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Antioxidant Intervention Studies Related to DNA Damage, DNA Repair and Gene Expression

STEFFEN LOFT [a,*] and HENRIK E. POULSEN [b]

[a]*Institute of Public Health, c/o Department of Pharmacology, The Panum Institute, Room 18-5-32, Panum, Blegdamsvej 3, DK-2200 Copenhagen N, Denmark;* [b]*Department of Clinical Pharmacology, Q7642, Rigshospitalet, 20 Tagensvej, DK-2200 Copenhagen N, Denmark*

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In human cells oxidatively modified nucleobases can be measured in the DNA and strand breaks can be detected by the Comet assay, optionally with the use of repair enzymes introducing breaks at oxidized bases. Oxidized bases and nucleosides from DNA repair, the nucleotide pool and cell turnover can be measured in urine. The excretion rate represents the average rate of damage in the body, whereas the level of oxidized bases in DNA is a concentration measurement in the specific cells. The expression of genes relevant for the defence against oxidative DNA damage, antioxidant and DNA repair enzymes can be assessed at the mRNA, protein and activity level. Functional assays can involve susceptibility to, and disappearance of, damage induced *ex vivo* in lymphocytes.

Vitamins C and E, β-carotene, coenzyme Q as well as various vegetables, fruit and carotenoid rich products, have been assessed with biomarkers mainly including damage in lymphocytes DNA by Comet and chemical assays and urinary excretion of oxidized bases and nucleosides. The basal levels as well as the reported effects of the interventions have been rather variable, possibly reflecting differences in the populations, regimens, functional correlates of the biomarkers as well as between laboratories and assays. However, the data suggest that a depleted state due to nutritional deficiency and/or an increased oxidative stress might facilitate demonstration of protective effects of antioxidants with respect to DNA damage. The effect of antioxidants on gene expression has been little studied in humans and only at the activity and protein level. New RT-PCR based techniques may improve this in the future.

The proper application of biomarkers of oxidative DNA damage in human studies may provide proof of causal relationships with cancer and aging and improve prevention.

Keywords: Biomarkers, oxidative DNA damage, gene expression, cancer, aging, antioxidants, intervention, human

Abbreviations: ROS, reactive oxygen species; 8-oxodG, 7-hydro-8-oxodeoxyguanosine; 8-oxoGua, 7-hydro-8-oxoguanine; 8-oxoG, 8-oxoguanosine; FapyGua, 2,6-diamino-4-hydroxy-5-formamidopyrimidine (ring opened guanine); 8-oxoAde, 8-oxoadenine; Tg/dTg, thymine/thymidine glycol; 5-OHmU, 5-hydroxymethyluracil; Fpg, FapyGua glycosylase; EndoIII, endonuclease III; HPLC-EC, high performance liquid chromatography with electrochemical detection; GC/MS, gas chromatography-mass spectrometry; ETS, environmental tobacco smoke; SOD, superoxide dismutase; GR, glutathione reductase; GST, glutathione S-transferase; GPX, glutathione peroxidase; ELISA, enzyme linked immuno sorbent assay

Oxidative modifications of DNA are abundant, mutagenic and thought to be important in carcinogenesis and aging as supported by experimental studies in animals and *in vitro*.[1–3] Indeed cells

*Corresponding author. Tel.: +45335327649. Fax: +4535327610. E-mail: s.loft@pubhealth.ku.dk.

are constantly exposed to oxidants from both physiological processes, such as mitochondrial respiration,[4] and pathophysiological conditions such as inflammation, ischemia/reperfusion, foreign compound metabolism and radiation.[1] The bodily defences against oxidants include an extensive system of antioxidant enzymes and radical scavengers and chain breakers, of which many are nutritionally dependent. Nevertheless, damage to DNA is continuously ongoing but is also repaired with high efficiency in the cells in the body.[5,6]

Descriptive epidemiological studies support important protective properties of antioxidants by a highly reproducible close relationship between a high dietary intake of vegetable and fruits rich in antioxidants and a low risk of epithelial cancers, particularly in the airways and upper gastrointestinal tract.[7–9] So far, however, the large scale intervention studies of the effects of single and combinations of antioxidants, vitamin E and β-carotene have been negative and even indicated a cancer promoting effect of the latter in smokers.[10–12] Due to the very large study populations and enormous costs required for such studies only a very limited number of supplements has been tried in these studies focusing on high risk groups, such as middle aged heavy smokers who may very well be beyond prevention. Accordingly, mechanistically based biomarkers of oxidative DNA damage and the involved defence systems may serve as intermediate endpoints in human intervention studies, which may target the optimum strategies for new large scale intervention.[13] Biomarker approaches may also provide support for a causal relationship between oxidative damage and cancer and aging.[14] In parallel, the biomarker approaches are applicable in mechanistic animal experiments and cancer bioassays as well as *in vitro*.[15] The present review focus on the use of biomarkers of oxidative DNA damage and repair and expression of the involved defence genes in relation to intervention studies involving antioxidants.

BIOMARKERS IN ANTIOXIDANT INTERVENTION STUDIES

Biomarkers of Oxidative Damage in Cellular DNA

In DNA more than 100 different oxidative modifications have been observed.[16,17] However, so far only a few of the base modifications have been used as biomarkers and of these the oxidative C-8 adduct of guanine is by far the most studied as either the nucleoside or base. In principle, the level in nuclear or mitochondrial DNA from target or surrogate tissues or cells or the excretion of repair products into the urine can be measured.

Oxidatively modified nucleobases in DNA can be measured by various chromatographic techniques, including high performance liquid chromatography with electrochemical detection (HPLC-EC) (or MS or UV), gas chromatography-mass spectrometry (GC/MS), thin layer chromatography with ^{32}P-postlabelling, and some immune assays. Recently, an ultra-sensitive capillary electrophoresis assay with immuno-extraction and laser based detection for Tg in DNA was published.[18] The required isolation of DNA and hydrolysis by enzymes or acid at high temperature may result in oxidation of the abundant unmodified nucleobases causing artificially high values. Particularly, the derivatization with silyl-groups for the GC/MS is prone to give rise to oxidation unless the unmodified base are removed[19,20] or possibly with control of temperature and other conditions.[21–23] New, approaches involving release of 7-hydro-8-oxo-guanine (8-oxoGua) and other modified bases from DNA by means of 2,6-diamino-4-hydroxy-5-formamidopyrimidine (ring open guanine) (FapyGua) glycosylase (Fpg), endonuclease III (EndoIII) and other enzymes before HPLC or GC/MS may solve that problem provided the reaction is complete.[24–26] Gamma-radiation from ^{32}P-phosphate used for post-labelling can oxidize guanine and thus explain some reported rather high

values.[27,28] Even with the HPLC-EC method the reported values for 7-hydro-8-oxodeoxyguanosine (8-oxodG) in leukocyte DNA vary from 0.1 to 13 per 10^5 dG.[2,29–31] Indeed, DNA extraction procedures may cause oxidation, particularly the use of impure phenol and drying of the DNA after ethanol precipitation.[32–36] The lowest values have been obtained with anaerobic DNA extraction and/or the use of sodium iodide and desferrioxamine or other antioxidants.[36–38] Work is in progress regarding interlaboratory validation of the measurement of 8-oxodG in DNA as reported elsewhere[39] and at the present conference. So far, the variation between different methods and laboratories is surprisingly large.

Oxidative damage to nucleobases can be assessed indirectly as strand breaks, DNA unwinding or relaxation of supercoiling induced by treatment of DNA by the relevant repair enzymes, i.e. Fpg and EndoIII for purines, including 8-oxodG, and pyrimidine lesions, respectively.[40,41] This approach is used in alkaline elution or unwinding of DNA as well as in alkaline single cell gel electrophoresis.[40–42] The 8-oxodG values estimated by those assays are around 0.3 per 10^6 dG in lymphocytes,[29] i.e. close to the lowest values obtained by HPLC-EC. So far, it is unknown whether the enzyme based assays are missing 8-oxodG in some of the DNA as suggested by a completeness of only 50% of Fpg repair of damaged DNA *in vitro*.[43] A strong correlation ($r = 0.89$, $p < 0.01$) has been shown between 8-oxodG concentration and the Comet assay value after 2-nitropropane induced DNA damage in rat bone marrow cells *in vivo*.[44] Recently a strong correlation between 8-oxodG in DNA and Fpg sensitive sites measured by the Comet assay was shown in monocytes exposed to different levels of gamma irradiation.[30] We have shown a fair correlation between 8-oxodG levels, strand breaks ($r = 0.50$) and Fpg sensitive sites ($r = 0.41$) in lymphocytes from 29 healthy subjects (unpublished data) whereas others have found poor correlations.[31] Interestingly, the Comet assay shows considerable seasonal variation, which is important to take into account in antioxidant intervention studies.[45] Recently, a highly sensitive slot blot assay was introduced for determination of apurinic/apyrimidinic sites.[46] However, there are as yet no reports on systematic interlaboratory validation of these enzyme based assays. Moreover, the application of the elegant ligation mediated PCR reaction for determination of hot spots for base oxidation at specific nucleotide positions in relevant genes for biomarker purposes requires further development.[47]

Urinary Excretion of Oxidation Products as Biomarkers of DNA Damage

Oxidized bases and nucleosides, are poor substrates for the enzymes involved in nucleotide synthesis, fairly water soluble, and generally excreted into the urine without further metabolism.[48,49] In animal experiments injected 8-oxodG is readily excreted unchanged into the urine without contamination from 8-oxodG in the diet or oxidation of dG.[48–50] Among the possible oxidation products from DNA 8-oxodG, 8-oxoGua, 8-oxoadenine (8-oxoAde), thymine glycol (Tg), thymidine glycol (dTg), 5-hydroxyuracil (5-OHU) and 5-hydroxymethyluracil (5-OHmU) have so far been identified in urine (Figure 1), Refs. [2,48,51–56]. Of these 8-oxodG and the thymine derivatives are the most intensively studied ones. Although interlaboratory validation of these urinary biomarkers has yet to be reported the levels of concentration and excretion of the oxidized bases and nucleosides obtained in different laboratories are generally in the same range.[2]

The assays for the urinary DNA repair products include HPLC with detection by electrochemistry for 8-oxodG and 8-oxoGua and by UV absorbance for dTg and Tg, whereas all the repair products can potentially be measured by GC/MS.[52,54,55,57,58] The major problem with all these assays involves separation of the very small amounts of analyte from urine that is a

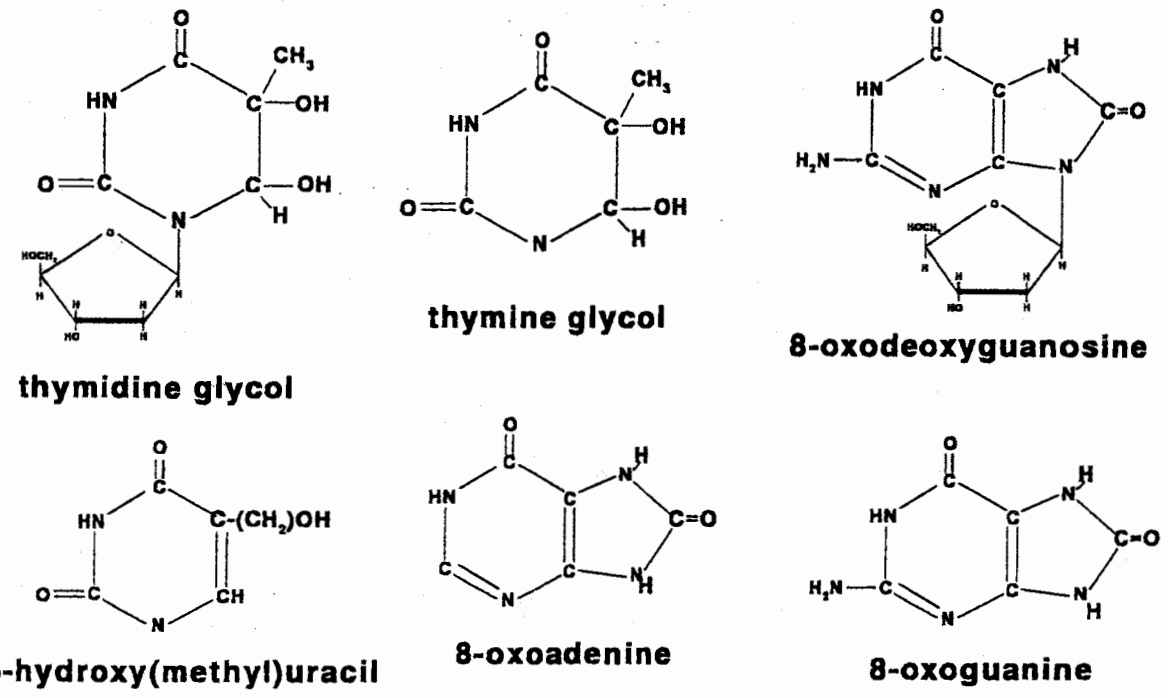

FIGURE 1 Oxidized bases and deoxynucleotides identified in urine.

very complicated matrix requiring extensive clean-up procedures.[48,50,51,59–64] Thus, quantitation of recovery may be difficult although this problem can be solved by the use of stable isotopes for GC/MS and LC/MS-MS assays.[58] With HPLC/MS-MS sample preparation required is minimum, providing high capacity, and true identity of the compound excreted is verified.[65] This technique yield similar values as HPLC-EC and may be the optimum assay at present. Enzyme linked immuno sorbent assay (ELISA) based on monoclonal antibodies for estimation of 8-oxodG in urine samples[66] generally yield 3–8 times higher values in human and rat urine than the chromatographic assays[2,67] and poor correlation with an HPLC assay has been reported in smokers.[68]

Collection of urine for 24 h may present some practical problems and spot urine samples collected for creatinine would be simpler. However, the excretion of 8-oxodG per kg body weight in 24 h correlated rather poorly ($r = 0.50$) with the ratio of 8-oxodG to creatinine in a spot urine sample from a subsequent voiding.[35] Thus, this approach is probably more valid for longitudinal studies where subjects are their own controls.

An important aspect of the urine assay is the great stability of 8-oxodG, i.e. at least 7 years at −20°C whereas DNA in cells and tissues oxidizes quickly even at very low temperatures.[35] Thus, for nested case control studies on biobank material only urine assays appear to be feasible. Little is known regarding the stability of the other urinary DNA damage products.

Interpretation of Nuclear Levels and Urinary Excretion of Oxidized DNA Products

In DNA from tissues or cells the level of oxidized bases is in a steady state determined by the balance between damage and repair rates as outlined in Figure 2. The major contributor is

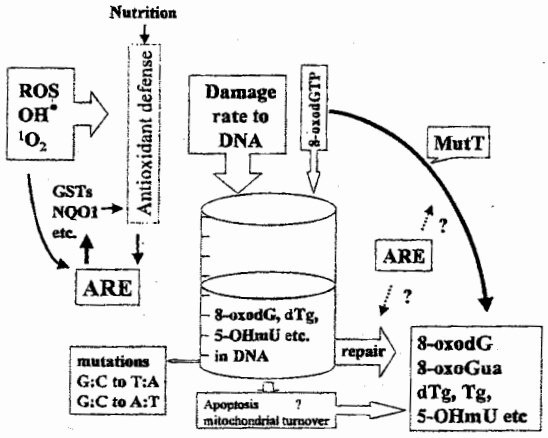

FIGURE 2 Possible role of antioxidants and the ARE for the mass balance of 8-oxodG formation in DNA and nucleotide pool and fates in terms of repair, cell and mitochondrial turnover and mutations. ROS are reactive oxygen species. GSTs and NQO1 are glutathione S-transferases and NAD(P)H:quinone reductase, respectively. MutT includes 8-oxodGTP phosphatase and 8-oxodGMP nucleotidase sanitizing the nucleotide pool (Mo *et al.*,[77] Hayakawa, H. *et al.*[78] Repair result in 8-oxodG or 8-oxoGua by nucleotide excision and base excision (OGG1 and OGG2).[74,76]

oxidation of bases within the DNA whereas incorporation of oxidized nucleotides is probably of minor quantitative importance although highly mutagenic and thus qualitatively important.[69] For the repair of the modified bases a number of enzymes are active.[6] The human 8-oxoGua glycosylase (OGG1) has been cloned and repairs 8-oxodG opposite pyrimidines[70–72] whereas another enzyme, OGG2, repairs 8-oxodG incorporated opposite purines.[73,74] Interestingly, overexpression of OGG1 in mammalian cell lines increases the repair rate of 8-oxodG residues induced by potassium bromate or a photosensitizer with light without affecting the mutation rate.[75] Nucleotide excision repair has been shown also to contribute to the repair of 8-oxodG in DNA.[76] 8-OxodG will also come from the highly specific 8-oxodGTP phosphatase (MutT) and 8-oxodGMP nucleotidase enzymes sanitizing the nucleotide pool.[77,78] This human MTH1 enzyme was recently shown to sanitize 2-hydroxy-dATP as well.[79] Digestion of DNA from apoptotic cells and turnover of mitochondria could also be a source of 8-oxodG. A few of the 8-oxodG lesions in DNA will after replication without repair or misrepair lead to mutations, 8-oxodG formed within DNA can lead to G to T transversions whereas incoporation of 8-oxodGTP an result in both G to T and G to A transversions.[69,80,81]

In theory an increase in the rate of damage to DNA due to oxidative stress should increase the nuclear level until an increased repair rate matches the damage rate and a new steady state is achieved. The increased repair rate can result just from the increased substrate availability as well as from increased enzyme activity as shown for OGG1 in lymphocytes from smokers.[82] The concept of steady state in DNA damage is supported by the limited accumulation with age shown in various human and animal cells and tissues investigated so far.[2] Moreover, in cultured human cells induced levels of oxidized nucleobases return to initial values within some hours.[83] In human and experimental animals exposed to radiation the increase in oxidized bases in DNA from leukocytes or liver as well as urinary excretion of repair products is temporary.[84–87] Indeed, the repair efficiency of 8-oxodG in DNA has been calculated to 99.4% and without repair the level of oxidized bases would double in DNA in about 20–66 days.[88]

The concept of a steady state indicate that efflux must equal influx, i.e. that the rate of damage can be estimated from the rate of excretion of the repair product, e.g. 8-oxodG (Figure 2). The unknowns in that equation are the contribution of the glycosylase pathway to the repair and the contribution from cellular and mitochondrial turnover to 8-oxodG formation. However, in a recent rat study the excess 8-oxodG excreted into urine accounted for most of the excess 8-oxodG induced in target organs by the liver carcinogen 2-nitropropane. It should be noted that the urinary excretion represent the cumulative body burden and that it usually cannot be determined if this originated from an impact to all body cells or from a much higher insult to one

or several organs. In contrast, tissue or cellular levels represent the measure of a concentration, reflecting the balance between rate of oxidation and the rate of repair. Moreover, in human studies the cellular levels are frequently measured in surrogate cells, such as lymphocytes, rather than in true target tissues. Accordingly, the levels of modified bases in DNA from cells or tissues and the urinary excretion of the corresponding bases or deoxynucleosides represent supplementary groups of biomarkers. However, much more knowledge regarding repair pathways and kinetics are warranted for the optimum interpretation of these biomarkers.

Biomarkers of DNA Repair and Gene Expression

Mechanistic consideration and knowledge on cell biology indicate that antioxidants and oxidative stress can trigger transcription of a battery of genes via the antioxidant responsive element (ARE).[89–91] Among the antioxidant-inducible genes are those encoding antioxidant enzyme activities such as glutathione S-transferases (GSTs) and NAD(P)H : quinone oxidoreductase (NQO1) active in detoxification of reactive substances such as oxygen radicals and redox cycling (semi)quinones (Figure 2). Enhanced expression of the antioxidant enzymes such as GSTs, γ-glutamylcysteine synthase and heme oxygenase as a response to oxidative stress has also been reported in experimental systems.[92–94] Recently, the importance of SOD in carcinogenesis was suggested by an increased risk of breast cancer found in women with a distinct MnSOD genotype and a low consumption of fruits and vegetables.[95] So far, however, few biomarkers of DNA repair capacity and gene expression have been employed in published studies on antioxidant interventions in humans. Moreover, interlaboratory validations have yet to be reported.

A few assays are available as biomarkers of repair of oxidative DNA damage. The direct activity of OGG1 can be assessed in extracts from lymphocytes[82] although the effects of antioxidants have yet to be studied in humans. The DNA repair capacity can also be estimated by means of the Comet assay in lymphocytes, e.g. by following the levels during incubation *ex vivo* or by applying cell extracts to gels of cells with defined oxidative damage.[96,97] By modifying the assay conditions the repair of, e.g. strand breaks, purines and pyrimidines may be studied.[40] Assays for the expression of the OGG1 protein and corresponding mRNA in human biomarker material have not yet been published although RT-PCR based blotting, chip, array or other techniques should allow the latter.

Simple, often kit based, spectrophotometric assays are available for assessment of activity of antioxidant enzymes, including GSTs, superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione reductase (GR), NQO1, and catalase in plasma, lymphocytes, red cells and/or available tissues. As discussed below such assays have been used in a number of antioxidant intervention studies activity but systematic interlaboratory validation efforts do not appear to have been published. At the level of enzyme protein some ELISA kits have also been used in antioxidant interventions.[97–99] RT-PCR based techniques should allow the study of the mRNAs of the enzymes in e.g. lymphocytes.

ANTIOXIDANT INTERVENTIONS AND BIOMARKERS OF DNA DAMAGE, REPAIR AND GENE EXPRESSION

The apparent cancer-protective effect of fruits and vegetables shown in epidemiological studies may partly be related to a number of agents with antioxidant capacity such as carotenoids, tocopherols, vitamin C, selenium and flavonoids.[9] Glucosinates and their many potent breakdown products found in cruciferous vegetables may have special protective effects.[100,101] A number of intervention studies with such compounds have been carried out in humans employing

biomarkers of oxidative damage to DNA and the enzymes involved in repair and antioxidant defence as end points, i.e. markers of a putative cancer risk (Table I). The results have not been completely consistent, possibly due to the differences in the employed biomarkers, their functional correlates and assays as well as in the populations and regimens.

Studies on DNA Damage

With HPLC-EC based assays depletion of or supplementation with up to 500 mg vitamin C had no effect on the level of 8-oxodG in lymphocyte DNA[102,103] whereas sperm cell DNA was highly susceptible to depletion.[104] In an uncontrolled sequential study, a cocktail of vitamins C, and E, β-carotene, selenium, zinc and copper brought increased levels of 8-oxodG in DNA extracted from whole blood found in subjects exposed to environmental tobacco smoke (ETS) down to levels found in unexposed subjects.[105] Similarly, after administration of vitamin E or red ginseng the 8-oxodG levels decreased in leukocytes in a small number of smokers.[103] However, effects of lycopene administered as tomato products on the 8-oxodG level in lymphocytes measured by HPLC-EC failed to reach significance. Interestingly, a recent study reported a north–south gradient in the levels of 8-oxodG in lymphocyte DNA from men from 5 European countries corresponding to the heart disease mortality whereas there was no such gradient among women.[106]

With GC/MS based assays complex patterns were reported after vitamin C interventions. In one study 8-oxoGua decreased while 8-oxoAde increased in lymphocytes.[107,108] In another study combining ascorbic acid with an iron supplement the sum of the levels of 13 different oxidative damaged bases DNA extracted from whole blood increased in subjects with initially high ascorbate levels in plasma whereas the levels decreased in subjects with low plasma levels.[109] However, even in the subjects with high initial vitamin C values the level of some oxidized bases, e.g. 8-oxoAde and 8-oxoguanine (8-oxoG) actually decreased whereas some others, such as FAPY-guanine, showed large increases.[109] It cannot be excluded that the iron supplement and its interaction with vitamin C were partly responsible for oxidant effects. It should also be noted that in both GC/MS based studies the reported levels of base damage, at least of 8-oxoGua, were 100 times higher than the levels reported by HPLC-EC recently.[29,31]

With the Comet assay on lymphocytes no effect of daily supplements of up to 60 mg vitamin C was shown on strand breaks and H_2O_2 resistance *ex vivo*[110] whereas increased *ex vivo* resistance to H_2O_2 and ionizing radiation was reported 2–4 h after a single dose of 1000–2000 mg vitamin C.[111,112] However, a daily cocktail of vitamins C and E and β-carotene decreased levels of oxidized pyrimidines assessed as EndoIII sensitive sites in the Comet assay and increased *ex vivo* resistance to H_2O_2.[113] Administration of individual carotenoids had no effect on strand breaks or Fpg or EndoIII sensitive sites in lymphocytes, although there initially were individual correlations between the levels of oxidized pyrimidines and plasma carotenoids and single doses increased H_2O_2 resistance *ex vivo*.[111,114] As compared to a period with carotenoid depletion, carotenoid rich vegetable products decreased strand breaks in the Comet assay and carrot juice also decreased EndoIII sensitive sites.[97,115]

The urinary excretions of 8-oxodG or 8-oxoG, an RNA oxidation product, have not been affected by antioxidants, including vitamins C and E, β-carotene and coenzyme Q10 in large well-controlled studies of smokers, non-smokers and in relation to exercise.[102,114,116–118] In contrast, increased levels were normalized by antioxidant combinations in AZT treated HIV patients.[119] Interestingly, after smoking cessation the decrease in urinary 8-oxodG excretion mirrored an increase in plasma ascorbate and the relationships of the two with smoking intensity

TABLE I Summary of antioxidant intervention studies on biomarkers of oxidative DNA damage and related gene expression

Intervention or dietary factor	Biomarker	Assay(s)	Summary of effects	References
Vitamin C depletion (5–20 mg/day) and supplementation (60–250 mg/day) ($n = 8$)	8-OxodG in sperm DNA and lymphocytes	HPLC-EC	Increased damage with depletion, reversed after supplementation in sperm, no effect in lymphocytes or on 24 h urinary excretion of 8-oxoG	[104,102]
Vitamin C 0, 6 or 60 mg/day for 2 weeks in a cross-over design in subjects with high and low cholesterol levels ($n = 48$)	Strand breaks and chromosome aberrations in lymphocytes	Comet ± H_2O_2 ± bleomycin challenge	No effect on strand breaks or resistance to H_2O_2, no effect on aberrations, decrease in bleomycin resistance to aberrations	[110]
Vitamin C 500 mg/day in sequential design ($n = 30$)	8-OxodG in urine, 8-oxoGua in lymphocyte DNA, 8-oxoGua + 8-oxoAde	ELISA, HPLC-EC, GC/MS	Ratio to cretinine increased in urine, decrease in 8-oxoGua, decrease in 8-oxoGua and increase in 8-oxoAde	[108,107]
Vitamin C 60 or 260 mg/day together with iron 14 mg/day in sequential design ($n = 40$)	13 oxidized bases in DNA from blood	GC/MS	Total base damage increased in subjects with initial high vitamin C, total base damage decreased in subjects with initial low vitamin C	[109]
Vitamin C 35 mg/kg in a single dose ($n = 6$)	Strand breaks in white blood cells induced *ex vivo*	Comet ± ionizing radiation	Increased resistance to ionising radiation	[112]
Vitamin C 1000 mg or α-tocopherol 1 g or β-carotene 45 mg in a single dose ($n = 12$)	Strand breaks in lymphocytes induced *ex vivo*	Comet ± H_2O_2	Increased resistance to H_2O_2 2–4 h after vitamin C and 18–24 h after vitamin E and β-carotene	[111]
Daily vitamin C 500 mg, vitamin E 200 U, β-carotene 9 mg, red ginseng or placebo in smokers for 4 weeks ($N = 3$/group)	8-OxodG in leukocyte DNA	HPLC-EC	Decreased after vitamin E (by 34%) or red ginseng (by 32%)	[103]
Vitamin C 1000 mg + α-tocopherol 600 mg + α/β-carotene 10 mg/day for 1 month ($n = 11$)	8-OxoG in urine	HPLC-EC	No effect on 24 h excretion in non-smokers	[118]
Vitamin C 500 mg, α-tocopherol 200 mg, coenzyme Q 90 mg/day or placebo for 2 months ($n = 140$)	8-OxodG in urine	HPLC-EC	No effect on 24 h excretion in smokers	[117]
Vitamin C 100 mg + α-tocopherol 280 mg + β-carotene 25 mg/day for 20 weeks ($n = 100$)	Strand breaks and oxidized pyrimidines in lymphocytes	Comet ± H_2O_2 ± EndoIII	No effect on strand breaks, decreased oxidized pyrimidines and increased resistance to H_2O_2 induced strand breaks *ex vivo*	[113]
Vitamin C 100 mg and α-tocopherol 600 mg/day in AZT treated HIV patients ($n = 5$)	8-OxodG in urine	HPLC-EC	70% lower values than in other AZT treated patients ($n = 8$) who had 46% increased levels compared to healthy controls ($n = 7$)	[119]

Vitamin C 60 mg, betaβ-carotene 3 mg, vitamin E 30 IU, Zn 40 μg, Se 40 μg and Cu 2 mg/day for 60 days in ETS exposed ($n = 30$; no control)	8-OxodG in DNA from whole blood, antioxidant enzymes	HPLC-EC Activity	Decreased 8-oxodG (by 62%) from initially increased levels SOD decreased by 18%, effects on GPX, GR and catalase not significant	[105]
β-Carotene 20 mg/day or placebo for 14 weeks in smokers ($n = 122$)	8-OxodG in urine	HPLC-EC	No effect on 8-oxodG excretion	[116]
15 mg/day of α/β-carotene, lutein, lycopene or placebo for 12 weeks ($n = 40$)	Oxidised bases in lymphocytes	Comet ± EndoIII/Fpg	No effect of interventions, negative correlation between initial plasma levels of carotenoids and oxidized pyrimidines (EndoIII sensitive sites)	[141]
β-Carotene 30 mg/day for 1 month or placebo before single exercise bout ($n = 14$)	8-OxodG in urine	HPLC-EC	No effect of β-carotene or exercise on 8-oxodG excretion	[114]
Diet low in carotenoids and tomato juice, carrot juice, or spinach powder for 2 weeks each in sequence ($n = 23$)	Strand breaks, base oxidation, DNA repair proteins, GSTPI, total protein	Comet ± EndoIII Comet ELISA	Strand breaks decreased by all products, decreased by carrot juice, increased by 6/23, increased in 9/21, increased 11/23	[115,97]
Lycopene 39–150 mg/day as tomato products for 1 week in cross-over design ($n = 19$)	8-OxodG in lymphocyte DNA	HPLC-EC	Effects in terms of a trend for protection was not significant	[142]
Brussels sprouts rich diet (300 g/day) compared with other vegetables ($n = 5 + 5$; $n = 10$)	8-OxodG, GSTs	HPLC-EC activity and protein	Decreased 24 excretion in non-smokers, effect only in men in second study GST-α increased in plasma, GST-α and GST-π increased in rectum	[124,125,99,127,98]
Black currant and apple juice 750, 1000 and 1500 ml/day in a cross over design ($n = 5$)	Antioxidant enzyme activity in erythrocytes	Spectrophotometric	Increased GPX	[128]
Relationships with plasma vitamin C, α-tocopherol, β-carotene, lycopene, coenzyme Q or GST ($n = 407$)	8-OxodG in urine	HPLC-EC	No significant correlations	[122]

were also mirror images.[120,121] However, there were no significant individual correlations in this study or other large studies combined between 8-oxodG excretion and plasma levels of vitamins C and E, carotenoids, or coenzyme Q.[122] Similarly, there was no significant difference with respect to 8-oxodG excretion between subjects with habitually high and low consumption of fruits and vegetables.[123] Nevertheless, intervention with a diet rich in Brussels sprouts compared to non-cruciferous vegetables reduced the urinary excretion of 8-oxodG by 30% in men[124,125] and in rats where an extract also prevented oxidative DNA damage induced by 2-nitropropane.[126]

Studies on DNA Repair and Gene Expression

A few studies have addressed the effect of antioxidants on the DNA repair capacity. In a study involving a single 1 g dose of vitamin C DNA repair assessed as the disappearance of strand breaks and EndoIII sensitive sites in the Comet assay was enhanced in two subjects out of eight with the highest response in plasma vitamin C.[96] Comparing carotenoids in terms of carrot juice with a carotenoid depleted diet the DNA repair capacity of a protein extract from lymphocytes assessed by the Comet assay on damaged indicator cells was enhanced in 9 out of 21 subjects although this effect was not statistically significant.[97] In another study involving administration of vitamin C 500 mg/day the concentrations of 8-oxodG estimated by an ELISA assay increased in plasma and urine whereas the levels decreased in lymphocyte DNA interpreted by the authors as indicating an increased repair rate.[108] However, in that study the reported basal plasma concentration of 8-oxodG were around 1 μM, i.e. 3–4 orders of magnitude higher than measured by HPLC-EC.[49,50] Moreover, it is not likely that plasma alone should contain 100 times more 8-oxodG than the total 24 h urinary excretion which is usually less than 30 nmol.[2] Similarly, in the vitamin C intervention study the urinary 8-oxodG to creatinine ratios were 6–8 times higher than reported from studies with HPLC-EC based assays.[2,108]

Antioxidant enzyme activities and other indications of expression have only been studied in a few antioxidant interventions although at least the former can be measured by relatively simple spectrophotometric assays. After a cocktail of antioxidants, each in modest doses, SOD activity decreased along with 8-oxodG levels in DNA from whole blood, whereas other antioxidant enzymes were unchanged.[105] GST activities and/or enzyme protein levels have been induced by different vegetable products. Thus, carrot juice administration increased GSTP1 level as measured by ELISA in lymphocytes from 7 out of 23 subjects when comparing with a carotenoid depleted period whereas the total protein level increased in all subjects.[97] Comparing a diet with 300 g of Brussels spouts with a diet with a similar amount of non-cruciferous vegetables the activity and protein level of GST-α increased in plasma and the protein level of GST-α and GTS-π increased in rectum whereas the levels were unchanged in duodenum and lymphocytes.[98,99,127] Supplementation of black currant and apple juice rich in flavonoids and vitamin C increased GPX activity in erythrocytes as well as protein oxidation in plasma whereas the activity of catalase, SOD and glutathione reductase were unchanged.[128]

Comments on Antioxidant Intervention Studies

The data could indicate that a depleted state due to nutritional deficiency and/or an increased oxidative stress might be required to show protective effects of antioxidants. Thus, effects of vitamin C depletion and supplementation in depleted subjects could be shown in sperm cells by HPLC-EC and in whole blood DNA by GC/MS.[109,129] Similarly, the effects of carotenoids

on the Comet assay were only shown in comparison with a depleted diet.[115] Positive effects of antioxidants and cocktails could be shown or were more pronounced on 8-oxodG in leukocyte DNA or EndoIII sensitive sites in ETS exposed subjects and smokers and in urine from ATZ treated HIV patients.[103,105,113,119] However, no effect of antioxidants has been shown on the urinary biomarkers in smokers or in relation to heavy exercise. Similarly, there are no correlations between plasma levels of antioxidants and the excretion of 8-oxodG.[122] Nevertheless, the urinary excretion of DNA oxidation products has consistently been elevated in subjects exposed to oxidative stress, e.g. in smokers, after radiation and chemotherapy and in relation oxygen consumption and occupational exposures.[2,121,130] With the Comet assay and the level of 8-oxodG in lymphocytes there have been inconsistent effects of smoking whereas some occupational exposures and radiation have in general shown the expected effects.[2,131–137] Thus, the urine based assays may be at least as sensitive as the lymphocyte based assays in detecting oxidative stress/damage and they are supplementary in their functional correlates in terms of damage rate and damage/repair balance, respectively.

The complex patterns of oxidized base alteration shown in the GC/MS based studies of vitamin C interventions indicate the need for measurements of other DNA oxidation products than 8-oxodG.[107,109] However, the problems of possible artefactual base oxidation during DNA extraction, hydrolysis and in particular during derivatization need to be solved. One possible solution to important parts of this problem involves the use of DNA repair enzymes to release the oxidized bases.

The *ex vivo* resistance to H_2O_2 appears to be more sensitive to antioxidant interventions than the basal level in the Comet assay. This suggests that the presence of antioxidants in samples may be important for possible artefactual oxidation of DNA after collection. In this context it remains to be investigated whether differences measured by the Comet assay or as oxidized bases in DNA apparently induced by, e.g. antioxidant manipulations actually reflects resistance to handling of samples *ex vivo*, e.g. oxidation during sample work-up or with respect to frozen storage. Moreover, it cannot be determined whether any change in the actual level of DNA damage in tissue or cell is a result of an altered damage rate, e.g. due to improved antioxidant defence or an altered DNA repair capacity.

In the interpretation of data related to these antioxidant and DNA repair enzymes it can be debated whether increased levels are good or bad, i.e. whether they are a signal of a response to oxidative stress or show an improved defence (Figure 2). Indeed, tobacco smoking appears to induce OGG1 in lymphocytes,[82] which could explain that the level of 8-oxodG is not consistently elevated in leukocyte DNA from smokers.[2,137] Similarly, antioxidant enzymes and markers of oxidative damage to DNA, protein and lipids are increased in bus drivers exposed to urban air pollution.[138–140] Moreover, SOD and 8-oxodG levels decreased concomitantly in ETS exposed subjects after antioxidant intervention.[105] In contrast, increases in GST protein and activities and decreases in 8-oxodG excretion resulted from intervention with Brussels sprouts.[98,99,124,125,127] A possible explanation could be that Brussels sprouts constituents turn on the ARE regulated genes without significant oxidative stress whereas the antioxidant cocktail can act as direct free radical scavengers. However, long term prospective or possibly nested case-control studies on biobank material are required to study and possibly link these biomarkers and mechanisms to risk of disease.

The differences between groups and effects of interventions seen in human studies of oxidized DNA bases and gene expression are usually rather small, less than two-fold and for urinary excretion less than 50%. Moreover, for the nuclear steady state levels, effects are obscured by the huge variation between and even within the various methods and laboratories. Thus, the

many negative results of antioxidant intervention trials could be due to the limited number of study subjects, i.e. a type 2 statistical error. Accordingly, power calculation should always be included in such trials. As an example we can assume that the variation with respect to the relevant biomarker is about 35% between subjects and set the level of statistical significance, α at 0.05 and the risk of a type 2 error, β at 0.2. A controlled trial with two parallel group would then require 190 persons in each group to detect an effect causing a difference of 10%, 86 persons to detect a difference of 15% and 47 persons to detect a difference of 20%. On the other hand, cross-over experiments with homogeneous, defined and controlled groups would show considerably less variation. If the intra-individual variation is 20%, differences of 10% or 20% can be detected within groups of 32 or 8 with similar α and β values, respectively. Thus a difference of 30% could be detected in a cross-over study of Brussels sprouts with 5 subjects.[124]

CONCLUSIONS

A number of different oxidatively modified nucleobases in cellular and tissue DNA and repair products excreted in urine can be measured with a variety of methods. However, problems remain with respect to the true values and intermethod and interlaboratory variation, in particular regarding the levels in nuclear DNA. Expression and activity of DNA repair and antioxidant enzymes can also be studied by a number of methods although rather few have been employed in intervention studies so far. In that area interlaboratory validation is even more scarce and interpretation more complex.

It should be emphasized that the levels of oxidized nucleobases in tissue/cells and the excretion of the repair products represent two fundamentally different estimates that are supplementary. The urine measurement represent the number of repaired bases summed from all organs and cells during a given time period, i.e. the rate of damage. The tissue measurement is a concentration measurement in the specific tissue/cells in the moment of sampling dependent on the balance between the rates of damage and repair. It has yet to be determined whether increased expression of antioxidant and DNA repair enzymes are good or bad, i.e. whether they are a signal of a response to oxidative stress or show an improved defense.

In human intervention studies, vitamins C and E, β-carotene, coenzyme Q as well as various vegetable and fruit regimens and products, particularly some rich in carotenoids, have been assessed with these biomarkers. The data could indicate that a depleted state due to nutritional deficiency and/or an increased oxidative stress might facilitate demonstration of protective effects of antioxidants with respect to DNA damage. The Comet assay involving *ex vivo* challenge with oxidants may be particularly sensitive to antioxidant intervention although the interpretation of the result of this assay is difficult. So far, no particular biomarker appears superior and the markers presented here have different functional correlates and thus supplement each other. Generally, the effects of antioxidants are small, in the order of 50% or much less, and statistical power is crucial. In the future the use of the biomarkers may provide further proof that endogenous and exogenous oxidative stress is involved in the pathogenesis of cancer and degenerative diseases of aging, as well as target preventive measures involving antioxidants. However, much more knowledge regarding the true sources, levels, pathways and kinetics of repair of the oxidized nucleobases and nucleosides as well as the interpretation of the expression of the corresponding repair and antioxidant genes is warranted.

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References

[1] B.N. Ames, L.S. Gold and W.C. Willett (1995) The causes and prevention of cancer. *Proceedings of the National Academy of Sciences of the United States of America* **92**, 5258–5265.

[2] S. Loft and H.E. Poulsen (1996) Cancer risk and oxidative DNA damage in man. *Journal of Molecular Medicine* **74**, 297–312.

[3] H. Wiseman and B. Halliwell (1996) Damage to DNA by reactive oxygen and nitrogen species: role on inflammatory disease and progression to cancer. *Biochemical Journal* **313**, 17–29.

[4] B. Chance, H. Sies and A. Boveris (1979) Hydroperoxide metabolism in mammalian organs. *Physiology Reviews* **59**, 527–605.

[5] B. Demple and L. Harrison (1994) Repair of oxidative damage to DNA: enzymology and biology. *Annual Reviews in Biochemistry* **63**, 915–948.

[6] J. Laval, J. Jurado, M. Saparbaev and O. Sidorkina (1998) Antimutagenic role of base-excision repair enzymes upon free radical-induced DNA damage. *Mutation Research* **402**, 93–102.

[7] G. Block, B. Patterson and A. Subar (1992) Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. *Nutrition and Cancer* **18**, 1–29.

[8] G. Block (1992) The data support a role for antioxidants in reducing cancer risk. *Nutrition Reviews* **50**, 207–213.

[9] World Cancer Research Fund and American Institute for Cancer Research (1997) *Food Nutrition and the Prevention of Cancer: A Global Perspective*. American Institute for Cancer Research, Washington, DC, 670pp.

[10] The Alpha-Tocopherol and 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. *New England Journal of Medicine* **330**, 1029–1035.

[11] G.S. Omenn, G.E. Goodman, M.D. Thornquist, J. Balmes, M.R. Cullen, A. Glass, J.P. Keogh, F.L. Meyskens, B. Valanis, J.H. Williams, S. Barnhart and S. Hammar (1996) Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. *New England Journal of Medicine* **334**, 1150–1155.

[12] C.H. Hennekens, J.E. Buring, J.E. Manson, M. Stampfer, B. Rosner, N.R. Cook, C. Belanger, F. LaMotte, M. Gaziano, P.M. Ridker, W. Willett and R. Peto (1996) Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. *New England Journal of Medicine* **334**, 1145–1149.

[13] R.M. Santella (1997) DNA damage as an intermediate biomarker in intervention studies. *Proceedings of the Society for Experimental Biology and Medicine* **216**, 166–171.

[14] P.A. Schulte and F.P. Perera (1993) *Molecular Epidemiology. Principles and Practices*. Academic Press, Inc. London. 588 pp.

[15] S. Loft, X.-S. Deng, J. Tuo, A. Wellejus, M. Sørensen and H.E. Poulsen (1998) Experimental study of oxidative DNA damage. *Free Radical Research* **29**, 525–539.

[16] M. Dizdaroglu (1991) Chemical determination of free radical-induced damage to DNA. *Free Radical Biology and Medicine* **10**, 225–242.

[17] J. Cadet, J.L. Ravanat, G.W. Buchko, H.C. Yeo and B.N. Ames (1994) Singlet oxygen DNA damage: chromatographic and mass spectrometric analysis of damage products. *Methods in Enzymology* **234**, 79–88.

[18] X.C. Le, J.Z. Xing, J. Lee, S.A. Leadon and M. Weinfeld (1998) Inducible repair of thymine glycol detected by an ultrasensitive assay for DNA damage. *Science* **280**, 1066–1069.

[19] J.-L. Ravanat, R.J. Turesky, E. Gremaud, L.J. Trudel and R.H. Stadler (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. *Chemical Research in Toxicology* **8**, 1039–1045.

[20] J. Cadet, C. D'Ham, T. Douki, J.-P. Pouget, J.-L. Ravanat and S. Sauvaigo (1998) Facts and artifacts in the measurement of oxidative base damage in DNA. *Free Radical Research* **29**, 541–550.

[21] M. Hamberg and L.Y. Zhang (1995) Quantitative determination of 8-hydroxyguanine and guanine by isotope dilution masss spectrometry. *Analytical Biochemistry* **229**, 336–344.

[22] A. Jenner, T.G. England, O.I. Aruoma and B. Halliwell (1998) Measurement of oxidative DNA damage by gas chromatography-mass spectrometry: ethanethiol prevents artifactual generation of oxidized DNA bases. *Biochemical Journal* **331**, 365–369.

[23] S. Senturker and M. Dizdaroglu (1999) The effect of experimental conditions on the levels of oxidatively modified bases in DNA as measured by gas chromatography-mass spectrometry: how many modified bases are involved? Prepurification or not? *Free Radical Biology and Medicine* **27**, 370–380.

[24] C. D'Ham, J.L. Ravanat and J. Cadet (1998) Gas chromatography-mass spectrometry with high-performance liquid chromatography prepurification for monitoring the endonuclease III-mediated excision of 5-hydroxy-5,6-dihydrothymine and 5,6-dihydrothymine from gamma-irradiated DNA. *Journal of Chromatography B, Biomedical Sciences and Applications* **710**, 67–74.

[25] K.B. Beckman and B.N. Ames (1999) Oxidative DNA damage and aging: the assay of 8-oxoguanine in DNA by FPG-HPLC. *4th Winter Research Conference*, Valloire, France.

[26] P. Jaruga, B. Tudek, E. Speina and R. Olinski (1999) Oxidative DNA base modifications analysed with repair enzymes and GC/MS technique. *4th Winter Research Conference*, Valloire, France.

[27] L. Moller and T. Hofer (1997) [32P]ATP mediates formation of 8-hydroxy-2′-deoxyguanosine from 2′-deoxyguanosine, a possible problem in the 32P-postlabeling assay. *Carcinogenesis* **18**, 2415–2419.

[28] D. Schuler, M. Otteneder, P. Sagelsdorff, E. Eder, R.C. Gupta and W.K. Lutz (1997) Comparative analysis of 8-oxo-2′-deoxyguanosine in DNA by ^{32}P- and ^{33}P-postlabeling and electrochemical detection. *Carcinogenesis* **18**, 2367–2371.

[29] A. Collins, J. Cadet, B. Epe and C. Gedik (1997) Problems in the measurement of 8-oxoguanine in human DNA. Report of a workshop, DNA Oxidation, held in Aberdeen, UK, 19–21 January, 1997. *Carcinogenesis* **18**, 1833–1836.

[30] J.P. Pouget, J.L. Ravanat, T. Douki, M.J. Richard and J. Cadet (1999) Measurement of DNA base damage in cells exposed to low doses of gamma-radiation: comparison between the HPLC-EC and comet assays. *International Journal of Radiation Biology* **75**, 51–58.

[31] C.M. Gedik, S.G. Wood and A.R. Collins (1998) Measuring oxidative damage to DNA; HPLC and the comet assay compared. *Free Radical Research* **29**, 609–616.

[32] R.A. Floyd, M.S. West, K.L. Eneff, J.E. Schneider, P.K. Wong, D.T. Tingey and W.E. Hogsett (1990) Conditions influencing yield and analysis of 8-hydroxy-2′deoxyguanosine in oxidatively damaged DNA. *Analytical Biochemistry* **188**, 155–158.

[33] H.G. Claycamp (1992) Phenol sensitization of DNA to subsequent oxidative damage in 8-hydroxyguanine assays. *Carcinogenesis* **13**, 1289–1292.

[34] S. Adachi, M. Zeisig and L. Moller (1995) Improvements in the analytical method for 8-hydroxydeoxyguanosine in nuclear DNA. *Carcinogenesis* **16**, 253–258.

[35] S. Loft and H.E. Poulsen (1999) Markers of oxidative damage to DNA: antioxidants and molecular damage. *Methods in Enymology* **300**, 166–184.

[36] H.J. Helbock, K.B. Beckman, M.K. Shigenaga, P.B. Walter, A.A. Woodall, H.C. Yeo and B.N. Ames (1998) DNA oxidation matters: the HPLC-electrochemical detection assay of 8-oxo-deoxyguanosine and 8-oxo-guanine. *Proceedings of the National Academy of Sciences of the United States of America* **95**, 288–293.

[37] A.R. Collins, M. Dusinska, C.M. Gedik and R. Stetina (1996) Oxidative damage to DNA: do we have a reliable biomarker? *Environmental Health Perspectives* **104**(Suppl. 3), 465–469.

[38] M. Nakajima, T. Takeuchi and K. Morimoto (1996) Determination of 8-hydroxydeoxyguanosine in human cells under oxygen-free conditions. *Carcinogenesis* **17**, 787–791.

[39] J. Lunec (1998) ESCODD: European standards committee on oxidative DNA damage. *Free Radical Research* **29**, 601–608.

[40] A.R. Collins, V.L. Dobson, M. Dusinska, G. Kennedy and R. Stetina (1997) The comet assay: what can it really tell us? *Mutation Research* **375**, 183–193.

[41] B. Epe (1995) DNA damage profiles induced by oxidizing agents. *Reviews in Physiology, Biochemistry and Pharmacology* **127**, 223–249.

[42] A. Hartwig, H. Dally and R. Schlepegrell (1996) Sensitive analysis of oxidative DNA damage in mammalian cells: use of the bacterial Fpg protein in combination with alkaline unwinding. *Toxicology Letters* **88**, 85–90.

[43] A. Karakaya, P. Jaruga, V.A. Bohr, A.P. Grollman and M. Dizdaroglu (1997) Kinetics of excision of purine lesions from DNA by *Escherichia coli* Fpg protein. *Nucleic Acids Research* **25**, 474–479.

[44] X.-S. Deng, J.-S. Tuo, H.E. Poulsen and S. Loft (1997) 2-Nitropropane induced DNA damage in rat bone marrow. *Mutation Research* **391**, 165–169.

[45] P. Møller, L.E. Knudsen, G. Frentz, M. Dybdahl, H. Wallin and B.A. Nexø (1998) Seasonal variation of DNA damage and repair in patients with non-melanoma skin cancer and referents with and without psoriasis. *Mutation Research* **407**, 25–34.

[46] J. Nakamura and J.A. Swenberg (1999) Endogenous apurinic/apyrimidinic sites in genomic DNA of mammalian tissues. *Cancer Research* **59**, 2522–2526.

[47] H. Rodriguez, R. Drouin, G.P. Holmquist and S.A. Akman (1997) A hot spot for hydrogen peroxide-induced damage in the human hypoxia-inducible factor 1 binding site of the PGK 1 gene. *Archives of Biochemistry and Biophysics* **338**, 207–212.

[48] M.K. Shigenaga, C.J. Gimeno and B.N. Ames (1989) Urinary 8-hydroxy-2′-deoxyguanosine as a biological marker of *in vivo* oxidative DNA damage. *Proceedings of the National Academy of Sciences of the United States of America* **86**, 9697–9701.

[49] S. Loft, P.N. Larsen, A. Rasmussen, A. Fischer-Nielsen, S. Bondesen, P. Kirkegaard, L.S. Rasmussen, E. Ejlersen, K. Tornøe, R. Bergholdt and H.E. Poulsen (1995) Oxidative DNA damage after transplantation of the liver and small intestine in pigs. *Transplantation* **59**, 16–20.

[50] E.-M. Park, M.K. Shigenaga, P. Degan, T.S. Korn, J.W. Kitzler, C.M. Wehr, P. Kolachana and B.N. Ames (1992) Assay of excised oxidative DNA lesions: isolation of 8-oxoguanine and its nucleoside derivatives from biological fluids with a monoclonal antibody column. *Proceedings of the National Academy of Sciences of the United States of America* **89**, 3375–3379.

[51] R. Cathcart, E. Schwiers, R.L. Saul and B.N. Ames (1984) Thymine glycol and thymidine glycol in human and rat urine: a possible assay for oxidative DNA damage. *Proceedings of the National Academy of Sciences of the United States of America* **81**, 5633–5637.

[52] H. Faure, M.F. Incardona, C. Boujet, J. Cadet, V. Ducros and A. Favier (1993) Gas chromatographic-mass spectrometric determination of 5-hydroxymethyluracil in human urine by stable isotope dilution. *Journal of Chromatography* **616**, 1–7.

[53] J. Suzuki, Y. Inoue and S. Suzuki (1995) Changes in urinary excretion level of 8-hydroxyguanine by exposure to reactive oxygen-generating substances. *Free Radical Biology and Medicine* **18**, 431–436.

[54] M.G. Simic and D.S. Bergtold (1991) Dietary modulation of DNA damage in human. *Mutation Research* **250**, 17–24.

[55] A.J. Teixeira, M.R. Ferreira, W.J. van Dijk, G. van de Werken and A.P. de Jong (1995) Analysis of 8-hydroxy-2′-deoxyguanosine in rat urine and liver DNA by stable isotope dilution gas chromatography/mass spectrometry. *Analytical Biochemistry* **226**, 307–319.

[56] J.L. Ravanat, P. Guicherd, Z. Tuce and J. Cadet (1999) Simultaneous determination of five oxidative DNA lesions in human urine. *Chemical Research in Toxicology* **12**, 802–808.

[57] M. Dizdaroglu (1994) Chemical determination of oxidative DNA damage by gas chromatography-mass spectrometry. *Methods in Enzymology* **234**, 3–16.

[58] I. Holmberg, P. Stal and M. Hamberg (1999) Quantitative determination of 8-hydroxy-2′-deoxyguanosine in human urine by isotope dilution mass spectrometry: normal levels in hemochromatosis. *Free Radical Biology and Medicine* **26**, 129–135.

[59] S. Loft, K. Vistisen, M. Ewertz, A. Tjønneland, K. Overvad and H.E. Poulsen (1992) Oxidative DNA-damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index. *Carcinogenesis* **13**, 2241–2247.

[60] C. Tagesson, M. Källberg and P. Leanderson (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. *Toxicology Methods* **1**, 242–251.

[61] M.K. Shigenaga, E.N. Aboujaoude, Q. Chen and B.N. Ames (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. *Methods in Enzymology* **234**, 16–33.

[62] R.K. Brown, A. McBurney, J. Lunec and F.J. Kelly (1995) Oxidative damage to DNA in patients with cystic fibrosis. *Free Radical Biology and Medicine* **18**, 801–806.

[63] C. Tagesson, M. Kallberg, C. Klintenberg and H. Starkhammar (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. *European Journal of Cancer* **31A**, 934–940.

[64] D. Germadnik, A. Pilger and H.W. Rudiger (1997) Assay for the determination of urinary 8-hydroxy-2′-deoxyguanosine by high-performance liquid chromatography with electrochemical detection. *Journal of Chromatography* **689**, 399–403.

[65] J.L. Ravanat, B. Duretz, A. Guiller, T. Douki and J. Cadet (1998) Isotope dilution high-performance liquid chromatography-electrospray tandem mass spectrometry assay for the measurement of 8-oxo-7,8-dihydro-2′-deoxyguanosine in biological samples. *Journal of Chromatography B, Biomedical Sciences and Applications* **715**, 349–356.

[66] T. Osawa, A. Yoshida, S. Kawakishi, K. Yamashita and H. Ochi (1995) Protective role of dietary antioxidants in oxidative stress. In: *Oxidative Stress and Aging* (Eds. R.G. Cutler, L. Packer, J. Bertram and A. Mori) Birkhauser Verlag, Basel, Switzerland, pp. 367–378.

[67] M. Erhola, S. Toyokuni, K. Okada, T. Tanaka, H. Hiai, H. Ochi, K. Uchida, T. Osawa, M.M. Nieminen, H. Alho and P. Kellokumpu-Lehtinen (1997) Biomarker evidence of DNA oxidation in lung cancer patients: association of urinary 8-hydroxy-2′-deoxyguanosine excretion with radiotherapy, chemotherapy, and response to treatment. *FEBS Letters* **409**, 287–291.

[68] H. Priemé, S. Loft, R.G. Cutler and H.E. Poulsen (1996) Measurement of oxidative DNA injury in humans: evaluation of a commercially available ELISA assay. In: *Natural Antioxidants and Food Quality in Atherosclerosis and Cancer Prevention* (Eds. J.T. Kumpulainen and J.T. Salonen) The Royal Society of Chemistry, London, pp. 78–82.

[69] T. Tajiri, H. Maki and M. Sekiguchi (1995) Functional cooperation of MutT, MutM and MutY proteins in preventing mutations caused by spontaneous oxidation of guanine nucleotide in *Escherichia coli*. *Mutation Research* **336**, 257–267.

[70] J.P. Radicella, C. Dherin, C. Desmaze, M.S. Fox and S. Boiteux (1997) Cloning and characterization of hOGG1, a human homolog of the OGG1 gene of *Saccharomyces cerevisiae*. *Proceedings of the National Academy of Sciences of the United States of America* **94**, 8010–8015.

[71] T. Roldan-Arjona, Y.F. Wei, K.C. Carter, A. Klungland, C. Anselmino, R.P. Wang, M. Augustus and T. Lindahl (1997) Molecular cloning and functional expression of a human cDNA encoding the antimutator enzyme 8-hydroxyguanine-DNA glycosylase. *Proceedings of the National Academy of Sciences of the United States of America* **94**, 8016–8020.

[72] P.M. Girard, C. D'Ham, J. Cadet and S. Boiteux (1998) Opposite base-dependent excision of 7,8-dihydro-8-oxoadenine by the Ogg1 protein of *Saccharomyces cerevisiae*. *Carcinogenesis* **19**, 1299–1305.

[73] H.M. Nash, S.D. Bruner, O.D. Scharer, T. Kawate, T.A. Addona, E. Spooner, W.S. Lane and G.L. Verdine (1996) Cloning of a yeast 8-oxoguanine DNA glycosylase reveals the existence of a base-excision DNA-repair protein superfamily. *Current Biology* **6**, 968–980.

[74] T.K. Hazra, T. Izumi, L. Maidt, R.A. Floyd and S. Mitra (1998) The presence of two distinct 8-oxoguanine repair enzymes in human cells: their potential complementary roles in preventing mutation. *Nucleic Acids Research* **26**, 5116–5122.

[75] S. Hollenbach, A. Dhenaut, I. Eckert, J.P. Radicella and B. Epe (1999) Overexpression of Ogg1 in mammalian cells: effects on induced and spontaneous oxidative DNA damage and mutagenesis. *Carcinogenesis* **20**, 1863–1868.

[76] J.T. Reardon, T. Bessho, H.C. Kung, P.H. Bolton and A. Sancar (1997) *In vitro* repair of oxidative DNA damage by human nucleotide excision repair system: possible explanation for neurodegeneration in Xeroderma pigmentosum patients. *Proceedings of the National Academy of Sciences of the United States of America* **94**, 9463–9468.

[77] J.Y. Mo, H. Maki and M. Sekiguchi (1992) Hydrolytic elimination of a mutagenic nucleotide, 8-oxodGTP, by human 18-kilodalton protein: sanitization of nucleotide pool. *Proceedings of the National Academy of Sciences of the United States of America* **89**, 11 021–11 025.

[78] H. Hayakawa, A. Taketomi, K. Sakumi, M. Kuwano and M. Sekiguchi (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.

[79] K. Fujikawa, H. Kamiya, H. Yakushiji, Y. Fujii, Y. Nakabeppu and H. Kasai (1999) The oxidized forms of dATP are substrates for the human MutT homologue, the hMTH1 protein. *Journal of Biological Chemistry* **274**, 18 201–18 205.

[80] Y. Kuchino, F. Mori, H. Kasai, H. Inoue, S. Iwai, K. Miura, E. Ohtsuka and S. Nishimura (1987) Misreading of DNA templates containing 8-hydroxydeoxyguanosine at the modified base and at adjacent residues. *Nature* **327**, 77–79.

[81] S. Shibutani, M. Takeshita and A.P. Grollman (1991) Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG. *Nature* **349**, 431–434.

[82] S. Asami, T. Hirano, R. Yamaguchi, Y. Tomioka, H. Itoh and H. Kasai (1996) Increase of a type of oxidative DNA damage, 8-hydroxyguanine, and its repair activity in human leukocytes by cigarette smoking. *Cancer Research* **56**, 2546–2549.

[83] P. Jaruga and M. Dizdaroglu (1996) Repair of products of oxidative DNA base damage in human cells. *Nucleic Acids Research* **24**, 1389–1394.

[84] D.S. Bergtold, C.D. Berg and M.G. Simic (1990) Urinary biomarkers in radiation therapy of cancer. *Advances in Experimental Medicine and Biology* **264**, 311–316.

[85] S. Blount, H.R. Griffiths and J. Lunec (1991) Reactive oxygen species damage to DNA and its role in systemic lupus erythematosus. *Molecular Aspects of Medicine* **12**, 93–105.

[86] R. Olinski, T.H. Zastawny, M. Foksinski, W. Windorbska, P. Jaruga and M. Dizdaroglu (1996) DNA base damage in lymphocytes of cancer patients undergoing radiation therapy. *Cancer Letters* **106**, 207–215.

[87] H. Kasai, P.F. Crain, Y. Kuchino, S. Nishimura, A. Ootsuyama and H. Tanooka (1986) Formation of

8-hydroxyguanine moiety in cellular DNA by agents producing oxygen radicals and evidence for its repair. *Carcinogenesis* **7**, 1849–1851.

[88] S. Loft and H.E. Poulsen (1998) Measurement of oxidative damage to DNA nucleobases *in vivo*: interpretation of nuclear levels and urinary excretion of repair products. In: *DNA Damage and Repair: Oxygen Radical Effects, Cellular Protection, and Biological Consequences* (Ed. M. Dizdaroglu) NATO ASI series, Plenum Press. New York, pp. 267–281.

[89] A.K. Jaiswal (1994) Antioxidant response element. *Biochemical Pharmacology* **48**, 439–444.

[90] R. Venugopal and A.K. Jaiswal (1998) Nrf2 and Nrf1 in association with Jun proteins regulate antioxidant response element-mediated expression and coordinated induction of genes encoding detoxifying enzymes. *Oncogene* **17**, 3145–3156.

[91] M.J. Jackson, A. McArdle and F. McArdle (1998) Antioxidant micronutrients and gene expression. *Proceedings ofthe Nutritional Society* **57**, 301–305.

[92] R. Pinkus, L.M. Weiner and V. Daniel (1995) Role of quinone-mediated generation of hydroxyl radicals in the induction of glutathione S-transferase gene expression. *Biochemistry* **34**, 81–88.

[93] R.M. Liu, M.M. Shi, C. Giulivi and H.J. Forman (1998) Quinones increase gamma-glutamyl transpeptidase expression by multiple mechanisms in rat lung epithelial cells. *American Journal of Physiology* **274**, L330–L336.

[94] S.W. Ryter and R.M. Tyrrell (1998) Singlet molecular oxygen ((1)O2): a possible effector of eukaryotic gene expression. *Free Radical Biology and Medicine* **24**, 1520–1534.

[95] C.B. Ambrosone, J.L. Freudenheim, P.A. Thompson, E. Bowman, J.E. Vena, J.R. Marshall, S. Graham, R. Laughlin, T. Nemoto and P.G. Shields (1999) Manganese superoxide dismutase (MnSOD) genetic polymorphisms, dietary antioxidants, and risk of breast cancer. *Cancer Research* **59**, 602–606.

[96] A.R. Collins, S.J. Duthie, L. Fillion, C.M. Gedik, N. Vaughan and S.G. Wood (1997) Oxidative DNA damage in human cells: the influence of antioxidants and DNA repair. *Biochemical Society Transactions* **25**, 326–331.

[97] B.L. Pool-Zobel, A. Bub, U.M. Liegibel, S. Treptow-van Lishaut and G. Rechkemmer (1998) Mechanisms by which vegetable consumption reduces genetic damage in humans. *Cancer Epidemiology Biomarkers and Prevention* **7**, 891–899.

[98] W.A. Nijhoff, M.J. Grubben, F.M. Nagengast, J.B. Jansen, H. Verhagen, G. van Poppel and W.H. Peters (1995) Effects of consumption of Brussels sprouts on intestinal and lymphocytic glutathione S-transferases in humans. *Carcinogenesis* **16**, 2125–2128.

[99] J.J. Bogaards, H. Verhagen, M.I. Willems, G. van Poppel and P.J. van Bladeren (1994) Consumption of Brussels sprouts results in elevated alpha-class glutathione S-transferase levels in human blood plasma. *Carcinogenesis* **15**, 1073–1075.

[100] D.T.H. Verhoeven, R.A. Goldbohm, G. van Poppel, H. Verhagen and P.A. van den Brandt (1996) Epidemiological studies on Brassica vegetables and cancer risk. *Cancer Epidemiology Biomarkers and Prevention* **5**, 733–748.

[101] D.T.H. Verhoeven, H. Verhagen, R.A. Goldbohm, P.A. van den Brandt and G. van Poppel (1997) A review of mechanisms underlying anticarcinogenicity by brasssica vegetables. *Chemical–Biological Interactions* **103**, 79–129.

[102] C.G. Fraga, P.A. Motchnik, M.K. Shigenaga, H.J. Helbock, R.A. Jacob and B.N. Ames (1991) Ascorbic acid protects against endogenous oxidative DNA damage in human sperm. *Proceedings of the National Academy of Sciences of the United States of America* **88**, 11 003–11 006.

[103] B.M. Lee, S.K. Lee and H.S. Kim (1998) Inhibition of oxidative DNA damage, 8-OHdG, and carbonyl contents in smokers treated with antioxidants (vitamin E, vitamin C, β-carotene and red ginseng). *Cancer Letters* **132**, 219–227.

[104] R.A. Jacob, D.S. Kelley, F.S. Pianalto, M.E. Swendseid, S.M. Henning, J.Z. Zhang, B.N. Ames, C.G. Fraga and J.H. Peters (1991) Immunocompetence and oxidant defence during ascorbate depletion of healthy men. *American Journal of Clinical Nutrition* **54**(Suppl. 6), 1302S–1309S.

[105] D.J. Howard, R.B. Ota, L.A. Briggs, M. Hampton and C.A. Pritsos (1998) Oxidative stress induced by environmental tobacco smoke in the workplace is mitigated by antioxidant supplementation. *Cancer Epidemiology Biomarkers and Prevention* **7**, 981–988.

[106] A.R. Collins, C.M. Gedik, B. Olmedilla, S. Southon and M. Bellizzi (1998) Oxidative DNA damage measured in human lymphocytes: large differences between sexes and between countries, and correlations with heart disease mortality rates. *FASEB Journal* **12**, 1397–1400.

[107] I.D. Podmore, H.R. Griffiths, K.E. Herbert, N. Mistry, P. Mistry and J. Lunec (1998) Vitamin C exhibits pro-oxidant properties. *Nature* **392**, 559.

[108] M.S. Cooke, M.D. Evans, I.D. Podmore, K.E. Herbert, N. Mistry, P. Mistry, P.T. Hickenbotham, A. Hussieni, H.R. Griffiths and J. Lunec (1998) Novel repair action of vitamin C upon *in vivo* oxidative DNA damage. *FEBS Letters* **439**, 363–367.

[109] A. Rehman, C.S. Collis, M. Yang, M. Kelly, A.T. Diplock, B. Halliwell and C. Rice-Evans (1998) The effects of iron and vitamin C co-supplementation on oxidative damage to DNA in healthy volunteers. *Biochemical and Biophysical Research Communications* **246**, 293–298.

[110] D. Anderson, B.J. Phillips, T.W. Yu, A.J. Edwards, R. Ayesh and K.R. Butterworth (1997) The effects of vitamin C supplementation on biomarkers of oxygen radical generated damage in human volunteers with "low" or "high" cholesterol levels. *Environmental and Molecular Mutagenesis* **30**, 161–174.

[111] M. Panayiotidis and A.R. Collins (1997) *Ex vivo* assessment of lymphocyte antioxidant status using the comet assay. *Free Radical Research* **27**, 533–537.

[112] M.H. Green, J.E. Lowe, A.P. Waugh, K.E. Aldridge, J. Cole and C.F. Arlett (1994) Effect of diet and vitamin C on DNA strand breakage in freshly-isolated human white blood cells. *Mutation Research* **316**, 91–102.

[113] S.J. Duthie, A. Ma, M.A. Ross and A.R. Collins (1996) Antioxidant supplementation decreases oxidative DNA damage in human lymphocytes. *Cancer Research* **56**, 1291–1295.

[114] S. Sumida, T. Doi, M. Sakurai, Y. Yoshioka and K. Okamura (1997) Effect of a single bout of exercise and beta-carotene supplementation on the urinary excretion of 8-hydroxy-deoxyguanosine in humans. *Free Radical Research* **27**, 607–618.

[115] B.L. Pool-Zobel, A. Bub, H. Muller, I. Wollowski and G. Rechkemmer (1997) Consumption of vegetables

reduces genetic damage in humans: first results of a human intervention trial with carotenoid-rich foods. *Carcinogenesis* **18**, 1847–1850.

[116] G. van Poppel, H. Poulsen, S. Loft and H. Verhagen (1995) No influence of beta carotene on oxidative DNA damage in male smokers. *Journal of the National Cancer Institute* **87**, 310–311.

[117] H. Priemé, S. Loft, K. Nyyssönen, J.T. Salonen and H.E. Poulsen (1997) 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. *American Journal of Clinical Nutrition* **65**, 503–507.

[118] E.H. Witt, A.Z. Reznick, C.A. Viguie, P. Starke-Reed and L. Packer (1992) Exercise, oxidative damage and effects of antioxidant manipulation. *Journal of Nutrition* **122**, 766–773.

[119] J.G. de la Asuncion, M.L. del Olmo, J. Sastre, A. Millan, A. Pellin, F.V. Pallardo and J. Vina (1998) AZT treatment induces molecular and ultrastructural oxidative damage to muscle mitochondria. Prevention by antioxidant vitamins. *Journal of Clinical Investigation* **102**, 4–9.

[120] J. Lykkesfeldt, H. Priemé, S. Loft and H.E. Poulsen (1996) Effect of smoking cessation on plasma ascorbic acid concentration. *British Medical Journal* **313**, 91.

[121] H. Prieme, S. Loft, M. Klarlund, K. Grønbæk, P. Tønnesen and H.E. Poulsen (1998) Effect of smoking cessation on oxidative DNA damage estimated by 8-oxo-7,8-dihydro-2′-deoxyguanosine. *Carcinogenesis* **19**, 347–351.

[122] H.E. Poulsen, S. Loft, H. Prieme, K. Vistisen, J. Lykkesfeldt, K. Nyssonen and J.T. Salonen (1998) Oxidative DNA damage *in vivo*, interpretation, relation to age, plasma antioxidants, drug metabolism, glutathione S-transferase activity and urinary creatinine correction. *Free Radical Research* **29**, 565–571.

[123] M.G.L. Hertog, A. de Vries, M.C. Ocké, A. Schouten, H. Bas Bueno-de-Mesquita and H. Verhagen (1997) Oxidative DNA damage in humans: comparison between high and low habitual fruit and vegetable consumption. *Biomarkers* **2**, 259–262.

[124] H. Verhagen, H.E. Poulsen, S. Loft, G. van Poppel, M.I. Willems and P.J. van Bladeren (1995) Reduction of oxidative DNA-damage in humans by Brussels sprouts. *Carcinogenesis* **16**, 969–970.

[125] H. Verhagen, A. de Vries, W.A. Nijhoff, A. Schouten, G. van Poppel, W.H.M. Peters and H. van den Berg (1997) Effect of Brussels sprouts on oxidative DNA-damage in man. *Cancer Letters* **114**, 127–130.

[126] X.-S. Deng, J. Tuo, H.E. Poulsen and S. Loft (1998) Prevention of oxidative DNA damage in rats by Brussels sprouts. *Free Radical Research* **28**, 323–333.

[127] W.A. Nijhoff, T.P. Mulder, H. Verhagen, G. van Poppel and W.H. Peters (1995) Effects of consumption of brussels sprouts on plasma and urinary glutathione S-transferase class-alpha and -pi in humans. *Carcinogenesis* **16**, 955–957.

[128] J.F. Young, S.E. Nielsen, J. Haraldsdottir, B. Daneshvar, S.T. Lauridsen, P. Knuthsen, A. Crozier, B. Sandstrom and L.O. Dragsted (1999) Effect of fruit juice intake on urinary quercetin excretion and biomarkers of antioxidative status. *American Journal of Clinical Nutrition* **69**, 87–94.

[129] D.W. Hein, T.J. Flammang, W.G. Kirlin, A. Trinidad and F. Ogolla (1987) Acetylator genotype-dependent metabolic activation of carcinogenic N-hydroxylaamines by S-acetyl coenzyme A-dependent enzymes of inbred hamster tissue cytosols: relationship to arylamine N-acetyltransferase. *Carcinogenesis* **8**, 1767–1774.

[130] F. Bianchini, F. Donato, H. Faure, J.L. Ravanat, J. Hall and J. Cadet (1998) Urinary excretion of 5-(hydroxymethyl) uracil in healthy volunteers: effect of active and passive tobacco smoke. *International Journal of Cancer* **77**, 40–46.

[131] G. Frenzilli, C. Betti, T. Davini, M. Desideri, E. Fornai, L. Giannessi, F. Maggiorelli, P. Paoletti and R. Barale (1997) Evaluation of DNA damage in leukocytes of ex-smokers by single cell gel electrophoresis. *Mutation Research* **375**, 117–123.

[132] S.M. Piperakis, E.E. Visvardis, M. Sagnou and A.M. Tassiou (1998) Effects of smoking and aging on oxidative DNA damage of human lymphocytes. *Carcinogenesis* **19**, 695–698.

[133] R.J. Sram, K. Podrazilova, J. Dejmek, G. Mrackova and T. Pilcik (1998) Single cell gel electrophoresis assay: sensitivity of peripheral white blood cells in human population studies. *Mutagenesis* **13**, 99–103.

[134] R.J. Sram, P. Rossner, K. Peltonen, K. Podrazilova, G. Mrackova, N.A. Demopoulos, G. Stephanou, D. Vlachodimitropoulos, F. Darroudi and A.D. Tates (1998) Chromosomal aberrations, sister-chromatid exchanges, cells with high frequency of SCE, micronuclei and comet assay parameters in 1,3-butadiene-exposed workers. *Mutation Research* **419**, 145–154.

[135] M. Wojewodzka, M. Kruszewski, T. Iwanenko, A.R. Collins and I. Szumiel (1999) Lack of adverse effect of smoking habit on DNA strand breakage and base damage, as revealed by the alkaline comet assay. *Mutation Research* **440**, 19–25.

[136] M. Wojewodzka, M. Kruszewski, T. Iwanenko, A.R. Collins and I. Szumiel (1998) Application of the comet assay for monitoring DNA damage in workers exposed to chronic low-dose irradiation. I. Strand breakage. *Mutation Research* **416**, 21–35.

[137] A.A. van Zeeland, A.J. de Groot, J. Hall and F. Donato (1999) 8-Hydroxydeoxyguanosine in DNA from leukocytes of healthy adults: relationship with cigarette smoking, environmental tobacco smoke, alcohol and coffee consumption. *Mutation Research* **439**, 249–257.

[138] S. Loft, H.E. Poulsen, K. Vistisen and L.E. Knudsen (1999) Increased urinary excretion of 8-oxo-2′-deoxyguanosine, a biomarker of oxidative DNA damage, in urban bus drivers. *Mutation Research* **441**, 11–19.

[139] B. Daneshvar (1997) Biomarkers for oxidative damage to proteins. Ph.D. Dissertation, University of Copenhagen, Denmark.

[140] H. Autrup, B. Daneshvar, L.O. Dragsted, M. Gamborg, Å.M. Hansen, S. Loft, H. Okkels, F. Nielsen, P.S. Nielsen, H. Wallin and L.E. Knudsen (1999) Biomarkers for exposure to ambient air pollution – comparison of carcinogen adduct levels with other exposure markers and markers for oxidative stress. *Environmental Health Perspectives* **107**, 233–238.

[141] A.R. Collins, B. Olmedilla, S. Southon, F. Granado and S.J. Duthie (1998) Serum carotenoids and oxidative DNA damage in human lymphocytes. *Carcinogenesis* **19**, 2159–2162.

[142] A.V. Rao and S. Agarwal (1998) Bioavailability and *in vivo* antioxidant properties of lycopene from tomato products and their possible role in the prevention of cancer. *Nutrition and Cancer* **31**, 199–203.