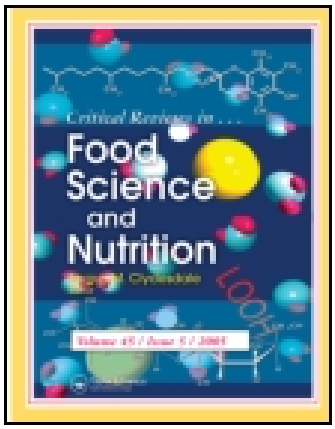

Critical Reviews in Food Science and Nutrition
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/bfsn20

DNA Damage, a Biomarker of Carcinogenesis: Its Measurement and Modulation by Diet and Environment

Eun-Sun Hwang [a] & Phyllis E. Bowen [a]
[a] Department of Human Nutrition , University of Illinois at Chicago , Chicago, 60612, IL
Published online: 06 Oct 2010.

To cite this article: Eun-Sun Hwang & Phyllis E. Bowen (2007) DNA Damage, a Biomarker of Carcinogenesis: Its Measurement and Modulation by Diet and Environment, Critical Reviews in Food Science and Nutrition, 47:1, 27-50, DOI: 10.1080/10408390600550299

To link to this article: http://dx.doi.org/10.1080/10408390600550299

Critical Reviews in Food Science and Nutrition, 47:27–50 (2007)

ISSN: 1040-8398
DOI: 10.1080/10408390600550299

Taylor & Francis
Taylor & Francis Group

DNA Damage, a Biomarker of Carcinogenesis: Its Measurement and Modulation by Diet and Environment

EUN-SUN HWANG[1] and PHYLLIS E. BOWEN

Department of Human Nutrition, University of Illinois at Chicago, Chicago, IL 60612

Free radicals and other reactive oxygen or nitrogen species are constantly generated in vivo and can cause oxidative damage to DNA. This damage has been implicated to be important in many diseases, including cancer. The assessment of damage in various biological matrices, such as tissues, cells, and urine, is vital to understanding this role and subsequently devising intervention strategies. During the last 20 years, many analytical techniques have been developed to monitor oxidative DNA base damage. High-performance liquid chromatography-electrochemical detection and gas chromatography-mass spectrometry are the two pioneering contributions to the field. Currently, the arsenal of methods available include the promising high-performance liquid chromatography-tandem mass spectrometry technique, capillary electrophoresis, ^{32}P-postlabeling, antibody-base immunoassays, and assays involving the use of DNA repair glycosylases such as the comet assay. The objective of this review is to discuss the biological significance of oxidative DNA damage, evaluate the effectiveness of several techniques for measurement of oxidative DNA damage in various biological samples and review current research on factors (dietary and non-dietary) that influence DNA oxidative damage using these techniques.

Keywords reactive oxygen species, fee radicals, DNA damage, cancer, diet

INTRODUCTION

Life styles that involve diets high in fruits and vegetables have been associated with reduced risk of cancer[1,2] and this association has motivated the National 5 A Day Program.[3] Although the bioactive ingredients in fruits and vegetables and the mechanism of action remains unclear, the focus has been on a number of antioxidants such as vitamin C, the carotenoids, and a vast array of polyphenolic compounds such as catechins, anthocyanins, flavonoids, and isoflavonoids. A plausible hypothesis is that these antioxidants may reduce the risk of cancer caused by oxidative stress. Oxidative stress has been classically defined as an imbalance between prooxidant and antioxidant activities leading to damage.[4] In the case of cancer the primary and secondary events are thought to be an oxidative attack on DNA causing point mutations.[5] Large-scale intervention studies of the effects of single and combinations of the antioxidants vitamin E and β-carotene have been negative for preventing cancer,[6] and two studies found that β-carotene increased the risk of lung cancers in smokers at pharmacological doses and similar doses in ferrets caused lung metaplasia with and without cigarette smoke exposure.[7,8] Accordingly, fruits and vegetables consumption may affect oxidative status via other bioactive compounds or oxidative stress may not be a prominent component in the continuing dynamics of cancer. Human intervention studies that include mechanistically based biomarkers of oxidative damage as intermediate end points, supported by animal and *in vitro* experiments, can explore mechanisms and target the optimum strategy for large-scale interventions. In addition, the use of such biomarkers may provide further proof of a causal relationship between oxidative damage to DNA and cancer. Cancer-causing DNA damage includes the loss of genes that act as tumor suppressors and the activation of oncogenes, which promote cancer.[9]

Since antioxidants undergo redox reactions they all can be found in the pro-oxidant state given the right environment and the

Address correspondence to Phyllis E. Bowen, Department of Human Nutrition, College of Applied Health Sciences, University of Illinois at Chicago, 1919 W. Taylor, Chicago, IL 60612, USA. E-mail: pbowen@uic.edu

[1]Present Address: Center for Agricultural Biomaterial, Seoul National University, San 56-1, Shillim-dong, Kwanak-gu, Seoul 151-921, Korea.

development of a prooxidative tissue environment can stimulate proliferative cell signaling pathways. This paper will give an overview of the current knowledge of oxidative DNA damage and cancer incidence and the approaches used to measure *in vivo* DNA damage. This will be followed by an exploration of environmental and dietary factors that have been found to influence DNA oxidative damage. Finally, an overall conclusion and suggestions for further research will be given.

OXIDATIVE DNA DAMAGE AND CANCER

Cells are constantly exposed to oxidants from normal metabolic reactions in the form of reactive oxygen and nitrogen species (ROS & RNS) as well as the environment. When carcinogens enter the body and are activated, they can react with the DNA to form DNA adducts, chemical compounds resulting from the attachment of the carcinogen to the DNA. ROS and RNS can also directly attack DNA causing oxygen-nitrogen adducts or chain breaks. If the body's natural defense systems do not properly repair the damage caused by these adducts and chain breaks, and additional damage to DNA that controls cell cycle (including proliferation and apoptosis) occurs, a critical first step in the development of cancer is taken. Failure of the system of enzymatic, endogenous, and nutritional antioxidants may lead to mutagenic oxidative DNA damage as well as dysregulation of cell cycle control, resulting in carcinogenesis. There is considerable evidence that ROS contributes to the endogenous or exogenous human carcinogenesis and circumstantial evidence that antioxidants may prevent or delay the onset of cancer.[10,11]

TYPES AND MECHANISMS OF OXIDATIVE DNA DAMAGE

Oxidants from metabolic activity, inflammation, radiation, or toxins can damage nucleic acids, generating lesions that appear to contribute to aging and cancer.[12] About 20 major oxidative DNA adducts have been characterized and some of the stable oxidative DNA base lesion are listed on Fig. 1.[13] One of these is 8-hydroxydeoxyguanosine (8-OHdG), an adduct for which specific cellular repair enzymes exist, and has been shown to cause G- to T transversions.[14] Damage to DNA by ROS has been suggested to: 1) cause structural alterations in DNA e.g. damaged bases, damage to deoxyribose, intra-DNA cyclic adducts, DNA interstrand crosslinks, and protein DNA crosslinks.

ROS is able to directly modify DNA bases include hydroxyl radical (OH*), singlet oxygen ($^1O_2$), peroxyl (RO_2*) and alkoxyl (RO*) radicals, and ozone (O_3) 2) ROS also activates cytoplasmic and nuclear signal transduction. 3) ROS modulates the activity of the proteins and genes that respond to stress and which act to regulate the effecter genes that are related to cell growth,

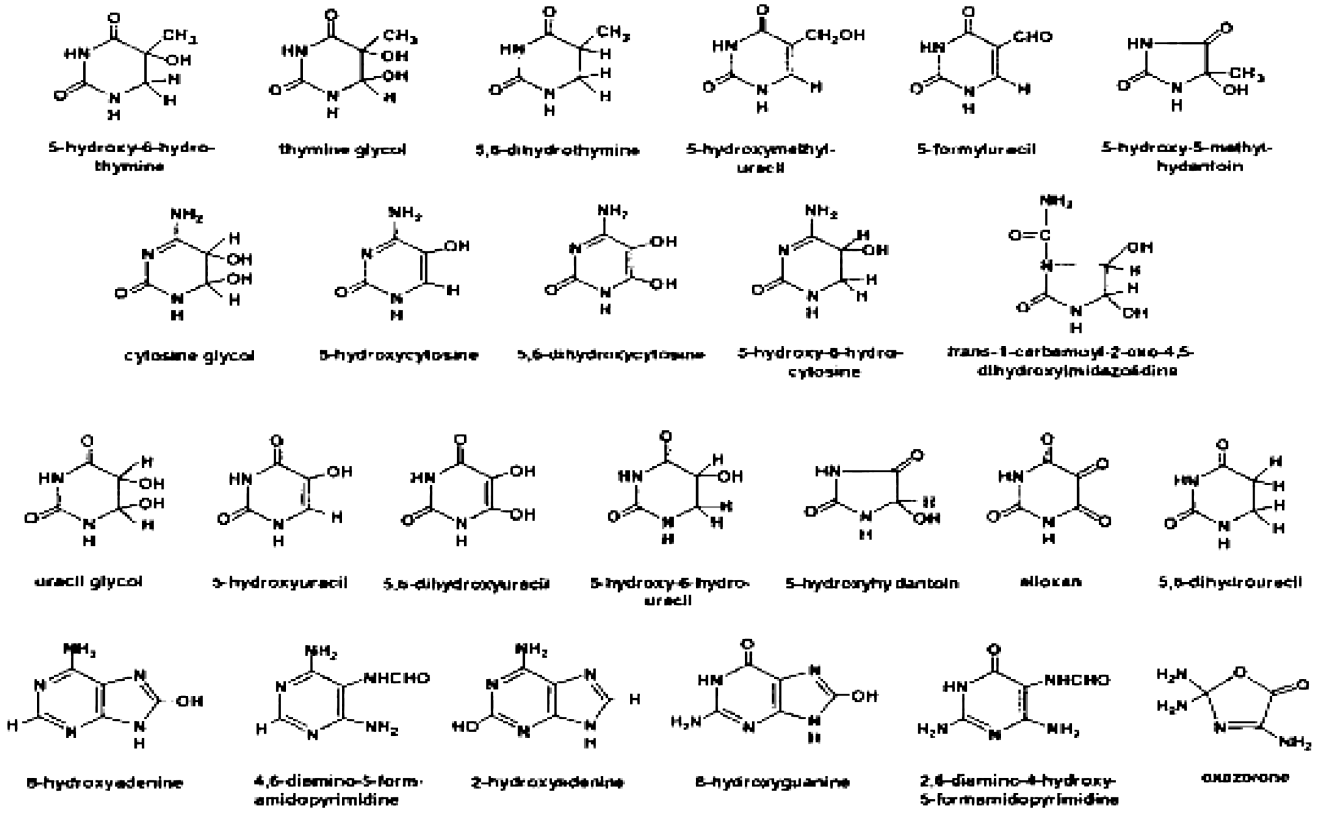

Figure 1 Chemical structures of modified DNA bases. These modified bases are formed in DNA as a result of interaction with reactive oxygen and free radical species. From Cooke et al. (2003).[13]

differentiation and death and 4) alters the activity of DNA polymerase to decrease fidelity.[11]

Several research groups have shown that DNA strand breaks develop rapidly in target animal and human tumor cells exposed to either activated neutrophils or hydrogen peroxide.[12,13] ROS and its toxic organic by-products (chloramines and lipid peroxidation products, such as aldehydes and peroxyl radicals) are mutagenic leading to point mutations in DNA bases.[14] The chemistry of DNA damage by several ROSs has been well characterized *in vitro*. Whereas superoxide and hydrogen peroxide do not react with DNA bases directly, hydroxyl radicals generate a multiplicity of products from all four DNA bases, especially in the presence of Ca^{+2} and Fe^{+2} via Fenton type reactions. By contrast, singlet oxygen is selective for guanine.[13] Most of the lesions identified after x-irradiation or other radical-forming treatments arise from secondary reactions after the initial radical formation. It is evident that most of the base lesions fall into families: hydroxylated bases (such as thymine glycol and 8-hydroxylguanine), ring-opened ruptured forms (formamidopyrimidine), contracted forms, and base fragments such as urea.[15] The thymine glycol (TG) residue constitutes a replication block for DNA polymerase I binding to DNA, in which the last nucleotide is inserted at the damaged base.[16,17] In contrast to TG, alkaline degradation of oxidized thymine to urea residues gives rise to sites that not only block DNA polymerase I, but also prevents the detectable incorporation of any deoxyribonucleoside monophosphate (dNMP) opposite the lesion. The formamidopyrimidine type of damage is a more potent block to DNA synthesis than TG.[18] Exposure of DNA in aqueous solution to conditions that generate active oxygen yields the most common base lesion, and the one most often measured as an index of oxidative damage, is 8-hydroxy-deoxyguaonosine (8-OHdG).[19]

Free-radical damage to DNA sugars has been known for many years, but has generally received less direct attention than the modified bases. The possible biological consequences of the radical-produced oxidized base-free (AP) sites and deoxyribose fragments are beginning to receive more attention. The deoxyribose fragments produced in DNA by free-radical attack are potent mutagens and block the action of DNA polymerase and DNA ligase *in vitro*.[20] The endogenous reactions that are likely to contribute to ongoing DNA damage in vivo are oxidation, methylation, depurination, and deamination. Methylation of cytosine in DNA is important for the regulation of gene expression and normal methylation can be altered during carcinogenesis. Conversion of guanine to 8-OHdG has been found to alter the enzymatic methylation of adjacent cytosines.[21,22] On the other hand, oxidative DNA damage can be repaired by the action of a series of enzymes and those enzymes may work close to capacity.

Mitochondrial DNA Damage

Mitochondrial DNA (mt DNA) is highly susceptible to oxidative damage.[23] Reasons for greater susceptibility are that mt DNA is located close to the inner mt membrane, where reactive oxygen species are generated.[23] It is small in size (16.5 kb) and is not protected by histone proteins as is the case for nuclear DNA. Also the mitochondria are characterized by the presence of incomplete repair mechanisms.[24–26] Mt DNA fragmentation seems to be closely related to cellular damage associated with aging.[27–29] Because mt DNA encodes for essential proteins involved in the processes of oxidative phosphorylation, this fragmentation leads to dysfunction of the mitochondrial respiratory chain, further stimulating mitochondrial ROS and RNS generation and oxidative mt DNA damage, in a sequence of events which is terminated by the induction of apoptosis (programmed cell death).[27,30]

In the mt respiration, superoxide anion is produced at two points in oxidative phosphorylation: the formation of ubisemiquinone and NAD reduction.[12,23] Instances have been reported with levels of 8-OHdG being $>1\%$ of the guanines present in the mt DNA although lower values (5 in 10^6) have been measured with different techniques.

mt DNA damage has been suggested to be important in several human diseases, and 8-OHdG has been detected in mt DNA at steady-state levels higher than in nuclear DNA.[26,31–34] The reactive species responsible for this damage has not been identified, since only limited information is available on levels of other base oxidation products in highly purified mt DNA. In addition, the elevated level of 8-OHdG could be due not only to increased oxidative DNA damage, but also to less effective repair of base lesions in mitochondria as compared with the nucleus.[26] Methodological questions are important here: it is difficult to isolate pure mt DNA and the multiple procedures involved run the risk of further oxidation, or contamination with oxidized fractions of nuclear DNA. More work on mt DNA oxidation is needed.[32,34]

Nuclear DNA Damage

Oxidation of nuclear DNA can occur through a variety of mechanisms.[12] Chief among these is an attack by ROS and RNS, mainly hydroxyl radical and singlet oxygen. The production of ROS and RNS occurs through a variety of endogenous processes. Endogenous DNA oxidation has been quantified either as increased oxidation products in the DNA of cells or as excretion of oxidized bases in urine,[35] the latter presumably released by excision repair of oxidized DNA. Measurement of excreted bases as an index of oxidation, however, is complicated by the possibility that they may be derived from the diet, intestinal flora, or dying cells releasing both nuclear and mt DNA. In the measurement of cellular DNA oxidation, differentiation must be made between oxidation of nuclear and mt DNA since oxidation of mt DNA is higher than that of the nucleus.[23] Nuclear DNA appears to have a degree of protection from oxidation by virtue of having a low oxygen tension and the radical-scavenging properties of associated chromatin. Nevertheless, nuclear DNA is clearly susceptible to oxidation. In measuring total tissue DNA

damage, mtDNA damage, though higher, is largely ignored because it makes up a small proportion of the total DNA of the cell.

Many environmental mutagens and carcinogens are known to react with components of DNA with or without metabolic activation, and to form adducts with adenine, guanine, cytosine, and thymine. Among these targets, guanine is known to be one of the most reactive.[36] Kasai and co-workers first observed formation of 8-OHdG from deoxyguanosine (dG) in high yield by a Fenton-type hydroxyl radical-generating system.[37] In addition, various oxygen radical-forming carcinogenic substances, such as cigarette smoke tar, asbestos plus H_2O_2, and ionization radiation are able to induce the formation of 8-OHdG in DNA *in vitro*.[38,39] In an *in vitro* DNA synthesis assay, 8-OHdG has been demonstrated as a potential mutagen due to misreading (producing a point mutation) of 8-OHdG during DNA replication. *In vitro* it has been shown that oxidized guanine bases in replication DNA will lead to G-T and A-C transversion. 8-OHdG has been reported in oxidized cancer cell and various carcinoma tissues. In human cells, oxidatively modified nucleobases such as 8-OHdG and strand breaks can be measured in the DNA from cells and tissues.[40] Oxidized bases and nucleosides liberated 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 specific cells. The expression of genes relevant for the defense against oxidative DNA damage, antioxidant and DNA repair enzymes can be assessed at the mRNA, protein and activity levels. Thus, 8-OHdG is a mutagenic adduct and a worthy marker of carcinogenesis.

DNA Repair Enzymes

Oxidative DNA damage can be repaired by the action of a series of enzymes, reviewed in and the existence of a low steady-state level of damage *in vivo* suggests that these enzymes may work close to capacity.[41,42] Some cancer may be the result of inadequate DNA repair.[43] A reduction in DNA repair capacity has been linked to lung cancer. Smokers with lung cancer were found to have a DNA repair capacity five times lower than healthy controls.[44] DNA damage and inadequate repair have also been linked to other chronic diseases of aging.[12] Therefore measurement of residual DNA damage by whatever approach will be a result of the balance between oxidative assault and DNA repair at the time of measurement.

Nuclear DNA contains billions of base pairs that encode the vast majority of biomolecules required for cellular function. DNA repair in the nucleus is essential for survival and evolution.[45]

For many years, it was thought that the mitochondria lacked an extensive or efficient DNA repair system comparable to the repair system maintaining nuclear DNA. However, recent studies have shown that mitochondria are capable of repairing oxidative damage to mt DNA (e.g., damage to bases and single-strand breaks) and that the base excision repair pathway plays a prominent role in mt DNA repair.[46] Mitochondria contain uracil DNA glycosylases, AP endonucleases, and UV endonuleases. Damage to mt DNA probably has more relevance to the mt theory of aging than direct damage to lipid or protein because damaged mt DNA can be propagated as mitochondria in dividing cells, thus allowing the physiological consequences of the damage to be amplified. In addition, damage to mt DNA could potentially be more important than deletions in nuclear DNA because the entire mt genome codes for genes that are expressed while nuclear DNA contains a large amount of non-transcribed sequences.

MEASUREMENT OF OXIDATIVE DNA DAMAGE

The measurement of oxidative DNA damage requires reliable analytical methods for measuring both ongoing and steady-state oxidative damage. A variety of methods have been proposed and applied. As shown in Fig. 1, oxidized bases have additional chiral centers introduced during the oxidation process although these isomers are seldom separated and the biological implication of isomarization is seldom considered.[47] Among the modified bases, 8-OHdG is the most abundant.[48] Reported levels of background DNA oxidation vary over 400-fold from about 0.0017 to 6 oxidized bases per 10^6 bases. To measure oxidative DNA damage by most of the currently available methods, one must first isolate DNA. The isolated DNA is then hydrolyzed and the hydrolysate prepared for analysis of oxidized bases. Five oxidized bases produced by oxygen free radical attack on DNA are being studied: thymine glycol, 5-hydroxylmethyluracil, 8-OHdG, formylamidopyrimidine, and 8-hydroxydeoxyadenine.[49] Counter arguments to the use of one oxidized base as a marker for DNA damage leading to point mutations and to carcinogenesis is the observation that ratio of 8-OH adenine plus 8-OHdG minus formamidopyridine-adenenine plus formamidopyridine-guanosine was more correlated with the aging prostate and predicted the maximal years for prostate cancer risk. Furthermore, 8-OHdA (although present in much smaller quantities) increased with age whereas 8-OHdG did not.[50] The reason for substracting the formamidopyridine damage products is that they arrest cell cycle when present and therefore prevent the replication of defective cells.

HPLC/Electrochemical Detection (ECD)

Analysis of 8-OHdG using HPLC coupled to electrochemical detection (ECD) with UV detection is a highly sensitive technique and most frequently used. At least 50 mg of tissues are needed to extract sufficient DNA although smaller samples have been used depending upon the extent of DNA damage in the tissue being measured. The 8-OHdG is released from the DNA in tissues, by enzymatic or acidic hydrolysis[22,51] and

detected by ECD via an electronically-driven redox reaction and deoxyguanosine (dG) is detected simultaneously by UV detection. This assay is not easily extended to measure other oxidized nucleosides or the oxidized bases because many are not electrochemically active. The extent of the oxidative damage is usually expressed as the ratio of 8-OHdG:dG.[13] However, the methodology has some limitations. Separation of 8-OHdG from other compounds in biological samples requires isolation of DNA and its hydrolysis to individual bases with high purity and maximal yield. Each step takes care and monitoring to eliminate bias. Common DNA extraction protocols and kits rely on the combined use of protease/phenol/organic solvent extractions, however, phenol exposure increases the amount of 8-OHdG in the sample. Hydrolysis exposes the liberated bases to ambient oxygen and transition metal ions and such metals are potent catalysts of free radical damage and can be present as contaminants in laboratory reagents[52] and equipment, e.g., dialysis membranes.[53] The result is a wide variation in reported residual ratios of 8-OHdG in various tissues with no agreed-upon criteria for determining the presence of artifactual levels.

A major problem with all the assays involves separation of the very small amounts of analyte from urine, which is a very complicated matrix. Thus, although several of the products are electrochemically active and high sensitivity is achievable, the HPLC methods require extensive cleanup procedures such as multiple solid-phase extractions, HPLC column switching techniques, or immunoaffinity columns.[54,55]

GC/MS and LC/MS Detection of Oxidized Bases

The gas chromatography-Mass Spectrometry (GC/MS) technique has also been extensively used to study DNA base damage by various free radical generation systems.[56] Since DNA must be extracted, purified, and bases must be liberated, the same artifactual issues pertain in sample preparation. High sensitivity of detection can be achieved by operating the mass spectrometer in the selected ion monitoring (SIM) mode.[56,57] GC/MS with SIM has been used to characterize oxidative DNA base damage by the identification of a spectrum of products, including thymine glycol, 5-hydroxymethyluracil and 8-OHdG, after acidic hydrolysis of DNA and derivatization (often by trimethylsilylation) to transform DNA bases and nucleosides into volatile products.[22,56,58] When GC/MS is used to measure modified DNA bases, a quantitative analysis of these bases in a DNA sample can be achieved by adding a suitable internal standard (usually the oxidized base labeled with stable isotope) to the sample at an early stage of the analysis, such as prior to the hydrolysis of the DNA. One advantage of the GC/MS approach is that measurement of a wide range of base damage products allows more descriptive quantitation of DNA damage and can help to identify the ROS species that cause damage.[58,59] However, DNA is hydrolyzed (usually by formic acid) and the sample derivertised (often by trimethylsilylation) to generate volatile products. It has been argued and also demonstrated that GC/MS might overestimate (usually 10 times higher values than HPLC/ECD) 8-OHdG as a result of its artificial formation during derivatization procedures and formic acid hydrolysis, thus causing the levels of 8-OHdG measured in a given sample by GC/MS to be higher than the levels of 8-OHdG measured by HPLC/ECD.[48,60] A mixture of endo- and exonucleases and alkaline phosphatase can also be used to hydrolyze DNA.[56]

Coupling liquid chromatography (LC) with MS is a way to avoid these problems. Because it not only can simultaneously detect several DNA oxidation products in one sample run with sufficient sensitivity (5 fmol) (just like GC-MS), but also has the advantage of avoiding artifactual DNA oxidation formed with GC. However, the peaks resulting from LC do not match the height and narrowness of those from GC, and the single quadruple approach is not sensitive and selective enough to match the sensitivity of HPLC/ECD.[61]

Immunochemical Determination of 8-OHdG

Immunochemical techniques may be employed to measure oxidative damage to cellular DNA. Test kits are commercially available. Following cell isolation, DNA is purified and subsequently digested to yield deoxynucleosides. The amount of 8-OHdG is then determined by competitive enzyme-linked immunosorbent assay (ELISA). Quantitation of the 8-OHdG is dependent upon measuring the colored end points resulting from assay of the samples and standards of known 8-OHdG concentration; since this is a competitive assay, the greater the amount of 8-OHdG within the test, the lower is the absorbance value. Yin et al.[62] developed an immunoaffinity chromatography-monoclonal antibody-based ELISA method. In the assay, two monoclonal antibodies have been developed and characterized by competitive ELISA. Immunoaffinity columns were prepared with a much more sensitive antibody 1F7 to isolate 8-OHdG from DNA hydrolysates followed by ELISA quantitation with another antibody 1F11. An ELISA based on monoclonal antibodies has been developed for estimation of 8-OHdG in urine samples.[63] However, the values obtained in rat and human urine samples were 3–5 times higher than other published values.[35,63] Similarly, in 4 smokers studied before and after smoking cessation, the urinary 8-OHdG excretion values estimated by the ELISA method were 8 times higher and showed only a weak correlation ($r = 0.42$) with the values obtained by HPLC.[64]

Immunohistochemistry using a monoclonal 8-OHdG antibody has also been shown to be a sensitive biomarker, especially when there is limited tissue availability. Toyokuni et al.[63] showed the 8-OHdG levels were higher in rat renal cells after carcinogen treatment compared to control using a monoclonal 8-OHdG antibody (N45.1) on paraffin sections. The specificity of N45.1 was also confirmed for immunohistochemistry based on the following observations: 1) procedures using non-immune mouse IgG of the same isotype or PBS instead of N45.1 showed no

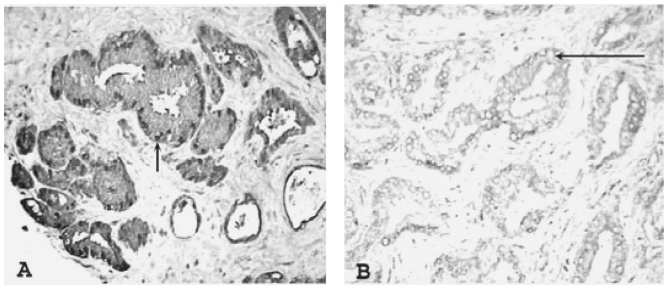

Figure 2 Immunoreactivity of the 8-OHdG monoclonal antibody on adenocarcinoma cells of human prostate from a single subject participating in a 3 wk intervention study. A) before and B) after tomato sauce supplementation. Arrows point to nuclei to of neoplastic epithelial cells and dark staining denotes presence of antibody action with 8-OHdG. From Chen (2000)[66].

positivity; 2) preincubation of N45.1 with 8-OHdG suppressed the nuclear staining. The 8-OHdG index of quantitative immunohistochemistry, as analyzed by NIH image freeware, correlated reasonably well with the 8-OHdG amount determined by HPLC/ECD.[63] Won et al.[65] also used this antibody to study oxidative DNA damage induced by ischemia-reperfusion insults in gerbil hippocampus. They found that the levels of 8-OHdG immunoreactivity (by immunodensity) increased by more than 3-fold at 30 min after ischemia. This increase remained elevated (up to 4-fold) for at least 12 h. These findings suggested that DNA damage may be involved in delayed neuronal death in the hippocampus.[65] We have also used this antibody to study the effect of tomato sauce consumption on oxidative DNA damage to prostate tissue cell populations in men with prostate cancer. Figure 2 shows immunoreactivity of the 8-OHdG monoclonal antibody on adenocarcinoma cells of human prostate before and after tomato sauce supplementation.[66] Although this technique provides more information on specific types of cell undergoing damage the methods must be considered semiquantitative. Also, of 18 literature citations using histochemical antibody techniques have used peroxidase instead of alkaline phosphatase. Preliminary studies in our laboratory demonstated the artifactual development of 8-OHdG with the use of peroxidase compared to alkaline phosphatase.

COMET Assay

The single-cell gel electrophoresis (comet) assay is a very simple, rapid, and sensitive technique to analyze DNA damage at the individual cell level and specifically for detecting DNA strand breaks. The principle of this assay is based upon the ability of denatured, cleaved DNA fragments to migrate out of the cell under the influence of an electric field. Undamaged DNA migrates slowly and remains within the confines of the nucleoid when a current is applied. Whereas damaged DNA which is often fragmented migrates further. Evaluation of the DNA "comet" tail (which is produced from fragmented DNA) shape and migration pattern allows for assessment of DNA damage. Figure 3 shows typical COMET fragmentation patterns.[67] In this assay,

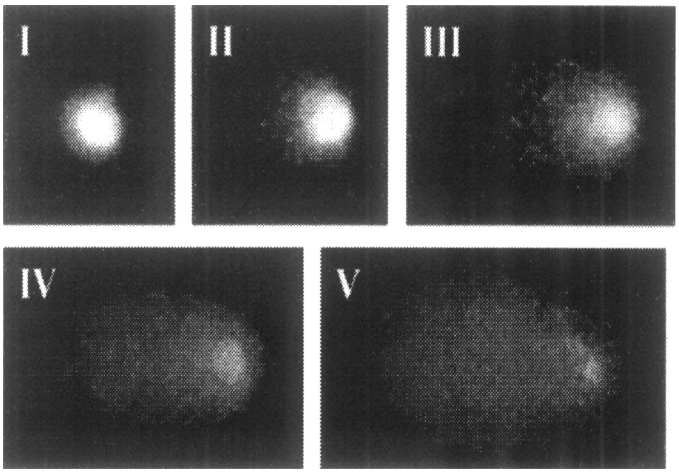

Figure 3 Typical COMET fragmentation patterns. DNA damage level increases from class I to V as indicated by the increasing tail migration (class I: 0–6%; class II: 6.1–17%; class III: 17.1–35%; class IV: 35.1–60%; class V: 60.1–100%). From Giovannelli et al. (2002)[47]

cells are immobilized in a bed of low melting point agarose. Following gentle cell lysis, samples are treated with alkali to unwind, denature the DNA, and hydrolyze sites of damage, and then subjected to electrophoresis. The cells are than stained with a fluorescent DNA intercalating dye. The sample is visualized under the microscope by epifluorescence. As an alternative, silver staining allows standard light microscopy to be used for data analysis.

The comet assay has been used in various studies to investigate the effect of ROS on DNA, and the protective effects of certain dietary antioxidants.[68,69] When peroxides are used the assay becomes an assessment of residual antioxidant activity which prevents peroxide attack on DNA. It is increasingly used in genotoxicity testing as well as in human biomonitoring studies.

^{32}P-Postlabeling

This method derived its name from the fact that the ^{32}P label is incorporated into the DNA after it has been isolated from tissue. In the original procedure, DNA is isolated from the tissues/cells and then digested enzymatically to deoxyribonucleaside 3′-monophosphates using micrococcal endonuclease and spleen phosphodiesterase. Deoxyribonucleoside 3′-monophosphates are then converted to their corresponding 5′−^{32}P-labelled 3′,5′-bisphosphates by transfer of ^{32}P from a molar excess of γ^{32}P-ATP using T4 polynucleotide kinase (PNK). Labelled bisphosphates are then separated using multidirectional anion-exchange TLC on polyethyleneimine (PEI) cellulose plates. In the first elution, labeled normal 3′,5′-bisphosphates, adenosine triphosphate (ATP) and inorganic phosphate migrate away from the origin onto a paper wick, this is subsequently removed, retaining any chemically modified nucleoside 3′,5′-bisphosphates at the origin. During the

Table 1 Published studies reporting oxidative DNA damage in pre-cancer or cancer patients

Experimental protocol	Source of DNA	Lesion	Assay	Mean ± SD (or range) per 10^5 base	Reference
211 gastric adenocarcinoma patients	Normal vs. tumor-adjacent vs. tumor gastric tissues	8-OHdG	HPLC-EC	2.86 ± 0.11 vs. 7.54 ± 0.43 vs. 6.29 ± 0.30	(71)
31 patients with renal cancer	Normal vs. renal cancer tissue	8-OHdG	HPLC-EC	3.6 ± 1.1 vs. 5.6 ± 2.3	(72)
22 patients with breast cancer	Normal vs. breast cancer tissue	8-OHdG	HPLC-EC	5.1 ± 3.3 vs. 3.6 ±1.9	(73)
11 liver metastasis patients, 15 chronic hepatitis patients,	Normal vs. inflamed liver	8-OHdG	HPLC-EC	1.6 ± 0.7 vs. 3.2 ± 2.1	(74)
13 liver cirrhosis patients, and 18 liver cancer patients	Cirrhotic vs. cancer tissue			2.3 ± 1.4 vs. 2.3 ± 1.6	
5 cervical cancer patients after undergoing brachytheraphy	Before vs. after brachytheraphy in biopsy tissue	8-OHdG 5-OHmU 8-OH-Ade	GC/MS-SIM	(20–40) vs. (30–82) (5–15) vs. (9–30) (12–30) vs. (15–83)	(75)
9 healthy children vs. 9 acute lymphoblastic leukemia (ALL)	Healthy children vs. lymphocyte DNA of all patients	8-OHdG 5-OHmU 8-OH-Ade	GC/MS-SIM	26.9 ± 7.7 vs. 62.1 ± 27.8 5.3 ± 3.0 vs. 6.4 ± 3.3 14.2 ± 3.9 vs. 30.4 ± 10.5	(76)
5 patients with lung cancer	Normal vs. lung cancer tissue	8-OHdG FapyGua 5-OHmU	GC/MS-SIM	(25–75) vs. (50–200) (25–33) vs. (50–120) (4–15) vs. (5–19)	(77)
38 healthy control vs. 25 breast cancer patients	Leukocyte	5-OHmU	GC/MS-SIM	8.3 ± 2.5 vs. 11.2 ± 4.6	(78)
14 various cancer patients after undergoing chemotherapy by adriamycin	Urinary excretion before vs. after chemotherapy	5-OHmU	GC/MS-SIM	74.9 ± 9.5 vs. 96.3 ± 8.7	(79)

next elutions (D3 and D4) chemically adducted nucleoside 3′,5′-bisphosphates are resolved. A fifth elution (D5) reduces background radioactivity on the plates. Chromatography conditions are varied depending on the type of adduct studied and optimum conditions need to be determined experimentally for each adduct. The adduct spots on the chromatography plates are located by screen-intensified autoradiography using high-speed X-ray film. The result of multidimensional TLC is the production of a unique fingerprint or profile of DNA adducts. The amount of radioactivity present in a particular spot or region can be determined by excising that part of the chromatographic plate and measuring its radioactivity by Cerenkov counting in a liquid scintillation counter (LSC).

^{32}P-postlabelling has several advantages over other methods. This technique is extremely sensitive and requires only small (1–10 μg) quantities of DNA. The sensitivity of the assay is as low as one adduct per 10^7–10^{10} unadducted nucleotides, corresponding to attomoles of DNA adduct per μg DNA and within the range of adducts routinely encountered in human samples. ^{32}P is widely used in molecular biology as a labeling agent. However, in ^{32}P-postlabelling larger quantities of δ^{32}P-ATP are routinely used. Since ^{32}P is a high-energy β-emitter, appropriate safety measures need to be taken before commencing work.

Cheng et al.[70] studied the role of the environment in Taiwanese females in lung cancer development in Taiwanese female nonsmokers, based on DNA adduct formation by both ^{32}P-postlabeling and ELISA. DNA adduct levels evaluated by ^{32}P-postlabeling (2.00–165.92 adducts/10^8 nucleotides) were relatively higher than those evaluated by ELISA (0–145.90 adducts/10^8 nucleotides) in all study subjects. The variations in DNA adduct levels evaluated by ^{32}P-postlabeling were 73- and 44-fold in lung cancer patients and noncancer control, respectively.

OXIDATIVE DAMAGE TO DNA IN VARIOUS TISSUES

Oxidative DNA adducts have been measured using animal and human tissues, urine, blood cells, specifically in circulating leukocytes. They can be categorized into two major types of studies: 1) to compare the DNA oxidative damage levels between cancer patients and healthy controls (Table 1); 2) to evaluate DNA oxidative damage levels after antioxidant intervention in a group of humans. Most studies have shown that cancerous tissues have higher DNA oxidative damage levels than noncancerous tissues. Intervention studies have shown that short-term (more than 1 month) supplementation with antioxidants in the form of vegetable components or diet modification can decrease DNA oxidative damage whereas antioxidant supplementation has failed to alter oxidative DNA damage. The following fluids and tissues are currently used to study the influence on the enzymatic defense system of the body and oxidative free radical damage to DNA.

Urine

Several base damage products are excreted in urine including the 8-OHdG, 8-OHadenine and 7-methyl-8-Ohguanine[10,54,80,81] but the one most exploited is 8-OHdG usually measured by a method involving HPLC/ECD.[82,83] Among the possible repair products from oxidative DNA modifications, 8-OHdG, 8-oxoguanine (8-oxoG), thymine glycol (Tg), thymidine glycol (dTg), and 5-hydroxymethyluracil (5-OHmU) have so far been identified in urine.[35,84] The repair products from oxidative DNA damage, i.e., oxidized bases and nucleosides, are poor substrates for the enzymes involved in nucleotide synthesis; they are fairly water soluble and generally are excreted into the urine

without further metabolism.[81,84] The validity of this urinary measurement is supported by the fact that the level of 8-OHdG is presumably not changed by the diet source of 8-OHdG, since nucleosides are not absorbed from the gut. Thus excretion is presumed to reflect integrated endogenous DNA damage which has been repaired with the damaged nucleosides excreted in the urine. However, it is possible that some or all of the 8-OHdG excreted in the urine may arise from deoxyGTP.[85]

In a study of 169 humans, the average 8-OHdG excretion was 200-300 pmol/kg BW/24 h, corresponding to 140–200 oxidative modifications of guanine per cell per day.[54,81] Furthermore, 32 smokers in this study excreted 50% more 8-OHdG than 53 non-smokers, indicating a 50% increased rate of oxidative DNA damage from smoking. GC/MS has also been used to measure 8-OHdG in urine and the limit of detection was 1.8 pmol corresponding to a level of 8-OHdG in urine of 35 mM.[86] The validity and meaning of these urinary measurements of oxidative DNA damage is also worth considering.

The assays for the urinary DNA repair products include HPLC with ECD for 8-OHdG and 8-oxoG and with UV absorbance detection for dTg and Tg, whereas all the repair products can potentially be measured by GC/MS.[82,87] The complicated extraction procedures cause recovery problems in both HPLC and GC/MS-SIM methods and may require labeled internal standards. Moreover, the complicated procedures limit the analytical capacity.

Blood (Leukocytes)

The utility of leukocyte DNA 8-OHdG as a reliable in vivo marker for DNA damage resulting from oxidative stress is dependent on vigorous control of artifactual development of 8-OHdG during the assay. Only limited leukocyte DNA 8-OHdG values are reported from healthy US human populations and range from 0.33 to 7.10×10^{-5}.[35] Takeuchi et al.[88] found no correlation between two repeated measures of leukocyte DNA 8-OHdG in 19 healthy subjects. These data suggest that this biomarker may have improved reliability when two or more repeated measures are performed, which is also true for serum cholesterol measurements.[89] Leukocyte 8-OHdG is a steady-state measure resulting from constant oxidative attack and repair, thus residual damage should appear fairy rapidly from the induction of oxidative stress.[90] The circulating leukocyte DNA damage would be a reflection of overall cellular DNA damage, although there are no data to compare DNA damage in various tissues. Analysis of 8-OHdG in DNA, most notably from lymphocytes, has been done but is hampered severely by the fact that artificial formation during sample clean-up overwhelms the background level (some say by up to 2 orders of magnitude.[83] It has been suggested that a straight extraction of DNA from whole blood may overcome artifact development.[91]

Duthie et al.[92] found that a 20-week supplementation with vitamin C (100 mg/d), vitamin E (280 mg/d), and β-carotene (25 mg/d) resulted in a 37.2% ($p < .002$) decrease in endogenous oxidative base damage in lymphocyte DNA of both smokers and non-smokers as assessed by the comet assay, which was not statistically significant by week 10.[54]

Oral Cells

Oral cells can be easily and repeatedly collected by a non-invasive method. Romano et al.[93] determined DNA damage in oral cells from 109 healthy volunteers by an immunohistochemical method. They found 1.43-fold increases in the DNA 8-OHdG adduct content from smokers versus non smokers ($p = 0.001$), showing that tabacco smoke led to a measurable increase of oxidative damage in oral cells. Feng et al.[94] assessed DNA damage in buccal cells from individuals chronically exposed to arsenic via drinking water in Ba Men, Inner Mongolia using the TUNEL assay. The results showed that the mean frequencies of position cells were 7.5-fold higher in the arsenic exposure group than in the controls. When buccal cells are harvested it makes a difference in the level of DNA damage using HPLC-EC detection of 8-OHdG. We found that buccal cells have more damage when harvested 7 days vs. 3 days after initial harvesting (unpublished), presumably because of longer exposure to oxidative damage.

Prostate and Mammary Gland

Whereas some LC-MS-MS assays are sufficiently sensitive to be able to measure the residual oxidized bases in samples as small as 10 ng.[95] Biopsies from prostate, mammary gland, etc provide about 10 mg of tissue. It would be logical to use an immunohistochemical method for the quantitation and localization of 8-OHdG on sections of prostate tissue. Chen et al.[96] determined resected prostate tissue oxidative damage in prostate cancer patients after 21-day, tomato sauce-based, whole-food intervention. Oxidative DNA damage was measured by HPLC/ECD. The 8-OHdG/dG ratios for 32 patients who participated in the study were compared with those of randomly selected prostate cancer patients who did not participate in the study. DNA damage in prostate tissue was roughly 2-fold higher than leukocyte damage and ther was a high correlation between two. They found that prostate tissue oxidative DNA damage was 28.3% lower in men who had the intervention than in the randomly selected patients. One of the problems in measuring whole tissues is that any sample will contain cells of varying types which may also differ greatly in the degree o DNA damage. In this same study we found that 8-OHdG, measured histochemically, was greatest in prostate epithelial cells where cancer starts and cancer cells had more damage than hyperplastic and normal epithelial cells.[97]

STUDIES EXAMINING EXOGENOUS CAUSES OF DNA DAMAGE

Not surprisingly, many of the factors that have emerged as modulators of cancer risk in epidemiological studies have also

been found to modulate DNA damage measured by the various measures discussed. These can be divided into non-dietary and dietary factors.

Non-Dietary Factors and DNA Damage

Exercise

Because exercise increases whole body and tissue rates of oxygen consumption, it has been hypothesized that exercise may cause oxidative stress and tissue damage.[98–100] During exercise, oxygen consumption can increase up to 10- to 15-fold above resting levels, thus temporarily increasing the rate of mitochondrial free radical production.[101] There have been many reports showing that exercise may cause oxidative stress, e.g., the direct detection of free radical generation in rat muscle and liver;[102–103] increases in oxidative damage biomarkers such as protein carbonyls and thiobarbituric acid reactive substances;[104,105] effects on mitochondrial function;[106,107] and decreases in levels of antioxidants and antioxidant enzymes in the heart,[102] blood,[108] lung,[109] liver,[108,110,111] brain,[103] and muscles.[104,108,112] However, there are also reports showing that exercise fails to result in a functionally significant level of oxidative stress in the heart.[113]

During intensive exercise, uptake and consumption of oxygen greatly increase, resulting in the production of oxygen radicals.[98] By using a 42 km marathon race as a model of massive aerobic exercise, Tsai et al.[101] demonstrated a concurrent 2-fold increase in the urinary excretion of 8-OHdG measured by ELISA kit (Bioxytech 8-OHdG-EIA kit, OXIS, Portland, OR).

The increase in oxygen consumption has been shown to be closely related to oxidative damage of DNA in humans.[114] There was a close association between oxidative DNA damage as assessed by the urinary excretion of 8-OHdG and oxygen consumption in 33 healthy premenopausal women. In the 12 women who smoked, 8-OHdG excretion was increased by 35%, although oxygen consumption increased only 10% compared with the 21 nonsmoking women. These findings raise the possibility that exercise induces oxidative damage of biomolecules including DNA in the body, thereby modifying the incidence of degenerative diseases and aging. However, epidemiologic studies suggest that physical activity is associated with decreased incidence of certain types of cancers.[115,116]

Some studies have observed exercise-induced DNA damage most associated with vigorous exercise conditions accompanied by muscle damage.[117,118] Hartmann et al.[117] studied the effect of physical activity on DNA damage with healthy volunteers. In a first multiple step test, the volunteers ran on a treadmill as long as possible with increasing speed. In a second test they had to run for 45 min with a fixed individual speed which was defined to ensure aerobic metabolism. In the first test, the white blood cells of all subjects showed increased DNA migration in the COMET assay. The effect was seen 6 hr after the end of the exercise and reached its maximum 24 hr later. After 72 hr, DNA migration decreased to about control level. The distribution of DNA migration among cells clearly demonstrated that the majority of white blood cells exhibited increased DNA migration and that the effect was not only due to a small fraction of damaged cells. In the second exercise, during aerobic metabolism, the effect on DNA migration was not seen. Sato et al.[118] measured the levels of white blood cell 8-OHdG by HPLC/ECD after mild acute exercise in healthy male subjects. The basal 8-OHdG levels of physically active subjects were significantly lower than those of sedentary subjects (1.5 8-OHdG/10^6dG vs. 2.8 8-OHdG/10^6dG). After mild exercise for 30 min, the 8-OHdG levels of the sedentary subjects significantly decreased (2.8 to 2.0 8-OHdG/10^6dG), suggesting that a mild exercise has a beneficial effect on the maintenance of low levels of 8-OHdG probably by keeping the repair system at higher levels.

However, some studies failed to detect DNA damage using moderate exercise conditions and/or trained subjects who may have acquired elevated antioxidant capacity through training.[82,119] Pilger et al.[82] examined the effect of a regular running exercise on the urinary levels of 8-OHdG in 32 long-distance runners and in a group of untrained healthy subjects. The range of 8-OHdG measured by HPLC/ECD in urine was 0.12–6.45 μmol/mol creatinine in both groups, and no significant difference in the mean excretion levels between runners and controls was observed. These data give no reason to believe that physical exercise in trained individuals may induce a disturbance of the oxidant-to-antioxidant balance. Sumida et al.[119] investigated the effects of acute exhaustive exercise and β-carotene supplementation on urinary 8-OHdG excretion by HPLC/ECD in healthy nonsmoking men. Fourteen untrained male (19–22 years old) volunteers participated in a double blind design. The subjects were randomly assigned to either the β-carotene or placebo supplement group. Eight subjects were given 30 mg of β-carotene per day for 1 month, while six subjects were given a placebo for the same period. All subjects performed incremental exercise to exhaustion on a bicycle ergometer both before and after the 1-month β-carotene supplementation period. The 24-hr urinary excretion of 8-OHdG was unchanged for the 3 days after their exercise either before or after supplementation in both groups. However, the baseline urinary excretion of 8-OHdG before exercise tended to be lower after β-carotene supplementation, suggesting that a single short period of incremental exercise does not induce oxidative DNA damage, while β-carotene supplementation may attenuate it. Muscle damage and the subsequent inflammation are involved in exercise-induced DNA damage, particularly in the case of leukocytes. In fact, exercise-induced DNA damage in blood cells has been shown to be closely related to an increase in plasma creatine phosphokinase (CPK) activity,[117] a marker of muscle damage. It has also been reported that intensive exercise induces an increase in plasma myeloperoxidase levels, a marker of neutrophil activation *in vivo*.[120] When the neutrophils are activated, they release reactive oxygen species (ROS) that damage the neutrophils themselves, in addition to damaging other cells or tissues.[88,121]

Smoking

Cigarette smoke contains about 3,800 chemicals, including many carcinogenic compounds such as polycyclic aromatic hydrocarbons (PAHs), tobacco specific nitrosamines, aromatic amines, and free radicals.[122] During metabolic processes, other compounds in cigarette smoke can be directly or indirectly metabolized into free radicals.[123] For example, the tar in each puff of smoke contains about 10^4 radicals;[124] benzo[*a*]pyrene (B[*a*]P) metabolically produces various types of B[*a*]P-quinones, which further generate free radicals and oxidatively damage DNA, protein, and antioxidant enzymes.[125–127] Reactive oxygen species (ROS) can be produced by cigarette smoke-induced phagocytic cells and cause oxidative damage to DNA, protein, and lipids.[10]

Since cigarette smoke lowers plasma levels of antioxidants such as vitamin C and β-carotene, smokers may need an additional intake of these antioxidants over the recommended dietary allowance for the general population.[128] However, in terms of chemoprevention of oxidative damage, the necessary dosage and the role of antioxidants are not yet clearly defined.

Asami et al.[129] compared the level of 8-OHdG by HPLC/ECD in lung tumor tissues and normal tissues from 30 previously untreated lung cancer patients (14 current smokers, 7 ex-smokers, and 9 non-smokers) by surgical lobectomy or pneumonectomy. The mean level of 8-OHdG/10^5 dG in the lung tissue from current smokers (0.74 ± 0.21) was 1.23- and 1.43-fold higher than that of the ex-smokers (0.60 ± 0.21) and non-smokers (0.52 ± 0.25), respectively. There was a positive correlation between the 8-OHdG levels in the lung tissues and the number of cigarettes smoked per day. Nia et al.[130] investigated the effects of smoking-induced oxidative stress in healthy volunteers (21 smokers vs. 24 non-smokers) by quantifying various markers of oxidative DNA damage and repair. Lymphocytic 8-OHdG levels measured by HPLC/ECD, were 0.76-fold lower in smokers as compared with non-smokers ($p = 0.05$). Urinary excretion of 8-OHdG assessed by ELISA did not differ significantly between smokers and non-smokers ($p = 0.3$).

There was a significant correlation between oxidative DNA damage and protein damage in smokers.[131] Fraga et al.[132] demonstrated that smokers had higher 8-OHdG levels in their sperm DNA than those of nonsmokers. Shen et al.[133] determined the level of 8-OHdG by HPLC/ECD in sperm DNA and cotinine concentration in seminal plasma in 60 healthy subjects (28 smokers vs. 32 nonsmokers). They found that the sperm DNA of smokers contained 1.6 times higher ratio of 8-OHdG than that of nonsmokers. The level of 8-OHdG in sperm DNA was also closely correlated to seminal cotinine concentration. Oxidative damage to sperm DNA could result in improper sperm function, infertility, birth defects, genetic disease, and cancer in offspring by increasing heritable mutation and chromosome abnormalities.[134,135]

Age

Cancer is a disease of old age, and the risk of carcinoma increases exponentially with age.[136] The age dependence has been explained by accumulation of mutations in the tumor-initiating cell that then expand to a clinical cancer.[137] Associated events include an age-dependent increase in oxidative damage to DNA and a decrease in DNA damage repair.[138,139] Ames and Saul[140] reported the first data on the effect of aging on DNA oxidation. They observed a significant (~2-fold) increase in 8-OHdG levels in nuclear DNA isolated from liver, kidney, and intestine of male rats between 2 and 24 months of age. Later, Ames et al.[141] reported that the levels of 8-OHdG in mt DNA isolated from male rat liver increased 2- to 3-fold with age. A number of researchers have observed an age-related increase in the level of 8-OHdG in both nuclear DNA and mt DNA in a variety of tissues of rats and mice.[142] On the other hand, many investigators have been unable to detect a significant increase in DNA oxidation in rodent tissues with increasing age.[143]

So far, no published study has systematically addressed age as a determinant of the markers of oxidative modification of DNA in humans. Mecocci et al.[144] measured markers of oxidative damage to DNA in sarcoplasmic reticulum membranes in muscle biopsies from vastus lateralis of 44 young ($\leq$35 years old; 22 males, 22 females) and 44 elderly ($\geq$70 years old; 23 males, 21 females) healthy subjects of both sexes using the HPLC/ECD. They found a 3.2-fold increase ($p < 0.001$) in 8-OHdG in the elderly group, more evident in males. Both young males and young females differed significantly from old males and females respectively. Furthermore, levels found in old males were significantly higher (1.7-fold) than in old females. From the studies involving different age groups it appears that the urinary excretion rate of the repair products decreases with age. Correspondingly, the 8-OHdG levels in leukocyte DNA appeared to increase linearly with age corresponding to $0.09 \pm 0.01/10^5$♣ deoxyguanosine per year.[145] Similarly, mt DNA from brain accumulated 8-OHdG with age.[144,146] The accumulation of 8-OHdG in n DNA from brain corresponds to approximately 0.04 modifications per 10^5 deoxyguanosine per year, or two modifications per cell per day.[144] Similar results regarding tissue DNA and urinary excretion of 8-OHdG have been obtained in aging rats.[147] Although the rate of repair appears to decrease with age, these data cannot distinguish whether the rate of oxidative damage also increases. Since aging mitochondria generate greater ROS, increased oxidative attack on DNA is a possibility.[148]

Gender

Gender differences have been found but the data is not consistent, probably related to the differences in study populations and the DNA damage measurement techniques employed. In addition, as part of another study, Cheng et al.[70] compared levels of prostate DNA adducts using both ^{32}P-postlabeling and ELISA techniques and data from both assays show remarkably higher DNA adduct levels in females compared to males

♣Due to the possible artifactual development of 8-OHdG during assay procedure these vales may be overestimated.

(^{32}P-postlabeling: 49.23 ± 37.67 adducts/10^8 nucleotides for females, 31.17 ± 25.51 adduct/10^8 nucleotides for males; ELISA: 27.29 ± 34.63 adduct/10^8 nucleotides for females, 9.45 ± 18.48 adduct/10^8 nucleotides for males). However, Mendoza-Nunez et al.[149] reported opposite results. They evaluated 160 subjects of over 60 years of age (44 males, 116 females) to determine gender and DNA damage in lymphocytes of elderly with the COMET assay. DNA damage was observed in 72 of the 160 study subjects (45%); 64% of males and 38% of females. Chen et al.[150] found higher leukocyte 8-OHdG/dG (16.8% in healthy young males compared to females using the HPLC-EC methodology while the women had a 15.6% lower kcal/kg BW energy intake. Proteggente et al.[151] also found gender differences in a variety of bases as measured by GC/MS.

DIETARY FACTORS AND DNA DAMAGE

Cancer prevalence among various populations around the world may be related to differences in their intake of specific dietary items. For example, the diet of Asian women has more soy and tea, with less fat, particularly saturated fat, than that of Western women. Each of these common food items may inhibit, or in the case of fat, promote the development of cancers of the breast and reproductive tract. In this part, we reviewed various dietary factors and their association with DNA damage.

Caloric Restriction

A calorie-restricted (CR) diet compared to *ad libitum* feeding has been shown to markedly decrease chemically induced tumor incidence in rodents,[152,153] and increase life span.[154–156] Although, enhanced detoxification of xenobiotics by calorie restriction may account for the marked effect of dietary manipulation on chemical carcinogenesis, many studies have indicated that reduced oxidative damage and altered rates of cell division and/or apoptosis may also be involved.[157–159]

The influence of calorie restriction on free radical metabolism has been described, and suggests that a decline in ROS is a likely mechanism by which calorie restriction mediates its protection.[157,159] Calorie restriction not only attenuates the generation of ROS by liver mitochondria, but also alters the activities of the electron transport chain complexes, I, III, and IV.[160] In a study of 83 healthy subjects lean/male subjects excreted more urinary 8-OHdG than obese/female subjects which was thought to be related to differences in metabolic rate. These investigators tested this hypothesis by placing subjects on a CR diet and observed a large decrease in 8-OHdG excretion.[81] That CR may produce decreases in oxidative DNA damage is demonstrated by animal studies. 8-OHdG/dG in skin dermal cells of adult male rats fed CR diets was directly proportional to daily caloric intake/g organ weight (a measure of whole-body metabolic rate) and glycated hemoglobin levels.[161] In another study,[162] 40% CR rats had 44% lower 5 hydroxymethyl uracil/thymine ratios (another DNA damage product) in liver and 32.3% lower in mammary gland compared to control animals. Low fat diets decreased oxidative damage but the differences were less and not statistically significant. Lopez-Torres et al.[148] studied long-term CR in rats and found that mt DNA damage, measured as HPLC-EC detection of 8-OHdG, was increased with age but abolished with CR whereas nDNA damage was unaffected by age or CR.

Fats

Although under some revision, dietary fat has been thought to be a major cancer risk factor based on reports of positive correlations between dietary fat intake and increased incidence and mortality especially in comparisons for cancers of the breast, colon and prostate.[163,164] Present epidemiological and experimental data have linked a high dietary intake of ω-6 polyunsaturated fatty acids (PUFAs) such as linoleic acid (C18:2), especially in association with a low intake of ω-3 PUFAs such as docosahexaenoic acid (C22:6), to increased risks for cancers of the breast, colon and possibly, prostate.[163,165] Linoleic acid is the only fatty acid that is carcinogenic in animal models.[166] Dietary fats, specially ω-6 and ω-3 PUFAs, affect a variety of steps in the multistage carcinogenesis process. Persistent cellular oxidative stress and enhanced peroxidation of PUFAs leads to macromolecular damage and disruption of signaling pathways.[167] *Trans*-4-Hydroxyl-2-nonenal, one of the major lipid peroxidation products, is formed by the oxidation of linoleic or arachidonic acid (ω-6 PUFAs) and is readily oxidized by fatty acid peroxides to form 2,3-epoxy-4-hydroxynonanal. This bifunctional alkylating agent can react with DNA to yield etheno adducts and other base adducts.[165] Etheno adducts are highly miscoding lesions in mammalian cells and are thought to initiate the carcinogenic process through specific point mutations, as shown with the known carcinogens vinyl chloride and urethane.[168]

Lipid peroxidation generates reactive DNA adducts in human cells and may contribute to diet-related cancer. Nair et al.[169] analyzed leukocyte DNA from volunteers in a carefully controlled dietary study and showed that a high intake (65%) of ω-6 PUFAs moderately increased the frequency of malondialdehyde (MDA)-derived DNA adducts, and to a somewhat greater degree in women than in men. In contrast, the frequency of etheno-DNA adducts in leukocytes was 40 times greater in women and not increased in men, with a huge interindividual variation in lipid peroxidation-derived DNA damage. Wang et al.[170] used the ^{32}P-post-labelling technique to quantify putative MDA-deoxyguanosine and MDA-deoxyadenosine adducts, and found a 3-fold increase in the level of these lipid peroxidation-derived adducts in normal breast tissue from breast cancer patients in comparison with cancer-free controls. Lipid peroxidation derived products and oxidative DNA base damage have been associated with increased breast cancer risk. Lipid peroxidation, as measured from MDA in urine, in pre-menopausal women with mammographic dysplasia was approximately double that in women without these radiological changes.[171]

The amount of fat consumption may reflect the levels of oxidative damage to DNA. Fats are readily oxidized upon storage, and a higher fat intake thus would be accompanied by a higher intake of oxidized fatty acids. Oxidized fatty acids could, in turn, cause production of ROS that can damage DNA. The effect of a low fat diet on levels of oxidative DNA damage in peripheral nucleated blood cells was studied in 21 women at high risk for breast cancer who were randomly assigned to a normal or low fat (15% of calories from fat) diet for 7 days.[172] The concentration of oxidized thymine, specifically 5-hydroxymethyluracil (5-OHmU), was 3-fold higher in the group who consumed their usual diet compared to those who consumed 20% fat as energy diet and there was a significant, linear relationship between daily total fat intake and leukocyte 5-OHmU. Djuric et al.[173] extended these studies to explore the interaction between low fat and lower energy diets in healthy premenopausal women by randomly assigning women to consume controlled, low-fat, low-energy or low-fat, low-energy combination diets for 12 wks. Leukocyte 5-OhmU was decreased in significantly more women consuming any of the experimental diets compared to the control group. The same research group[174] examined the effects of five different dietary fat intakes on levels of oxidative DNA damage in rats. Animals fed diets containing 3, 5, 10, 15, or 20% corn oil for 20 weeks. 5-Hydroxymethyl-2-deoxyuridine (5-OHmdU) was measured in lymphocytes and mammary gland using GC/MS. Levels of 5-OHmdU in lymphocytes increased with dietary fat levels, and mean levels were significantly higher in animals fed 20% oil than in all other groups. Mammary gland 5-OHmdU levels in animals fed 5%, 10%, or 15% fat were significantly higher than in animals fed 3% fat. Increasing polyunsaturated fatty acid intake (PUFA) from 5% of energy to 15% of energy for 2 wks 7 significantly increased leukocyte DNA damage as measured by the COMET assay in 21 healthy non-smoking men. The effect was abolished by vitamin supplementation to 80 mg/d compared to 5–7 mg/d.[175] Other studies have also shown that the type of oil and the level of its antioxidant capacity modulates DNA damage.[176,177] These data suggest that oxidative DNA damage may be a mechanistic link between dietary fatty acids and cancer risk. However, lard intake with a large increase in fat energy decreased DNA-adduct levels as measured by ^{32}P-postlabeling but increased DNA repair mechanisms and resulted in no increase in oxidative DNA damage in liver, colon, or the urine of the experimental rats.[178]

Antioxidant Vitamins and Minerals

Epidemiological studies indicate that a diet rich in fruit and vegetables is associated with lower incidence of various forms of cancer.[179–180] This protection is commonly attributed to the dietary content of antioxidants such as vitamin C, vitamin E, and various carotenoids.[181–183] Antioxidant supplementation could be expected to reduce the rate of oxidative DNA modification, but as yet the data from intervention studies 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-OHdG in smokers.[184] Similarly, the urinary excretion of the RNA damage product, 8-oxoguanosine, was not affected by the daily administration of vitamin C (1000 mg) and E (533 mg) and β-carotene (10 mg) for 1 month.[185] These data are in agreement with the observation that neither β-carotene nor vitamin E reduced the risk of lung cancer in a large-scale clinical intervention study involving approximately 30,000 smokers.[186] These trials do not address the possibility that correction of DNA-damaging, oxidative stress by antioxidant supplementation may only be effective for a subpopulation possessing poor antioxidant status since diets containing high amounts of β-carotene or vitamin E are linked with lower risk in numerous case-control and cohort studies. Furthermore, antioxidant vitamins can also be considered to possess pro-oxidant properties, which has been confirmed experimentally.[187–189]

Despite the failure of antioxidant supplement clinical trials to show decreases in DNA damage and cancer incidence, antioxidant vitamins have been shown to modulate oxidative DNA damage in cells, cell free systems, and in animals. More extensive reviews of these substances are necessary.

Vitamin C

Addition of vitamin C to purified DNA or isolated nuclei in the presence of redox active metal ions results in single-strand breaks and base modifications such as 8-OHdG.[190–192] This is thought to be due to binding of reduced metal ions to DNA (ascorbate maintains them in a reduced state) and resultant site-specific hydroxyl radical production and oxidative damage.[190] In the absence of added metal ions, however, vitamin C inhibits the formation of 8-OHdG in purified DNA exposed to peroxynitrite or UV light.[191,193,194] Vitamin C also acts as an antioxidant in cells, inhibiting oxidative DNA damage in isolated and cultured cells exposed to hydrogen peroxide and UV-visible light.[195,196] In contrast, several studies have shown increased formation of oxidative DNA damage in cultured cells and isolated human lymphocytes in the presence of added vitamin C.[197–198] In view of the above findings, the pro-oxidant effect of vitamin C is most likely due to the presence of contaminating metal ions in the media.[190–192]

Vitamin C supplementation studies have been conducted in animals to determine the effects on oxidative DNA damage.[199–200] Vitamin C manipulation in guinea pigs, equivalent to marginal deficiency, optimum intake, and megadose intake, had no effect on the hepatic steady-state levels of 8-OHdG, as determined by HPLC/ECD, despite up to a 60-fold variation in vitamin C levels in the liver.[199] In contrast, a UV challenge to the eyes of guinea pigs and rats showed a decrease in single strand breaks in lens epithelium in vitamin C-supplemented animals and a corresponding increase in vitamin C-deficient animals.[200]

Various experimental studies, in which cellular markers of DNA damage were measured in humans, have been conducted. Fraga et al.[201] found that an intake of 60 or 250 mg/d vitamin C for 28 days decreased sperm 8-OHdG levels, measured by HPLC/ECD, in 10 male subjects but did not affect lymphocyte

or urine 8-OHdG or DNA strand breaks. In contrast, Anderson et al.[202] showed no effect of either 60 or 6000 mg/d vitamin C on lymphocyte DNA or chromosome damage, as measured by the COMET assay, in 48 nonsmoking subjects supplemented for 14 days. Podmore et al.[203] found 2.5-fold decreased levels of 8-oxo-7,8-dihydroguanine (8-oxo-guanine) and 4-fold increased in 8-oxo-dihydroadenine (8-oxo-adenine), as measured by GC-MS, in lymphocyte DNA of 30 healthy subjects supplemented with 500 mg/d vitamin C for 6 weeks. In a following report on the same study the investigators showed increases in serum and urinary 8-OHdG, suggesting a possible repair action of vitamin C on oxidative DNA damage.[203] Other studies have used supplementation with vitamin C together with other micronutrients. Duthie et al.[92] supplemented 50 male smokers and 50 non smokers with 100 mg/d vitamin C and 280 mg/d vitamin E and 25 mg/d β-carotene for 20 weeks and showed a 156% and 36% decrease in oxidized pyrimidines both smokers and nonsmokers, respectively, as measured by the COMET assay. In contrast, Prieme et al.[64] showed a 10.2% increase in 8-OHdG excretion following supplementation with 250 mg/d vitamin C, both alone or in combination with 100 mg/d vitamin E, in smokers, again suggesting that vitamin C may affect repair. On the other hand, the study by Rehman et al.[204] in which two groups of subjects, one with low (50 μmol/L) and one with high (72 μmol/L) initial plasma vitamin C levels, were supplemented with 14 mg/d iron and either 60 or 260 mg/d vitamin C for 2 months, exhibited a complex outcome. In the higher initial plasma vitamin C group there was 50% and 35% decreased in urinary 8-OHdG (measured by GC/MS), respectively, in 60 and 260 mg/d vitamin C supplemented groups. In the group with the lower initial plasma ascorbate, there was a 60% and 71% decreased in urinary 8-OHdG with low and high vitamin C supplementation, respectively. These results suggest that vitamin C status is important and should be taken into consideration when evaluating the efficacy of vitamin C supplementation for the prevention of DNA damage in various tissues. Thus, studies testing the effects of supplemental vitamin C intake on markers of oxidative damage to DNA have given mixed results. A recent review suggested no clear support for the concept of a pro-oxidant action of ascorbate in vivo, either alone or in association with iron, under physiological conditions,[205] but the questions concerning the efficacy of vitamin C supplementation to reduce oxidative damage in humans remain unresolved.[206]

In summary, most of the studies reviewed showed a vitamin C-dependent reduction in oxidative DNA damage, whereas some studies found either no change or an increase in the levels of selected DNA lesions. Experiments using purified DNA or isolated nuclei[190–192] confirm that in the presence of added metal irons, vitamin C acts as a pro-oxidant in vitro. In the absence of added metal ions, however, vitamin C inhibits oxidative DNA damage in purified DNA and cells.[191,193–196] The contradictory results are likely explained by 'contaminating' metal ions in the cell culture media.

Of the two animal supplementation studies discussed, one showed protection by vitamin C against UV-induced DNA damage in the eye[200] and the other reported no change in the steady-state levels of oxidative DNA damage in the liver.[199] Of nine human vitamin C supplementation studies, four showed a reduction in *ex vivo* or *in vivo* DNA oxidation,[198,201,207] whereas two showed no change;[64,202] another three showed a decrease in some markers and an increase in others.[203,204,208] Two of the latter studies,[203,204] however, suffer from serious shortcomings, primarily artifactual DNA oxidation during GC/MS analysis and high base line levels of vitamin C.

Vitamin E

Vitamin E (α-tocopherol) is widely recognized as the most important biological antioxidant.[209,210] Although there is considerable interest in the possibility that vitamin E may be protective against cancer[92] several human studies have demonstrated that the vitamin can act as a promoter of carcinogenesis, at both the initiation and promotion stages.[211,212]

Cadenas et al.[199] determined the effect of dietary supplementation with vitamin C and E on hepatic 8-OHdG by HPLC/ECD of normal unstressed guinea-pigs at dietary doses that are known to effectively modify hepatic ascorbate and vitamin E levels. In one experiment, three groups of 6–8 guniea-pigs were fed diets containing 15 mg of vitamin E/kg chow and three different amounts of vitamin C (33, 660, or 13200 mg/kg) for 5 weeks. In a second experiment, three groups of seven guinea-pigs were fed diets containing 600 mg of vitamin C/kg and three different amounts of vitamin E (15, 150, or 1500 mg/kg) for 5 weeks. The level of 8-OHdG/10^5 dG in the liver DNA was 1.89 ± 0.32, 1.94 ± 0.78, and 1.93 ± 0.65 in the low, medium and high dose ascorbate groups (no effect: $p > 0.05$). In the low, medium, and high dose vitamin E groups, 8-OHdG/10^5dG level in the liver DNA was 2.85 ± 0.70, 2.74 ± 0.66, and 2.61 ± 0.92 (no effect: $p > 0.05$). It is concluded that even very large variations in the content of the antioxidant vitamins C and E in the diet and liver have no influence on the steady-state level of oxidative damage to guanine in the liver DNA of normal unstressed guinea-pigs. Likewise, Duthie et al.[213] found no increase in 8-OHdG levels in lymphocytes, liver, or colon in rats placed on a vitamin E deficient diet for 12 wks despite increases in lipid peroixdation biomarkers. Also, when 1000 mg/day of vitamin E was given to melanoma patients for 3 months there were no overall differences in the plasma antibody titer for 5-OHmdU between those that received the supplement and those tat did not. However, those that had less aggressive melanoma had a 48% decrease in 5-OHmdU plasma antibody levels in response to supplementation.[214] This was a small preliminary study.

Carotenoids

Carotenoids as biological antioxidants have been the focus of numerous investigations. Those carotenoids with nine or more conjugated double bonds are able to quench singlet oxygen with increasing activity depending on the number of conjugated double bonds. The most prominent dietary ones include β-carotene,

lycopene, lutein, β-crytoxanthin, and α-carotene. Several studies point to carotenoid-rich green leafy vegetables being able to lower cancer risks, and β-carotene in particular is considered to be an important protective ingredient.[215,216] β-Carotene has antioxidant properties and may inhibit carcinogenesis by several mechanisms, for example, prevention of DNA damage induced by free radicals, interference with the metabolic activation of carcinogens, or prevention of the binding of carcinogens to DNA.[217] β-Carotene given as a supplement, however, increased lung cancer incidence and mortality rates in smokers[218] or was without effect in healthy subjects.[71] Several factors could explain the opposite effects of carotenoid-rich diets on the one hand and β-carotene supplementation on the other. Foods contain not only β-carotene but many hundreds of carotenoids. Also, the bioavailability is lower from ingested vegetables than from supplements. Finally, current smokers may be more susceptible to prooxidant activities of β-carotene than nonsmokers.[219,220]

Tomatoes and tomato products are the major source of lycopene in the American diet providing about 80%. Among these carotenoids, lycopene in particular, has received the most attention in the prevention of DNA damage. Rao and Agarwal[221] evaluated the effect of dietary supplementation of lycopene from 6 different tomato products including placebo, tomato juice (50.4 mg lycopene/day), 2 different spaghetti sauces (20.5 or 39.2 mg lycopene/day), and 2 levels of lycopene as tomato oleoresin (75 or 150 mg lycopene/day) with 19 healthy human subjects. Each treatment was for one week each with a one-week washout phase. They assayed lipid, protein, and lymphocyte DNA oxidation, measured by 8-OHdG using HPLC/ECD. All treatments resulted in significantly lower ($p < 0.001$) lipid oxidation than in the placebo group. Although not statistically significant, a tendency of lowered protein and DNA oxidation was observed with each treatment except placebo.

Riso et al.[222] found that the daily consumption of 60 g of tomato puree per day, containing 16.5 mg lycopene and 0.6 mg β-carotene, for 3 weeks decreased lymphocyte DNA damage by 42%, quantified as the COMET assay. The same investigators found that the consumption of 25 g of tomato puree per day, containing 7 mg lycopene and 0.3 mg β-carotene, for 2 weeks, reduced lymphocyte DNA damage by $\sim$50% based on the COMET assay.[223] Both these studies involved healthy nonsmoking subjects. These results indicate that a small amount of tomato puree added to the diet over a short period of time can increase the resistance of lymphocytes to oxidative stress and DNA damage.

Our research group supplemented 32 newly diagnosed prostate cancer patients with 200 g of tomato spaghetti sauce baked into pasta entrees (30 mg lycopene/day) for 3 weeks prior to prostatectomy. Oxidative DNA damage measured by HPLC/ECD as 8-OHdG/dG in leukocytes decreased by 21%, in men consuming tomato sauce and in prostate decreased 28% compared to a control group of prostatectomy patients not participating in the study. A histochemical evaluation of 8-OHdG showed that the decreased DNA damage was greatest in prostate cancer cells and histochemically measured 8-OHdG decreased 46%.[97] Although this study demonstrated that tomatoes have an *in vivo* protective effect against oxidative DNA damage, it is not clear whether a decrease in DNA damage to cancer cells is a positive or negative outcome, if this promotes the greater survival of these cells.[224]

So far antioxidant supplementation has failed to alter oxidative DNA damage except that vitamin C appears to be important for the protection of sperm DNA and lycopene is readily absorbed from tomato products and may act as an in vivo antioxidant to protect from DNA damage.

Selenium

Selenium (Se) is an essential dietary nutrient for all mammalian species and a key component of various enzymes. Selenoproteins such as glutathione peroxidases (GPx), thioredoxin reductases (TrxR), and selenoprotein P (SePP) contain molecular selenium in the form of selenocysteines within their active center. They are involved in the defense of reactive oxygen species, which otherwise may cause DNA damage and alterations of protein function. However it is also toxic *in vivo* and to cells in culture.[225] Toxicity of Se is now thought to occur due to its prooxidant ability to catalyze the oxidation of thiols and simultaneously generate superoxide ($O_2^{\bullet -}$).[226] Humans receive Se as selenoamino acids (selenomethionine, selenocysteine, and selenocystine). Selenoamino acids, in particular selenomethionine, have a higher bioavailability than the inorganic species.

Se has been shown to prevent cancer in numerous animal model systems when fed at levels exceeding the nutritional requirements.[227,228] Stewart et al.[229] evaluated prooxidative effects of three different Se compounds; selenite, selenocystamine (SeC), and selenomethionine (SeM) at final concentrations of Se 5–10 μg/ml in BALB/c MK-2 mouse keratinocyte cell line. 8-OHdG adducts were measured by HPLC/ECD after 24 h incubation with Se compounds. DNA adducts were 5.5-fold greater with the SeC at 5 μg Se/ml treatment compared to control. Selenite treated cells at the 5 μg Se/ml level also showed significant elevations (4.6-fold) of 8-OHdG. However, greater detachment of cells had occurred with the more toxic selenite treatment at 10 μg Se/ml. No statistical difference in adducts between control cells and SeM treated cells was observed. This indicates that SeM has a low toxicity and does not participate in redox cycle production of superoxide.

Cruciferous Vegetables

Epidemiological studies have shown that increased consumption of cruciferous vegetables such as broccoli, Brussels sprouts (BS), and cabbage, can lower the risk of various developing cancers.[230,231] It is considered that much of this chemopreventive effect can be attributed to the anticarcinogenic effects of glucosinolates, which are relatively unique to cruciferous vegetables.[232] Glucosinolates themselves exhibit low bioactivities, but on

autolysis they are hydrolyzed to release a number of products, mainly isothiocyanates and nitriles.[233] The proposed mechanism of action is by inducing enzymes which scavenge electrophiles or by mimicking the cellular protective response to oxidative stress.[234,235]

Two studies investigating the effect of BS in the human diet have been conducted.[83,184] In the first study, healthy male, nonsmoking volunteers continued on a diet free of cruciferous vegetables and another 5 men consumed 300 g of cooked BS per day. In the control group there was no difference between the two periods in levels of 8-OHdG in urine measured by HPLC/ECD. In contrast, the levels of 8-OHdG were significantly decreased by 28% in BS group after a 3-week intervention period. In a second study, 10 men and women were randomly assigned to only glucosinolate-free meals whereas the other five consumed an additional 300 g of cooked BS per day. After 1 week, the dietary regimes were reversed. In four of five men a 55% reduction in 8-OHdG was found during the BS supplementation period, whereas in the fifth male the 8-OHdG excretion was high in the control period and was even much higher (4-fold) in the BS period. Consumption of BS had no effect on the excretion of 8-OHdG in females. Because urinary excretion could be confounded by modulation of DNA repair. Zhu et al.[236] investigated the effect of an aqueous extract of cooked BS on the formation of 8-OHdG in calf thymus DNA *in vitro.* Damage was induced by a Fenton reaction, short ultraviolet (VUC, 254 nm), long ultraviolet (UVA, 365 nm), sunlamp light, and methylene blue with visible light. The BS extract inhibited 8-OHdG formation up to 60–90% in all systems except the visible light with methylene blue. However, further investigation of oxidative DNA damage in specific rat organs shows that treatment with BS may result in an increase in 8-OHdG formation. Sorensen et al.[237] measured DNA damage using HPLC/ECD in rat liver after administration of BS extract for 3 or 7 days. They found increased levels of 8-OHdG from 16% to 23% after 3 or 7 day treatment, respectively. The observed increase in oxidative DNA damage raises the question whether the decreased urinary excretion levels in the human studies were a result of changes in DNA repair.

Soy

Epidemiological studies indicate that consumption of a soybean-containing diet is associated with a lower incidence of certain human cancers in Asian vs. Caucasian population.[238–240] Based on soy food consumption in Japan, typical daily isoflavone intake has been estimated at approximately 50 mg/person.[241] Genistein and daizein are the two principal components in soy products, and have been the focus of studies investigating the role of soy isoflavones in cancer prevention.[242]

One of the mechanisms by which soy isoflavones are thought to prevent cancer is via their antioxidant properties.[243,244] Soy isoflavones have been shown to exhibit antioxidant effects both *in vitro* and *in vivo*. Isoflavones have direct free radical quenching ability, with genistein and daizein being particularly effective.[243,245] Isoflavones may also produce decreased oxidative damage in cells via indirect mechanisms, such as induction of antioxidant scavenging enzymes.[246]

Mitchell and Collins[247] determined the effect of soy, in the form of soy milk, in 10 healthy men assigned to one of three groups consuming 1 L of either soy milk, rice dream (vegetable protein control), or semi-skimmed cow's milk (animal protein control) each day for 4 wks. The supplement decreased oxidative damage to DNA bases detected using the COMET assay compared with controls. Djuric et al.[174] examined levels of 5-OHmdU in lymphocytes of six women (50 mg isoflavones once daily) and six men (50 mg isoflavones twice daily) by GC/MS before and during soy supplementation using Novasoy tablets (Archer Daniels Midland Company, Decatur, IL, USA). Mean levels of leukocyte 5-OhmdU decreased after 1 week of supplementation in the women, with a decrease of 47% after 3 weeks. In men, mean leukocyte 5-OHmdU levels did not decrease until after 3 weeks of supplementation, at which there was a 61% decrease. Another pilot study has been conducted by Davis et al.[242] Six healthy males took one tablet containing a 50 mg mixture of soy isoflavones (Novasoy) twice daily with meals for 3 weeks. The mean value of 5-OHmdU measured by GC/MS was 2.6-fold lower ($p < .01$) after 3 weeks of supplementation. These results demonstrates that soy isoflavones may protect cells from oxidative stress by decreasing DNA adduct levels.

Foods High in Polyphenolic Compounds

Phenolics are naturally occurring secondary metabolites from plants and considered as nonnutrient biologically active compounds.[248] They are present in fruits, vegetables, leaves, nuts, seeds, and flowers. A direct relationship between the total phenolic content and the antioxidant activities in fruits and vegetables has been found.[170,249] These compounds act as inhibitors or activators for a large variety of mammalian enzyme systems, and as metal chelators and scavengers of free oxygen radicals.[250,251] Phenolics interfere with the pathways that regulate cell division and proliferation, detoxification, and inflammatory and immune response.[252,253]

Teas

Tea, which is one of the most popular beverages consumed in the world, contains polyphenols including (−)-epicatechin (EC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), (−)-epigallocatechin gallate (EGCG) and aflavins. Tea can block the formation of mutagens and carcinogens from precursors.[254] Tea and its polyphenols inhibit the biochemical activation of genotoxic carcinogens. They increase their detoxification through the induction of higher levels of glucuronosyl transferases.[255] Tea polyphenols influence molecular events at the level of the gene such as AP-1 or NF-κB, leading to control of the activity of the transforming growth factors TGF-α

and TGF-β.[150,256,257] Tea polyphenols inhibit xanthine oxidase associated with the formation of reactive oxygen species and radicals.[258] The effect of tumor promoters involves the blockage of cellular growth control messages through cell gap junctions, and the tea polyphenols restore effective gap junction communication and hence inhibit the action of promoters.[257,259] In many animal studies, the polyphenolic fraction isolated from green tea, the water extract of green tea, or individual polyphenolic catechins present in green tea have shown protective effects against chemically induced carcinogenesis in lung, liver, esophagus, forestomach, duodenum, pancreas, colon, and breast.[260,261] It is believed that much of the cancer chemopreventive properties of green tea are mediated by EGCG.[252,262] A single cup of brewed green tea contains up to 400 mg of polyphenolic antioxidants, of which 200 mg is EGCG.

Wei et al.[263] compared the effects of different extractable fractions of green and black teas (0–100 μg/mL) on UV irradiation-induced formation of 8-OHdG by HPLC/ECD in calf thymus DNA. Total fractions of green and black teas inhibited the induction of 8-OHdG from 5.6 to 24 fold depending on the tea fractions, their concentration and source of UV (UVA, B, and C). There were no significant differences between green and black teas which implies that tea processing, or tea fermentation does not alter the overall antioxidant activity of tea, as was expected, although the polyphenol profile of the two are different. Addition of EGCG to a low concentration of green and black tea extracts substantially enhanced the quenching of 8-OHdG production, suggesting the important role of EGCG in the antioxidant activities of tea extracts. Klaunig et al.[264] investigated smokers and nonsmokers in two volunteer groups (one in China and the other in Indiana, United States). 40 healthy male subjects in China received either 3 g/day of green tea extract or no tea for 1 day or 7 days, 27 female and male smokers in Indiana received 32 oz of green tea/day for 7 days. Leukocyte and urinary 8-OHdG were measured by HPLC/ECD. The China study showed a 40% decrease in leukocyte and urinary 8-OHdG after 7 days of green tea treatment. Indiana study smokers experienced a 500% and 250% decrease in leukocyte 8-OHdG and urinary 8-OHdG excretions, respectively after 7-day intervention. A more recent study showed that green tea consumption over a 4 month period decreased 8-OHdG urinary excretion in smokers who were positive for the glutathione-*S*-transferase (GST) polymorphism, GSTM1 or GSTT1, both of which render the entire gene absent.[265] GST is involved in phase II xenobiotic metabolism and is part of the endogenous antioxidant system. The effect of tea constituents on the apparent prevention of DNA damoge may be through the elimination of cells with DNA damage. Smokers and non-smokers drank 5 cups of green tea per day for 4 wks and human oral cells (buccal cells) were collected weekly and assessed for bulky adducts, oxidizaed bases, cell growth, DNA content, and apoptosis (programmed cell death). During the course of this small study smoking-induced DNA damage was decreased, cell growth was inhibited, and the cell cycle was arrested while markers of apoptosis were increased.[266] By reducing the number of DNA damaged cell through cell cycle arrest and apoptosis the amount of DNA damage in the tissue sample will have decreased.

Grapes

Grapes contain a number of antioxidant compounds including resveratrol, catechin, epicatechin, and proanthrocyanidins.[267] Resveratrol is a natural phytoalexin that is found in large quantities in berry skins of most grape cultivars. It has been reported that diet supplementation with red wine solids can inhibit tumor formation in mice.[268,269] Mizutani et al.[270] examined the effect of resveratrol on oxidative DNA damage in male and female stroke-prone spontaneously hypertensive rats (SHRSP). Five-week old male and female SHRSP were divided into control and resveratrol groups. The resveratrol group was given 1 mg/kg of resvertrol per day, orally, by gastric intubation once a day. Following an 8 week feeding period, the levels of 8-OHdG in the urine were measured by an 8-OHdG ELISA kit. Treatment with resveratrol resulted in a 35 and 37% reduction in urine 8-OHdG in male and female SHRSP, respectively, compared with controls. Leighton et al.[271] performed an intervention study to evaluate the influence of a Mediterranean diet, a high fat diet, and their supplementation with red wine in moderate amounts, on DNA damage. For 3 months, two groups of 21 men each, received either a Mediterranean diet or a high fat diet; during the second month, red wine (240 mL/day) was added isocalorically. Oxidative DNA damage, detected by 8-OHdG levels in blood leukocyte DNA using HPLC/ECD, was markedly increased by the high fat diet; however, it was strongly reduced, to approximately 50% of basal values, after wine supplementation, both in the high fat diet and Mediterranean diet groups. Taken together these results suggest that red wine or resveratrol can act as an antimutagenic/anticarcinogenic agent perhaps, by preventing oxidative DNA damage.

CONCLUSIONS

Oxidative DNA damage has been implicated in the pathogenesis of many diseases, particularly cancer. The assessment of damage products in various biological matrices, such as tissue DNA, serum, and urine, could be important to understanding its role and subsequently devising intervention strategies. Despite the numerous techniques to measure oxidative DNA damage products, it remains unclear what these measurements truly represent, since even direct in vivo measurements represent a combination of the level of ROS attack, antioxidant status and activity of the DNA repair mechanisms. Several micronutrients are required as co-factors in DNA maintenance reactions including DNA synthesis, DNA repair, DNA methylation, and apoptosis.[272] Deficiencies of these micronutrients may lead to high levels of DNA damage in the same way as exposure to a carcinogen. These deficiencies have not been investigated in animal or human studies. DNA damage is also important later

in the cancer process. The induction of specific metabolizing enzymes by bioactive compounds found at high levels in certain vegetables, fruits, and cereals may increase the likelihood of detoxifying and excreting DNA damaging compounds.[273]

It is clear that age and gender as well as environmental factors can modulate levels of DNA damage in humans as well as animals. Most salient is the DNA damaging habit of smoking whereas exercise effects are dependent on the intensity, timing of sample collection, and the level of training.

A number of studies have explored the role of dietary factors, especially antioxidants, for their ability to reduce residual DNA damage. Overall, there are too few studies to come to any conclusions regarding a single food antioxidant or a food containing antioxidants. Furthermore, in areas where there have been several studies, there is little concordance in outcome. This state of the science likely reflects the variety of measurement techniques used, the design of the studies, the nutritional status of the animals or subjects and whether the antioxidants used might have affected the rate of ROS attack or the rate of repair. Most human studies have used pharmacological doses in study subjects having presumably adequate antioxidant status. It is not clear whether additional benefit can be obtained by supra-normal antioxidant status whereas deficiency states have not been explored except in the case of vitamin E. Of the antioxidants, vitamin C has been the most researched because it can promote as well as prevent DNA damage in vitro. Some studies have shown a reduction in DNA damage where others have not, but most importantly, vitamin C supplementation does not appear to increase DNA damage in vivo. Surprisingly, there is no evidence of a protective effect of vitamin E even in deficiency or combined with vitamin C. The two carotenoids which have been evaluated are β-carotene and lycopene. The ability of lycopene to decrease DNA damage in healthy and prostate cancer patients is promising whereas there is no evidence of a protective effect for β-carotene supplemented at pharmacological doses. The most dramatic reductions in DNA damage in humans have been reported for tea drinking with epigallocatechin gallate thought to be the most bioactive of the mixture of phenolic compounds in green and black tea. Red wine also contains a mixture of catechins and anthrocyanidins and DNA damage was substantially decreased with red wine supplementated in both high and low fat diets. Resveratrol, a highly variable component of red wine was shown to reduce 8OHdG excretion which may reflect improved DNA repair as well as a decreased ROS attack. Soy isoflavonoids have antioxidant and estrogenic/antiestrogenic properties. Both soy milk and soy isoflavonoid supplements reduce DNA damage in humans. Cruciferous vegetables which contain glucosinolates that induce enzymes that either scavenge electrophiles or protect against oxidative stress also decrease DNA damage in humans. Caloric restriction has been shown to reduce DNA damage in animals. The role of total fat or polyunsaturated fat in increasing DNA damage is unclear. The variance in study results may be related to not only the proportion and type of unsaturated fatty acid exposure but also to the relative amount of vitamin E in the diets.

Although there is insufficient evidence that any of these food antioxidants alone can affect long term residual amounts of DNA damage in tissues that are targets for cancer, it is somewhat surprising that so many studies have been able to modulate DNA damage levels in healthy people. Whether these small changes in DNA damage translate into reductions in cancer risk await the use of DNA damage measures in population studies and clinical trials where specific cancers are the endpoint.

ACKNOWLEDGEMENT

This work was supported by a Korea Research Foundation Grants (KRF-2004-005-F00055 and KRF-2005-206-F00008) funded by the Korean Government (MOEHRD).

REFERENCES

[1] Go, V.L., Wong, D.A., Wang, Y., Butrum, R.R., Norman, H.A., and Wilkerson, L. 2004. Diet and cancer prevention: evidence-based medicine to genomic medicine. *J. Nutr.*, **134**:3513S–3516S.

[2] Hung, H.C., Joshipura, K.J., Jiang, R., Hu, F.B., Hunter, D., Smith-Warner, S.A., Colditz, G.A., Rosner, B., Spiegelman, D., and Willett, W.C. 2004. Fruit and vegetable intake and risk of major chronic disease. *J. Natl. Cancer Inst.*, **96**:1577–1584.

[3] Van Duyn, M.A., Kristal, A.R., Dodd, K., Campbell, M.K., Subar, A.F., Stables, G., Nebeling, L., and Glanz, K. 2001. Association of awareness, intrapersonal and interpersonal factors, and stage of dietary change with fruit and vegetable consumption: a national survey. *Am. J. Health Promot*., **16**:69–78.

[4] Halliwell, B. 1994. Free radicals, antioxidants and human disease: Curiosity, cause or consequence. *Lancet*, **344**:721–724.

[5] Marnett, L.J. 2000. Oxyradicals and DNA damage. *Carcinogenesis*, **21**:361–370.

[6] Giovannucci, E., Rimm, E.B., Liu, Y., Stampfer, M.J., and Willett, W.C. 2002. A prospective study of tomato products, lycopene, and prostate cancer risk. *J. Natl. Cancer Inst.*, **94**:391–398.

[7] Wang, X.D., Liu, C., Bronson, R.T., Smith, D.E., Krinsky, N.I., and Russell, M. 1999. Retinoid signaling and activator protein-1 expression in ferrets given beta-carotene supplements and exposed to tobacco smoke. *J. Natl. Cancer Inst.*, **91**:60–66.

[8] Liu, C., Wang, X.D., Bronson, R.T., Smith, D.E., Krinsky, N.I., and Russell, R.M. 2000. Effects of physiological versus pharmacological beta-carotene supplementation on cell proliferation and histopathological changes in the lungs of cigarette smoke-exposed ferrets. *Carcinogenesis*, **21**:2245–2253.

[9] Fernandez, V., Hartmann, E., Ott, G., Campo, E., and Rosenwald, A. 2005. D Pathogenesis of mantle-cell lymphoma: all oncogenic roads lead to dysregulation of cell cycle and DNA damage response pathways. *J. Clin. Oncol*. **23**:6364–6369.

[10] Trueba, G.P., Sanchez, G.M., and Giuliani, A. 2004. Oxygen free radical and antioxidant defense mechanism in cancer. *Front Biosci.*, **9**:2029–2044.

[11] Laurent, A., Nicco, C., Chereau, C., Goulvestre, C., Alexandre, J., Alves, A., Levy, E., Goldwasser, F., Panis, Y., Soubrane, O., Weill, B., and Batteux, F. 2005. Controlling tumor growth by modulating endogenous production of reactive oxygen species. *Cancer Res*., **65**:948–956.

[12] Ji, L.L., and Peterson, D.M. 2004. Aging, exercise, and phytochemicals: promises and pitfalls. *Ann. N. Y. Acad. Sci*., **1019**:453–461.

[13] Cooke, M.S., Evans, M.D., Dizdaroglu, M., and Lunec, J. 2003. Oxidative DNA damage: mechanisms, mutant, and disease. *FASEB J.* **17**:1195–1214.

[14] Unfried, K., Schurkes, C., and Abel, J. 2002. Distinct spectrum of mutations induced by crocidolite asbestos: clue for 8-hydroxydeoxyguanosine-dependent mutagenesis *in vivo*. *Cancer Res*., **62**:99–104.

[15] Muniz, P., Saez, P., Iradi, A., Vina, J., Oliva, M.R., and Saez, G.T. 2001. Differences between cysteine and homocysteine in the induction of deoxyribose degradation and DNA damage. *Free Radic. Biol. Med*., **30**:354–362.

[16] Purma, A.A., Lampman, G.W., Bond, J.P., Hatahet, Z., and Wallace, S.S. 1998. Enzymatic processing of uracil glycol, a major oxidative product of DNA cytosine. *J. Biol. Chem*., **273**:10026–10035.

[17] Graziewicz, M.A., Zastawny, T.H., Olinski, R., Speina, E., Siedlecki, J., and Tudek, B. 2000. Fapyadenine is a moderately efficient chain terminator for prokaryotic DNA polymerases. *Free Radic Biol. Med*., **28**:75–83.

[18] Faure, V., Saparbaev, M., Dumy, P., and Constant, J.F. 2005. Action of multiple base excision repair enzymes on the 2′-deoxyribonolactone. *Biochem. Biophys. Res. Commun*., **328**:1188–1195.

[19] Valavanidis, A., Vlahoyianni, T., and Fiotakis, K. 2005. Comparative study of the formation of oxidative damage marker 8-hydroxy-2′-deoxyguanosine (8-OHdG) adduct from the nucleoside 2′-deoxyguanosine by transition metals and suspensions of particulate matter in relation to metal content and redox reactivity. *Free Radic. Res*., **39**:1071–1081.

[20] Fortini, P., Pascucci, B., parlanti, E., D'Errico, M., Simonelli, V., and Dogliotti, E. 2003. 8-Oxoguanine DNA damage: at the crossroad of alternative repair pathways. *Mutat. Res*., **531**:127–139.

[21] Sakano, K., Oikawa, S., Hasegawa, K., and Kawanishi, S. 2001. Hydroxyurea induces site-specific DNA damage via formation of hydrogen peroxide and nitric oxide. *Jpn. J. Cancer Res.*, **92**:1166–1174.

[22] Lin, H., and Hollenberg, P.E. 2001. N-nitrosodimethylamine-mediated formation of oxidized and methylated dna bases in a cytochrome P450 2E1 expressing cell line. *Chem. Res. Toxicol*., **14**:562–566.

[23] Mandavilli, B.S., Santos, J.H., and Van Houten, B. 2002. Mitochondrial DNA repair and aging, *Mutat. Res.*, **509**:127–151.

[24] Yates, F.M., and Van Houten, B. 1997. Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. *Proc. Natl. Acad. Sci. USA*., **94**:514–519.

[25] Ishibashi, Y., Sugimoto, T., Ichikawa, Y., Akatsuka, A., Miyata, T., Nangaku, M., Tagawa, H., and Kurokawa, K. 2002. Glucose dialysate induces mitochondrial DNA damage in peritoneal mesothelial cells. *Perit. Dial. Int*., **22**:11–21.

[26] Grishko, V.I., Rachek, L.I., Spitz, D.R., Wilson, G.L., and LeDoux, S.P. 2005. Contribution of mitochondrial DNA repair to cell resistance from oxidative stress. *J. Biol. Chem*., **280**:8901–8905.

[27] Lin, P.H., Lee, S.H., Su, C.P., and Wei, Y.H. 2003. Oxidative damage to mitochondrial DNA in atrial muscle of patients with atrial fibrillation. *Free Radic. Biol. Med*., **35**:1310–1318.

[28] Mawrin, C., Kirches, E., Krause, G., Schneider-Stock, R., Bogerts, B., Vorwerk, C.K., and Dietzmann, K. 2004. Region-specific analysis of mitochondrial DNA deletions in neurodegenerative disorders in humans. *Neurosci. Lett*., **357**:111–114.

[29] Kang, D., and Hamasaki, N. 2005. Alterations of mitochondrial DNA in common diseases and disease states: aging, neurodegeneration, heart failure, diabetes, and cancer. *Curr. Med. Chem*., **12**:429–441.

[30] Green, P.S., and Leuwenburgh, C. 2002. Mitochondrial dysfunction is an early indicator of doxorubicin-induced apoptosis. *Biochim. Biophys. Acta.*, **1588**:94–101.

[31] Zastawny, T.H., Dabrowska, M., Jaskolski, T., Klimarczyk, M., Kulinski, L., Koszela, A., Szczesniewicz, M., Sliwinska, M., Witkowski, P., and Olinski, R. 1998. Comparison of oxidative base damage in mitochondrial and nuclear DNA. *Free Radic. Biol. Med*., **24**:722–725.

[32] Suter, M., and Richter, C. 1999. Fragmented mitochondrial DNA is the predominant carrier of oxidative DNA bases. *Biochem.*, **38**:459–464.

[33] Lim, P.S., Ma, Y.S., Cheng, Y.M., Chai, H., Lee, C.F., Chen, T.L., and Wei, Y.H. 2002. Mitochondrial DNA mutations and oxidative damage in skeletal muscle of patients with chronic uremia. *J. Biomed. Sci*., **9**:549–560.

[34] Bohr, V.A. 2002. Repair of oxidative DNA damage in nuclear and mitochondrial DNA, and some changes with aging in mammalian cells. *Free Radic. Biol. Med*., **32**:804–812.

[35] Lifshitz, J., and McIntosh, T.K. 2003. Age-associated mitochondrial DNA deletions are not evident chronically after experimental brain injury in the rat. *J. Neurotrauma*., **20**:139–149.

[36] Kang, D., and Hamasaki, N. 2005. Alterations of mitochondrial DNA in common diseases and disease states: aging, neurodegeneration, heart failure, diabetes, and cancer. *Curr. Med. Chem*., **12**:429–441.

[37] Kasai, H., Chung, M.H., Jones, D.S., Inoue, H., Ishikawa, H., Kamiya, H., and Ohtsuka, E. 1991. 8-Hydroxyguanine, a DNA adduct formed by oxygen radicals: its implication on oxygen radical-involved mutagenesis/carcinogenesis. *J. Toxicol. Sci.*, **16**(Suppl 1):95–105.

[38] Shukla, A., Jung, M., Stern, M., Fukagawa, N.K., Taatjes, D.J., Sawyer, D., Van Houten, B., and Mossman, B.T. 2003. Asbestos induces mitochondrial DNA damage and dysfunction linked to the development of apoptosis. *Am. J. Physiol. Lung Cell Mol. Physiol*., **285**:L1018–L1025.

[39] Narayan, S., Jaiswal, A.S., Kang, D., Srivastava, P., Das, G.M., and Gairola, C.G. 2004. Cigarette smoke condensate-induced transformation of normal human breast epithelial cells *in vitro*. *Oncogene*, **23**:5880–5889.

[40] Miyake, H., Hara, I., Kamidono, S., and Eto, H. 2004. Prognostic significance of oxidative DNA damage evaluated by 8-hydroxy-2′-deoxyguanosine in patients undergoing radical nephrectomy for renal cell carcinoma. *Urology*, **64**:1057–1061.

[41] Lunec, J., Holloway, K.A., Cooke, M.S., Faux, S., Griffiths, H.R., and Evans, M.D. 2002. Urinary 8-oxo-2′-deoxyguanosine: redox regulation of DNA repair *in vivo*? *Free Radic. Biol. Med*., **33**:875–885.

[42] Powell, C.L., Swenberg, J.A., and Rusyn, I. 2005. Expression of base excision DNA repair genes as a biomarker of oxidative DNA damage. *Cancer Lett*., **229**:1–11.

[43] McMurray, C.T. 2005. To die or not to die: DNA repair in neurons. *Mutat. Res*., **577**:260–274.

[44] Gackowski, D., Speina, E., Zielinska, M., Kowalewski, J., Rozalski, R., Siomek, A., Paciorek, T., Tudek, B., and Olinski, R. 2003. Products of oxidative DNA damage and repair as possible biomarkers of susceptibility to lung cancer. *Cancer Res*., **63**:4899–4902.

[45] Bellacosa, A. 2001. Functional interactions and signaling properties of mammalian DNA mismatch repair proteins. *Cell Death Differ*., **8**:1076–1092.

[46] Croteau, D.L., and Bohr, V.A. 1997. Repair of oxidative damage to nuclear and mitochondrial DNA in mammalian cells. *J. Biochem*., **272**:25409–25412.

[47] Guetens, G., De Boeck, G., Highley, M., van Oosterom, A.T., and de Bruijin, E.A. 2002. Oxidative DNA damage: biological significance and methods of analysis. *Crit. Rev. Clin. Lab. Sci*., **39**:331–457.

[48] Wu, L.L., Chiou, C.C., Chang, P.Y., and Wu, J.T. 2004. Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics. *Clin. Chim. Acta*., **339**:1–9.

[49] Floyd, R. 1991. DNA damage and repair., In: *Oxidative Damage and Repair: chemical, biological and medical aspects*., Davies KJ, Ed. Pergamon Press, NY, USA.

[50] Malins, D.C., Johnson, P.H., Wheeler, T.M., Barker, E.A., Polissar, N.L., and Vinson, M.A. 2001. Age-related radical-induced DNA damage is linked to prostate cancer. *Cancer Res.*, **61**:6025–6028.

[51] Abu-Shakra, A., and Zeiger, E. 1997. Formation of 8-hydroxy-2′-deoxyguanosine following treatment of 2′-deoxyguanosine or DNA by hydrogen peroxide or glutathione. *Mutat Res.*, **390**:45–50.

[52] Gutteridge, J.M., and Halliwell, B. 2000. Free radicals and antioxidants in the year 2000. A historical look to the future. *Ann. NY. Acad Sci.*, **899**:136–147.

[53] Gedik, C.M., Wood, S.G., and Collins, A.R. 1998. Measuring oxidative damage to DNA: HPLC and the comet assay compared. *Free Radic. Res*., **29**:609–615.

[54] Germadnik, D., Pilger, A., and Rudiger, H.W. 1997. Assay for the determination of urinary 8-hydroxy-2′-deoxyguanosine by high-performance liquid chromatography with electrochemical detection. *J. Chromatogr B. Biomed Sci. Appl*., **689**:399–403.

[55] De Martinis, B.S., and de Lourdes Pires Bianchi, M. 2002. Methodology for urinary 8-hydroxy-2′-deoxyguanosine analysis by HPLC with electrochemical detection. *Pharmacol. Res*., **46**:129–131.

[56] Mei, S., Yao, Q., Wu, C., and Xu, G. 2005. Determination of urinary 8-hydroxy-2′-deoxyguanosine by two approaches-capillary electrophoresis and GC/MS: An assay for in *vivo* oxidative DNA damage in cancer patients. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci*., **827**:83–87.

[57] Allgayer, H., Kolb, M., Stuber, V., and Kruis, W. 2002. Effects of bile acids on base hydroxylation in a model of human colonic mucosal DNA. *Cancer Detect. Prev*., **26**:85–89.

[58] Halliwell, B. 1996. Commentary; Oxidative stress, nutrition and health. Experimental strategies for optimization of nutritional antioxidant intake in humans. *Free Radic. Res*., **25**:57–74.

[59] Ghiselli, A. D., Amicis, A., and Giacosa, A. 1997. The antioxidant potential of the Mediterranean diet. *Eur. J. Cancer Prev*., **6**:S15–S19.

[60] Orhan, H., van Holland, B., Krab, B., Moeken, J., Vermeulen, N. P., Hollander, P., and Meerman, J. H. 2004. Evaluation of a multi-parameter biomarker set for oxidative damage in man: increased urinary excretion of lipid, protein and DNA oxidation products after one hour of exercise. *Free Radic Res*., **38**:1269–1279.

[61] Van Breemen, R.B., Xu, X., Viana, M.A., Chen, L., Stacewicz-Sapuntzakis, M., Duncan, C., Bowen, P.E., and Sharifi, R. 2002. Liquid chromatography-mass spectrometry cis- and all trans-lycopene in human serum and prostate tissue after dietary supplementation with tomato sauce. *J. Agric. Food Chem*., **50**:2214–2219.

[62] Yin, B., Whyatt, R.M., Perera, F.P., and Randall, M.C. 1995. Determination of 8-hydroxyldeoxyguanosine by an immunoaffinity chromatography-monoclonal antibody-based ELISA. *Free Radic. Biol. Med*., **18**:1023–1032.

[63] Toyokuni, S., Tanaka, T., Hattori, Y., Nishiyama, Y., Yoshida, A., Uchida, K., Hiai, H., Ochi, H., and Osawa, T. 1997. Quantitative immunohistochemical determination of 8-hydroxy-2′-deoxyguanosine by a monoclonal antibody N45.1: its application to ferric nitrilotriacetate-induced renal carcinogenesis model. *Lab Invest.*, **76:**365–374.

[64] Prieme, H., Loft, S., Nyyossonen, K., Salonen, J. T., and Poulsen, H. E. 1997. No effect of supplementation with vitamin E, ascorbic acid, or coenyme Q10 on oxidative DNA damage estimated by 8-oxo-7,8-dihydro-2′-deoxyguanosine excretion in smokers. *Am. J. Clin. Nutr*., **65**:503–507.

[65] Won, M.H., Kang, T.C., Jeon, G.S., Lee, K.H., and Cho, S.S. 1999. Immunohistochemical detection of oxidative DNA damage induced by ischemia-reperfusion insults in gerbil hippocampus in vivo. *Brain Res*., **836**:70–78.

[66] Chen, L. 2000. Tomato sauce supplementation reduces DNA damage in men with prostate cancer. *Dissertation*, University of Illinois at Chicago.

[67] Giovannelli, L., Cozzi, A., Guarnieri, I., Dolara, P., and Moroni, F. 2002. Comet assay as a novel approach for studying DNA damage in focal cerebral ischemia: Differential effects of NMDA receptor antagonists and poly(ADP-ribose) polymerase inhibitors. *J. Cerebral Blood Flow & Metabol*., **22**:697–704.

[68] Boyle, S. P., Dobson, V. L., Duthie, S. J., Kyle, J. A., and Collins, A. R. 2000. Absorption and DNA protective effects of flavonoid glycosides from an onion meal. *Eur. J. Nutr*., **39**:213–223.

[69] Collins, A. R., Harrington, V., Drew, J., and Melvin, R. 2002. Nutritional modulation of DNA repair in a human intervention study. *Carcinogenesis*, **24**:511–515.

[70] Cheng, Y.-W., Hsieh, L.-L., Lin, P.-P., Chen, C.-P., Chen, C.-Y., Lin, T.-S., Su, J.-M., and Lee, H. 2001. Gender difference in DNA adduct levels among nonsmoking lung cancer patients. *Environ. Mol. Mutagen.*, **37**:304–310.

[71] Lee, B. M., Jang, J. J., and Kim, H. S. 1999. Benzo[a]pyrene diol-epoxide-I-DNA and oxidative DNA adducts associated with gastric adenocarcinoma. *Cancer Lett*., **125**:61–68.

[72] Okamoto, K., Toyokuni, S., Uchida, K., and 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.

[73] Nagashima, M., Tsuda, H., Takenoshita, S., and Kasai, H. 1995. 8-hydroxy-2′-deoxyguanosine levels in DNA of human breast cancer are not significantly different from those of non-cancerous breast tissues by HPLC-ECD method. *Cancer Lett*., **90**:157–162.

[74] Shimoda, R., Nagashima, M., Yokota, K., and Kasai, H. 1995. Increased formation of oxidative DNA damage, 8-hydroxydeoxyguanosine, in human livers with chronic hepatitis. *Cancer Res*., **54**:3171–3172.

[75] Foksinski, M., Jaruga, P., Makarewicz, R., and Olinski, R. 1998. Oxidative DNA base damage in cancerous tissues of patients undergoing brachytherapy. *Cancer Lett*., **132**:169–173.

[76] Senturker, S., Karahalil, B., Inal, M., and Dizdaroglu, M. 1997. Oxidative DNA base damage and antioxidant enzyme levels in childhood acute lymphoblastic leukemia. *FEBS Lett.*, **416**:286–290.

[77] Jaruga, P., Zastaway, T. H., Dizdaroglu, M., and Olinski, R. 1994. Oxidative DNA base damage and antioxidant enzyme activities in human lung cancer. *FEBS Lett*., **341**:59–64.

[78] Djuric, Z., Heilbrun, L. K., Simon, M. S., Smith, D., and Martino, S. 1996. Levels of 5-hydroxymethyl-2′-deoxyrindine in DNA from blood as a marker of breast cancer. *Cancer*, **77**:691–696.

[79] Faure, H., Coudray, C., Mousseau, M., Douki, T., and Favier, A. 1996. 5-Hydroxymethyluracil excretion, plasma TBARS, and plasma antioxidant vitamins in adriamycin-treated patients. *Free Radic. Biol. Med*., **20**:979–983.

[80] Pilger, A., Ivancsits, S., Germadnik, D., and Rudiger, H. W. 2002. Urinary excretion of 8-hydroxy-2′-deoxyguanosine measured by high-performance liquid chromatography with electrochemical detection. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci*., **778**:393–401.

[81] Wu, L. L., Chiou, C. C., Chang, P. Y., and Wu, J. T. 2004. Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics. *Clin. Chim. Acta*., **339**:1–9.

[82] Kristenson, M., Kucinskiene, Z., Schafer-Elinder, L., Leanderson, P., and Tagesson, C. 2003. Lower serum levels of β-carotene in Lithuanian men are accompanied by higher urinary excretion of the oxidative DNA adduct, 8-hydroxydeoxyguanosine. The LiVicordia study. *Nutrition*, **19**:11–15.

[83] Chen, C., Qu, L., Li, B., Xing, L., Jia, G., Wang, T., Gao, Y., Zhang, P., Li, M., Chen, W., and Cai, Z. 2005. Increased oxidative DNA damage, as assessed by urinary 8-hydroxy-2′-deoxyguanosine concentrations, and serum redox status in persons exposed to mercury. *Clin. Chem*., **51**:759–767.

[84] Helbock, H. J., Beckman, K. B., Shigenaga, M. K., Walter, P. B., Woodall, A. A., Yeo, H. C., and Ames, B. N. 1998. DNA oxidation matters: the HPLC-electrochemical detection assay of 8-oxo-deoxyguanosine and 8-oxo-guanine, *Proc. Natl. Acad. Sci. USA*, **95**:288–293.

[85] Huang, H. Y., Helzlsouer, K. J., and Appel, L. J. 2000. The effects of vitamin C and vitamin E on oxidative DNA damage: results from a randomized controlled trial. *Cancer Epidemiol Biomarkers Prev*., **9**:647–652.

[86] Teixeira, A. J. R., Gommers-Ampt, J. H., van de Werken, G., Westra, J. G., Stavenuiter, J. F. C., and de Jong, A. P. J. M. 1993. Method for the analysis of oxidized nucleosides by gas chromatography/mass spectrometry. *Anal. Biochem*., **214**:474–483.

[87] Dizdaroglu, M., Jaruga, P., and Rodriguez, H. 2001. Measurement of 8-hydroxy-2′ deoxyguanosine in DNA by high performance liquid chromatography-mass spectrometry: comparison with measurement by gas chromatography-mass spectrometry. *Nucleic. Acids Res.*, **29**:E12.

[88] Zhang, J., Ichiba, M., Hanaoka, T., Pan, G., Yamano, Y., Hara, K., Takahashi, K., and Tomokuni, K. 2003. Leukocyte 8-hydroxydeoxyguanosine and aromatic DNA adduct in coke-oven workers with polycyclic aromatic hydrocarbon exposure. *Int. Arch. Occup. Environ. Health*, **76**:499–504.

[89] Galan, P., Viteri, F. E., Bertrais, S., Czernichow, S., Gaure, H., Arnaud, J., Ruffieux, D., Chenal, S., Arnault, N., Favier, A., Roussel, A. M., and Hercberg, S. 2005. Serum concentrations of beta-carotene, vitamins C and E, zinc and selenium are influenced by sex, age, diet, smoking status, alcohol consumption and corpulence in a general French adult population. *Eur. J. Clin. Nutr*., **59**:1181–1190.

[90] Akcay, T., Saygili, I., Andican, G., and Yalcin, V. 2003. Increased formation of 8-hydroxy-2′-deoxyguanosine in peripheral blood leukocytes in bladder cancer. *Urol. Int*., **71**:271–274.

[91] Halliwell, B. 2000. Why and how should we measure oxidative DNA damage in nutritional studies? How far have we come? *Am. J. Clin. Nutr*., **72**:1082–1087.

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

[93] Romano, G., Sgambato, A., Flamini, G., Boninsegna, A., Milito, S., Ardito, R., and Cittadini, A. 2000. Evaluation of 8-hydroxydeoxyguanosine in human oral cells: the importance of tobacco smoke and urban environment. *Anticancer Res*., **20**:3801–3805.

[94] Feng, Z., Xia, Y., Tian, D., Wu, K., Schmitt, M., Kwok, P. K., and Mumford, J. L. 2001. DNA damage in buccal epithelial cells from individual chronically exposed to arsenic via drinking water in Inner Mongolia, China. *Anticancer Res.*, **21**:51–57.

[95] Hua, Y., Wainhaus, S. B., Yang, Y., Shen, L., Xiong, Y., Xu, X., Zhang, F., Bolton, J. L., and van Breemen, R. 2001. Comparison of negative and positive ion electrospray tandem mass spectrometry for the liquid chromatography tandem mass spectrometry analysis of oxidized deoxynucleosides. *Am. Soc. Mass Spec.*, **12**:80–87.

[96] Chen, L., Sapuntzakis, M. S., Duncan, C., Sharifi, R., Ghost, L., van Breeman, R., Ashton, D., and Bowen, P. E. 2001. Oxidative DNA damage in prostate cancer patients consuming tomato sauce-based entrees as a whole-food intervention. *J. Natl. Cancer Inst.*, **93**:1872–1879.

[97] Bowen, P., Chen, L., Stacewicz-Sapuntzakis, M., Duncan, C., Sharifi, R., Ghosh, L., Kim, H. S., Christove-Tzelkov, K., and van Breemen, R. 2002. Tomato sauce supplementation and prostate cancer: lycopene accumulation and modulation of biomarkers of carcinogenesis. *Exp. Biol. Med. (Maywood).*, **227**:886–893.

[98] Sacheck, J. M., Milbury, P. E., Cannon, J. G., Roubenoff, R., and Blumberg, J. B. 2003. Effect of vitamin E and eccentric exercise on selected biomarkers of oxidative stress in young and elderly men. *Free Radic Biol. Med*., **34**:1575–1588.

[99] Radak, Z., Apor, P., Pucsok, J., Berkes, I., Ogonovszky, H., Pavlik, G., Nakamoto, H., and Goto, S. 2003. Marathon running alters the DNA base excision repair in human skeletal muscle. *Life Sci*., **72**:1627–1633.

[100] Ogonovszky, H., Berkes, I., Kumagai, S., Kaneko, T., Tahara, S., Goto, S., and Radak, Z. 2005. The effects of moderate-, strenuous- and overtraining on oxidative stress markers, DNA repair, and memory, in rat brain. *Neurochem. Int*., **46**:635–640.

[101] Tsai, K., Hsu, T.-G., Hsu, K.-M., Cheng, H., Liu, T.-Y., Hsu, C.-F., and Kong, C.-W. 2001. Oxidative DNA damage in human peripheral leukocytes induced by massive aerobic exercise. *Free Radic. Biol. Med.*, **31**:1465–1472.

[102] Fehrenbach, E., Veith, R., Schmid, M., Dickhuth, H. H., Northoff, H., and Niess, A. M. 2003. Inverse response of leukocyte heat shock proteins and DNA damage to exercise and heat. *Free Radic. Res*., **37**:975–982.

[103] Radak, Z., Naito, H., Kaneko, T., Tahara, S., Nakamoto, H., Takahashi, R., Cardozo-Pelaez, F., and Goto, S. 2002. Exercise training decreases DNA damage and increases DNA repair and resistance against oxidative stress of proteins in aged rat skeletal muscle. *Pflugers Arch*., **445**:273–278.

[104] Siu, P. M., Bryner, R. W., Martyn, J. K., and Always, S. E. 2004. Apoptotic adaptations from exercise training in skeletal and cardiac muscles. *FASEB J.*, **18**:1150–1152.

[105] Ogonovszky, H., Berkes, I., Kumagai, S., Kaneko, T., Tahara, S., Goto, S., and Radak, Z. 2005. The effects of moderate-, strenuous- and overtraining on oxidative stress markers, DNA repair, and memory, in rat brain. *Neurochem. Int*., **46**:635–640.

[106] Parise, G., Brose, A.N., and Tarnopolsky, M.A. 2005. Resistance exercise training decrease oxidative damage to DNA and increases cytochrome oxidase activity in older adults. *Exp. Gerontol*., **40**:173–180.

[107] Mitsui, T., Azuma, H., Nagasawa, M., Iuchi, T., Akaike, M., Odomi, M., and Matsumoto, T. 2002. Chronic corticosteroid administration causes mitochondrial dysfunction in skeletal muscle. *J. Neurol*., **249**:1004–1009.

[108] Parise, G., Brose, A.N., and Tarnopolsky, M.A. 2005. Resistance exercise training decreases oxidative damage to DNA and increases cytochrome oxidase activity in older adults. *Exp Gerontol*., **40**:173–180.

[109] Moller, P. 2005. Genotoxicity of environmental agents assessed by the alkaline comet assay. *Basic Clin. Pharmacol. Toxicol*., **96** (Suppl 1):1–42.

[110] Ohkuwa, T., Itoh, H., Yamamoto, T., Minami, C., Yamazaki, Y., Kimoto, S., and Yoshida, R. 2004. Effects of hypoxia and hypoxic training on 8-hydroxydeoxyguanosine and glutathione levels in the liver. *Metabolism*, **53**:716–719.

[111] Asami, S., Hirano, T., Yamaguchi, R., Tsurudome, Y., Itoh, H., and Kasai, H. 1998. Effects of forced and spontaneous exercise on 8-hydroxydeoxyguanosine levels in rat organs. *Biochem Biophys Res Commun*., **243**:678–682.

[112] Palazzetti, S., Richard, M.J., Favier, A., and Margaritis, I. 2003. Overloaded training increases exercise -induced oxidative stress and damage. *Can. J. Appl. Physiol*., **28**:588–604.

[113] Almar, M., Villa, J.G., Cuevas, M.J., Rodriguez-Marroyo, J.A., Avila, C., and Gonzalez-Gallego, J. 2002. Urinary levels of 8-hydroxydeoxyguanosine as a marker of oxidative damage in road cycling. *Free Radic Res*., **36**:247–253.

[114] Pagano, G., Degan, P., d'Ischia, M., Kelly, F.J., Pallardo, F.V., Zatterale, A., Anak, S.S., Akisik, E.E., Beneduce, G., Calzone, R., De Nicola, E., Dunster, C., Lloret, A., Manini, P., Nobili, B., Saviano, A., Vuttariello, E., and Warnau., M. 2004. Gender- and age-related distinctions for the *in vivo* prooxidant state in Fanconi anaemia patients. *Carcinogenesis*, **25**:1899–1909.

[115] Smith, M.R. 2003. Diagnosis and management of treatment-related osteoporosis in men with prostate carcinoma. *Cancer*, **97**:789–795.

[116] Calle, E.E., Rodriguez, C., Jacobs, E.J., Almon, M.L., Chao, A., McCullough, M.L., Feigelson, H. S., and Thun, M.J. 2002. The American Cancer Society Cancer Prevention Study II Nutrition Cohort: rationale, study design, and baseline characteristics. *Cancer*, **94**:500–511.

[117] Hartmann, A., Plappert, U., Raddatz, K., Grunert-Fuchs, M., and Speit, G. 1994. Does physical activity induce DNA damage? *Mutagenesis*, **9**:269–272.

[118] Sato, Y., Nanri, H., Ohta, M., Kasai, H., and Ikeda, M. 2003. Increase of human MTH1 and decrease of 8-hydroxydeoxyguanosine in leukocyte DNA by acute and chronic exercise in healthy male subjects. *Biochem. Biophys. Res. Commun*., **305**:333–338.

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

[120] Oh-ishi, S., Kizaki, T., Ookawara, T., Sakurai, T., Izawa, T., Nagata, N., and Ohno, H. 1997. Endurance training improves the resistance of rat diaphragm to exercise-induced oxidative stress. *Am. J. Respir. Crit. Care Med*., **156**:1579–1585.

[121] Peake, J., and Suzuki, K. 2004 Neutrophil activation, antioxidant supplements and exercise-induced oxidative stress. *Exerc. Immunol. Rev*., **10**:129–141.

[122] Hoffmann, D., and Hecht, S.S. 1989. Advances in tobacco carcinogenesis. In: *Handbook of Experimental Pharmacology*, pp. 63–109. Copper, C.S., and Grover, P.L. (Eds.), Springer-Verlan, New York.

[123] Pryor, W.A. 1997. Cigarette smoke radicals and the role of free radicals in chemical carcinogenicity. *Environ. Health Perspect*., **105** (Suppl 4):875–882.

[124] Spencer, J.P., Jenner, A., Chimel, K., Aruoma, O.I., Cross, C.E., Wu, R., and Halliwell, B. 1995. DNA damage in human respiratory tract epithelial

cells: damage by gas phase cigarette smoke apparently involves attack by reactive nitrogen species in addition to oxygen radicals. *FEBS Lett*., **375:**179–182.

[125] Kim, H.S., Kwack, S.J., and Lee, B.M. 2000. Lipid peroxidation, antioxidant enzymes, and benzo[a]pyrene-quinones in blood of rats treated with benzo[a]pyrene. *Chem. Biol. Interact.*, **127**:139–150.

[126] Speit, G., Witton-Davies, T., Heepchantree, W., Trenz, K., and Hoffmann, H. 2003. Investigations on the effect of cigarette smoking in the comet assay. *Mutat Res*., **542**:33–42.

[127] Mahadevan, B., Luch, A., Bravo, C.F., Atkin, J., Steppan, L.B., Pereira, C., Kerkvliet, N.I., and Baird, W. M. 2005. Dibenzo[a]pyrene induced DNA adduct formation in lung tissue *in vivo*. *Cancer Lett*., **227**:25–32.

[128] Schneider, M., Diemer, K., Engelhart, K., Zankl, H., Trommer, W.E., and Biesalski, H.K. 2001. Protective effects of vitamins C and E on the number of micronuclei in lymphocytes in smokers and their role in ascorbate free radical formation in plasma. *Free Radic Res*., **34**:209–219.

[129] Asami, S., Hideo, M., Miyake, J., Tsurudome, Y., Hirano, T., Yamaguchi, R., Itoh, H., and Kasai, H. 1997. Cigarette smoking induces an increase in oxidative DNA damage, 8-hydroxydeoxyguanosine, in a central site of the human lung. *Carcinogenesis*, **18**:1763–1766.

[130] Nia, A.B., Van Schooten, F.J., Schilderman, P.A.E.L., De Kok, T.M.C.M., Haenen, G.R., Van Herwijnen, M.H.M., Van Agen, E., Pachen, D., and Kleinjans, J.C.S. 2001. A multi-biomarker approach to study the effects of smoking on oxidative DNA damage and repair and antioxidative defense mechanisms. *Carcinogenesis*, **21**:395–401.

[131] Hoffmann, H., Isner, C., Hogel, J., and Speit, G. 2005. Genetic polymorphisms and the effect of cigarette smoking in the comet assay. *Mutagenesis*, **20**:359–364.

[132] Fraga, C.G., Motchnik, P.A., Wyrobek, A.J., Rempel, D.M., and Ames, B.N. 1996. Smoking and low antioxidant levels increase oxidative damage to sperm DNA. *Mutat Res*., **351**:199–203.

[133] Shen, H.M., Chia, S.E., Ni, Z.Y., New, A.L., Lee, B.L., and Ong, C.N. 1997. Detection of oxidative DNA damage in human sperm and the association with cigarette smoking. *Reprod Toxicol.*, **11**:675–680.

[134] Ames, B. N., Motchnik, P.A., Fraga, C.G., Shigenaga, M.K., and Hagen, T.M. 1994. Antioxidants prevention of birth defects and cancer. In: *Male-mediated development toxicology,*. pp. 242–259. Mattison, D. R., Olshan, A. Eds. New York: Plenum Publishing Co.

[135] Cummins, J.M., Jequier, A.M., and Kan, R. 1994. Molecular biology of human male infertility: linked with aging, mitochondrial genetics, and oxidative stress? *Mol. Reprod. Dev*., **37**:345–362.

[136] DeVries, E., Bray, F.I., Coebergh, J.W., and Parkin, D.M. 2003. Changing epidemiology of malignant cutaneous melanoma in Europe 1953–1997: Rising trends in incidence and mortality but recent stabilization in Western Europe and decreases in Scandinavia. *Int. J. Cancer*, **107**:119–126.

[137] Herrero-Jimenez, P., Tomita-Mitchell, A., Furth, E.E., Morgenthaler, S., and Thilly, W.G. 2000. Population risk and physiological rate parameters for colon cancer. The union of an explicit model for carcinogenesis with the public health records of the United States. *Mutat. Res*., **447**:73–116.

[138] Atamna, H., Cheung, I., and Ames, B. N. 2000. A method for detecting abasic sites in living cells: age-dependent changes in base excision repair. *Proc. Natl. Acad. Sci. USA*, **97**:686–691.

[139] Hoelzl, C., Bichler, J., Ferk, F., Simic, T., Nersesyan, A., Elbling, L., Ehrlich, V., Chakraborty, A., and Knasmuller, S. 2005. Methods for the detection of antioxidants which prevent age related diseases: a critical review with particular emphasis on human intervention studies. *J. Physiol. Pharmacol*., **56**(Suppl 2):49–64.

[140] Ames, B.N., and Saul, R.L. 1986. Oxidative DNA damage as related to cancer and aging. *Proc. Clin. Biol. Res.*, **209A**:11–26.

[141] Ames, B.N., Shigenaga, M.K., and Hagen, T.M. 1995. Mitochondrial decay in aging. *Biochim. Biophys. Acta*., **1271**:165–170.

[142] Hamilton, M.L., Van Remmen, H., Drake, J.A., Yang, H., Guo, Z.M., Kewitt, K., Walter, C.A., and Richardson, A. 2001. Does oxidative damage to DNA increase with age? *Proc. Natl. Acad. Sci. USA*, **18**:10469–10474.

[143] Bohr, V., Anson, R.M., Mazur, S., and Dianov, G. 1998. Oxidative DNA damage processing and changes with aging. *Toxicol Lett*., **102**:47–52.

[144] Mecocci, P., MacGarvey, U., Kaufman, A.E., Koontz, D., Shoffner, J.M., Wallace, D.C., and Beal, M.F. 1993. Oxidative damage to mitochondrial DNA shows marked age-dependent increases in human brain. *Ann. Neurol*., **34**:609–616.

[145] Lagorio, S., Tagesson, C., Forastiere, F., Axelson, O., and 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.

[146] Hayakawa, M., Hattori, K., Sugiyama, S., and Ozawa, T. 1992. Age-associated oxygen damage and mutations in mitochondrial DNA in human hearts. *Biochem. Biophys. Res. Commun*., **189**:979–985.

[147] Jia, G., Tohyama, C., and Sone, H. 2002. DNA damage triggers imbalance of proliferation and apoptosis during development of preneoplastic foci in the liver of Long-Evans Cinnamon rats. *Int. J. Oncol*., **21**:755–761.

[148] Lopez-Torres, M., Gredilla, R., Sanz, A., and Barja, G. 2002. Influence of aging and long-term caloric restriction on oxygen radical generation and oxidative DNA damage in rat liver mitochondria. *Free Rad. Biol. Med.*, **32**:882–889.

[149] Mendoza-Nunez, V.M., Sanchez-Rodriguez, M.A., Retana-Ugalde, R., Vargas-Guadarrama, L.A., and Altamirano-Lozano, M.A. 2001. Total antioxidant levels, gender, and age as risk factors for DNA damage in lymphocytes of the elderly. *Mech. Aging Devel.*, **122**:835–847.

[150] Chen, Y.C., Liang, Y.C., Lin-Shiau, S.Y., Ho, C.T., and Lin, J.K. 1999. Inhibition of T.P.A.-induced protein kinase C and transcription activator protein-1 binding activities by theaflavin-3,3′-digallate from black tea in NIH3T3 cells. *J. Agric. Food Chem*., **47**:1416–1421.

[151] Proteggente, A.R., England, T.G., Rehman, A., Rice-Evans, C.A., and Halliwell, B. 2002. Gender differences in steady-state levels of oxidative damage to DNA in healthy individuals. *Free Radic Res*., **36**:157–162.

[152] Weindruch, R., Kayo, T., Lee, C.K., and Prolla, T.A. 2001. Microarray profiling of gene expression in aging and its alteration by caloric restriction in mice. *J. Nutr*., **131**:918S–923S.

[153] Djuric, Z., Lewis, S.M., Lu, M.H., Mayhugh, M., Naegeli, L., Tang, N., and Hart, R.W. 2002. Effect of varying caloric restriction levels on female rat growth and 5-hydroxymethyl-2′-deoxyuridine in DNA. *Toxicol Sci*., **66**:125–130.

[154] Yu, B.P. 1994. How diet influences the aging process of the rat. *Proc. Soc. Exp. Biol. Med*., **205**:97–105.

[155] Aidoo, A., Desai, V.G., Lyn-Cook, L.E., Chen, J.J., Feuers, R.J., and Casciano, D.A. 1999. Attenuation of bleomycin-induced Hprt mutant frequency in female and male rats by calorie restriction. *Mutat Res*., **430**:155–163.

[156] Weindruch, R., Kayo, T., Lee, C.K., and Prolla, T.A. Microarray profiling of gene expression in aging and its alteration by caloric restriction in mice. *J. Nutr*., **131**:918S–923S.

[157] Chung, M.H., Kasai, H., Nishimura, S., and Yu, B.P. 1992. Protection of DNA damage by dietary restriction. *Free Radic. Biol. Med.*, **12**:523–525.

[158] Li, Y., Yan, Q., and Wolf, N.S. 1997. Long-term caloric restriction delays age-related decline in proliferation capacity of murine lens epithelial cell in vitro and in vivo. *Invest. Ophthalmol. Visual Sci*., **38**:100–106.

[159] Fischer, W.H., and Lutz, W.K. 1998. Influence of diet restriction and tumor promoter dose on cell proliferation, oxidative DNA damage and rate of papilloma appearance in the mouse skin after initiation with DMBA and promotion with TPA. *Toxicol. Lett.*, **98**:59–69.

[160] Desai, V.G., Weindruch, R., Hart, R.W., and Feuers, R.J. 1996. Influences of age and dietary restriction on gastronemius electron transport system activities in mice. *Arch. Biochem. Biophys.*, **333**:145–157.

[161] Greenberg, J.A., Wei, H., Ward, K., and Boozer, C.N. 2000. Whole-body metabolic rate appears to determine the rate of DNA oxidative damage and glycation involved in aging. *Mech Ageing Dev.*, **115**:107–117.

[162] Djuric, Z., Lu, M.H., Lewis, S.M., Luongo, D.A., Chen, X.W., Heilbrun, L.K., and Reading, B.S. 1992. Oxidative DNA damage levels in rats fed low-fat, high-fat, or calorie-restricted diets. *Toxicol. Appl. Pharmacol.*, **115**:156–160.

[163] Coffey, D.S. 2001. Similarities of prostate and breast cancer: Evolution, diet, and estrogens. *Urology*, **57**:31–38.

[164] Bartsch, H., Nair, J., and Owen, R.W. 2002. Exocyclic DNA adducts as oxidative stress markers in colon carcinogenesis: potential role of lipid peroxidation, dietary fat and antioxidants. *Biol. Chem*., **383**:915–921.

[165] Bartsch, H., Nair, J., and Owen, R.W. 1999. Dietary polyunsaturated fatty acids and cancers of the breast and colorectum: emerging evidence for their role as risk modifiers. *Carcinogenesis*, **20**:2209–2218.

[166] Ritskes-Hoitinga, J., Meijers, M., Timmer, W.G., Wiersma, A., Meijer, G.W., and Weststrate, J.A. 1996. Effects of two dietary fat levels and four dietary linleic acid levels on mammary tumor development in Balb/c-MMTV mice under ad libitum feeding conditions. *Nutr. Cancer*, **25**:161–172.

[167] Dreher, D., and Junod, A.F. 1996. Role of oxygen free radicals in cancer development. *Eur. J. Cancer*, **32A**:30–38 .

[168] Bartsch, H., Barbin, A., Marion, M.J., Nair, J., and Cuichard, Y. 1994. Formation, detection, and role in carcinogenesis of ethenobases. *Drug Metab. Rev.*, **26**:349–371.

[169] Nair, J., Vaca, C.E., Velic, I., Mutanen, M., Valsta, L.M., and Barsch, H. 1997. High dietary ω-6 polyunsaturated fatty acids drastically increase the formation of etheno-DNA base adducts in white blood cells of female subjects. *Cancer Epidemiol. Biomarkers Prev*., **6**:597–601.

[170] Wang, H., Cao, G.H., and Prior, R.L. 1996. Total antioxidant capacity of fruits. *J. Agric. Food Chem*., **44**:701–705.

[171] Kang, D.H. 2002. Oxidative stress, DNA damage, and breast cancer. *AACN Clin. Issues*. **13**:540–549.

[172] Djuric, Z., Heilbrun, L.K., Reading, B.A., Boomer, A., Valeriote, F.A., and 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.

[173] Djuric, Z., Lababidi, S., Uhley, V.E., and Heilbrun, L.K. 2004. Levels of 5-hydroxymethyl-2′-deoxyuridine in DNA from women participating in an intervention trial of low-fat and low-energy diets. *Biomarkers*, **9**:93–101.

[174] Djuric, Z., Chen, G., Doerge, D.R., Heilbrun, L.K., and Kucuk, O. 2001. Effect of soy isoflavone supplementation on markers of oxidative stress in men and women. *Cancer Lett.*, **172**:1–6.

[175] Jenkinson, A.M., Collins, A.R., Duthie, S.J., Wahle, K.W., and Duthie, G.G. 1999. The effect of increased intakes of polyunsaturated fatty acids and vitamin E on DNA damage in human lymphocytes. *FASEB J.*, **13**:2138–2142.

[176] Quiles, J.L., Ochoa, J.J., Ramirez-Tortosa, C., Battino, M., Huertas, J.R., Martin, Y., and Mataix, J. 2004. Dietary fat type (virgin olive vs. sunflower oils) affects age-related changes in DNA double-strand-breaks, antioxidant capacity and blood lipids in rats. *Exp. Geronotol*., **39**:1189–1198.

[177] Elmadfa, I., and Park, E. 1999. Impact of diets with corn oil or olive/sunflower oils on DNA damage in healthy young men. *Eur. J. Nutr.*, **38**:286–292.

[178] Vogel, U., Danesvar, B., Autrup, H., Risom, L., Weiman, A., Poulsen, H.E., Moller, P., Loft, S., Wallin, H., and Dragsted, L.O. 2003. Effect of increased intake of dietary animal fat and fat energy on oxidative damage, mutation frequency, DNA adduct level and DNA repair in rat colon and liver. *Free Radic Res*., **37**:947–956.

[179] Ahn, J., Gammon, M.D., Santella, R.M., Gaudet, M.M., Britton, J.A., Teitelbaum, S.L., Terry, M.B., Nowell, S., Davis, W., Garza, C., Neugut, A.I., and Ambrosone, C.B. 2005. Associations between breast cancer risk and the catalase genotype, fruit and vegetable consumption, and supplement use. *Am. J. Epidemiol*., **162**:943–952.

[180] Ferriari, P., Al-Delaimy, W.K., Slimani, N., Boshuizen, H.C., Roddam, A., Orfanos, P., Skeie, G., Rodriguez-Barranco, M., Thiebaut, A., Johansson, G., Palli, D., Boeing, H., Overvad, K., and Riboli, E. 2005. An approach to estimate between- and within-group correlation coefficients in multicenter studies: plasma carotenoids as biomarkers of intake of fruits and vegetables. *Am. J. Epidemiol*., **162**:591–598.

[181] Michaud, D.S., Pietinen, P., Taylor, P.R., Virtanen, M., Virtamo, J., and Albanes, D. 2002. Intakes of fruits and vegetables, carotenoids and vitamins A, E, C in relation to the risk of bladder cancer in the ATBC cohort study. *Br. J. Cancer*, **87**:960–965.

[182] Genkinger, J.M., Platz, E.A., Hoffman, S.C., Comstock, G.W., and Helzlsouer, K.J. 2004. Fruit, vegetable, and antioxidant intake and all-cause, cancer, and cardiovascular disease mortality in a community-dwelling population in Washington County, Maryland. *Am. J. Epidemiol*., **160**:1223–1233.

[183] Kiefer, I., Prock, P., Lawrence, C., Wise, J., Bieger, W., Bayer, P., Rathmanner, T., Kunze, M., and Rieder, A. 2004. Supplementation with mixed fruit and vegetable juice concentrates increased serum antioxidants and folate in healthy adults. *J. Am. Coll. Nutr*., **23**:205–211.

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

[185] Witt, E.H., Reznick, A.Z., Viguie, C.A., Starke-Reed, P., and Packer, L. 1992. Exercise, oxidative damage and effects of antioxidant manipulation. *J. Nutr*., **122**:766–773.

[186] The alpha-tocopherol, beta-carotene cancer prevention study 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.

[187] Van Hoydonck, P.G., Temme, E.H., and Schouten, E.G. 2002. A dietary oxidative balance score of vitamin C, beta-carotene and iron intakes and mortality risk in male smoking Belgians. *J. Nutr*., **132**:756–761.

[188] Palozza, P., Serini, S., Di Nicuolo, F., Piccioni, E., and Calviello, G. 2003. Prooxidant effects of beta-carotene in cultured cells. *Mol Aspects Med*., **24**:353–362.

[189] Hwang, E.S., and Bowen, P.E. 2005. Effects of lycopene and tomato paste extracts on DNA and lipid oxidation in LNCaP human prostate cancer cells. *Biofactors*, **23**:97–105.

[190] Drouin, R., Rodriguez, H., Gao, S.W., Gebreyes, A., O'Connor, T.R., Holmquist, G.P., and Akman, S.A. 1996. Cupric ion/ascorbate/hydrogen peroxide-induced DNA damage: DNA-bound copper ion primarily induces base modifications. *Free Radic. Biol. Med*., **21**:261–273.

[191] Yatsuzuka, M., Tazawa, K., Yasuda, T., Yoshii, M., Ogawa, K., and Wada, S. 2004. Identification of hydroxyl radicals after UV irradiation of aqueous extracts of cigarette smoke and evaluation of urinary 8-OHdG in smokers. *Asian Pac. J. Cancer Prev*., **5**:279–283.

[192] Hu, M.L., and Shih, M.K. 1997. Ascorbic acid inhibits lipid peroxidation but enhances DNA damage in rat liver nuclei incubated with iron ions. *Free Radic. Res*., **26**:585–592.

[193] Fiala, E.S., Sodum, R.S., Bhattacharya, M., and Li, H. 1996. (−)-Epigallocatechin gallate, a polyphenolic tea antioxidant, inhibits peroxynitrite-mediated formation of 8-oxodeoxyguanosine and 3-nitrotyrosine. *Experientia*, **52**:922–926.

[194] Wei, H., Cai, Q., Tian, L., and Lebwohl, M. 1998. Tzmoxifen reduces endogenous and UV light-induced oxidative damage to DNA, lipid and protein in vitro and *in vivo*. *Carcinogenesis*, **19**:1013–1018.

[195] Noroozi, M., Angerson, W.J., and Lean, M.E.J. 1998. Effects of flavonoids and vitamin C on oxidative DNA damage to human lymphocytes. *Am J. Clin. Nutr*., **67**:1210–1218.

[196] Pflaum, M., Kielbassa, C., and Garmyn, M. 1998. Oxidative DNA damage induced by visible light in mammalian cells: extent, inhibition by antioxidants and genotoxic effects. *Mutat. Res*., **408**:137–146.

[197] Singh, N. 1997. Sodium ascorbate induces DNA single-strand breaks in human cells in vitro. *Mutat. Res*., **375**:195–203.

[198] Green, M.H.L., Lowe, J.E., Waugh, A.P.W., Aldridge, K.E., Cole, J., and Arlett, C.F. 1994. Effect of diet and vitamin C on DNA strand breakage in freshly-isolated human white blood cells. *Mutat. Res.*, **316**:91–102.

[199] Cadenas, S., Barja, G., Poulsen, H.E., and Loft, S. 1997. Oxidative DNA damage estimated by oxo8dG in the liver of guinea-pigs supplemented

with graded dietary doses of ascorbic acid and α-tochpherol. *Carcinogenesis*, **18**:2372–2377.

[200] Reddy, V.N., Giblin, F.J., Lin, J.R, and Chakrapani, B. 1998. The effect of aqueous humor ascorbate on ultraviolet-B-induced DNA damage in lens epithelium. *Invest. Opthalmol. Vis. Sci*., **39**:344–350.

[201] Fraga, C.G., Motchnik, P.A., Shigenaga, M.K., Helbock, H.J., Jacob, R.A., and Ames, B.N. 1991. Ascorbic acid protects against endogenous oxidative DNA damage in human sperm. *Proc. Natl. Acad. Sci. USA*, **88**:1100–11006.

[202] Anderson, D., Phillips, B.J., Yu, T., Edwards, A.J., Ayesh, R., and Butterworth, K.R. 1997. The effects of vitamin C supplementation on biomarkers of oxygen radical generated damage in human volunteers with "low" or "high" colesterol levels. *Environ. Mol. Mutagen*., **30**:161–174.

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

[204] Rehman, A., Collis, C.S., Yang, M., Kelly, M., Diplock, A.T., Halliwell, B., and Rice-Evans, C. 1998. The effects of iron and vitamin C co-supplementation on oxidative damage to DNA in healthy volunteers. *Biochem. Biophys. Res. Commun*., **246**:293–298.

[205] Carr, A., and Frei, B. 1999. Does vitamin C act as a pro-oxidant under physiological conditions? *FASEB J.*, **13**:1007–1024.

[206] McCall, M.R., and Frei, B. 1999. Can antioxidant vitamins materially reduce oxidative damage in humans? *Free Rad. Biol. Med.*, **26**:1034–1053.

[207] Lee, B.M., Lee, S.K., and Kim, H.S. 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 Lett*., **132**:219–227.

[208] Cooke, M.S., Evans, M.D., Podmore, I.D., Herber, K.E., Mistry, N., Mistry, P., Hickenbotham, P.T., Hussieni, A., Griffiths, H.R., and Lunec, J. 1998. Novel repair action of vitamin C upon in vivo oxidative DNA damage. *FEBS Lett.*, **139**:363–367.

[209] Jiang, J., Suzuki, S., Xiang, K., Kuriki, K., Hosono, A., Arakawa, K., Wang, J., Nagaya, T., Kojima, M., Katsuda, N., and Tokudome, S. 2005. Plasma carotenoid, alpha-tocopherol and retinol concentrations and risk of colorectal adenomas: A case-control study in Japan. *Cancer Lett.*, **226**:133–141.

[210] Kim, J., Sun, P., Lam, Y.W., Troncoso, P., Sabichi, A.L., Babaian, R.J., Pisters, L.L., Pettaway, C.A., Wood, C.G., Lippmann, S.M., McDonnell, T.J., Lieberman, R., Logothets, C., and Ho, S.M. 2005. Changes in serum proteomic patterns by presurgical alpha-tocopherol and L-selenomethionine supplementation in prostate cancer. *Cancer Epidemiol Biomarkers Prev*., **14**:1697–1702.

[211] Chung, H., Wu, D., Han, S.N., Gay, R., Goldin, B., Bronson, R.E., Mason, J.B., Smith, D.E., and Meydani, S.N. 2003. Vitamin E supplementation does not alter azoxymethane-induced colonic aberrant crypt foci formation in young or old mice. *J. Nutr*., **133**:528–532.

[212] Lunec, J., Halligan, E., Mistry, N., and Karakoula, K. 2004. Effect of vitamin E on gene expression changes in diet-related carcinogenesis. *Ann. NY. Acad. Sci*., **1031**:169–183.

[213] Duthie, S.J., Gardner, P.T., Morrice, P.C., Wood, S.G., Pirie, L., Bestwick, C.C., Milne, L., and Duthie, G.G. 2004. DNA stability and lipid peroxidation in vitamin E-deficient rats in vivo and colon cells in vitro. Modulation by the dietary anthocyanin, cyanding-3-glycoside. *Eur J. Nutr.*, **44**:195–203.

[214] Mahabir, S., Frenkel, K., Brady, M.S., Coit, D., Leibes, L., Karkoszka, J., and Berwick, M. 2004. Randomized, placebo-controlled pilot trial of the effects of alpha-tocopherol supplementation on levels of autoantibdies against 5-hydroxymethyl-2-deoxyuridine in melanoma patients. *Melanoma Res*., **14**:49–56.

[215] Adzersen, K.H., Jess, P., Freivogel, K.W., Gerhard, I., and Bastert, G. 2003. Raw and cooked vegetables, fruits, selected micronutrients, and breast cancer risk: a case-control study in Germany. *Nutr. Cancer*, **46:**131–137.

[216] Nkondjock, A., and Ghadirian, P. 2004. Dietary carotenoids and risk of colon cancer: case-control study. *Int. J. Cancer*, **110**:110–116.

[217] Prakash, P., Liu, C., Hu, K.Q., Krinsky, N.I., Russell, R.M., and Wang, X.D. 2004. Beta-carotene and beta-apo-14′-carotenoic acid prevent the reduction of retinoic acid receptor beta in benzo[a]pyrene-treated normal human bronchial epithelial cells. *J. Nutr.*, **134**:667–673.

[218] Trobs, M., Renner, T., Scherer, G., Heller, W.D., Geiss, H.C., Wolfram, G., Hass, G.M., and Schwandt, P. 2002. Nutrition, antioxidants, and risk factor profile of nonsmokers, passive smokers and smokers of the Prevention Education Program (PEP) in Nuremberg, Germany. *Prev. Med.*, **34**:600–607.

[219] Speit, G., Witton-Davies, T., Heepchantree, W., Trenz, K., and Hoffmann, H. 2003. Investigations on the effect of cigarette smoking in the comet assay. *Mutat. Res*., **542**:33–42.

[220] Zhang, P., and Omaye, S.T. 2001. Antioxidant and prooxidant roles for beta-carotene, alpha-tocopherol and ascorbic acid in human lung cells. *Toxicol. In Vitro*. **15**:13–24.

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

[222] Riso, P., Pinder, A., Santangelo, A., and Porrini, M. 1999. Does tomato consumption effectively increase the resistance of lymphocyte DNA to oxidative damage? *Am. J. Clin. Nutr.*, **69**:712–718.

[223] Porrini, M., and Riso, P. 2000. Lymphocyte lycopene concentration and DNA protection from oxidative damage is increased in women after a short period of tomato consumption. *J. Nutri*., **130**:189–192.

[224] Hwang, E., and Bowen, P.E. 2004. Effects of lycopene on lipid peroxidation and oxidative DNA damage in LNCaP human prostate cancer cells. *Food Sci. Biotechnol*., **13**:297–301.

[225] Weiller, M., Latta, M., Kresse, M., Lucas, R., and Wendel, A. 2004. Toxicity of nutritionally available selenium compounds in primary and transformed hepatocytes. *Toxicology*, **201**:21–30.

[226] Spallholz, J.E. 1994. On the nature of selenium toxicity and carcinostatic activity. *Free Radic. Biol. Med*., **17**:45–64.

[227] Combs, G.F. Jr., and Gray, W. P. 1998. Chemopreventive agents: selenium. *Pharmacol. Ther.*, **79**:179–192.

[228] Ip, C. 1998. Lessons from basic research in selenium and cancer prevention. *J. Nutr*., **128**:1845–1854.

[229] Stewart, M.S., Spallholz, J.E., Neldner, K.H., and Pence, B.C. 1999. Selenium compounds have disparate abilities to impose oxidative stress and induce apoptosis. *Free Radic. Biol. Med*., **26**:42–48.

[230] Giovannucci, E., Rimm, E.B., Liu, Y., Stampfer, M.J., and Willett, W.C. 2003. A prospective study of cruciferous vegetables and prostate cancer. *Cancer Epidemiol. Biomarkers Prev.*, **12**:1403–1409.

[231] Brennan, P., Hsu, C.C., Moullan, N., Szeszenia-Dabrowska, N., Lissowska, J., Zaridze, D., Rudnai, P., Fabianova, E., Mates, D., Bencko, V., Foretova, L., Janout, V., Gemignani, F., Chabrier, A., Hall, J., Hung, R.J., Boffetta, P., and Canzian, F. 2005. Effect of cruciferous vegetables on lung cancer in patients stratified by genetic status: a mendelian randomisation approach. *Lancet*, **366**:1558–1560.

[232] McNaughton, S.A., and Marks, G.C. 2003. Development of a food composition database for the estimation of dietary intakes of glucosinolates, the biologically active constituents of cruciferous vegetables. *Br. J. Nutr.*, **90**:687–697.

[233] Matusheski, N.V., and Jeffery, E.H. 2001. Comparison of the bioactivity of two glucoraphanin hydrolysis products found in broccoli, sulforaphane and sulforaphane nitrile. *J. Agric. Food Chem*., **49**:5743–5749.

[234] Joseph, M.A., Moysich, K.B., Freudenheim, J.L., Shields, P.G., Bowman, E.D., Zhang, Y., Marshall, J.R., and Ambrosone, C.B. 2004. Cruciferous vegetables, genetic polymorphisms in glutathione S-transferases M1 and T1, and prostate cancer risk. *Nutr. Cancer*, **50**:206–213.

[235] Paolini, M., Perocco, P., Canistro, D., Valgimigli, L., Pedulli, G.F., Iori, R., Croce, C.D., Cantelli-Forti, G., Legator, M. S., and Abdel-Rahman, S. Z. 2004. Induction of cytochrome P450, generation of oxidative stress and in vitro cell-transforming and DNA-damaging activities by gluco-

raphanin, the bioprecursor of the chemopreventive agent sulforaphane found in broccoli. *Carcinogenesis*, **25**:61–67.

[236] Zhu, C., Poulsen, E., and Loft, S. 2000. Inhibition of oxidative DNA damage in vitro by extract of Brussels sprouts. *Free Radic. Res.*, **33**:187–196.

[237] Sorensen, M., Jensen, B.R., Poulsen, H.E., Deng, X-S., Tygstrup, N., Dalhoff, K., and Loft, S. 2001. Effects of a Brussels sprouts extract on oxidative DNA damage and metabolizing enzymes in rat liver. Food and Chem. *Toxicol.*, **39**:533–540.

[238] Horn-Ross, P.L., John, E.M., Lee, M., Stewart, S.L., Koo, J., Sakoda, L.C., Shiau, A.C., Goldstein, J., Davis, P., and Perez-Stable, E.J. 2001. Phytoestrogen consumption and breast cancer risk in a multiethnic population: the Bay Area Breast Cancer Study. *Am. J. Epidemiol.*, **154**:434–441.

[239] Zhang, M., Xie, X., Lee, A. H., and Binns, C.W. 2004. Soy and isoflavone intake are associated with reduced risk of ovarian cancer in southeast china. *Nutr. Cancer*, **49**:125–130.

[240] Shannon, J., Ray, R., Wu, C., Nelson, Z., Gao, D.L., Li, W., Hu, W., Lampe, J., Horner, N., Satia, J., Patterson, R., Fitzgibbons, D., Porter, P., and Thomas, D. 2005. Food and botanical groupings and risk of breast cancer: a case-control study in Shanghai, China. *Cancer Epidemiol. Biomarkers Prev.*, **14**:81–90.

[241] Messina, M. 1995. Isoflavone intakes by Japanese were overestimated. *Am. J. Clin. Nutr.*, **62**:645–649.

[242] Davis, J.N., Kucuk, O., Djuric, Z., and Sarkar, F.H. 2001. Soy isoflavone supplementation in healthy men prevents NF-kB activation by TNF-α in bloos lymphocytes. *Free Radic. Biol. Med.*, **30**:1293–1302.

[243] Ruiz-Larrea, M.B., Mohan, A.R., Paganga, G., Miller, N.J., Bolwell, G.P., and Rice-Evans, C.A. 1997. Antioxidant activity of phytoestrogenic isoflavones. *Free Radic. Res.*, **26**:63–70.

[244] Stoll, B.A. 1997. Eating to beat breast cancer: potential role for soy supplements. *Ann. Oncol.*, **8**:223–225.

[245] Arora, A., Nair, M.G., and Strasburg, G.M. 1998. Antioxidant activities of isoflavones and their biological metabolites in a liposomal system. *Arch. Biochem. Biophys.*, **356**:133–141.

[246] Cai, Q., and Wei, H. 1996. Effect of dietary genistein on antioxidant enzyme activities in SENCAR mice. *Nutr. Cancer*, **25**:1–7.

[247] Mitchell, J.H., and Collins, A.R. 1999. Effects of a soy milk supplement on plasma cholesterol levels and oxidative DNA damage in men-a pilot study. *Eur. J. Nutr.*, **38**:143–148.

[248] Shahidi, F., and Naczk, M. 1995. *Food Phenoilics: Source, Chemistry, Effects, Applications*. Technomic Publishing Company, Inc.: Lancaster, PA, USA.

[249] Liu, M., Li, X. Q., Weber, C., and Liu, R.H. 2002. Antioxidant and antiproliferative activities of raspberries. *J. Agric. Food Chem.*, **50**:2926–2930.

[250] Garbisa, S., Sartor, L., Biggins, S., Salvato, B., Benelli, R., and Albini, A. 2001. Tumor gelatinases and invasion inhibited by the green tea flavanol epigallocatechin-3-gallate. *Cancer*, **91**:822–832.

[251] Russo, A., Acquaviva, R., Campisi, A., Sorrenti, V., Di Giacomo, C., Virgata, G., Barcellona, M.L., and Vanella, A. 2000. Bioflavonoids as antiradicals, antioxidants and DNA cleavage protectors. *Cell Biol. Technol.*, **16**:91–98.

[252] Mukhtar, H., and Ahmad, N. 2000. Tea polyphenols: prevention of cancer and optimizing health. *Am. J. Clin. Nutr.*, **71**:1698S–1702S.

[253] Bravo, L. 1998. Polyphenols: chemistry, dietary source, metabolism, and nutritional significance. *Nutr. Rev.*, **56**:317–333.

[254] Ohe, T., Marutani, K., and Nakase, S. 2001. Catechins are not major components responsible for anti-genotoxic effects of tea extracts against nitroarenes. *Mutat. Res.*, **496**: 75–81.

[255] Embola, C.W., Weisburger, M.C., and Weisburger, J.H. 2001. Green tea and the metabolism of 2-amino-3-methylimidazo. *Food Chem. Toxicol.*, **39**:629–633.

[256] Ripple, M.O., Henry, W.F., Schwarze, S.R., Wilding, G., and Weindruch, R. 1999. Effect of antioxidants on androgen-induced AP-1 and NF-κB DNA-binding activity in prostate carcinoma cells. *J. Natl. Cancer Inst.*, **91**:1227–1232.

[257] Fujiki, H., Suganuma, M., Okabe, S., Sueoka, E., Suga, K., Imai, K., and Nakachi, K. 2000. A new concenpt of tumor promotion by tumor necrosis factor-alpha, and cancer preventive agents (−)-epigallocatechin gallate and green tea: a review. *Cancer Detection Prev.*, **24**:91–99.

[258] Aucamp, J., Gaspar, A., and Hara, Y. 1996. Apostolides Z. Inhibition of xanthine oxidase by catechins from tea (Camellia sinensis). *Anticancer Research*, **17**:4381–4385.

[259] Trosko, J.E. 2001. Commentary: is the concept of "tumor promotion" a useful paradigm? *Molecular Carcinogenesis*, **30**:131–137.

[260] Ahmad, N., Feyes, D.K., Nieminen, A.L., Agarwal, R., and Mukhtar, H. 1997. Green tea constituent epigallocatechin-3-gallate and induction of apoptosis and cell cycle arrest in human carcinoma cells. *J. Natl. Cancer Inst.*, **89**:1881–1886.

[261] Katiyar, S.K., Perez, A., and Mukhtar, H. 2000. Green tea polyphenol treatment to human skin prevents formation of ultraviolet light B-induced pyrimidine dimers in DNA. *Clin Cancer Res.*, **6**:3864–3869.

[262] Lin, J.K., and Liang, Y.C. 2000. Cancer chemoprevention by tea polyphenols. *Proc. Natl. Sci. Counc. Repub. China B.*, **24**:1–13.

[263] Wei, H., Zhang, X., Zhao, J.-F., Wang, Z.-Y., Bickers, D., and Lebwohl, M. 1999. Scavenging of hydrogen peroxide and inhibition of ultraviolet light-induced oxidative DNA damage by aqueous extracts from green and black teas. *Free Radic. Biol. Med.*, **26**:1427–1435.

[264] Klaunig, J.E., Xu, Y., Han, C., Kamendulis, L.M., Chen, J., Heiser, C., Gordon, M.S., and Mohler, E. R. 1999. The effect of tea consumption on oxidative stress in smokers and nonsmokers. *Proc. Soc. Exp. Biol. Med.*, **220**:249–254.

[265] Hakim, I.A., Harris, R.B., Chow, H.H., Dean, M., Brown, S., and Ali, I.U. 2004. Effect of a 4-month tea intervetion on oxidative DNA damage among heavy smokers: role of glutathione S-transferase genotype. *Cancer Epidemiol. Biomarkers Prev.*, **13**:242–249.

[266] Schwartz, J.L., Muscat, J.E., Baker, V., Larios, E., Stephenson, G.D., Guo, W., Xie, T., Gu, Z., and Chung, F.L. 2004. Oral cytology assessment by flow cytometry of DNA adducts, aneuploidy, proliferation and apoptosis shows differences between smokers and non-smokers. *Oral Oncol.*, **39**:842–854.

[267] Das, D.K., Sato, M., Ray, P.S., Maulik, G., Engleman, R.M., Bertelli, A.A.E., and Bertelli, A. 1999. Cardioprotection of red wine: role of polyphenolic antioxidants. *Drugs Under Experimental and Clinical Research*, **25**: 115–120.

[268] Clifford, A.J., Ebeler, S.E., Ebeler, J.D., Bills, N.D., Hinrichs, S.H., Teissedre, P. L., and Waterhouse, A.L. 1996. Delayed tumor onset in transgenic mice fed an amino acid-based diet supplemented with red wine solids. *Am. J. Clin. Nutr.*, **64**:748–756.

[269] Uenobe, F., Nakamura, S., and Miyazawa, M. 1997. Antimutagenic effect of resveratrol against Trp-P-1. *Mutat. Res.*, **373**:197–200.

[270] Mizutani, K., Ikeda, K., Kawai, Y., and Yamori, Y. 2001. Protective effect of resveratrol on oxidative damage in male and female stroke-prone spontaneously hypertensive rats. *Clin. Exp. Pharmacol. Physiol.*, **28**: 55–59.

[271] Leighton, F., Cuevas, A., Guasch, V., Perez, D. D., Strobel, P., San Martin, A., Urzua, U., Diez, M. S., Fonces, R., Castillo, O., Mizon, C., Espinoza, M.A., Urquiaga, I., Rozowski, J., Maiz, A., and Germain, A. 1999. Plasma polyphenols and antioxidants, oxidative DNA damage and endothelial function in a diet and wine intervention study in humans. *Drugs Exp. Clin. Res.*, **25**:133–141.

[272] Fenech, M., and Ferguson, L.R. 2001. Vitamins/minerals and genomic stability in humans. *Mutation Res.*, **475**:1–6.

[273] Helsby, N.A., Zhu, S., Pearson, A.E., Tingle, M.D., and Ferguson, L.R. 2000. Antimutagenic effects of wheat bran diet through modification of xenobiotic metabolizing enzymes. *Mutat. Res.*, **454**:77–88.