could be related to the recruitment of young lymphocytes, as shown by increased counts after exercise [128]. In fact, 10 h after a massive exercise effort in a marathon run a 130% increase in the urinary 8-oxodG to creatinine ratio has been reported [130]. Moreover, data from the authors′ laboratory show a significant increase in the 8-oxodG to creatinine ratio in 20 soldiers after a period of 30 days with extensive exercise 8–11 h per day [131].

In rodents energy restriction consistently increases life span and reduces the incidence of both spontaneous and well as carcinogen induced tumors [132, 133]. This effect may be related to a decreased metabolic rate and improved coupling of mitochondrial respiration with less ROS generation [133, 134], in addition to increased activity or expression of antioxidant and DNA repair enzymes [133–135]. Accordingly, in rodents energy restriction is associated with decreased levels of oxidative modifications in nuclear and mitochondrial DNA from liver and mammary gland [136, 137] and with decreased lipid peroxidation and oxidative modification of protein [133, 134, 138, 139]. Similarly, in a single human subject energy restriction by 40–50% for periods of 10 days reduced the urinary excretion rate of 8-oxodG and dTg by 50–80% [103]. However, in a controlled study of 16 subjects on a diet containing 80% of their weight-maintaining energy for 10 weeks there was a slight increase in the rate of urinary 8-oxodG excretion and no change in the 8-oxodG levels in lymphocyte DNA (Table 3) [113, 140]. In this study a control of 8 subjects continued on a diet with 100% of their weight-maintaining energy.

The reduction in fat intake to a target of 15% of the energy for 3–24 months reduced the level of 5-hydroxyuracil in DNA of circulating lymphocytes by 68% in 9 of 21 women at risk of breast cancer, compared to the remaining 12 who continued with a fat intake in excess of 30% of the energy [141]. However, a cross-sectional study observed no relationship between the percentage

of energy intake from fat and the excretion of 8-oxodG[74].

With regard to the effect of metabolism, exercise, and diet on the rate of oxidative DNA modification a clear relationship has been shown for oxygen uptake, and massive exercise appears to increase the damage whereas limited exercise may have no effect. Possible effects of energy restriction and dietary fat have not been finally established.

Tobacco smoking and other environmental exposures

Tobacco smoking has consistently been shown to increase the urinary excretion rate of 8-oxodG by 30–50% (Table 3) [74, 104, 120, 128]. Similarly, tobacco smoking increased the creatinine-corrected concentration of the alternative repair product 8-oxoguanine in the urine [102]. With regard to leukocyte DNA two studies have shown similar effects on the levels of 8-oxodG while one study failed to show a relationship with smoking status (Table 2) [93, 117, 118]. In DNA isolated from placentas the 8-oxodG values estimated by ELISA were significantly higher in smokers than in nonsmokers as determined by questionnaires although there was no significant correlation with plasma cotinine levels [85].

The mechanism of oxidative stress from tobacco smoking may involve both an increase in the rate of metabolism with an increase in mitochondrial production of ROS in the cells, and the presence of ROS and ROS-generating compound in the smoke. Tobacco smoke and several of its constituents, including hydroquinone and cathecol, have been shown in vitro to generate ROS and to induce oxidative damage to isolated DNA and to produce 8-oxodG in cell cultures [142, 143]. The volatile phase of tobacco smoke induces lipid peroxidation and lipoprotein oxidation in human plasma in vitro [23]. In accordance, low concentrations of the important antioxidants, ascorbic acid and β-carotene, have been found in the plasma of smokers, suggesting increased consumption and reduced antioxidant capacity [23, 144, 145].

A number of toxic occupational and other environmental exposures could be expected to act partly by inducing oxidative damage to DNA. The biomarkers discussed in the present review seem well suited for the study of such hypotheses. The leukemogenic effect of benzene, a component of gasoline and an industrial chemical, may be related to its metabolism and to induction of oxidative DNA damage, as shown by the formation of 8-oxodG both in cell cultures and in bone marrow in vivo [25]. Indeed, the creatinine-corrected urinary concentration of 8-oxodG was found to be correlated significantly (r=0.34) with benzene exposure in 65 fuel filling attendants [118]. Although asbestos induces 8-oxodG *in vitro* [146], there was no significant difference between patients with asbestosis and a hospital control without exposure [147]. Nevertheless, rubber, azo dye, and asbestos-exposed workers from Poland have been reported to have increased ratios of 8-oxodG to creatinine in the urine [148]. Exposure to heavily polluted urban air for 3 h increased the urinary creatinine corrected concentration of 8-oxoguanine, the alternative repair product of 8-oxodG in DNA [102].

So far, tobacco smoking and occupational or other environmental exposures known to be carcinogenic appear to induce oxidative DNA damage. Whether this is an important part of their mechanism in man has yet to be demonstrated.

Antioxidant and phytochemicals

Antioxidant supplementation could be expected to reduce the rate of oxidative DNA modification, but as yet the data have provided limited support for this notion. The daily administration of 20 mg β-carotene for 20 weeks had no effect on the excretion rate of 8-oxodG [149] in smokers. In agreement, neither β-carotene nor vitamin E reduced the risk of lung cancer in a large-scale clinical intervention study involving approximately 30’000 smokers [15]. Similarly, the urinary excretion of the RNA damage product, 8-oxoguanosine, was not affected by the daily administration of vitamins C (1000 mg) and E (533 mg) and β-carotene (10 mg) for 1 month [112]. In fact, a positive correlation between the creatinine corrected 8-oxodG levels in urine and the plasma levels of α-tocopherol has been reported in patients with cystic fibrosis [87], although a biological mechanism for such an association is hard to conceive. In contrast, the 8-oxodG levels in DNA from human sperm depend on the concentration of vitamin C in the seminal plasma, as shown both in relation to the spontaneous variation and as a result of vitamin C depletion [150]. In this context sperm DNA may be a special case, where vitamin C is the dominating antioxidant.

A potential DNA protective effect of specific vegetable components was suggested by a 28% reduction in the rate of urinary 8-oxodG excretion after a diet with 300 g Brussels sprouts in comparison with 300 g noncruciferous vegetables [151]. Indeed, cruciferous vegetables such as Brussels sprouts and broccoli contain certain phytochemicals which are anticarcinogenic possibly by inducing enzymes scavenging electrophiles and by mimicking the cellular protective response to oxidative stress [152, 153].

So far antioxidant supplementations have failed to alter the estimated rate of oxidative DNA damage except that vitamin C appears to be important for sperm DNA. Similarly, controlled trials of antioxidants have generally failed to show protection from cancer. Specific plant constituents, for example, from Brussels sprouts, may be responsible for the beneficial effects of vegetable- and fruit-rich diets indicated by epidemiological studies [8, 13, 14]. The use of biomarkers of oxidative DNA damage in intervention studies may target optimum combinations of phytochemicals and antioxidant supplementation for use in large scale cancer prevention trials.

Diseases

The biomarkers of oxidative DNA modifications have been studied in relation to a variety of diseases, in particular cancers. A number of cancers from lung, colon, ovary, and breast have been studied with the GC/MS-SIM assays for a range of oxidatively modified DNA bases. Most of these were found to be higher in cancerous tissue than in the surrounding histologically normal tissue (Table 2) [70, 154–156]. Moreover, in lung cancer tissues decreased activities of the antioxidant enzymes superoxide dismutase and catalase were also found [156]. However, although the comparisons are relative between cancerous and noncancerous tissue, the data obtained by GC/MS analysis should be interpreted with caution due to the potential artifactual formation of oxidative modifications during sample preparation, as previously discussed. With the HPLC-EC assay for comparison of 8-oxodG in cancerous and cancer-free tissues the levels were not increased in breast or liver cancer [157, 158] whereas the levels were increased by 54% in renal cell carcinoma [159].

Two studies have found an increased urinary excretion rate of 8-oxodG or 8-oxodG to creatinine ratio in patients with malignant diseases [104, 107], whereas a third showed rates of Tg and dTg excretion similar to those of healthy control subjects [160] (Table 3). Various cancer treatments, including radiation and certain chemotherapeutic agents, have been reported to increase the 8-oxodG excretion rate [107, 114].

Higher levels have recently been reported of several oxidative DNA modifications, including 8-oxodG, in hyperplastic tissue than in surrounding normal tissue in patients with benign prostatic hyperplastic [161]. The excess oxidative modification was apparently found in hyperplastic tissues with decreased activities of superoxide dismutase and catalase, supporting a preventive role of these antioxidant enzymes [161].

Transition metals such as iron and copper catalyze the production of hydroxyl radicals in Fenton-type reactions. Thus, Wilson's disease and hemochromatosis with defect storage and accumulation of copper and iron resulting in liver cirrhosis would be expected to involve oxidative DNA damage. Surprisingly, the levels of 8-oxodG were not increased in liver tissue from patients with end-stage disease requiring transplantation as compared with liver tissue from donor organs (Table 2) [162]. However, it should be noted that the 8-oxodG levels in the donor liver tissue were much higher than the values reported by other investigators (Table 2), and it may not be the proper control. In fact, other yet unidentified bulky DNA adducts were found in the liver tissue from the patients with the storage diseases [162]. Moreover, Long-Evans cinnamon rats have a copper storage defect and develop hepatic cancer preceded by increased 8-oxodG the liver DNA [163].

The level of 8-oxodG in liver tissue from patients with chronic hepatitis is higher than normal tissue obtained at liver resection for metastases [158]. Indeed, chronic hepatitis is an important risk factor for development of hepatocellular carcinoma, which frequently contains GT transversions potentially arising from 8-oxodG, in the p53 tumor suppressor gene. Moreover, transgenic mice with chronic hepatitis destined to develop carcinoma accumulate 8-oxodG progressively in the liver DNA [164].

Fanconi anemia is an autosomal recessive disease characterized by chromosomal instability, cellular susceptibility to cross-linking agents, and oxygen and an increased risk of malignant diseases, suggesting a link to oxidative DNA damage [94, 165]. Leukocyte DNA from these patients and to a lesser extent their siblings and parents contains substantially increased levels of 8-oxodG [94]. Moreover, transformed lymphoblasts from the patients show increased susceptibility to 8-oxodG induction by incubation with hydrogen peroxide, possibly due to a deficiency in catalase activity [165].

An important role of oxidative damage has been suggested in autoimmune diseases including rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). Lymphocytes isolated from patients suffering from either disease contain increased levels of 8-oxodG in the DNA [79]. In the urine the concentration of 8-oxodG per creatinine is increased in RA patients compared to that in healthy controls whereas SLE patients excrete virtually no 8-oxodG [87]. A study on cultured blood monocytes isolated from SLE patients showed reduced capacity for repair of 8-oxodG induced by incubation with hydrogen peroxide [87]. In DNA isolated from immuncomplexes precipitated from plasma 8-oxodG was a 100-fold more abundant in SLE patients than in RA patients and compared to usual values of nuclear DNA [87] (Table 2). This oxidatively modified DNA may be a source of the DNA antibodies characteristic for SLE. Accordingly, both RA and SLE patients may suffer from an increased rate of oxidative DNA damage, and the latter also from deficient repair. This may contribute to the pathogenesis and even to the increased risk of malignant diseases in these patients [79, 87].

In patients with cystic fibrosis the creatinine-corrected concentration of urinary 8-oxodG in urine was higher than that in healthy controls, offering a possible explanation of the increased cancer risk in these patients [166]. However, as creatinine production may be affected by this disease, these data require confirmation from a study with quantitative urine collection.

In summary, most studies involving cancer tissue or other samples from patients with malignant diseases or diseases associated with an increased risk of cancer show signs of an increased rate of oxidative DNA modification or in some instances deficient repair. This supports the experimentally based notion of oxidative DNA damage as an important mutagenic and apparently carcinogenic factor. However, the proof of a causal relationship in humans is still lacking. This could be possibly be supported by demonstration of the rate of oxidative DNA damage as an independent risk factor for cancer in a prospective study of biobank material using a nested case control design.

Conclusion

Exposure to ROS and cellular production of ROS are facts of life. Although a large number of protective mechanisms, from antioxidants to DNA repair enzymes, have been described, resulting oxidative damage to DNA is abundant in human tissues. Accordingly, a concept of balance between physiological/metabolic functions and deleterious effects has emerged as the basis for development of degenerative diseases, including cancer.

The most abundant oxidative DNA modification, 8-oxodG, is also the most mutagenic one, resulting in GT transversions frequently found in tumor-relevant genes. A plethora of other oxidative modifications of base and sugar residues frequently occurs in DNA, but they are less well studied and their biological significance less apparent. Several methods for invasive and noninvasive study of oxidative DNA modifications in humans have emerged. The biomarkers used in this context include urinary excretion of oxidized nucleosides and bases as repair products and modification in DNA isolated from surrogate cells, such as lymphocytes, or in target cells. These biomarkers will reflect the rate of damage and the balance between the damage and repair rate, respectively.

The data from human studies obtained so far support the experimentally based notion of oxidative DNA damage as an important mutagenic and apparently carcinogenic factor. However, the proof of a causal relationships in humans is still lacking. In the future the use of the biomarkers may provide this evidence and allow further investigation of the qualitative and quantitative importance of oxidative DNA modification and carcinogenesis in humans and also elucidate possible preventive measures.

References

1. Spector A, William HG (1981) Hydrogen peroxide and human cataract. Exp Eye Res 33:673–681
2. Ames BN (1989) Endogenous oxidative DNA damage, aging, and cancer. Free Radic Res Commun 7:121–128
3. Fraga C, Shigenaga MK, Park J-W, Degan P, Ames BN (1990) Oxidative damage to DNA during aging: 8-hydroxy-2′-deoxyguanosine in rat organ DNA and urine. Proc Natl Acad Sci USA 87:4533–4537
4. Lunec J (1990) Free radicals: their involvement in disease processes. Ann Clin Biochem 27:173–182
5. Reilly PM, Schiller HJ, Bulkley GB (1991) Pharmacologic approach to tissue injury mediated be free radicals and other reactive oxygen metabolites. Am J Surg 161:488–503
6. Greenwald RA (1991) Oxygen radicals, inflammation, and arthritis: pathophysiological considerations and implications for treatment. Semin Arthritis Rheum 20:219–240
7. Ames BN, Shigenaga MK, Hagen TM (1993) Oxidants, antioxidants, and the degenerative diseases of aging. Proc Natl Acad Sci USA 90:7915–7922
8. Ames BN, Gold LS, Willett WC (1995) The causes and prevention of cancer. Proc Natl Acad Sci USA 92:5258–5265
9. Clayson DB, Mehta R, Iverson F (1994) Oxidative DNA damage – the effects of certain genotoxic and operationally nongenotoxic carcinogens. Mutation Res 317:25–42
10. Cerutti P, Ghosh R, Oya Y, Amstad P (1994) The role of the cellular antioxidant defense in oxidant carcinogenesis. Environ Health Perspect 102 Suppl 10:123–129
11. Cerutti P, Shah G, Peskin A, Amstad P (1992) Oxidant carcinogenesis and antioxidant defense. Ann N Y Acad Sci 663:158–166
12. Ames BN, Shigenaga MK, Gold LS (1993) DNA lesions, inducible DNA repair, and cell division: three key factors in mutagenesis and carcinogenesis. Environ Health Perspec 101 [Suppl 5]:35–44
13. Block G, Patterson B, Subar A (1992) Fruit, vegetables, and cancer prevention: a review of the epidemilogical evidence. Nutr Cancer 18:1–29
14. Block G (1992) The data support a role for antioxidants in reducing cancer risk. Nutr Rev 50:207–213
15. The Alpha-Tocopherol, Beta Carotene Cancer Prevention Group (1994) The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. N Engl J Med 330:1029–1035
16. Smigel K, Edmonds S (1996) Beta carotene and vitamin A halted in lung cancer prevention trial. CancerNet News. Natl Cancer Inst
17. Omenn GS, Goodman GE, Thornquist MD, Rosenstock L, Barnhart S, Gylys-Colwell I, Metch B, Lund B (1993) The Carotene and Retinol Efficacy Trial (CARET) to prevent lung cancer in high-risk populations: pilot study with asbestos-exposed workers. Cancer Epidemiol Biomarkers Prev 2:381–387
18. Greenberg ER, Baron JA, Tosteson TD, Freeman DH, Beck GJ, Bond JH, Colacchio TA, Coller JA, Frankl HD, Haile RW, Mandel JS, Nierenberg DW, Rothstein R, Snover DC, Stevens MM, Summers RW, van Stolk RU (1994) A clinical trial of antioxidant vitamins to prevent colorectal adenoma. N Engl J Med 331:141–147
19. Blot WJ, Li JY, Taylor PR, Guo W, Dawsey S, Wang GQ, Yang CS, Zheng SF, Gail M, Li GY, et al. (1993) Nutrition intervention trials in Linxian, China: supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population. J Natl Cancer Inst 85:1483–1492
20. Halliwell B, Gutteridge JMC (1989) Free radicals in biology and medicine. Clarendon, Oxford 2:1–543
21. Halliwell B (1994) Free radicals, antioxidants, and human disease: curiosity, cause or consequence. Lancet 344:721–724
22. Chance B, Sies H, Boveris A (1979) Hydroperoxide metabolism in mammalian organs. Physiol Rev 59:527–605
23. Frei B, Forte TM, Ames BN, Cross CE (1991) Gas phase oxidants of cigarette smoke induce lipid peroxidation and changes in lipoprotein properties in human blood plasma. Biochem J 277:133–138
24. Nagashima M, Kasai H, Yokota J, Nagamachi Y, Ichinose T, Sagai M (1995) Formation of an oxidative DNA damage, 8-hydroxydeoxyguanosine, in mouse lung DNA after intratracheal instillation of diesel exhaust particles and effects of high dietary fat and beta-carotene on this process. Carcinogenesis 16:1441–1445
25. Kolachana P, Subrahmanyam VV, Meyer KB, Zhang L, Smith MT (1993) Benzene and its phenolic metabolites produce oxidative DNA damage in HL60 cells in vitro and in the bone marrow in vivo. Cancer Res 53:1023–1026
26. Shen HM, Ong CN, Lee BL, Shi CY (1995) Aflatoxin B1-induced 8-hydroxydeoxyguanosine formation in rat hepatic DNA. Carcinogenesis 16:419–422
27. Mauthe RJ, Cook VM, Coffing SL, Baird WM (1995) Exposure of mammalian cell cultures to benzo[a]pyrene and light results in oxidative DNA damage as measured by 8-hydroxydeoxyguanosine formation. Carcinogenesis 16:133–137
28. Sussman MS, Bulkley GB (1990) Oxygen-derived free radicals in reperfusion injury. Meth Enzymol 186:711–723
29. Zimmerman BJ, Granger DN (1992) Reperfusion injury. Surg Clin North Am 72:65–83
30. Moncada S, Higgs A (1993) The L-arginine-nitric oxide pathway. N Engl J Med 329:2002–2012
31. deRojas-Walker T, Tamir S, Ji H, Wishnok JS, Tannenbaum SR (1995) Nitric oxide induces oxidative damage in addition to deamination in macrophage DNA. Chem Res Toxicol 8:473–477

32. Davies KJA (1987) Protein damage and degradation by oxygen radicals. J Biol Chem 262:9895–9901
33. Epe B (1991) Genotoxicity of singlet oxygen. Chem-Biol Interact 80:239–260
34. Feig DI, Reid TM, Loeb LA (1994) Reactive oxygen species in tumorigenesis. Cancer Res suppl. 54:1890s-1894s
35. Dizdaroglu M (1991) Chemical determination of free radical-induced damage to DNA. Free Radic Biol Med 10:225–242
36. Dizdaroglu M (1994) Chemical determination of oxidative DNA damage by gas chromatography–mass spectrometry. Meth Enzymol 234:3–16
37. Breen AP, Murphy A (1995) Reactions of oxyl radical with DNA. Free Radic Biol Med 18:1033–1077
38. Shigenaga MK, Gimeno CJ, Ames BN (1989) Urinary 8-hydroxy-2′-deoxyguanosine as a biological marker of in vivo oxidative DNA damage. Proc Natl Acad Sci USA 86:9697–9701
39. Floyd RA, Watson JJ, Wong PK, Altmiller DH, Rickard RC (1986) Hydroxyl free radical adduct of deoxyguanosine: sensitive detection and mechanisms of formation. Free Radic Res Commun 1:163–172
40. Floyd RA (1990) The role of 8-hydroxyguanine in carcinogenesis. Carcinogenesis 11:1447–1450
41. Fischer-Nielsen A, Jeding IB, Loft S (1994) Radiation induced formation of 8-hydroxy-2′deoxyguanosine and its prevention by scavengers. Carcinogenesis 15:1609–1612
42. Ramotar D, Demple B (1993) Enzymes that repair oxidative damage to DNA. In: Halliwell B, Aruoma OI (eds) DNA and free radicals. Horwood, Chichester
43. Bemple B, Harrison L (1994) Repair of oxidative damage to DNA: enzymology and biology. Annu Rev Biochem 63:915–948
44. Boiteux S, Gajewski E, Laval J, Dizdaroglu M (1992) Substrate specificity of the Escherischia coli Fpg protein (formamidopyrimidine-DNA glycosylase): excision of purine lesions in DNA produced by ionizing radiation or photosensitization. Biochemistry 31:106–110
45. Bohr VA, Taffe BG, Larminat F (1995) DNA repair, oxidative stress and aging. In: Cutler RG, Packer L, Bertram J, Mori A (eds) Oxidative stress and aging. Birkhauser, Basel
46. Bessho T, Tano K, Kasai H, Ohtsuka E, Nishimura S (1993) Evidence for two DNA repair enzymes for 8-hydroxyguanine (7,8-dihydro-guanine) in human cells. J Biol Chem 268:19416–19421
47. Czeczot H, Tudek B, Lambeert B, Laval J, Boiteux S (1991) *Escherischia coli* fpg protein and UvrABC endonuclease repair DNA damage induced by methylene blue plus visible light in vivo and in vitro. J Bacteriol 173:3419–3424
48. Shibutani S, Takeshita M, Grollman AP (1991) Insertion of specific bases during DNA synthesis past the oxidation damaged base 8-oxodG. Nature 349:431–434
49. Kamiya H, Miura K, Ishikawa H, Nishimura S, Ohtsuka E (1992) c-Ha-*ras* containing 8-hydroxyguanine at codon 12 induces point mutations at the modified and adjacent positions. Cancer Res 52:3483–3485
50. Mo JY, Maki H, Sekiguchi M (1992) Hydrolytic elimination of a mutagenic nucleotide, 8-oxodGTP, by human 18-kilodalton protein: sanitization of nucleotide pool. Proc Natl Acad Sci USA 89:11021–11025
51. Sakumi K, Furuichi M, Tsuzuki T, Kakuman T, Kawabata S, Maki H, Sekiguchi M (1993) Cloning and expression of a cDNA for a human enzyme that hydrolyzes 8-oxo-dGTP, a mutagenic substrate for DNA synthesis. J Biol Chem 268:23524–23530
52. Furuichi M, Yoshida MC, Oda H, Tajiri T, Nakabeppu Y, Tsuzuki T, Sekiguchi M (1994) Genomic structure and chromosome location of the human mutT homologue gene MTH1 encoding 8-oxo-dGTPase for prevention of A:T to C:G transversion. Genomics 24:485–490
53. Wani G, D'Ambrosia SM (1995) Cell type-specific expression of human 8-oxo-7,8-dihydroguanosine triphosphatase in normal breast and skin tissues in vivo. Carcinogenesis 16:277–283
54. Tajiri T, Maki H, Sekiguchi M (1995) Functional cooperation of MutT, MutM and MutY proteins in preventing mutations caused by spontaneous oxidation of guanine nucleotide in *Escherichia coli*. Mutation Res 336:257–267
55. Digweed M (1993) Human genetic instability syndromes: single gene defects with increased risk of cancer. Toxicol Lett 67:259–281
56. Hansson J (1992) Inherited defects in DNA repair and susceptibility to DNA-damaging agents. Toxicol Lett 64–65:141–148
57. Retèl J, Hoebee B, Braun JEF, Lutgerink JT, van den Akker E, Wanamarta AH, Joneje H, Lafleur MVM (1993) Mutational specificity of oxidative DNA damage. Mutation Res 299:165–182
58. McBride TJ, Schneider JE, Floyd RA, Loeb LA (1992) Mutations induced by methylene blue plus light in single-stranded M13mp2. Proc Natl Acad Sci USA 89:6866–6870
59. Emmert S, Epe B, Saha-Moller CR, Adam W, Runger TM (1995) Assessment of genotoxicity and mutagenicity of 1,2-dioxetanes in human cells using a plasmid shuttle vector. Photochem Photobiol 61:136–141
60. Kuchino Y, Mori F, Kasai H, Inoue H, Iwai S, Miura K, Ohtsuka E, Nishimura S (1987) Misreading of DNA templates containing 8-hydroxydeoxyguanosine at the modified base and at adjacent residues. Nature 327:77–79
61. Kamiya H, Murata-Kamiya N, Fujimuro M, Kido K, Inoue H, Nishimura S, Masutani C, Hanaoka F, Ohtsuka E (1995) Comparison of incorporation and extension of nucleotides in vitro opposite 8-hydroxyguanine (7,8-dihydro-8-oxoguanine) in hot spots of the c-Ha-*ras* gene. Jpn J Cancer Res 86:270–276
62. Shibutani (1993) Quantitation of base substitutions and deletions induced by chemical mutagens during DNA synthesis in vitro. Chem Res Toxicol 6:625–629
63. Cheng KC, Cahill DS, Kasai H, Nishimura S, Loeb LA (1992) 8-Hydroxyguanine, an abundant form of oxidative DNA damage, causes G-T and A-C substitutions. J Biol Chem 267:166–172
64. Higinbotham KG, Rice JM, Divan BA, Kasprzak KS, Reed CD, Perantoni AO (1992) GGT to GTT transversion in codon 12 of the K-*ras* oncogene in rat renal sarcomas induced with nickel subsulfide or nickel subsulfiede/iron are consistent with oxidative damage to DNA. Cancer Res 52:4747–4751
65. Hollstein M, Sidransky D, Vogelstein B, Harris CC (1991) p53 mutations in human cancers. Science 253:49–52
66. Harris CC, Hollstein M (1993) Clinical implications of the p53 tumor-suppressor gene. N Engl J Med 329:1318–1327
67. Hussain SP, Aguilar F, Amstad P, Cerutti P (1994) Oxy-radical induced mutagenesis of hotspot codons 248 and 249 of the human p53 gene. Oncogene 9:2277–2281
68. Feig DI, Loeb LA (1993) Mechanisms of mutation by oxidative DNA damage: reduced fidelity of mammalian DNA polymerase. Biochemistry 32:4466–4473
69. Weitzman SA, Turk PW, Milkowaki DH, Kozlowski K (1994) Free radical adducts induce alterations in DNA cytosine methylation. Proc Natl Acad Sci USA 91:1261–1264
70. Olinski R, Zastawny T, Budzbon J, Skokowski J, Zegarski W, Dizdaroglu M (1992) DNA base modification in chromatin of human cancerous tissues. FEBS Lett 309:193–198
71. Bartsch H, Petruzelli S, De Flora S, Hietanen E, Camus A-M, Castegnaro M, Geneste O, Camoirano A, Saracci R, Giuntini C (1991) Carcinogen metabolism and DNA adducts in human lung tissues as affected by tobacco smoking or metabolic phenotype: a case-control study on lung cancer. Mutation Res 250:103–114
72. Cathcart R, Schwiers E, Saul RL, Ames BN (1984) Thymine glycol and thymidine glycol in human and rat urine: a possible assay for oxidative DNA damage. Proc Natl Acad Sci USA 81:5633–5637
73. Adelman R, Saul RL, Ames BN (1988) Oxidative damage to DNA: relation to species metabolic rate and life span. Proc Natl Acad Sci USA 85:2706–2708
74. Loft S, Vistisen K, Ewertz M, Tjønneland A, Overvad K, Poulsen HE (1992) Oxidative DNA-damage estimated by 8-hy-

droxydeoxyguanosine excretion in man: influence of smoking, gender and body mass index. Carcinogenesis 13:2241–2247
75. Wang YJ, Ho YS, Lo MJ, Lin JK (1995) Oxidative modification of DNA bases in rat liver and lung during chemical carcinogenesis and aging. Chem-Biol Interact 94:135–145
76. Ogawa T, Higashi S, Kawarada Y, Mizumoto R (1995) Role of reactive oxygen in synthetic estrogen induction of hepatocellular carcinomas in rats and preventive effect of vitamins. Carcinogenesis 16:831–836
77. Hayakawa M, Hattori K, Sugiyama S, Ozawa T (1992) Age-associated oxygen damage and mutations in mitochondrial DNA in human hearts. Biochem Biophys Res Commun 189:979–985
78. Mecocci P, MacGarvey U, Kaufman AE, Koontz D, Shoffner JM, Wallace DC, Beal MF (1993) Oxidative damage to mitochondrial DNA shows marked age-dependent increases in human brain. Ann Neurol 34:609–616
79. Bashir S, Harris G, Denman MA, Blake DR, Winyard PG (1993) Oxidative DNA damage and cellular sensitivity to oxidative stress in human autoimmune diseases. Ann Rheum Dis 52:659–666
80. Frenkel K, Zhong Z, Wei H, Karkokoszka J, Patel U, Rashid K, Georgescu M, Solomon JJ (1991) Quantitative high-performance liquid chromatography analysis of DNA oxidized in vitro and in vivo. Anal Biochem 196:126–136
81. Cadet J, Weinfeld M (1993) Detecting DNA damage. Anal Biochem 65:675A-682A
82. Povey AC, Wilson VL, Weston A, Doan VT, Wood ML, Essigmann JM, Shields PG (1993) Detection of oxidative damage by 32P-postlabelling: 8-hydroxydeoxyguanosine as a marker of exposure. In: Phillips DH, Castegnaro M, Bartsch H (eds) Postlabelling methods for detection of DNA adducts. IARC, Lyon
83. Cajigas A, Gayer M, Beam C, Steinberg JJ (1994) Ozonation of DNA forms adducts: a 32P-DNA labelling and thin layer chromatography technique to measure DNA environmental biomarkers. Arch Environ Health 49:25–36
84. Musarrat J, Wani AA (1994) Qunatitative immunoanalysis of promutagenic 8-hydroxy-2′-deoxyguanosine in oxidized DNA. Carcinogenesis 15:2037–2043
85. Yin B, Whyatt RM, Perera FP, Randall MC, Cooper TB, Santella RM (1995) Determination of 8-hydroxydeoxyguanosine by an immunoaffinity chromatography-monoclonal antibody-based ELISA. Free Radic Biol Med 18:1023–1032
86. Halliwell B, Dizdaroglu M (1992) The measurement of oxidative damage to DNA by HPLC and GC/MS techniques. Free Radic Res Commun 16:75–87
87. Lunec J, Herbert K, Blount S, Griffiths HR, Emery P (1994) 8-Hydroxydeoxyguanosine. A marker of oxidative DNA damage in systemic lupus erythematosus. FEBS Lett 348:131–138
88. Teixeira AJ, Ferreira MR, van Dijk WJ, van de Werken G, de Jong AP (1995) Analysis of 8-hydroxy-2′-deoxyguanosine in rat urine and liver DNA by stable isotope dilution gas chromatography/mass spectrometry. Anal Biochem 226:307–319
89. Ravanat J-L, Turesky RJ, Gremaud E, Trudel LJ, Stader RH (1995) Determination of 8-oxoguanine in DNA by gas chromatography-mass spectrometry and HPLC-electrochemical detection: overestimation of the background level of the oxidized base by the gas chromatography-mass spectrometry assay. Chem Res Toxicol (in press)
90. Hamberg M, Zhang LY (1995) Quantitative determination of 8-hydroxyguanine and guanine by isotope dilution masss spectrometry. Anal Biochem 229:336–344
91. Claycamp HG (1992) Phenol sensitization of DNA to subsequent oxidative damage in 8-hydroxyguanine assays. Carcinogenesis 13:1289–1292
92. Adachi S, Zeisig M, Moller L (1995) Improvements in the analytical method for 8-hydroxydeoxyguanosine in nuclear DNA. Carcinogenesis 16:253–258
93. Takeuchi T, Nakajima M, Ohta Y, Mure K, Takeshita T, Morimot K (1994) Evaluation of 8-hydroxydeoxyguanosine, a typical oxidative DNA damage, in human leukocytes. Carcinogenesis 15:1519–1523
94. Degan P, Bonassi S, De Caterina M, Korkina LG, Pinto L, Scopacasa F, Zatterale A, Calzone R, Pagano G (1995) In vivo accumulation of 8-hydroxy-2′-deoxyguanosine in DNA correlates with release of reactive oxygen species in Fanconi's anaemia families. Carcinogenesis 16:735–741
95. Epe B, Pflaum M, Haring M, Hegler J, Rudiger H (1993) Use of repair endonucleases to characterize DNA damage induced by reactive oxygen species in cellular and cell-free systems. Toxicol Lett 67:57–72
96. Gedik CM, Ewen SW, Collins AR (1992) Single-cell gel electrophoresis applied to the analysis of UV-C damage and its repair in human cells. Inter J Rad Biol 62:313–320
97. McKelvey-Martin VJ, Green MH, Schmezer P, Pool-Zobel BL, De Meo MP, Collins A (1993) The single cell gel electrophoresis assay (comet assay): a European review. Mutation Res 288:47–63
98. Epe B (1995) DNA damage profiles induced by oxidizing agents. Rev Physiol Biochem Pharmacol 127:223–249
99. Collins AR, Ai-guo M, Duthie SJ (1995) The kinetics of repair of oxidative DNA damage (strand breaks and oxidised pyrimidines) in human cells. Mutation Res 336:69–77
100. Loft S, Larsen PN, Rasmussen A, Fischer-Nielsen A, Bondesen S, Kirkegaard P, Rasmussen LS, Ejlersen E, Tornøe K, Bergholdt R, Poulsen HE (1995) Oxidative DNA damage after transplantation of the liver and small intestine in pigs. Transplantation 59:16–20
101. Faure H, Incardona MF, Boujet C, Cadet J, Ducros V, Favier A (1993) Gas chromatographic-mass spectrometric determination of 5-hydroxymethyluracil in human urine by stable isotope dilution. J Chromatogr 616:1–7
102. Suzuki J, Inoue Y, Suzuki S (1995) Changes in urinary level of 8-hydroxyguanine by exposure to reactive oxygen-generating substances. Free Radic Biol Med 18:431–436
103. Simic MG, Bergtold DS (1991) Dietary modulation of DNA damage in human. Mutation Res 250:17–24
104. Tagesson C, Källberg M, Leanderson P (1992) Determination of urinary 8-hydroxydeoxyguanosine by coupled-column high-perfomance liquid chromatography with electrochemical detection: a noninvasive assay for in vivo oxidative DNA damage in humans. Toxicol Meth 1:242–251
105. Park E-M, Shigenaga MK, Degan P, Korn T, Kitzler JW, Wehr CM, Kolachana P, Ames BN (1992) Assay of excised oxidative DNA lesions: isolation of 8-oxoguanine and its nucleoside derivatives from biological fluids with a monoclonal antibody column. Proc Natl Acad Sci USA 89:3375–3379
106. Shigenaga MK, Aboujaoude EN, Chen Q, Ames BN (1994) Assays of oxidative DNA damage biomarkers 8-oxo-2′-deoxyguanosine and 8-oxoguanine in nuclear DNA and biological fluids by high-performance liquid chromatography with electrochemical detection. Meth Enzymol 234:16–33
107. Tagesson C, Kallberg M, Klintenberg C, Starkhammar H (1995) Determination of urinary 8-hydroxydeoxyguanosine by automated coupled-column high performance liquid chromatography: a powerful technique for assaying in vivo oxidative DNA damage in cancer patients. Eur J Cancer 31A:934–940
108. Osawa T, Yoshida A, Kawakishi S, Yamashita K, Ochi H (1995) Protective role of dietary antioxidants in oxidative stress. In: Cutler RG, Packer L, Bertram J, Mori A (eds) Oxidative stress and aging. Birkhauser, Basel
109. Prieme H, Loft S, Cutler RG, Poulsen HE (1995) Measurement of oxidative DNA injury in humans: evaluation of a comercially available ELISA assay. In: International Conference on Natural Antioxidants and Lipid Peroxidation in Atherosclerosis and Cancer. (in press)
110. Klein JC, Bleeker MJ, Saris CP, Roelen HCPF, Brugghe HF, van den Elst H, van der Marel GA, van Boom JH, Westra JG, Kriek E, Berns AJM (1992) Repair and replication of plasmids with site-specific 8-oxodG and 8-AAFdG residues in normal and repair-deficient human cells. Nucleic Acids Res 20:4437–4443
111. Hayakawa H, Taketomi A, Sakumi K, Kuwano M, Sekiguchi M (1995) Generation and elimination of 8-oxo-7,8-dihydro-

2′-deoxyguanosine 5′-triphosphate, a mutagenic substrate for DNA synthesis, in human cells. Biochemistry 34:89–95
112. Witt EH, Reznick AZ, Viguie CA, Starke-Reed P, Packer L (1992) Exercise, oxidative damage and effects of antioxidant manipulation. J Nutr 122:766–773
113. Loft S, Wierik EJMV, van der Berg H, Poulsen HE (1995) Energy restriction and oxidative DNA damage. Cancer Epidemiol Biomarkers Prev 4:515–519
114. Bergtold DS, Berg CD, Simic MG (1990) Urinary biomarkers in radiation therapy of cancer. Adv Exp Med Biol 264:311–316
115. Blount S, Griffiths HR, Lunec J (1991) Reactive oxygen species damage to DNA and its role in systemic lupus erythematosus. Mol Aspects Med 12:93–105
116. Kasai H, Crain PF, Kuchino Y, Nishimura S, Ootsuyama A, Tanooka H (1986) Formation of 8-hydroxyguanine moiety in cellular DNA by agents producing oxygen radicals and evidence for its repair. Carcinogenesis 7:1849–1851
117. Kiyosawa H, Suko M, Okudaira H, Murata K, Miyamoto T, Chung MH, Kasai H, Nishimura S (1990) Cigarette smokeing induces formation of 8-hydroxydeoxyguanosine, one of the oxidative DNA damages in human peripheral leucocytes. Free Radic Res Commun 11:23–27
118. Lagorio S, Tagesson C, Forastiere F, Axelson O, Carere A (1994) Exposure to benzene and urinary concentrations of 8-hydroxydeoxyguanosine, a biological marker of oxidative damage to DNA. Occup Environ Med 51:739–743
119. Wilson VL, Taffe BG, Shields PG, Powey AC, Harris CC (1993) Detection and quantification of 8-hydroxydeoxyguanosine addducts in peripheral blood of people exposed to ionizing radiation. Environ Health Perspec 99:261–263
120. Loft S, Astrup A, Buemann B, Poulsen HE (1994) Oxidative DNA damage correlates with oxygen consumption in humans. FASEB J 8:534–537
121. Cutler RG (1991) Human longevity and aging: possible role of reactive oxygen species. Ann N Y Acad Sci 621:1–28
122. Loft S, Fischer-Nielsen A, Jeding IB, Vistisen K, Poulsen HE (1993) 8-Hydroxydeoxyguanosine as a biomarker of oxidative DNA damage. J Toxicol Environ Health 40:391–404
123. Sohal RS, Orr WC (1992) Relationship between antioxidants, prooxidants, and the aging process. Ann N Y Acad Sci 663:74–84
124. Else PL, Hulbert AJ (1985) Mammals: an allometric study of metabolism at tissue and mitochondrial level. Am J Physiol 248:R415-R421
125. Richter C, Park J-W, Ames BN (1988) Normal oxidative damage to mitochondrial and nuclear DNA is extensive. Proc Natl Acad Sci USA 85:6465–6467
126. Richter C (1995) Oxidative damage to mitochondrial DNA and its relationship to ageing. Int J Biochem Cell Biol 27:647–653
127. Haramaki N, Packer L (1994) Oxidative stress indices in exercise. In: Sen CK, Packer L, Hänninen O (eds) Exercise and oxygen toxicity. Elsevier, Amsterdam
128. Inoue T, Mu Z, Sumikawa K, Adachi K, Okochi T (1993) Effect of physical exercise on the content of 8-hydroxydeoxyguanosine in nuclear DNA prepared from human lymphocytes. Jpn J Cancer Res 84:720–725
129. Nielsen HB, Hanel B, Loft S, Poulsen HE, Pedersen BK, Diamant M, Vistisen K, Secher NH (1995) Restricted pulmonary diffusion capacity after exercise is not an ARDS-like injury. J Sport Sci 13:109–113
130. Alessio HM (1993) Exercise-induced oxidative stress. Med Sci Sports Exerc 25:218–224
131. Poulsen HE, Loft S, Vistisen K (1995) Increased oxidative DNA damage after 30 days of vigorous exercise. J Sport Sci (in press)
132. Weindruch R, Albanes D, Kritchevsky D (1991) The role of calories and caloric restriction in carcinogenesis. Hematol Oncol Clin North Am 5:79–89
133. Masoro EJ, Shimokawa I, Yu BP (1991) Retardation of the aging processes in rats by food restriction. Ann N Y Acad Sci 621:337–352
134. Turturro A, Hart RW (1991) Longevity-assurance meachanism in caloric restriction. Ann N Y Acad Sci 621:363–372
135. Weraarchakull N, Strong R, Wood G, Richardson A (1989) The effect of aging and energy restriction on DNA repair. Exp Cell Res 181:197–204
136. Chung MH, Kasai H, Nishimura S, Yu BP (1992) Protection of DNA damage by dietary restriction. Free Radic Biol Med 12:523–525
137. Djuric Z, Lu MH, Lewis SM, Luongo DA, Chen XW, Heilbrun LK, Reading BA, Duffy PH, Hart RW (1992) Oxidative DNA damage levels in rats fed low-fat, high-fat or calorie-restricted diets. Toxicol Appl Pharmacol 115:156–160
138. Semsei I, Rao G, Richardson A (1989) Changes in the expression of superoxide dismutase and catalase as a function of age and dietary restriction. Biochem Biophys Res Commun 164:620–625
139. Youngman LD, Park J-YK, Ames BN (1992) Protein oxidation associated with aging is reduced by dietary restriction of proetin or calories. Proc Natl Acad Sci USA 89:9112–9116
140. Wieric EJMV, Leeuwen REW van, Hendriks HFJ, Verhagen H, Loft H, Poulsen HE, Berg H van der (1995) Moderate energy restriction does not affect parameters of oxidative stress and genotoxicity in man. J Nutr 125:2631–2639
141. Djuric Z, Heilbrun LK, Reading BA, Boomer A, Valeriote FA, Martino S (1991) Effects of low-fat diet on levels of oxidative damage to DNA in human peripheral nucleated blood cells. J Natl Cancer Inst 83:766–769
142. Leanderson P, Tagesson C (1990) Cigarette smoke-induced DNA-damage: role of hydroquinone and catechol in the formation of the oxidative DNA-adduct, 8-hydroxydeoxyguanosine. Chem-Biol Interact 75:71–81
143. Leanderson P, Tagesson C (1992) Cigarette smoke-induced DNA damage in cultured human lung cells: role of hydroxyl radicals and enduclease activation. Chem-Biol Interact 810:197–208
144. Ziegler RG (1989) A review of the epidemiologic evidence that carotenoids reduce the risk of cancer. J Nutr 119:116–122
145. Riemersma RA, Wood DA, Macintyre CCA, Elton RA, Gey KF, Oliver MF (1991) Risk of angina pectoris and plasma concentration of vitamin A, C, and E and carotene. Lancet 337:1–5
146. Adachi S, Yoshida S, Kawamura K, Takahashi M, Uchida H, Odagiri Y, Takemoto K (1994) Inductions of oxidative DNA damage and mesothelioma by crocidolite, with special reference to the presence of iron inside and outside of asbestos fiber. Carcinogenesis 15:753–758
147. Hanaoka T, Tsugane S, Yamano Y, Takahashi T, Kasai H, Natori Y, Watanabe S (1993) Quantitative analysis of 8-hydroxyguanine in peripheral blood cells: an application for asbestosis patients. Int Arch Occup Environ Health 65:S215-S217
148. Tagesson C, Chabiuk D, Axelson O, Baranski B, Palus J, Wyszynska K (1993) Increased urinary excretion of the oxidative DNA adduct, 8-hydroxydeoxyguanosine, as a possible early indicator of occupational cancer hazards in the asbestos, rubber, and azo-dye industries. Polish J Occup Med Environ Health 6:357–368
149. Verhagen H, Poulsen HE, Loft S, van Poppel G, Willems MI, van Bladeren PJ (1995) Reduction of oxidative DNA-damage in man by Brussels sprouts. Carcinogenesis 16:969–970
150. Fraga CG, Motchnik PA, Shigenaga MK, Helbock HJ, Jacob RA, Ames BN (1991) Ascorbic acid protects against endogenous oxidative DNA damage in human sperm. Proc Natl Acad Sci USA 88:11003–11006
151. van Poppel G, Poulsen HE, Loft S, Verhagen H (1995) No influence of beta-carotene on oxidative DNA damage in male smokers. J Natl Cancer Inst 87:310–311
152. Prestera T, Holtzclaw WD, Zhang Y, Talalay P (1993) Chemical and molecular regulation of enzymes that detoxify carcinogens. Proc Natl Acad Sci USA 90:2965–2969
153. Zhang Y, Kensler TW, Cho CG, Posner GH, Talalay P (1994) Anticarcinogenic activities of sulforaphane and structurally

related synthetic norbornyl isothiocyanates. Proc Natl Acad Sci USA 91:3147–3150
154. Malins DC, Haimanot R (1991) Major alterations in the nucleotide structure of DNA in cancer of the female breast. Cancer Res 51:5430–5432
155. Malins DC, Holmes EH, Polissar NL, Gunselman S (1993) The etiology of breast cancer. Characteristic alterations in hydroxyl radical-induced DNA base lesions during oncogenesis with potential for evaluating incidence risk. Cancer Lett 71:3036–3042
156. Jaruga P, Zastawny TH, Skokowski J, Dizdaroglu M, Olinski R (1994) Oxidative DNA base damage and antioxidant enzyme activities in human lung cancer. FEBS Lett 341:59–64
157. Nagashima M, Tsuda H, Takenoshita S, Nagamachi Y, Hirohashi S, Yokota J, Kasai H (1995) 8-hydroxydeoxyguanosine levels in DNA of human breast cancer are not significantly different from those of non-cancerous breast tissues by the HPLC-ECD method. Cancer Lett 90:157–162
158. Shimoda R, Nagashima M, Sakamoto M, Yamaguchi N, Hirohashi S, Yokota J, Kasai H (1994) Increased formation of oxidative DNA damage, 8-hydroxydeoxyguanosine, in human livers with chronic hepatitis. Cancer Res 54:3171–3172
159. Okamoto K, Toyokuni S, Uchida K, Ogawa O, Takenewa J, Kahehi Y, Kinoshita H, Hattori-Nakakuki Y, Hiai H, Yoshida O (1994) Formation of 8-hydroxy-2′deoxyguanosine and 4-hydroxy-2-nonenal-modified proteins in human renal-cell carcinoma. Int J Cancer 58:825–829
160. Cao E-H, Wang J-J (1993) Oxidative damage to DNA: levels of thymine glycol and thymidine glycol in neoplastic human urines. Carcinogenesis 14:1359–1362
161. Olinski R, Zastawny TH, Foksinski M, Barecki A, Dizdaroglu M (1995) DNA base modifications and antioxidant enzyme activities in human benign prostatic hyperplasia. Free Radic Biol Med 18:807–813
162. Carmichael PL, Hewer A, Osborne MR, Strain AJ, Phillips DH (1995) Detection of bulky DNA lesions in the liver of patients with Wilson's disease and primary haemochromatosis. Mutation Res 326:235–243
163. Yamamoto F, Kasai H, Togashi Y, Takeichi N, Hori T, Nishimura S (1993) Elevated level of 8-hydroxydeoxyguanosine in DNA of liver, kidney, and brain of Long-Evans cinnamon rats. Jpn J Cancer Res 84:508–511
164. Hagen TM, Huang S, Curnutte J, Fowler P, Martinez V, Wehr CM, Ames BN, Chisari FV (1994) Extensive oxidative DNA damage in hepatocytes of transgenic mice with chronic active hepatitis destined to develop hepatocellular carcinoma. Proc Natl Acad Sci USA 91:12808–12812
165. Takeuchi T, Morimoto K (1993) Increased formation of 8-hydroxydeoxyguanosine, an oxidative DNA damage, in lymphoblasts Fanconi's anemia pateints due to possible catalase deficiency. Carcinogenesis 14:1115–1120
166. Brown RK, McBurney A, Lunec J, Kelly FJ (1995) Oxidative damage to DNA in patients with cystic fibrosis. Free Radic Biol Med 18:801–806
167. Taylor A, Lipman RD, Jahngen-Hodge J, Plamer V, Smith D, Padhye N, Dallal GE, Cyr DE, Laxman E, Shepard D, Morrow F, Salomon R, Perrone G, Asmundsson G, Meydani M, Blumberg J, Mune M, Harrison DE, Archer JR, Shigenaga M (1995) Dietary calorie restriction in the Emory mouse: effects on lifespan, eye lens cataract prevalence and progression, levels of ascorbate, glutathione, glucose, and glycohemoglobin, tail collagen break time, DNA and RNA oxidation, skin integrity, fecundity, and cancer. Mech Ageing Dev 79:33–57