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Use of conventional and -omics based methods for health claims of dietary antioxidants: a critical overview

Siegfried Knasmüller[1]*†, Armen Nersesyan[1]†, Miroslav Mišík[1], Christopher Gerner[1], Wolfgang Mikulits[1], Veronika Ehrlich[1], Christine Hoelzl[1], Akos Szakmary[1] and Karl-Heinz Wagner[2]

[1]*Institute of Cancer Research, Inner Medicine I, Medical University of Vienna, Borschkegasse 8a, A-1090 Vienna, Austria*
[2]*Department of Nutritional Sciences, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria*

This article describes the principles and limitations of methods used to investigate reactive oxygen species (ROS) protective properties of dietary constituents and is aimed at providing a better understanding of the requirements for science based health claims of antioxidant (AO) effects of foods. A number of currently used biochemical measurements aimed of determining the total antioxidant capacity and oxidised lipids and proteins are carried out under unphysiologcial conditions and are prone to artefact formation. Probably the most reliable approaches are measurements of isoprostanes as a parameter of lipid peroxidation and determination of oxidative DNA damage. Also the design of the experimental models has a strong impact on the reliability of AO studies: the common strategy is the identification of AO by *in vitro* screening with cell lines. This approach is based on the assumption that protection towards ROS is due to scavenging, but recent findings indicate that activation of transcription factors which regulate genes involved in antioxidant defence plays a key role in the mode of action of AO. These processes are not adequately represented in cell lines. Another shortcoming of *in vitro* experiments is that AO are metabolised *in vivo* and that most cell lines are lacking enzymes which catalyse these reactions. Compounds with large molecular configurations (chlorophylls, anthocyans and polyphenolics) are potent AO *in vitro*, but weak or no effects were observed in animal/human studies with realistic doses as they are poorly absorbed. The development of -omics approaches will improve the scientific basis for health claims. The evaluation of results from microarray and proteomics studies shows that it is not possible to establish a general signature of alterations of transcription and protein patterns by AO. However, it was shown that alterations of gene expression and protein levels caused by experimentally induced oxidative stress and ROS related diseases can be normalised by dietary AO.

Dietary antioxidants: -Omics methods: Health claims

It is well documented that oxidative stress, defined by Blomhoff[1] as a "condition that is characterised by the accumulation of non-enzymatic oxidative damage to molecules that threaten the normal functions of a cell or the organism" is involved in the aetiology of a large number of human diseases. Typical examples are various forms of cancer (breast, colon and liver), neuropathological disorders such as Parkinson's and Altzheimer's disease, inflammations including hepatitis and inflammatory bowel diseases, different types of dermatitis as well as bacterial and viral infections (HBV, sepsis), diabetes, and rheumatoid arthritis (for review see[2]). Also coronary heart diseases which are the major cause of death in industrialised countries[2,3] and idiopathic infertility[4,5], which has increased over the last decades in Western countries seem to be causally related to reactive oxygen species (ROS) meditated damage and it has been stressed that oxidative damage is also involved in several diseases of ageing, e.g. Werner's syndrome[6], progeria[7], amyotrophic lateral sclerosis, cataract formation and decreased immune functions[8–10].

Already half a century ago it was found that the acute toxic, DNA-damaging and carcinogenic effects of ionising radiation which are predominantly caused by the formation of ROS can be reduced by antioxidant vitamins such as C, E, and A[11–13]. In the following decades, it became apparent that plant derived foods as well as beverages contain a large number of compounds which protect against oxidative damage and its consequences. Typical examples for such antioxidants which have been defined as "redox-active compounds that reduce pro-oxidative stress by reacting non-enzymatically with a reactive oxidant"[1] are flavonoids and phenolic acids contained in fruits and vegetables[14–17], allylslulfides in Allium species[18], hydroxycinnamic acids in coffee[19,20], phenolic compounds in wines[21] and vegetable oils[22], catechins in teas[23], specific ingredients of common spices such as capsaicin in chillies[24], gingerol[25] and curcumin[26], chlorophylls[27], anthocyanins in berries[28] as well as carotenoids[29] to name only a few.

The increasing evidence of the strong impact of the redox status on human health has stimulated intense research activities in this field. It has been estimated that around 10 papers dealing with oxidative stress and/or antioxidants are published daily[1] and many of them concern the identification of dietary compounds in the diet and investigations concerning their mode of action. The results of these efforts have a strong

* **Corresponding author:** Professor Siegfried Knasmüller, fax +431 4277 9651, email siegfried.knasmueller@mediuniwien.ac.at
† Contributed equally

impact on the development of nutritional recommendations and led to the development of supplements which contain high levels of food derived antioxidants[30] and to the production of functional foods. A broad variety of different methods are currently used to study antioxidants in human foods and to identify and characterise their active principles. The models include chemical–analytical and physical measurements, experiments with subcellular fractions and intact cells, animal studies as well as human intervention trails. In the last decade, new biomarkers have been developed and validated which can be used in human studies and the rapid development of -omics techniques (in particular the use of microarrays and two dimensional gel electrophoresis) offers the possibility to explore the effects of antioxidants on gene expression and protein levels and to study alterations of disease related patterns[31–33].

The aim of the present article is it to give a critical overview on the advantages and limitations of the different approaches which are currently used with particular emphasis on the newly developed methods. We anticipate that it will help in the interpretation of existing data and lead to the development of improved strategies concerning the detection of antioxidants.

The formation of ROS as well as their physical and chemical properties, their reactions with organic molecules and their inactivation by antioxidants have been extensively described in the scientific literature[34–36]. Therefore, these topics are confined in the present article to short descriptions which are essential to understand the subsequent chapters.

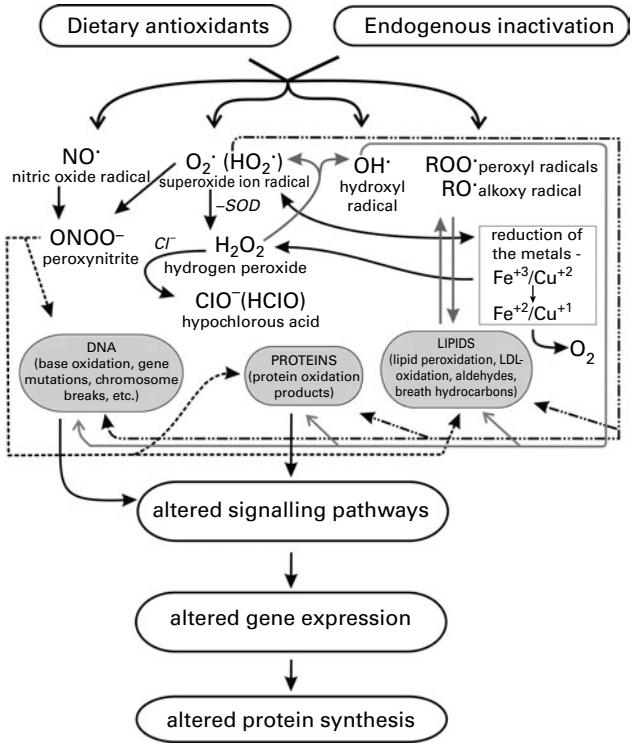

Fig. 1. Different form of reactive oxygen species and their interaction with organic molecules.

Formation of reactive oxygen species (ROS)

Pro-oxidants (often termed as reactive oxygen species) can be classified in two groups, namely radicals and non-radicals. Radicals (O_2, O_2^-, $OH^{\cdot}$, $ROO^{\cdot}$, $RO^{\cdot}$, and $NO^{\cdot}$) contain unpaired electrons in the shells around the nucleus which causes the high reactivity of these species (except O_2), due to their ability to donate or receive other atoms to obtain stability. Important non-radicals comprise hyperchlorous acid (HOCl), hydrogen peroxide (H_2O_2), organic peroxides, aldehydes and ozone (O_3). The most relevant ROS as well as some of their main reactions are shown in Fig. 1.

Superoxide (O_2^-), which is formed for example during respiration in mitochondria (as a consequence of reduction of oxygen required for APT production) forms at low pH hydroperoxyl (HO_2) which penetrates the cell membranes more easily than the charged form[37,38]. Enzymatic as well as non-enzymatic dismutation leads to formation of hydrogen peroxide (H_2O_2) which can be detoxified enzymatically (catalase, glutathion peroxidase). H_2O_2 molecules can damage cells at low concentrations and degrade haem proteins and oxidise DNA, enzymes, –SH groups and keto acids and are also the source of more deleterious species such as HOCl and $OH^{\cdot}$. The latter radical is short lived and reacts at a high rate with most organic molecules (DNA, amino acids, sugars, proteins, lipids). Transition metals (first row of the D block of the periodic table) contain unpaired electrons (except Zn) and can therefore be considered as radicals. In particular copper and iron are contained at relatively high concentrations in many organisms. At physiological pH, most of the metals are present in oxidised forms ($Fe^{3+\cdot}$, Fe^{2+}), but after reduction (e.g. by ascorbic acid or via the Haber-Weiss reaction $O_2^- + Fe^{3+} \rightarrow O_2 + Fe^{2+}$), they can undergo "Fenton type" reactions (e.g. $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH + OH^-$). These two processes explain the formation of $OH^{\cdot}$ *in vivo*. However, it is notable that in organisms, metals are always bound to proteins and membranes and it has been shown that they can undergo in this state the aforementioned reactions and produce $OH^{\cdot}$ at a single site and convert non-reactive radicals to highly reactive species.

Nitric oxide ($NO^{\cdot}$) is produced by oxydation of the terminal guanidine-nitrogen atoms of arginine[38–40]. This reaction is catalysed by nitric oxide synthetases (NOS, i.e. neuronal NOS, endothelial NOS and inducible NOS). $NO^{\cdot}$ can react with different radicals, the most important reaction under physiological conditions is the formation of peroxynitirite ($ONOO^-$) in which O_2^- is involved[38,40]. $ONOO^-$ can cause damage similar to that induced by $OH^{\cdot}$[39].

The biological significance of the different ROS species has been discussed quite controversially. Some authors suggested that O_2^- and $NO^{\cdot}$ are the most relevant ones, while other stressed that peroxyl radicals may be even more important[8].

Several exogenous factors contribute to oxidative stress. Ionising radiation causes toxic effects in organisms primarily via ionisation of intracellular water[41]. Also non-ionising radiation (UV light) can indirectly produce a variety of ROS species including O_3[42]. Other major sources of exposure are air pollutants such as cigarette smoke[43] and car exhausts[44], drugs (bleomycin, doxorubicine)[45] as well as pesticides and herbicides[46] and industrial chemicals[47]. Also pathogenic microorganisms may produce oxygen species, but the most relevant external source is nutrition as most of the foods we

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consume are oxidised and contain oxidants such as peroxides, aldehydes, fatty acids and transition metals[48,49].

The most important endogenous processes of ROS production are respiration processes in the mitochondria and the massive continuous production of radicals is even increased in ageing cells[50,51]. Another source are white blood cells involved in immune responses which can undergo a respiratory bust that is characterised by an up to 20-fold increase of oxygen production[52,53]. During this reaction, NAPDH serves as a donor of electrons which results in the production of O_2^- from oxygen. The enzyme myeloperoxidase catalyses the production of HOCl by interaction between H_2O_2 peroxides and chlorides[54,55].

Targets of oxidative damage

The continuous exposure to ROS from exogenous and endogenous sources results in oxidative damage of many cell components and alterations of cellular functions; some of these changes can be used as markers of oxidative stress and to investigate putative protective effects of phytochemicals.

Proteins. Radicals react in particular with nucleophilic amino acids for example with tryptophane, histidine and cysteine[56,57]. Apart from direct oxidation of SH-groups by H_2O_2 and O_2^-, organic radicals may bind covalently to cellular proteins which are part of cell membranes or have enzymatic functions. One of the major adducts which can be easily detected is 3-nitrotyrosine which is produced by interactions between $ONOO^-$ and other nitrogen reactive radicals with the amino acid tyrosine[58]. Also relatively resistant amino acids such as lysine and proline can be hydroxylated non-enzymatically by $OH^{\cdot}$[59].

It is also known that ROS can destroy peptide bonds and cause drastic alterations of their structures resulting in changes of their cellular functions (for review see[36]). $NO^{\cdot}$ reacts in particular with Fe–S centres of proteins which transport electrons and this affects the functions of mitochondria[60]. Another important feature is their reaction with thiol groups of proteins; a typical example is the *S*-nitrosylation of caspases which are part of cell signalling processes[61].

Lipids. All cellular membranes are vulnerable to oxidation due to their high concentrations of unsaturated fatty acids. Damage of lipids by ROS (lipid peroxidation, LP) occurs in three stages. In the first ("initiation phase"), double bonds of fatty acids are attacked by radicals which leads to formation of fatty acids radicals. During the "propagation", a chain reaction takes place which leads to continuous formation of these radicals. The last stage (chain determination) occurs following interactions $ROO^{\cdot}$ or with other radical types and/or antioxidants (for a detailed description of LP see[38,62,63]). Important marker molecules formed during LP are aldeydes and ketones, for example malonedialdehyde (MDA) and 4-hydroxynonenal (4-HNE) as well as the family of isoprostanes which are excreted in the urine. Oxidation of fatty acids can be measured with the relative change in the fatty acid pattern and the formation of conjugated dienes.

Blood cholesterols can be oxidised by ROS to form oxidised low density lipoproteins (LDL). The oxidative modification hypothesis of atherosclerosis assumes that circulating LDL particles are modified by oxidation and that these particles are then taken up by macrophages inside the arterial wall and form the start of atherosclerotic plaques[64,65].

DNA damage. ROS can damage DNA either directly or indirectly (via LP). The major part of damage is attributable to $OH^{\cdot}$ radicals which interact either with the sugar phosphate chain or oxidise bases and form reaction products such as thymine glycol, 5-hydroxy-uracil and 8-hydoxy-desoxyguanosine (8-OH-dG)[66,67]. Also $NO^{\cdot}$ can damage DNA indirectly via formation of peroxynitrite, another pathway of damage is the formation of covalent bonds between radicals and nucleobases[38,68,69].

The most important indirect form of DNA damage is caused by LP (for review see[70]). Lipid radicals formed during the chain reaction cause adduct formation, strand breaks and DNA-protein crosslinks. The former lesions are also induced by specific end products of the reaction such as alkenes and alkanes[71,72] and numerous studies have been published which concern the genotoxic effects of reaction products such a MDA and 4-HNE[70]. Nuclelotide- as well as base excision repair mechanisms (BER and NER) prevent the persistence of oxidative lesions. Oxidised guanosine is removed by the action of glycosylases such as 8-oxoguanine DNA glycosylase 1 and 2 (OGG1 and OGG2); mispaired adenines by MYH and MTH1. NEIL glycosylases containing β/δ-elimination activities excise a broad range of oxidatively damaged bases, including 5-hydroxyuracil (5-OHU), thymine glycol (Tg), uracil, 8-oxoguanine (8-oxoG) and ring-fragmented purines[73]. Also pyrimidine derived lesions are a substrate of this latter enzyme, important uracil specific enzymes are for example UNG and SMUG1. A detailed description of repair of oxidised DNA can be found in the articles of Sanderson *et al.* [74], Krokan *et al.* [75] and Cooke *et al.* [76].

Oxidative stress, cell signalling and activation of transcription factors

The exposure of organisms to ROS causes dramatic changes in gene regulation patterns and protein synthesis. In lower eucaryotes, e.g. in yeast the expression of up to 1/3 of the genes is affected by oxidative stress[77], mammalian cells are somewhat less flexible but still hundreds of genes have been identified which react towards oxidative damage. During the last decades, complex signalling pathways have been discovered which activate transcription factors involved in gene regulation. The networks involved in ROS mediated cellular responses have been described in a number of reviews[78–84]. In the present article we will give a short overview on the most important processes since the mode of action of dietary antioxidants may involve changes of signalling pathways which cause activation of cellular defence systems[85].

Alterations of cell signalling pathways. Oxidants can impinge signalling either by influencing redox dependent protein–protein interactions or via altering enzyme activities, i.e. oxidations leading to inhibition of phosophor/serine/threonine-, phosphothyrosine- and phospholipid-phosphatases[82]. The key reactions which have been identified are interactions with sulphydryl groups on their cysteine residues[86].

The intracellular responses can be grouped in two categories namely receptor-mediated ones and non-receptor effects. Fig. 2 gives a schematic overview on the different processes.

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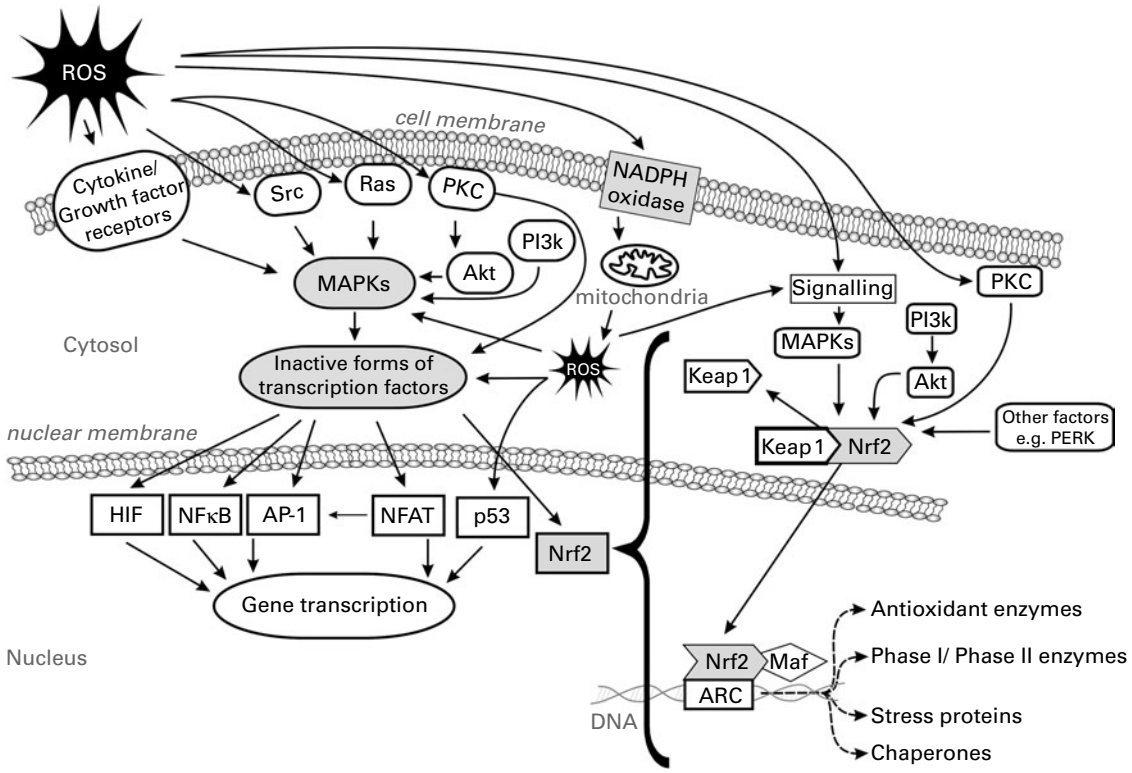

Fig. 2. Impact of ROS on cell signalling and activation of transcription factors.

Growth factors and cytokines (e.g. TNF-α, IL-1) cause ROS production in non-pathogenic cells and activate intracellular receptor mediated signalling which affect mitogen activated protein kinases (MAPKs). Growth factor receptors are tyrosine kinases (RTLs), apart from these, also non-receptor protein kinases have been identified which are also activated by ROS and belong for example to the Src family[87,88]. Activated Src binds to membranes and initiates MAPKs, NfκB and PI3K signalling pathways[78]. Other important targets involved in signalling are Ras (membrane bound G proteins involved in the regulation of cell growth), protein tyrosine phosophatases (PTP) and seronine/threonine kinases. The most important representative of the latter group is protein kinase C (PKC), its catalytic site is a zinc finger domain containing several cysteine rich regions which can be modified by various oxidants[89].

MAPKs (for a detailed description see[90]) relay signals generated by exogenous or endogenous stimuli to intracellular space via phosphorylation of proteins. During this process, the kinases interact also with downstream mediators including transcription factors[91]. Studies on the upregulation of MAPKs have shown that these processes are type and stimuli specific. For example, it was found that endogenous H_2O_2 production by respiratory burst induces ERK but not p38 kinase[92] while exogenous peroxide treatment activates the latter enzyme[93].

Activation of transcription factors by ROS. The most significant effects of ROS on MAPKs concern the activation of transcription factors which control the expression of protective genes, arrest division of damaged cells and induce apoptosis (programmed cell death).

AP-1 is a collection of dimeric basic region-leucine zipper proteins which are for example induced by metals and H_2O_2 [94,95] and regulate cell growth, differentiation and apoptosis.

NFκB is a DNA binding protein which is sequestered in the cytoplasm because of an interaction with a member of the inhibitory IκB family. Activation via ROS causes dissociation and allows NFκB to enter the nucleus and activate genes involved in inflammatory responses, transformation and angiogenesis[96]. A number of investigations showed that that activation by different stimuli can be blocked by antioxidants including *N*-acetlylcysteine, cysteine, vitamin E, thiols and green tea polyphenolics[78].

Another important factor which plays a key role in protecting cells from malignant transformation is p53, also termed a "tumour suppressor" since it arrests cell cycle and induces apoptosis[97]. p53 is directly activated by oxidants and its overexpression leads to increase of intracellular ROS levels. One of the important functions of p53 is the up regulation of proteins that play a role in ROS mediated apoptosis namely ferrodoxin reductase (FDXR) and a novel stress-response gene Redd1/HIF-1 originally isolated as an HIF-1-response gene[98]. It was shown in a number of *in vitro* studies that antioxidants reduce apoptosis rates due to interaction with p53[99,100]. In a recent human intervention study we observed a drastic reduction of the apoptosis frequencies in lymphocytes after consumption of wheat sprouts which is probably due to antioxidant effects[101].

Two other transcription factors affected by ROS are nuclear factor of activated T cells (NFAT) and HIF. The former family regulates muscle growth and differentiation as well

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as cytokine formation and angiogenesis[94,102]; while the latter is a heterodimer controlling genes encoding for vascular endothelial growth factor (VEGF), aldolase, enolase and lactate dehydrogenase[103].

Probably the most important contribution to cell defence against oxidative stress is mediated through transcriptional activation of genes via a cis-acting enhancer known as antioxidant responsive element (ARE) which was discovered by Pickett and co-workers[104–106] and identified in the 5′flanking flanking regions of many genes. A number of studies showed that the transcription factor Nrf2 which belongs to the CNC (cap'N'collar) basic leucine zipper family and is represented in many tissues is the key mediator of ARE dependent activation[107,108]. Comparative investigations with genetically altered rodents (Nrf2 + /+ and Nrf2 − /−) showed that numerous genes are regulated by the element including those which encode for protection against ROS such as glutamate cystein ligase (GCL) which catalyses the rate limiting step of glutathione synthesis, NADPH quinone oxidoreductase (NQO), glutathione *S*-transferase (GST), aldehyde dehydrogenase (ADH), glutathione peroxidase, glutathione reductase, peroxiredoxin I (PrxI), superoxide dismutase (SOD), catalase, and thioredoxin[109,110]. Also enzymes which are involved in the supply of reducing equivalents (e.g. glucose-6-phosphate dedydrogenase) as well as xenobiotic drug metabolising enzymes (e.g. CYPs), chaperones, and stress response proteins are regulated by Nrf2[111,112].

Recent investigations showed that the actin binding protein Kelch-like Ech-associated protein (Keap1) regulates transcription factor Nrf2 by controlling its stability and subcellular localisation[113–115]. The disruption of the Keap-Nrf2 complex by oxidative stress leads to Nrf2 accumulation in the nucleus where it is associated with small MAF transcription factors and mediates ARE dependent gene expression (see Fig. 2).

It is well documented that chemicals which release ROS such as metals[116,117] and vascular diseases (arteriosclerosis, diabetes, chronic renal failure, preeclampsia) both cause induction of the different transcription factors described above[118].

The interaction of phytochemicals with these processes has been reviewed in several articles[118–121]. It was shown that phenolics such as EGCG and resveratrol and spice ingredients (e.g. capsaicin, curcumin) inhibit the transcription factors NFkB, AP-1 and β-catenin-TcF signalling via interaction with upstream signalling pathways (IKK phosphorylation, MAPK phosphorylation and PI3K/Akt phosphorylation), in parallel proinflammatory mediators (TNF-α, IL, PGE2 and NO) and the activities of proinflammatory enzymes (COX 2, iNOS) were reduced[119]. In the case of COX 2, it is known that inhibition by synthetic compounds such as non-steroidal anti-inflammatory drugs (NSAIDS) is paralleled by decreased rates of colon and colorectal cancers in humans[122,123]. On the contrary, the induction of detoxifying enzymes (including those which inactivate ROS) is due to activation of the transcription factor Nrf2. Typical example for dietary antioxidants which cause an induction of this transcription factor are synthetic and tea specific phenolics, isothicoyanates and sulforaphane[124], curcumin[125] and flavonoids[126,127].

The molecular mechanisms by which phytochemicals interact with signal transmission cascades are not precisely known. It is supposed that the downregulation of transcription factors may be due to the direct scavenging of ROS. In the case of Nrf2 it was shown that the activation by sulforaphane and synthetic alkylating compounds is due to modifications of cysteine residues of Keap1, a sensor protein which regulates Nrf2[128–131]. However it cannot be excluded that Nrf2 activation seen with certain phytochemicals may be due to release of ROS; it is known that antioxidants (in particular phenolics) can act under certain conditions as pro-oxidants.

Conventional and new methods for the detection of dietary antioxidants

A large number of different techniques have been developed to monitor oxidative damage and its consequences; these approaches can be also used to identify dietary antioxidants and their mode of action. These methods are often applied in experimental systems in which oxidative stress is induced by specific treatments or diseases. The most frequently employed models are described in the next chapter; the following sections describe physics–based and biochemical methods, techniques for the detection of oxidative DNA-damage, approaches used to investigate alterations of signalling pathways as well as the advantages and disadvantages of -omics techniques (Fig. 3).

Induction of oxidative stress in biological systems

In experiments with subcellular fractions and in *in vitro* experiments with cells, ROS are in most cases generated by chemical reactions, for example with the xanthine/xanthine oxidase system by hydroquinone oxidation[32,132,133] which generate O_2^-. Transition metals such as copper and iron play a major role in Fenton type reactions thereby forming mainly $OH^{\cdot}$ radicals. Another frequently used approach is the use of chemicals such as H_2O_2, *t*-butyl-hydrogenperoxide or bleomycin which release O_2^- and $OH^{\cdot}$ or of compounds such as menadione[134], paraquat[135], and plumbagin[136] which form O_2.

Activated phagocytic cells produce oxygen radicals as part of their defence system and a burst of ROS can be induced by exposing such cells to bacteria, particles or certain chemicals; one of the most powerful responses can be evoked with the tumour promotor phorbol myristate acetate[137].

Chemicals which generate ROS are rarely used in animal studies due to their high reactivity. A more convenient way to cause oxidative damage which has been also used in numerous *in vitro* experiments is ionising radiation. Indirect approaches are feeding of vitamin E deficient diets[138], iron overload[139] or inhalation of oxygen[140].

Organ specific inflammations can be induced with certain chemicals; for example liver cirrhosis with CCl_4[141] or thioacetamide[142]. 2,4,6-Trinitrobenzene sulfonic acid, oxazolone and dextran sodium sulfate are used in models for inflammatory bowel diseases[143]; diabetes can be caused by the antibiotic streptozotocin.

In the last years, a variety of genetically altered mice and rat strains have been developed as models for ROS-related diseases for example animals which are deficient in specific SOD forms[144] and GST isozymes[144].

Disease related models include those of ataxia-telangiectasia[145], Alzheimer's[146] and Parkinson's[147] disease, ageing

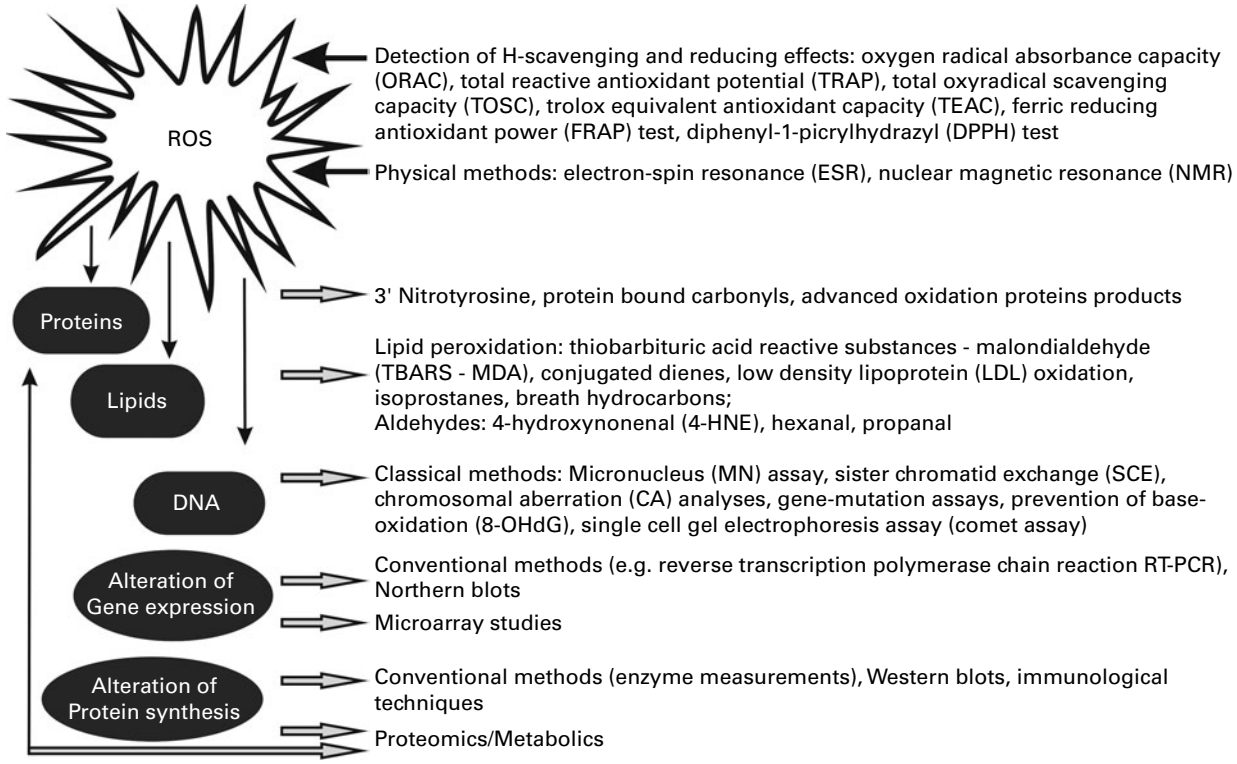

Fig. 3. Overview of different methods used for the detection of dietary antioxidants.

models[148]. In addition, also rodent species have been developed which are deficient in specific genes encoding for repair of oxidative DNA damage such as *Ogg1* [149] and *Myh* [150].

Most human studies on dietary antioxidants are carried out with healthy volunteers. In this context it is notable that a number of parameters such as age, sex, body mass index, and seasonal variations were found to affect the redox status and should be taken into consideration in dietary studies[151]. In the last years, a number of antioxidant studies has been conducted in which oxidative stress was induced by physical exercise[152,153] or hyperbaric treatment[154] and also with patients with ROS related diseases such as diabetes[155–157], HIV[158], atherosclerosis and coronary artery diseases[159], cancer[160,161], uremia[162] or systemic lupus erythematosus[163].

Conventional methods used for the detection of oxidative stress or for the identification of antioxidative dietary components

This part of the review will focus on conventional methods used to describe oxidative stress, and will be divided into five parts namely (1) physics based approaches, (2) methods used for the determination of the antioxidant compounds; (3) biochemical methods used to monitor the oxidation of macromolecules and their oxidation products, (4) approaches for the detection of ROS induced DNA-damage and (5) methods used to measure antioxidant enzymes and transcriptional factors.

Trapping of reactive species. The only technique that can measure free radicals directly and specify them is electron spin resonance (ESR), because it detects the presence of unpaired electrons. However, ESR can only be used to monitor only fairly unreactive radicals, since reactive ones do not accumulate at high-enough levels. One solution to this problem is to add 'traps' or 'probes', i.e. agents that intercept reactive radicals reacting with them to form a stable radical that can be detected by ESR. Whole-body ESR techniques are being used with rodents[164] but are currently not applicable to humans due to the lack of human safety data on the probes. A wide range of traps is available for use in animals and cell culture systems, not only *N*-tertbutyl-*p*-phenylnitrone (PBN) and 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO)[165] which were frequently used, but also "newcomers" as 1,1,3-trimethyl-isoindole *N*-oxide (TMINO)[166], 5,5-diethylcarbonyl-1-pyrroline *N*-oxide (DECPO)[167], *N*-2-(2-ethoxycarbonyl-propyl)-*a*-phenyl-nitrone (EPPN)[168], 5-diet hoxyphosphoryl-5-methyl-1-pyrroline *N*-oxide (DEPMPO)[165] and 5-*tert*-butoxycarbonyl-5-methyl-1-pyrroline-*N*-oxide (BMPO)[169]. A generally underestimated problem is, that the reaction products giving an ESR signal, can be rapidly removed *in vivo* and in cultured cells, both by enzymic metabolism and by direct reduction by agents such as ascorbate[170].

Approaches to determine the total antioxidant capacity. Two main approaches have been developed to evaluate the antioxidant capacity in foods and human material (in particular plasma and LDL). The first measures the ability of a substance to transfer one electron to reduce compounds like radicals, carbonyls or metals. The most popular tests which belong to this category are the ferric iron reducing antioxidant parameter (FRAP), the Trolox equivalent antioxidant capacity (TEAC), and the diphenyl-1-picrylhydrazyl test (DPPH). Methods which fall into the second category are based on their ability to quench free radicals by hydrogen donation.

Some scientists believe that these reactions are similar to the reaction mechanisms of antioxidants[171]. The most popular methods are the oxygen radical absorbance capacity test (ORAC), the total radical trapping antioxidant parameter (TRAP), the total oxidant scavenging capacity (TOSC) method (all measuring effects in the hydrophilic compartment of the plasma) and the inhibition of linoleic acid and LDL oxidation.

Free radical quenching methods. The *ORAC assay*, which is based on the work of Ghiselli *et al.* [172], Glazer[173] and Cao *et al.* [174] measures the antioxidant inhibition of $ROO^{\cdot}$ induced oxidations. Therefore, it reflects the classical H donating ability of antioxidants in the hydrophilic compartment. The peroxyl-radical reacts with a fluorescent probe thereby forming a non-fluorescent product which can be quantitated by following the fluorescence over time. In earlier studies, β-phycoerythrin was used as the fluorescent agent emitting in the visible region (Exc 495 nm, Em 595 nm), but due to shortcomings and inconsistencies of the results, fluorescein or dichlorofluorescein are currently used, since they are less reactive and more stable[175]. The antioxidative activity can be expressed as the lag time or the net integrated area under the fluorescence curve (AUC). ORAC values are reported as Trolox equivalents. Originally, the ORAC assay was limited to the measurement of hydrophilic chain breaking antioxidant capacity. A newer protocol, in which lipophilic and hydrophilic compounds are selectively separated by extraction, allows now also the quantification of lipophilic antioxidants using a mixture of acetone and water[176]. The advantage of the ORAC assay is that it can be automated. Convincing results have been obtained with 48 or 96 well plates coupled with a microplate reader[177]. One important parameter is the temperature control (37°C), as small temperature differences decrease the reproducibility of the test[178]. A principal drawback of the test is, that the effect of oxidation of the photoreceptor of the protein used does not necessarily reflect protection against oxidative damage of the protein itself[179].

The *TRAP assay*, proposed by Wayner *et al.* [180] is based on the use of 2,2′-azobis(2,4-amidinopropane)dihydrochloride (AAPH), a hydrophilic azo-compound which generates peroxyl-radicals. AAPH decomposes at 37°C spontaneously with a known rate. Various substances like 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), R-phycoerythrin or 2′-7′-dichlorofluorescin (DCFH)[181–183] have been used as oxidizable agents. A comprehensive review on the different modifications has been published by Ghiselli *et al.* [184]. The basic reactions of the procedure are similar to those of the ORAC assay. The probe reacts with $ROO^{\cdot}$ radicals at low concentrations with a significant spectroscopic change in between the native and the oxidized sample and no radical chain reaction beyond sample oxidation should occur. The antioxidant capacity is determined as the time required to consume all antioxidants by extension of the lag time for appearance of the oxidized probe when antioxidants are present. TRAP values are usually expressed as the lag time of the sample compared to the corresponding times for Trolox. The test is relatively complex to perform, requires experience and is time-consuming. The use of the lag phase is based on the assumption that all antioxidants show a lag phase and that the lag phase corresponds to the antioxidative capacity.

One drawback of TRAP and ORAC is the interference of proteins which contribute by $\geqslant 80\,\%$ to the total antioxidant capacity[175,184]. Therefore, Trolox can be used as an internal standard or the samples must be deproteinized prior to the measurements.

The *TOSC assay* was initially used for environment related studies on marine organisms[185,186]. It is based on the inhibition of the radical-dependent formation of ethylene from ketomethiolbutyric acid by antioxidants. This procedure permits testing against three different ROS species (i.e. peroxyl-, hydroxyl-radicals as well as peroxynitrite) with physiological relevance and different reactivities. It can be conducted at physiological temperature and pH; non-linear concentration-dependent activity variations can be examined easily and different types of antioxidant reactions (retardant or fast-acting) can be distinguished. However, high throughput analyses are not possible and multiple injections of each sample are required in order to observe ethylene formation. Further limitations are the multiple endpoints of calculated 20, 50 and 80 % TOSC and the DT_{50} (first derivative of TOSC of 50 %) since it was shown that there is no linear relationship between the different multiple endpoints[187].

The *chemiluminescence assay (CL)* is a modification of the TRAP assay. Radical formation is followed by CL or photo-CL (PCL). CL is characterized by low emission intensity and by the fact that reactions with oxidants emit CL. The most widely used marker is luminol[188,189], but also biuminescent proteins like pholasin are becoming popular[190–192]. The antioxidant capacity is the time of depressed light emission, which is measured at 10 % recovery of light output.

Recently Popov *et al.* [193] have described the PCL, a commercial test system termed PHOTOCHEM for the determination of the integral antioxidative capacity towards O_2[193]. In a strict sense, the method measures antiradical capacity. In contrast to many other assays used to determine AOC, this procedure requires no standardisation of the pH and of the temperature. However, to date, the system is only marketed by one company (Analytic Jena, Germany) and reagents for the hydrophilic and lipophilic assays are only available from the manufacturer; furthermore, a high throughput is not possible. Ascorbic acid is normally used for the determination of hydrophilic and Trolox for the lipophilic antioxidative capacity.

Low-density lipoprotein (LDL) oxidation is based on the autoxidation of linoleic acid or LDL which is induced *in vitro* mainly by Cu^{2+} or some other azo-initiators[194–196]. LDL-oxidation is of higher physiological relevance when tested under *in vivo* conditions and not *ex vivo*. The oxidation is monitored at 234 nm for conjugated dienes or by peroxide values for lipid hydroperoxides. LDL has to be freshly isolated from blood which is a time- and material-consuming procedure which requires ultracentrifugation. During the preparation, low temperature and light protection are essential[197]. Further, conjugated dienes can be formed in presence of polyunsaturated fatty acids.

Recently, fluorescence and UV based ELISA assays with plasma became available, for which no complicated and time consuming LDL isolation is needed. This methods can also be used for larger human trials. Also the procedure developed by Holvoet *et al.* [198] who measured oxidized LDL levels by a competitive ELISA utilizing a specific murine

monoclonal antibody (mAb-4E6) based on UV is employed quite often at present[198]. The AOC is determined in all these experiments either as AUC or as the lag time until the antioxidants are consumed. An important modification was developed by Frankel *et al.* [199] who determined the secondary oxidation product hexanal from LDL. Hexanal was chosen, since it is the major oxidation product of *n*-6 fatty acids and is monitored with head space gas chromatography, the percentage inhibition of hexanal formation is used as a parameter for AOC. In many *ex vivo* studies LDL was isolated, subsequently the substances were added and tested on their ability to delay oxidation. This scenario does not reflect *in vivo* conditions. Furthermore, not all oxidation inducers which are used *ex vivo* can be used for *in vivo* testing[179].

The *Crocin bleaching assay* monitors the protection of AAPH-induced crocin bleaching, by antioxidants[200]. Crocin is a mix of natural pigments and absorbs, similar to carotenoids, at 450 nm. Therefore, the interpretation of the results can be complicated in experiments with food samples. Initially, the test was used for the analysis of plasma samples[201]. One of its limitations is that crocin is not commercially available, but high sample throughput with microplates is possible.

Single electron transfer methods. In these assays, the sample itself is an oxidant that abstracts an electron from the antioxidant, thereby causing colour changes which are proportional to the AOC. When the change of absorbance is plotted against the antioxidant concentration, the slope of the curve reflects the total reducing capacity. In contrast to the methods described in the last chapter, no oxygen radicals are present in the system; therefore, it can be assumed that the reducing capacity is equal to the antioxidant capacity.

The *TEAC-assay* is a spectrophotometric test which was developed by Miller *et al.* [202]. 2,2-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) is oxidized by $ROO^{\cdot}$ to a green-blue radical cation. The ability of antioxidants to delay colour formation is expressed relative to Trolox. Originally, the test used metmyoglobin and H_2O_2, and the ABTS radical was measured at 734 nm. Meanwhile, various modifications have been developed[203,204]. After generation of the ABTS radical, the sample to be tested is added, subsequently other chemicals like manganese dioxide, ABAP, potassium persulfate or enzymes are used to generate the ABTS radical[175,202,203,205]. Temperatures higher than 37°C, which are not physiological and different absorption maxima (415, 645 734 or 815 nm) are frequently used. Depending on the protocol, the decrease or increase in ABTS radical absorbance in presence of the test sample or Trolox at a fixed time point is measured and the antioxidant capacity is calculated as Trolox equivalents. ABTS is not a physiological substance. It reacts fast with aqueous and organic solvents and substances with a low redox potential show a good response. Therefore, phenolic compounds or ascorbic acid react quite well with ABTS whereas lipophilic compounds respond more weakly. The test can be adapted to microplates and is not restricted to a narrow pH range, but high haemoglobin concentration in the plasma may interfere with the measurements.

The *FRAP assay* determines the reduction of 2,4,6-tripyridyl-*s*-triazine (TPTZ) in plasma to a coloured product[206] and has also been adapted for food samples[207,208]. Similar to the TEAC assay, compounds with a redox potential $< 0{\cdot}7$ V are detected. FRAP not enable to monitor compounds that quench radicals like proteins or thiol compounds such as glutathione (similar to all the "reducing assays") and can therefore underestimate the AOC. In order to maintain the solubility of Fe, the assay is conducted at acidic conditions (pH of 3·6). Most redox reactions take place within a few minutes, therefore both tests consider most of the substance effects (like polyphenols) but not all, since some substances have longer reaction times[175]. This was recently shown for polyphenols like caffeic-, tannic-, ferulic- or ascorbic acids, where the absorption steadily increased for hours[209]. Since the test reflects only the reducing potential and does not consider H transfer, it should only be considered in combination with other methods to give a more complete picture. Similar to the TEAC, it is a relatively easy test procedure, can be done manually or fully automated and requires no expensive equipment.

The *copper reducing assay* (CUPRAC, AOP-90) is a modification of the FRAP in which iron is replaced by Cu; Cu^{2+} is reduced to Cu^{1+}[210]. The assay is conducted at 490 nm with bathocuprine, or at 450 nm with neocuproine. Results are expressed as uric acid equivalents. Since copper has a lower redox potential than iron, but enhances the redox cycling potential, its pro-oxidative potential can be more sensitive[175]. The limitations regarding the underestimation of slower reactive molecules is similar to the other assays but one of it advantages is that almost every antioxidant including thiols can be detected[175].

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical used in the *DPPH-assay* is stable, deep purple, commercially available and has not to be generated. The test was developed by Brand-Williams *et al.* [211]. The loss of DPPH colour at 515 nm after reaction with test compounds is measured either by decrease of absorbance or by electron spin resonance[212]. The concentration of a 50 % decrease of the DPPH radical is defined as EC_{50}, the time to reach it as T_{EC50}. Since the DPPH assay uses a wavelength of 515 nm, it can interfere with substances with a similar spectrum like carotenoids[213]. The test considers both, electron transfer as well as hydrogen (H) transfer reactions with the focus on the prior. Again, smaller molecules have better access to the radical site and contribute to a higher extent to the AOC[175]. The test procedure itself is quite simple and fast and requires only a spectrophotometric device.

For all the electron transfer tests it can be assumed that they "overestimate" smaller molecules and hydrophilic substances like ascorbic acid, uric acid or polyphenols and reflects not always the situation in the organism[214].

From the evaluation of the different assays it becomes clear that no single test reflects the overall antioxidative capacity of antioxidants. Both hydrophilic and lipophilic activities must be considered, as well as H transfer and single electron transfer mechanisms and additional tests which reflects the inactivation of various reactive oxygen/nitrogen species are needed to fully estimate the AOC (Table 1).

Oxidation of macromolecules

Biomarkers of lipid oxidation. Cell membranes are highly susceptible to LP due to their specific composition which is charactesized by high amounts of polyunsaturated fatty acids (PUFAs)[215]. LP oxidation is a chain reaction (see above) and leads to structural and functional damage of membranes

Table 1. Comparison of methods used to determine the total antioxidant capacity (TAC)

Test systems	Principle	Endpoint	Biological relevance	Simplicity/throughput*	Number of studies with dietary antioxidants†
ORAC	H-transfer	Lag time AUC	+++	++/+++	156
TRAP	H-transfer	Lag time	+++	− − −/− −	270 TRAP
TOSC	H-transfer	AUC DT_{50}	++	−/− − −	23 TOSC
CL/PCL	Not known	Lag time AUC	++	+/− −	CL 2445 PHOTOCHEM 11
LDL Oxidation	H-transfer	Lag time	+++ − (*in vitro*)	UC: − −/− − ELISA: ++/++	LDL-oxidation 2357
TEAC	Electron-transfer	Δ Optical density	−	++/++	TEAC 217 TAC 133
FRAP	Electron-transfer	Δ Optical density	− −	+++/+++	FRAP 173
CUPRAC	Electron-transfer	Δ Optical density	−	+++/+++	6
DPPH	Electron-transfer	Δ Optical density	−	+/+	DPPH 1061

CL, chemiluminescence assay; CUPRAC, cupric reducing antioxidant capacity; DPPH, 2,2-diphenyl-2-picrylhydrazyl assay; ELISA, enzyme-linked immunosorbent assay; FRAP, ferric reducing ability of plasma; LDL, low density lipoprotein; ORAC, oxygen radical absorbance capacity test; PCL, photo-chemiluminescence assay; TEAC, trolox equivalent antioxidative capacity; TOSC, total oxidant scavenging capacity; TRAP, total radical trapping antioxidant parameter.

* +,++,+++, positive; −, − −, − − −, negative; UC, ultracentrifugation.

† Number of antioxidant studies conducted with the different methods identified by use of a computer aided search (Scopus database).

as well as to the formation of lipid hydroperoxides which are unstable and degrade to various secondary oxidation products.

The formation of malondialdehyde (MDA) is the most widely used parameter of PUFA peroxidation in *the thiobarbituric acid-reacting substances (TBARS) assay*. One of the oldest and still most widely used methods is based on the precipitation of protein nearly at boiling conditions[216]. The samples are heated for 1 h with TBA at low pH and the pink chromogen formed absorbs at 532 nm. The sample preparation has been criticized since it is far from physiological conditions and not the free MDA in the original sample is measured but the amount generated by decomposition of lipid peroxides during heating[217]. Furthermore, it is known that also other compounds like sugars, amino acids or bilirubin are able to react with TBA[218]. The sensitivity of the test can be increased by combining it with HPLC to separate such compounds before acidic heating[219].

In the last few years, several innovations have been introduced to improve the specificity of the test and to reduce known bias. In particular the temperature at the deprotenization step has been reduced to physiological conditions. In addition, several methods have been developed which do not require derivatization[220,221] or new derivatization agents like 2,4-dinitrophenylhydrazine[222] or diaminonaphthalene[223]. Very recently, GC/MS based methods have been developed which possess high sensitivity showing an overestimation of MDA levels[224,225]. Although it is questionable whether MDA measurements are a reliable method for LP, it is well documented by a large number of studies that increased levels are found in patients with ROS related diseases[226].

The first step of PUFA oxidation is the conjugation of double bonds leading to formation of *conjugated dienes* (CD) which absorb at around 234 nm. They can either be absorbed by lipids but also in plasma samples. The plasma preparation is more physiological and requires no heat treatment. In plasma samples, CD are usually analysed with HPLC–UV detection[227]. Determination of the diene levels cannot be used alone to describe oxidative stress, but when measured with HPLC–CD, the findings can support results obtained with other more reliable parameters of oxidative stress. Although MDA and CD are both primary oxidation products, they can develop differently in the same sample due to different mechanisms of formation[228].

Isoprostanes are stable oxidation products from arachidonic acid, initially formed from phospholipids and released into circulation before the hydrolyzed form is excreted in urine[229]. A large number of endproducts can theoretically be generated but interest has focused mainly on $F_{2\alpha}$-isoprostanes[230]; the must promising marker for oxidative stress/injury being 8-prostaglandin $F_{2\alpha}$[231]. At present it is regarded as one of the most reliable markers of oxidative stress, although the presence of detectable concentrations of isoprostanes in biological fluids requires continuous lipid peroxidation[232]. Several favourable characteristics make isoprostanes attractive as reliable markers for oxidative stress, i.e. they are specific oxidation products, stable, present in detectable quantities, increased strongly at *in vivo* oxidative stress, and their formation is modulated by antioxidants[231]. Various approaches such as gas chromatography–mass spectrometry (GC–MS), GC–tandem MS, liquid chromatography–tandem MS and immunoassays are available for the detection of $F_{2\alpha}$-isoprostanes[233]. The first results were produced by use of the MS technique, and various isoprostanes can be separated with this method[233]. Recently, immunoassays have been developed which correlate apparently quite well with the results obtained with GC–MS measurements in urine but some discrepancies might occur with plasma samples when they were not tested on cross reactivity with other prostaglandin metabolites[232]. Nevertheless, their use might be appropriate in intervention studies with various blood samplings from the same subject, thereby focusing not on absolute levels but on relative changes.

The measurement of *breath hydrocarbons* is a non-invasive method which allows to determine LP through exhaled breath by measuring trace volatile hydrocarbons[234]. Ethane formation results from *n*-3 oxidation, pentane formation is caused by *n*-6 oxidation. Although the data reported on their consistency to describe LP are quite convincing, the limiting factor is their detection. They are mainly employed with GC–FID, but one concern is the background level in the breath since bacteria were shown to produce significant amounts of theses

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hydrocarbons *in vivo* [235]. Furthermore, the separation of different hydrocarbons is not easy due to similar boiling points[236].

Aldehydes represent stable products of PUFA oxidation. 4-Hydroxynonenal (4-HNE) and hexanal are mainly formed by n-6 fatty acid oxidation, while propanol and 4-hydroxyhexenal result from *n*-3 fatty acid oxidation[199,237,238]. High concentrations of 4-HNE have been shown to trigger well-known toxic pathways such as induction of caspases, the laddering of genomic DNA, and release of cytochrome c from mitochondria, which may lead to cell death[239]. The most frequently used methods for determination of the aldehydes are GC–MS, GC–head space or HPLC[240]. Also polyclonal or monoclonal antibodies directed against 4-HNE-protein conjugates are now frequently used for 4-HNE measurements[241].

Biomarkers of protein oxidation. Markers of protein oxidation are less frequently used than lipid oxidation parameters (Table 2). They are mainly applied in combination with LP markers, although their formation has been associated with several diseases[242].

Formation of *protein bound carbonyls* is most abundant endpoint used to monitor protein oxidation[243,244] by a conventional colorimetric assay using 2,4-dinitrophenylhdrazine[245]. The test is easy to perform, but large quantities of solvents are required. Recently, an ELISA method has been developed and the results correlated well with the spectrophotometric method[246].

Advanced oxidation protein products (AOPP) are predominantly albumin and its aggregates damaged by oxidative stress[247]. They contain abundantly dityrosines which cause crosslinking, disulfide bridges and carbonyl groups and are formed mainly by chlorinated oxidants such as hypochloric acid and chloramines resulting from myeloperoxidase activity[248]. AOPP have several similar characteristics as advanced glycation endproducts-modified proteins. Induction of proinflammatory activities, adhesive molecules and cytokines is even more intensive than that caused by advanced glycation end products (AGEs). They are referred to as markers of oxidative stress as well as markers of neutrophil activation[249]. Protein oxidation products mediated by chlorinated species (HOCl) generated by the enzyme myeloperoxidase were found in the extracellular matrix of human atherosclerotic plaques and increased levels of advanced oxidation protein products were postulated to be an independent risk factor for coronary artery disease[250,251]. AOPPs are expressed as chloramine-T equivalents by measuring absorbance in acidic conditions at 340 nm in presence of potassium iodide. The test is easy to perform and can be carried out with microprobes.

Markers of oxidative DNA damage used in studies with dietary antioxidants. During the last fifty years, a broad variety of genotoxicity test procedures have been developed which are used for routine testing of chemicals, in environmental research and also in human studies concerning the impact of occupational exposure, lifestyle factors and nutrition on DNA-integrity. Due to the conservative structure of the genetic material, mutagenicity experiments can be carried out with a broad variety of indicator organisms including bacteria, yeasts, plants, invertebrates including Drosophila, laboratory rodents and also with cultured mammalian cells[252–254]. The advantages and limitations of the different methods for the detection of DNA-protective dietary factors have been described by Knasmüller and coworkers[255–257]. One of the

Table 2. Main biomarkers for lipid and protein oxidation

Test	Marker	Methods	Biological system	Simplicity/throughput/importance	Number of studies with dietary antioxidants*
TBARS/MDA	Lipid oxidation	HPLC GC–MS	Plasma/serum, cells	HPLC: +/+/– GC–MS: –/+/+	TBARS-3570; 16 %iv; 53 %an; 31 %hu MDA-16 255; 14 %iv; 53 %an; 33 %hu
Conjugated dienes $F_{2\alpha}$-Iso-prostanes	Lipid oxidation Lipid oxidation	HPLC GC–MS ELISA	Foods, plasma, cells Plasma/serum, urine	+/+/– GC–MS: –/–/+ ELISA: –/+/+	Conjugated dienes with HPLC-68; 29 %iv; 23 %an; 48 %hu F2-Isoprostane-260; 12 %iv; 34 %an; 54 %hu
Breath hydrocarbons Aldehydes	Lipid oxidation Lipid oxidation	GC HPLC GC–MS	Exhaled air Plasma, LDL, urine	+/–/+ HPLC: +/+/+ GC–MS: –/–/+	299; 24 %an; 76 %hu 4-HNE-389; 12 %iv; 52 %an; 36 %hu
Protein bound carbonyls	Protein oxidation	Photom ELISA	Plasma	Photom: +/+/– ELISA: +/+/–	Protein carbonyls-355; 26 %iv; 31 %an; 43 %hu
AOPP	Protein oxidation	ELISA	Plasma, urine	+/+/+	AOPP-196; 19 %iv; 12 %an; 69 %hu

AOPP, advanced oxidation protein products; CD, conjugated dienes; GC/MS, gas chromatography–mass spectrometry; ELISA, enzyme-linked immunosorbent assay; 4-HNE, 4-hydroxynonenal; HPLC, high-performance liquid chromatography; MDA, malondialdehyde; TBARS, thiobarbituric acid reactive substances. Distribution of the total studies to *in vitro* (iv), animal (an) and human (hu) studies: iv%, percentage *in vitro*; an%, percentage animal studies; %hu, percentage human studies.

*The number of antioxidant studies conducted with the different methods was assessed by use of a computer aided search (Scopus database).

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main problems (which is encountered also relevant for studies of the effects of dietary antioxidants) encountered in experiments with mammalian cells and lower organisms concerns the inadequate representation of the metabolism of the test compounds which may lead to results which cannot be extrapolated to humans. Nevertheless, all these methods have been used to study oxidative DNA-damage and to investigate putative protective effects of phytochemicals. At present, the most widely used endpoints are gene mutation assays with bacteria and mammalian cells (*Salmonella typhimurium*/microsome test, HPRT gene mutation assay), chromosome analyses in metaphase cells which can be conducted with stable cell lines (e.g. CHO, V79) and lymphocytes *in vitro* but can be also scored in *in vivo* and *ex vivo* experiments with blood cells. Another important endpoint are micronuclei which are formed as a consequence of chromosome breakage (clastogenicity) and aneuploidy and are less time consuming to evaluate as chromosomal aberrations[256,258]. The most frequently used approaches to monitor antioxidant effects of dietary factors are described in the subsequent chapters.

The most widely used *bacterial* mutagenicity test procedure is the *Salmonella*/microsome assay, which has been developed by B. Ames in the 1970s[259]. The test is based on the detection of back mutations in specific genes which encode for histidine biosynthesis. One of the disadvantages of the initial set of tester strains was, that none of them was highly sensitive towards oxidative effects, therefore new polyplasmid strains (TA102 and TA104) were constructed which are in particular suitable for the detection of mutagenic effects caused by ROS[260]. Since the target gene is located in these strains (in contrast to the classical tester strains) on plasmids, it can be easily lost and due to the high spontaneous reversion rates many groups encountered difficulties with these derivatives. Nevertheless, the *Salmonella* strains are at present widely used in antioxidant experiments; mutations are induced either by radiation or chemically and putative protective compounds or complex mixtures are plated on histidine free selective media plates together with the indicator bacteria. After incubation, the differences in the numbers of his^+ revertants serves as an indication of protective effects. The test procedure has been standardised for routine testing of chemicals (see for example OECD guideline 471[261]) and the criteria which have been defined (sufficient number of plates, inclusion of positive and negative controls, testing of different doses) can also be applied for the detection of antioxidants. One of the problems which affects the reliability of the test results, concerns the fact that false positive effects may be obtained with compounds which cause bactericidal or bacteriostatic effects since these parameters are not monitored under standard conditions. A typical example are the protective effects seen with cinnamaldehyde[262]. With complex dietary mixtures, difficulties may be encountered due to their histidine contents[263].

Apart from the *Salmonella*/microsome assay, also a number of other bacterial genotoxicity tests have been developed which are less frequently used, e.g. assays, based on the scoring of backward or forward mutations with *E. coli* strains, tests based on the induction of repair processes, such as umu and SOS chromotest or differential DNA-repair assays based on the comparison of the survival of strains differing in their DNA-repair capacity[256].

Single cell gel electrophoresis (SCGE) assays are based on the determination of DNA migration in an electric field which leads to formation of COMET shaped images. In the initial version, the experiments were carried out under neutral conditions which allowed only the detection of double strand breaks[264]. Subsequently, Tice *et al.*[265] and Singh *et al.*[266] developed protocols for experiments in which the cells are lysed under alkaline conditions (pH $>$ 13), which enables the additional detection of single strand breaks and apurinic sites. One of the main advantages of this test procedure is, that it does not require cell divisions (which are a prerequisite for gene mutation and micronucleus experiments), therefore only short incubation periods are required so that not only stable cell lines can be used for *in vitro* studies but additionally also primary cells from different organs. Furthermore, it is also possible to carry out *in vivo* experiments with rodents and to study effects in a broad variety of tissues[267]. In most human studies, peripheral lymphocytes have been used as target cells, few experiments were conducted with exfoliated epithelial cells[268–271] which are problematic due to their low viability. Very recently, also results from a human intervention trail with antioxidants were reported in which bioptic material from the colon was analysed[272].

Collins *et al.* developed in the 1990s[273] a protocol in which isolated nuclei are treated with lesion specific enzymes (endonuclease III, formadidopyrimidine glycosylase, FPG). This approach has been used intensely to study the prevention of endogenous formation of oxidised purines and pyrimidines. However, it is crucial in these experiments to determine the optimal amounts of the enzymes due to their instability. More recently, Collins and coworkers published an additional modified version of the SCGE test which enables to monitor the impact of compounds on the repair capacity of the cells[274,275] and it was shown in a few model studies that antioxidants may alter DNA-repair processes[276,277]. In order to obtain information concerning alterations of the sensitivity towards exogenous ROS mediated damage, it is possible to treat the cells with H_2O_2 other radical generating chemicals or radiation (ROS-challenge).

In the guidelines published by Tice *et al.*[265] and Hartmann *et al.*[278], a number of criteria are defined which are essential to obtain reliable results. They concern for example the number of parallel cultures and cells which are required in *in vitro* studies, the number of animals and treatment periods and also adequate statistical methods. It was generally agreed, that different parameters such as tail lengths of the comets, as well as percentage DNA in tail and tail moment are acceptable and that apart from automated image analysis systems also manual scoring methods are acceptable[279]. An important point which is also relevant for antioxidant studies concerns the fact that multiple doses should be tested to substantiate effects and that it is necessary to monitor acute toxic effects. It is well documented that cell death leads to degradation of DNA, therefore it is essential to determine the viability of the cells after treatment with appropriate methods in *in vitro* experiments and to monitor toxic effects in inner organs by histopathology[279] and exclude conditions under which strong acute effects are observed in animal experiments. These criteria are fulfilled in most recent studies, but not in a number of older investigations.

The SGCE technique has been used in numerous *in vitro* investigations to study antioxidant effects of individual compounds and complex mixtures in stable cell lines and in human lymphocytes. Typical examples are investigations of flavonoids by Anderson and coworkers with blood and sperm cells[280,281], experiments concerning antioxidant effects of coffee and its constituents[282], studies on the protective effects of tea catechins[283,284] to name only a few. Also quite a substantial number of *in vivo* studies with laboratory animals have been published, e.g. with carotenoids[285], quercetin[286], vitamins E and C[287] and garlic oil[288].

The results of human studies are described in the reviews of Moller & Loft[289–291]. Most of them were conducted as intervention trails which have the advantage that inter-individual variations can be reduced. In total, 76 investigations have been published since the first trial was conducted by Pool-Zobel *et al.* in 1997[292]. Evidence for antioxidant effects was observed in approximately 64 % of the studies (25/39); the highest number of protective effects concerned the prevention of endogenous formation of oxidised pyrimidines, (39 % of the trials, 7/19), while in few studies evidence for reduced sensitivity towards ROS and protection against formation of oxidised purines was found. The lowest rate of protective effects was seen when the SCGE analyses were carried out under standard conditions (i.e. without ROS challenge and enzymes). Clear evidence for protective effects was observed for example in experiments with lycopene and tomatoes[293], cruciferous and leguminous sprouts[294] and carotenoid supplementation[295]. In our own experiments we found strong protective properties of sumach (a common spice) and its main active principle gallic acid[296], wheat sprouts[101], after consumption of coffee[282] and in experiments with Brussels sprouts[297]. The latter observation is of particular interest; since no effects were seen in a large intervention trial in which the participants consumed 600 g of fruits and vegetables/d[298] our findings may be taken as an indication that coffee intake may contribute to a higher extent to ROS protection than plant derived foods.

Moller & Loft (2004) discuss in their papers also the possible shortcomings of intervention trails and emphasize the importance of controlled study designs which should include a sufficient number of participants and additionally also either placebo groups or washout periods[290]. These latter criteria are not fulfilled in some of the older studies.

Micronucleus (MN) experiments have nowadays largely replaced conventional *chromosomal aberration* (CA) analyses (which have been used extensively in the past to study protective effects of antioxidants towards radiation and chemically induced DNA-damage) as they are less time consuming and laborious. Both endpoints can be used in *in vitro* experiments with cell lines and lymphocytes; one of the most promising newer developments is the use of human derived liver cell lines (HepG2, HepG3 etc). HepG2 cells have retained the activities of drug metabolising enzymes including those which metabolise plant specific antioxidants and thus may reflect the effects in humans more adequately than cell lines which are commonly used in genetic toxicology (for reviews see[299–301]).

The most widely procedure used in animal experiments is the bone marrow MN assay. Since it is problematic to induce MN with chemical oxidants *in vivo*, most experiments were conducted with radiation. Standardised guidelines have been published for this test system, e.g. by OECD[302], and one of the most important parameters relevant for AO studies is the treatment period. It is also notable, that one of the main shortcomings of this test is due to the fact that reactive molecules may not reach the target cells; this explains why negative results were obtained with a number of potent genotoxic carcinogens, also antioxidant effects may not be detected due to this limitation. Typical examples for AO studies in which this assay was used are experiments with epigallocatechin gallate, vitamin C, lipoic acid, ubidecarenone or coenzyme Q(10)[303–308].

The development of the cytokinesis block micronucleus (CBMN) method by Fenech *et al.* [309,310] allows to monitor MN formation in peripheral blood cells *in vitro* and can be also used in dietary human studies with lymphocytes. It is based on the use of the mitogen phytohaemagglutinin (which stimulates nuclear division), in combination with cytochalasin B (which stops cellular division), so that MN can be scored in binucleated cells. Typical examples for the use of this approach are supplementation studies with vitamin C and intervention trials with red wine[311–313], *in vitro* experiments with wine specific phenolic compounds[314] as well as tests with the antioxidant drugs amifostine and melatonin[315].

More than 100 different oxidative modifications of DNA have been described in the literature[316] and it is notable that oxidative adducts occur at a frequency of 1 or more orders of magnitude higher than non-oxidative adducts[317]. The dominant oxidative modification of DNA is 8-hydroxylation of guanine, which leads to formation of *8-oxo-7,8-dihydro-2-deoxyguanosine* (*8-oxodG*). In most studies, this oxidation product was measured in urine and in leukocytes, but it is also possible to monitor it in various inner organs, e.g. in liver, lung and kidneys[318–321]. The first investigations were conducted in the late 1980s[322] and a number of different methods have been developed namely gas chromatography coupled with mass spectrometry[323], liquid chromatography prepurification followed by gas chromatography coupled with mass spectrometry[324], liquid chromatography coupled with tandem mass spectrometry[325], high-performance liquid chromatography with electrochemical detection[326–328], high-performance liquid chromatography with mass spectrometry[329] and enzyme-linked immunosorbent assay based methods[330].

It is well known that guanine is easily prone to oxidation if stringent precautions are not taken during preparation of samples for analysis[331], therefore much of what is measured could be artefacts. In order to prepare compounds for analysis by gas chromatography coupled with mass spectrometry which enables the simultaneous analysis of a number of different adducts[332], DNA is chemically hydrolysed and converted into a volatile form by derivatization with a suitable reagent. This reaction is normally carried out at high temperature. During the derivatization reaction, oxidation occurs to an extent that overwhelms the amount originally present[333–335]. Generally speaking, gas chromatography coupled with mass spectrometry estimates of DNA oxidation have been higher than high-performance liquid chromatography with electrochemical detection estimates by about a factor of 10[333]. The cause of this difference (an overestimate due to artificial oxidation with gas chromatography coupled with

mass spectrometry *vs* an underestimate due to inefficient enzymatic digestion with high-performance liquid chromatography with electrochemical detection) has now been settled[335]. In addition, GC/MS analyses are difficult to perform, labour-intensive and exhibit poor sensitivity or inadequate specificity when they are used to test urinary samples[336]. After enzymatic hydrolysis of DNA to nucleosides it is possible to analyse the probes with chromatography by high-performance liquid chromatography[328]. 8-Oxo-dG and its corresponding base 8-oxo-Gua are electrochemically active, lending themselves to sensitive electrochemical detection. The relative simplicity and sensitivity of high-performance liquid chromatography with electrochemical detection of 8-oxo-dG have made it the most popular method for monitoring of DNA-oxidation[337]. The high-performance liquid chromatography with electrochemical detection method itself has been criticized for its high variability[338,339]. Estimates of the ratio of 8-oxodG to dG have ranged from approximately $0{\cdot}25 \times 10^{-5}$ to $\geqslant 10^{-4}$, possibly due to artificial oxidation[337,338]. Dreher & Junod[340] described in detail how the formation of artefacts that occurs with high-performance liquid chromatography with electrochemical detection can be avoided. Also this method is prone to oxidation of samples during preparation for analysis but by use of antioxidants and anaerobic conditions during isolation and hydrolysis of DNA, these effects can be minimised.

Immunoassays have, by far, demonstrated the greatest versatility in terms of the matrices to which they can be applied (urine, serum, plasma, cell culture medium, DNA hydrolysates), and simplicity of use[330], but comparisons with urinary 8-oxodG levels by chromatographic techniques revealed strong discrepancies of the results[341–343]. The precise reason for the higher enzyme-linked immunosorbent assay based methods values remains unknown although there is a correlation between the data obtained by this and HPLC based methods[341–343]. Nevertheless, this method can be applied to studies comparing relative urinary 8-oxo-dG values among several groups if the determination of the exact concentratins of 8-oxo-dG in urine is not required[342,343].

The European Standards Committee on Oxidative DNA Damage[261,344–347] studied systematically the reasons for the discrepancies of the results obtained with different methods. Comparative analyses of baseline 8-oxo-dG levels in mammalian cell DNA, by different methods in different laboratories showed that estimates of 8-oxo-dG in pig liver, using chromatographic techniques, ranged between 2.23 and 441 per 10^6 guanines, and in HeLa cells DNA between 1.84 and 214. In the case of chromatographic methods, it was argued that spurious oxidation during work-up has been a problem and the trustworthiest results are the lowest. Most laboratories employing HPLC–ECD were able to measure chemically induced damage with similar efficiency and dose response gradients for seven of the eight sets of results were almost identical. GC–MS and HPLC–MS/MS, employed in three laboratories, did not convincingly detect dose response effects. In another study, Gedik & Collins[347] evaluated data obtained in various laboratories in studies on 8-oxodG in calf thymus DNA, pig liver, oligonucleotides, HeLa cells and lymphocytes. The authors conclude that HPLC–ECD is capable of measuring 8-oxo-dG induced experimentally in the different types of samples. On the contrary, GC–MS failed to detect a dose response of physically (photosensitizer Ro 19-8022 and visible light) induced 8-oxodG formation and was not regarded as a reliable method for measuring low levels of damage; also HPLC–MS/MS was not found capable of detecting low levels of oxidative DNA damage.

8-Oxo-dG studies were used in a number of animal studies with laboratory rodents for example with cyanidin-3-glycoside[348], curcumin[349], grape juice[350], green tea extract, catechins[351,352] and coffee[353] to name only a few.

The formation of oxidised guanine was also monitored extensively in human studies. It was shown that certain diseases such as cancer, Parkinson's and Alzheimer's diseases, multiple sclerosis, HIV, cystic fibrosis of lung, muscular dystrophy, diabetes mellitus, rheumatoid arthritis[325] as well as occupational exposure to coal dust[354], polyaromatic hydrocarbons and aromatic amines[355,356], asbestos[357], arsenic[336] and smoking[336,358] lead to increased urinary excretion.

The results of dietary intervention trials are summarised in the reviews of Moller & Loft[289,290]. At present results of 23 studies are available, and in 12, protective effects were found. Evidence for reduced formation was seen for example in intervention trials with individual vitamins (C and E) and with supplements containing different vitamins, red ginseng, green tea and red wine and in newer investigations with specific vegetables[359,360]. Machowetz *et al.* [361] published recently an interesting study in which they found a significant (13 %) reduction of the formation of oxidised bases after consumption of olive oil which could be not explained by the phenolic contents of the different oils. Examples for studies in which no effects were detected are intervention trials with β-carotene, low vitamin E levels in combination with polyunsaturated fatty acids and cranberry juice[348,362].

Correlations between different markers of oxidative stress. The questions if and to which extent relationships exist between the sensitivity of commonly used biomarkers of oxidative stress and endpoints of oxidative DNA damage, is addressed in the review of Dotan *et al.* [363]. The authors analysed the results of studies in which two or more methods were used under identical experimental conditions and conclude that good correlations exist between measurements of peroxidation products such as MDA, lipid hydroperoxides, F-2 isoprostanes, conjugated dienes, glutathione and protein carbonyls but not with other criteria of the oxidative status such as the concentrations of antioxidants (TAC) and DNA migration (SCGE assay).

The evaluation of recent studies with dietary antioxidants shows that in general good correlations are observed between different biomarkers in *in vitro* experiments while this is not the case for animal and human studies. Results obtained with curcurmin, lycopene and ellagic acid in X-radiation experiments *in vitro* showed that the antioxidants increased the levels of GSH and reduced lipid peroxidation (MDA formation), in parallel a decrease of MN formation was observed[364–366]. Also in a study with colon derived cell lines (HT29, CaCo2), and phenolic apple juice extracts, improvement of the intracellular redox status (measured with dichloroflurecein assay) was paralleled by decreased DNA-damage in SCGE experiments[367].

In a recent Indian study[368] with mice, a clear correlation was observed between protection against radiation induced

DNA-migration in lymphocytes and TBARS formation by gallic acid, while no such relations could be seen between these parameters in experiments with vitamin E deficient rats. Although the TBARS levels were significantly increased, formation of 8-oxodG and comet formation were not affected[369].

Also in human studies controversial results were obtained in multiple endpoint studies. Consumption of anthocyanin/polyphenolic rich fruit juices reduced in an intervention trial ($n = 18$) DNA-migration in lymphocytes and increased the levels of reduced GSH, while other parameters (MDA in plasma, excretion of isoprostanes in urine) were not affected[370]. Also in an earlier study ($n = 27$) with two juices containing either cyanidin glycoside or EGCG, a significant decrease of ENDO III lesions was observed in COMET assays, while other markers of the redox status (FRAP, TBARS, and FOX2) were not altered[371]. In another larger study ($n = 36$), significant decreases of plasma antioxidant capacity and reduced ratio of GSH:GGSG were found while the concentration of reduced GSH and 8-oxo-dG in lymphocytes were not altered[372].

Also changes of markers of oxidative DNA-damage did not correlate in all studies. In workers exposed to coke ovens and of graphite electrode producing plants significant increases of both 8-oxo-dG concentrations and FPG lesions were observed in white blood cells[356]. Also Gedik *et al.*[373] found a good overall correlation between 8-oxo-dG excretion in urine and FPG lesions in lymphocytes in unexposed individuals ($n = 8$), while no associations were detected at the individual level. On the contrary, no correlations between these two endpoints were found in a much larger study ($n = 99$)[374]. Only few investigations have been conducted in which MN and comet formation were studied in parallel. In *in vitro* experiments with radiated lymphocytes both parameters were reduced by several dietary antioxidants[364–366], but in a recent trial with wheat sprouts ($n = 13$), a significant decrease of FPG lesions was detected in lymphocytes whereas no differences in the comets were detected under standard conditions with Endo III and the frequencies of MN were also not altered in the same target cells[101]. It is notable that the differences between formation of 8-oxo-dG and comet formation may be due to the fact that the latter parameter detects a broader spectrum of lesions. The discrepancies between results of MN assays and comet measurements may be due to the fact that DNA oxidation is efficiently repaired and does not necessarily lead to double strand breaks which cause formation of chromosomal aberrations and MN.

The discrepancies seen in comparative experiments raise the question which of the parameters is the most reliable. In terms of prevention of diseases the most reliable endpoints are probably those which are directly related to specific pathologic conditions. For example, oxidized LDL is known to be related to coronary heart disease[375] while for chromosomal aberrations and micronuclei it is well known that they are predictive for increased cancer risks[376,377]. The association between comet formation in lymphocytes and human diseases is not fully understood at present but it is known that occupationally exposed individuals as well as individuals with diseases which are associated with elevated cancer risks show increased comet formation[257,378,379].

Measurement of antioxidant enzymes and of proteins involved in cell signalling and transcription

In many AO studies, the activities of enzymes were measured, which inactivate ROS and are part of the endogenous defence system. Table 3 lists up examples of such studies, a detailed description of currently used methods for the measurement of SOD, GPx and CAT can be found in the paper of Vives-Bauza *et al.*[380].

In many investigations it was found, that dietary factors cause an induction of SOD. For example, significant effects were seen in human trials with coffee[282], Brussels sprouts[297], gallic acid[381], plant extract supplements[382] and phenolics rich diets[383]. However, in some studies e.g. in an animal study by Akturk and coworkers[384], a decrease of its activity was found with antioxidants. Also in the case of heme oxygenase-1 (HO-1) induction effects were observed in most investigations (see Table 3), while glutathione peroxidase (GPx) was not altered in the majority of human and animal studies[282,381,384–386], one exception being experiments with *Ginkgo biloba* extracts administered to ethanol fed rats, in which an increase was detected[387]. It can be seen in Table 3 that the results of catalase (CAT) measurements with different dietary the antioxidants are inconsistent. Antioxidant diets caused an induction of CAT in a human study[382] while garlic feeding to rats caused a decrease of its activity[386].

NADPH-quinone reductase (NADPH-QR) was used as a marker enzyme for the general induction of phase I and phase II enzymes. Prochaska & Talalay[388] developed a protocol for high throughput measurements of food derived enzyme inducers with a murine hepatoma cell line (Hepa 1c1c7), subsequently, an improved protocol was developed, which enables the discrimination between monofunctional and bifunctional inducers[389] i.e. between compounds which cause an induction of both, phase I and phase II enzymes and those which only affect the latter group. In a large number of studies, induction of NADPH-QR was observed with a variety of different dietary compounds in this experimental system; one of the most potent inducers identified was sulforaphane, which was used as a model compound in numerous subsequent experiments[390]. However, comparative measurements in human derived cells (Hep G2) showed, that the measurement of NADPH-QR does not correlate quantitatively with the induction of GST, a key enzyme involved in the detoxification of many carcinogens[391].

Gamma glutamylcysteine synthetase (GCS) can be regarded as an "indirect" AO enzyme. It catalyses the rate limiting step of glutathione (GSH) synthesis[392] and its activity can be measured with the protocol of Nardi and coworkers[393]. This mehtod is based on the formation of c-glutamylcysteine from cysteine and glutamic acid which is quantified by derivatization with bromobimane and fluorimetric detection after separation by high performance liquid chromatography. It was shown that coffee diterpenoids and also other antioxidants such as the phenolic food additive butylated hydroxyanisole and vitamin E induce GCS[394–396]. GSH itself is a potent antioxidant and scavenges predominantly O_2^-, $^{\cdot}OH$, $RO^{\cdot}$ and $ROO^{\cdot}$[397]. In a number of dietary studies the ratio between oxidised and reduced GSH was monitored as a parameter of antioxidant effects. The method is based on spectrophotometric measurements with Ellmans reagent[398]

Table 3. Overview on currently used methods for the detection of antioxidant enzymes and examples of their induction by food constituents

Detection method*	Example/results†	Reference
Superoxide dismutase (SOD)		
SM of SOD activites: Bioxytech SOD-525™ kit (OXIS International): tetrahydro 3,9,10-trihydroxy-benzofluorene is converted to a chromophore, $\lambda = 525$ nm	Untreated hypertensive (SHR) rats ($n = 6$/group): significant ↑ of immunodetectable SOD in the renal cortex and ↓ in the medulla	(585)
WB analysis of protein levels with immunodetection (using a primary antibody for Cu Zn SOD and Mn SOD)	A diet enriched with α-tocopherol, ascorbic acid, selenium, zinc reversed: the effects on SOD on the protein level	
t.o. kidney	In control rats no effect of the AO-diet was seen ↔ SOD activity (same in all groups)	
SM (McCord & Fridovich[586]) ferricytochrome c as indicating scavenger (SOD activities were assayed by their capacity to compete with native or partially succinylated ferricytochrome c for superoxide radicals generated by the xanthine/xanthine oxidase system); $\lambda = 550$ nm	Plant extracts in capsules (675 mg/d for 120 days Protandim: *B. monniera*, *S. marianum*, *W. somnifera*, green tea polyphenols, EGCG, tumeric) were administered to healthy volunteers ($n = 20$) and caused a significant ↑ in erythrocyte SOD after 120 days	(382,586)
t.o. blood		
SM of SOD-activity[587] in the supernatant of tissue homogenates: a red formazan dye formed by superoxide radicals (generated by the xanthine/xanthine oxidase system) reacting with iodophenyl-nitrophenol phenyltetrazolium	Diazinone (an insecticide causing ROS and lipid peroxidation) treated Wistar albino rats ($n = 6–8$/group): ↑ levels of SOD Diazinone + Vit C + Vit E: ↓ SOD activity and ↓ lipidperoxidation	(384,587)
t.o. heart		
SM[588]; modified from[589] of erythrocyte Cu Zn SOD activity by monitoring the inhibition of autooxidation of pyrogallol; $\lambda = 320$ nm	Soy isoflavone-rich capsules (138 mg isoflavones/day for 24 days or placebo, cross-over design) were given to postmenopausal breast cancer survivors ($n = 7$); a significant ↑ of erythrocyte Cu Zn SOD levels was found	(588,590)
t.o. blood		
Catalase (CAT)		
SM[591]: variation of absorbance due to dismutation of H_2O_2 by CAT, $\lambda = 240$ nm	Untreated hypertensive (SHR) rats: ↑ levels of immunodetectable CAT compared to control rats, but no ↑ in enzyme activity	(585)
WB: quantification of post translational protein levels (immunodetection with primary antibodies)	Antioxidant therapy (see above): ↑ CAT activity	
t.o. kidney		
SM of erythrocyte CAT[592] following breakdown of H_2O_2 based on UV-absorption of peroxide, $\lambda = 240$ nm	Supplementation with plant extract (675 mg/d for 120 days; see above): ↑ erythrocyte CAT activity in healthy volunteers ($n = 20$)	(382,592)
t.o. blood		
SM of the rate constant of decomposition of H_2O_2[593]; $\lambda = 240$ nm	Diazinone (see above) treatment in Wistar rats: ↑ CAT activity	(384,593)
t.o. heart	Diazinone + Vit C + Vit E: ↔ CAT activity	
SM of H_2O_2[593]	Effect of a 2 % garlic diet (2 weeks) on renal and hepatic CAT expression of Wistar rats ($n = 10$/group)	(386)
WB analysis of protein levels (immunodetection with rabbit anti-human CAT antibody)	CAT activity ↓ and CAT protein level ↓ (Western blot) in garlic fed rats in kidney and liver	(593,594)
Northern blot analysis of m-RNA levels	CAT m-RNA level ↔ (Northern blot)	
CAT synthesis and degradation determined by kinetic of reappearance of CAT activity after aminotriazole injection according to Price *et al.*[594]	Rate of CAT synthesis ↓ in kidney and liver Rate of CAT degradation ↔	
t.o. kidney and liver		
Glutathione peroxidase (GPx)		
SM of GPx activity[595]: GSH oxidation by cumene hydroperoxide is catalysed by GPx; GSH-reductase recovers GSSG with concomitant oxidation of NADPH to $NADP^+$; decrease of absorbance by NADPH is measured at $\lambda = 340$ nm	Diazinone treatment in Wistar rats: ↔ GPx activity; diazinone + Vit C + Vit E: ↔ GPx activity; both groups had the same GPx activity as the control group	(384)
t.o. heart		(595)
SM of GPx activity[596] using the same substrates as Paglia & Valentine[595], $\lambda = 340$ nm	2 % garlic diet had no effect on GPx activity in kidneys and livers of Wistar rats ($n = 10$/group)	(386)
t.o. kidney and liver		
SM of GPx activity: Bioxytech GPX-340™ kit (OXIS International): the assay is based on the assay of Paglia & Valentine[595] but *t*-BOH is used instead of cumene hydroperoxide	Untreated hypertensive (SHR) rats ($n = 10$): ↑ of immunodetectable GPx, ↔ GPx activity compared to controls (WKY) AO treated (diet enriched with α-tocopherol, ascorbic acid, selenium, zinc) SHR rats: ↓ of immunodetectable GPx to the levels seen in controls, slight ↓ GPx activity	(585)
WB analysis of protein levels (immunodetection with primary antibodies)		
t.o. kidney		
SM of GPx activity[597] with slight medications: incubation with H_2O_2 and GSH, addition of trichlor-acetic acid; after centrifugation disodium hydrogen phosphate and DTNB are added to the supernatant and absorbance measured, $\lambda = 412$ nm	Ethanol fed rats (2·4 g/kg, $n = 8$/group): ↓ GPx activity After administration of a *Ginkgo biloba* extract (48 or 96 mg/kg) for 90 days before ethanol treatment: ↑ GPx activity No effect on GPx without ethanol treatment	(387,597)
t.o. liver		

Table 3. *Continued*

Detection method*	Example/results†	Reference
Heme oxygenase-1 (HO-1)		
RT-PCR for m-RNA level determination	Chronic ethanol administration to rats (see above):	(599)
Flow cytometry for determining HO-1 protein levels according to Chow *et al.*[598]: fluorescent emission caused by binding of HO-1 antibodies was measured with a flow cytometer	↓ HO-1 expression on m-RNA and protein level *Ginkgo biloba* extract without ethanol treatment: ↑ of HO-1 m-RNA and protein levels *v.* controls	(598)
t.o. liver		
SM of HO-1 activity[600]: determination of the conversion rate of heme to bilirubin (Δ absorbance between $\lambda = 464$ and 530 nm)	Coincubation of human hepatocytes with ethanol (100 mM) and quercetin (10–200 μM) for 24 h *in vitro*: ↑ of HO-1 activity and protection from ethanol derived oxidative stress	(599,600)
Cells: human hepatocytes		
SM of HO-1 activity (see above)	Exposure of cells to curcumin (5–15 μM, for 18 h): ↑ of HO-1 m-RNA, ↑ of HO-1 protein level, ↑ of HO-1 activity	(601)
WB analysis of protein levels (immunodetection with a polyclonal rabbit anti-HO-1 antibody)	Hypoxic culture conditions (for 18 h): ↑ of HO-1 m-RNA, ↑ of HO-1 activity, effect potentiated by curcumin (5 μM)	
Northern blot analysis of m-RNA levels		
Cells: bovine aortic endothelial cells		
NADPH-quinone reductase (NAPHD-QR)		
Luciferase assay (Dual-Luciferase Assay System, Promega): rat QR luciferase promoter construct transfected into human hepatoma cells; luciferase activity determined as ratio of firefly luminescence to renilla luminescene by use of a luminometer	Sulphoraphane treated Hep G2 cells transfected with QR reporter constructs: ↑ of NADPH-QR transcription	(391)
Cells: human hepatoma cell line (HepG2)		
SM of NADPH-QR activity[602]: dicumorol sensitive reduction of DCPP, $\lambda = 600$ nm	CDF 344 (crl/BR) male rats ($n = 4$/group) received crambene (50 mg/kg bw) or I3C (56 mg/kg bw) by gavage for 7 days: ↑ of NADPH-QR and GST activity	(602,603)
t.o. liver	Co-treatment of crambene and I3C potentiated activation of NADPH-QR and GST	
Quantitative bioassay of inducer potency (adapted from De Long *et al.*[604] as described in Prochaska *et al.*[388]) in Hepa 1c1c7 cells grown in 96-well microtiter plates; SM of the reduction of 2,6-dichloroindophenol by NADH; $\lambda = 610$ nm	Hepa 1c1c7 murine hepatoma cells treatment with extract of mature fresh broccoli or of broccoli sprouts: ↑ of NADPH-QR activity; see also Talalay *et al.*[605] for results with different compounds	(390,606)
Cells: Hepa 1c1c7 murine hepatoma cell line		
Inducible nitric oxygen synthetase (iNOS)		
RT-PCR for m-RNA level determination	*In vitro* treatment of LPS-stimulated mouse macrophage cells (RAW 264.7) with lutein: ↓ LPS-induced NO-production; ↓ iNOS m-RNA levels; ↓ iNOS protein levels	(607)
WB analysis of protein levels		
Cells: mouse macrophage (RAW 264.7)		
RT-PCR for m-RNA level determination	Transgenic mice (Alb c-Myc/TGF-α) show ↑ iNOS levels; dietary supplementaion with Vit E (2000 units/kg diet for 9 or 26 weeks, $n = 8$–15/group): ↓ iNOS expression	(608)
WB analysis of protein levels		
Immunohistochemistry (using a mouse monoclonal anti-iNOS antibody)		
t.o. liver		
RT-PCR for m-RNA level determination	Curcumin (1–20 μM) treatment caused of *ex vivo* cultured mouse macrophages: ↓ iNOS m-RNA level	(609,610)
Citrulline assay (according to Hevel & Marletta[609]) enzyme activity measured by the conversion rate of [^{3}H]arginine to [^{3}H]citrulline, the level of radioacitivity correlates to enzyme activity	Oral administration (2 × 92 ng curcumin/g bw) to LPS pretreated mice: ↓ iNOS m-RNA ↔ in *ad libitum* fed mice	
t.o. liver, *cells*: BALB/c mouse peritoneal macrophages		
NAD(P)H: quinone oxidoreductase 1 (NQO1)		
RT-PCR for m-RNA level determination	Treatment of cells with the phenolic antioxidant butylated hydroxyanisole (5–200 μM for 24 h) and its metabolite *tert*-butylhydroquinone (200 μM for 24 h): ↑of NQO1 protein level and ↑ of NQO1 transcription	(611)
WB analysis of protein levels		
Cells: primary cultured rat and human hepatocytes		
SM of NQO1 activity[388,389]: based on the coupling of the oxidation of menadione to the reduction of a tetrazolium salt and measuring the formation of the resulting blue formazan dye with a microplate reader; $\lambda = 595$ nm	6-methylsulfinyl hexyl isothiocyanate (an active principle of wasabi) treatment (0·1–5 μM for 24 h) of cells ↑ of NQO1 protein level and ↑ of NQO1 activity	(612)
RT-PCR for m-RNA level determination		
Cells: Hepa 1c1c7 murine hepatoma cells		
SM of NQO1 activity[613] using 2,6 dichlorophenol indophenol as a substrate	Oral treatment of A/J mice ($n = 4$–5/group) with garlic organosulifdes (2 × 25 μM): ↑ of forestomach NQO1 activity, ↑ of forestomach NQO1 protein levels	(614)
WB analysis of protein levels (due to low pulmonary expression of NQO1, WB analysis could not be performed in the lung)	DATS and DAS treatment: ↑ of lung NQO1 activity	
t.o. forestomach and lung		

* SM, spectrophotometric measurement; t.o., target organ; GSH, glutathione; GSSG, oxidised form of glutathione; NADPH, nicotinamide adenine dinucleotide phosphate; n.i., not indicated; RT-PCR, real time polymerase chain reaction; WB, Western blot.

† ↑, increase; ↓, decrease; ↔, unchanged; bw, body weight; h, hours; AP-1, activating protein-1; CAT, catalase; DAS, diallyl sulphide; DATS, diallyl trisulfide; EGCG, epigallocatechin-3-gallate; GPx, glutathione peroxidase; HO-1, heme oxigenase-1 protein; LPS, lipopolysaccharide; iNOS, inducible nitric oxide synthase; NAD(P)H, nicotinamide adenine dinucleotide phosphate; QR, quinone reductase; SOD, superoxide dismutase; TGF-α, transforming growth factor-α.

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and changes were observed in newer *in vivo* studies for example with kawheol/cafestol[394], polyphenol rich cereals[399] antioxidant (vitamins C and E, L-carnitine, and lipoic acid or additional bioflavonoids, polyphenols, and carotenoids) enriched diets[400] and vitamin E[401]. GSH is also the substrate of glutathione-*S*-transferases (GSTs), which are regulated by Nrf2 and catalyse the detoxification of a broad variety of xenobiotics including food specific carcinogens (for reviews see[402,403]) and it has been postulated that GSTs are also involved in the inactivation of radicals[397,402].

Increased levels of the antioxidant enzymes listed in Table 3 are also seen under oxidative stress conditions and in inflammatory and ROS-related diseases and in tumour tissues[404,405]. This holds also true for GCS which is increased for example by NO and in meta- and dysplastic cells[404]. Therefore the induction of these enzymes cannot be taken as a firm evidence for protective properties of dietary constituents and results of such measurements should be supported by additional data.

A plethora of methods has been developed to monitor the impact of antioxidants on proteins involved in cell signalling and on the activation of transcription factors. A detailed description of the currently used techniques is beyond the scope of the present article. In general, biochemical standard methods, such as Western blots, Northern blots, real time PCR and ELISA-based techniques are employed. For the measurement of kinases, e.g. mitogen activated protein kinases (MAPKs) and others, like protein kinase C (PKC) as well as phosphoinositide 3 kinase (PI3K), which are also important targets of phytochemicals[121,406] mainly Western blots with phosphorylation specific antibodies[407,408] and more recently also more expensive ELISA techniques are used (for commercially available test kits and description of methods see also www.biocompare.com or www.cellsignal.com). Transcription factors like the nuclear factor kappa B (NFκB) can be determined by the luciferin/luciferase system, which is a sensitive reporter assay for gene expression. D-Luciferin is the substrate for firefly luciferase catalyzing the oxidation of luciferin to oxyluciferin in presence of ATP and magnesium, resulting in bioluminescence[409]. By use of this assay, expression of NFκB can be measured *in vivo* simultaneously in different organs[410] or in stable cell cultures[391,411].

Two other enzymes, which are often monitored in antioxidant studies are cyclooxygenase 2 (COX 2) and ornithine decarboxylase (ODC). They are regarded as important makers of inflammation and tumour development.

COX 2 is an inducible enzyme which can be activated by NFκB and catalyses the cyclooxygenase pathway of prostaglandin synthesis from arachidonic acid. It is abundant in activated macrophages and other cells at sites of inflammation. COX 2 overexpression has been found in various types of human cancers[412]. Protein and m-RNA levels of COX 2 are measured with Western blots, SDS-PAGE, and Northern blots. COX 2 activity can be determined in various ways; a) oxygen consumption can be monitored using an oxygen electrode[413], b) by measurement of COX 2 produced prostaglandines via enzyme immunoassays (EIA)[414], c) In addition, several methods for the determination of peroxidase activity of COX 2 with luminescent detection have been developed (for a detailed description of methods and commercially available test kits see e.g. www.caymanchem.com/app/template/cur-rents,010.vm/a/z; ELISA kits also can be found on www.calbiochem.com). The results obtained in experiments with dietary antioxidants on COX 2 inhibition are reviewed in the article of Surh and coworkers[415].

ODC catalyses the decarboxylation of ornithine into putrescine and is involved in cell proliferation. Its activity is rapidly modified in response to various chemical or physical stimuli[416]. ODC is a marker enzyme for tumour promotion and is upregulated in transformed cells[417]. The reduction of its activity as well as reversal of the malignant state of cells by antioxidants has been shown in many studies[418,419]. Various methods for ODC activity measurements were developed, including radiometric assays based on trapping the $[^{14}C]O_2$ generated from the decarboxylation of $[1^{-14}C]$ornithine[420], the retention of ^{3}H-putrescine from tritiated ornithine on a strong cation-exchange paper[421] and the determination of the radiolabelled form of the enzyme-activated suicide inhibitor of ODC, difluoro-methylornithine (DFMO)[422]. A radioimmunoassay using purified polyclonal antibodies has also been employed in some experiments[423]. More recently, chemiluminescence and spectrophotometry based methods have became available[416,424].

Use of -omics based approaches for the investigation of oxidant and antioxidant effects

During the last decade, a number of new techniques has been developed and optimised which enable the simultaneous detection of a large number of alterations of biological functions. The use of these approaches in nutritional sciences has led to the formation of a new discipline termed "Nutrigenomics"[425]. The overall aim of this new field of research is to find out how dietary factors alter gene transcription, protein expression and metabolism[426] in regard to health effects. The area covers three interrelated main areas namely transcriptomics (gene expression analyses), proteomics (global protein analysis) and metabolomics (metabolite profiling). Details on the basic concepts can be found in the article of Kussmann *et al.* [427].

In the following chapters we will focus on the description of results which have been obtained in studies concerning the effects of oxidative stress and with dietary antioxidants in transcriptomics, proteomics and metabolomics. The latter approach are based on the chemical characterisation of metabolites by use of nuclear magnetic resonance and/or mass spectrometry[428,429]. The use of these techniques for dietary studies is described in recent reviews, e.g.[430,431]. Of particular interest for the detection of oxidant/antioxidant effects are analyses of the lipidome (i.e. of the lipids in biological liquids).

Only few dietary studies have been carried out in which the effects of plant foods, beverages and phytochemicals were investigated. Urinary patterns of three types of diets – vegetarian, low meat and high meat consumption in humans were compared, and a specific vegetarian metabolic signatures (i.e. a metabolite of microbial metabolism) were found[432]. In other dietary studies with humans, e.g. with camomile tea[433] and also with black and green teas[434] changes were registered; e.g. in the latter trial a change of the excretion of dihydroxyphenylsulphates (end products of flavonoid degradation by colonic bacteria) was seen.

Other studies which are notable are the one of Solanky *et al.* [435] who investigated the impact of isoflavones on plasma profiles, and the investigations of Ito *et al.* [436,437] who studied metabolites of dietary polyphenolics. Fardet *et al.* studied the excretion profiles after consumption of whole-grain and refined wheat flours[438]. Overall, none of these investigations is clearly indicative for antioxidant properties of the food factors studies and the changes detected concern alterations of the composition of the gut microflora and of energy metabolism, or the identification of compound specific metabolites.

Transcriptomics

Basic principles. Subtractive and differential hybridization of messenger RNAs (mRNAs) have been the most frequently employed techniques to determine disparities in the steady state expression levels of transcripts in cells at a given time, the latter in general referred to as the transcriptome. Starting in the early eighties, cDNA (complementary DNA to mRNA) libraries have been used to identify clones representative of genes whose changes in transcription and/or mRNA stability result from variations in the proliferation state[439], the stage of development and disease[440,441], or the consequences of an agent[442]. Although these techniques allow to reveal the whole transcriptome and to select for both, known and unknown differentially expressed mRNAs characteristic to open systems, these methods showed limitations due to the preferential identification of highly to moderately abundant mRNAs[443]. Subsequently, several more efficient procedures emerged, among them the reverse transcription-polymerase chain reaction (RT-PCR)-based representational difference analysis (RDA)[444], the differential display (DD) technique and the serial analysis of gene expression (SAGE)[445,446], which both represent open screening techniques even allowing to detect and quantify differentially expressed mRNA species of low abundance. Yet, these methods are subject to the criticism that they require considerable sequencing efforts and may yield false positive signals which demand additional experimental arrangements to get rid of them. In the mid to late nineties, DNA microarrays have been developed for the high throughput expression profiling of the transcriptome which facilitates to dissect the genetic flow of information upon various (patho)physiological stages and to identify suitable drug targets[447,448].

DNA microarrays are currently widely used and provide the unique opportunity to detect hybridization signals to sequenced clones and collections of partially sequenced cDNAs known as expressed sequence tags (ESTs)[449,450]. A variety of different cDNA microarrays with high density hybridization targets immobilized on nitrocellulose or nylon surfaces are available and frequently applied in basic research and clinical settings[451]. Advances in the microarray technology are associated with the development of different techniques to synthesize DNA probes and to deliver them by immobilization on solid surfaces[452]. Beside mechanical microspotting and ink jetting of non-synthetic cDNA probes with various lengths on coated glass slides which were prepared by biochemical methods such as PCR, the photolithography technologies employs *in situ* synthesis of oligonucleotides.

The isolation of total RNA or mRNA by standard biochemical methods requires quality control by analyzing the respective integrity. Solid techniques are available to amplify even low amounts of the extracted RNA for subsequent labeling and hybridization on microarrays. Particular efforts have been made to separate RNA in polysome-associated species which are involved in protein synthesis and to profile translated mRNA in order to more closely depict the proteome and to identify both transciptionally and translationally controled mRNAs[453–455]. In all experimental settings, standard operating procedures are recommended for the RNA extraction and labeling, the hybridization procedure and the subsequent monitoring of hybridization signals to obtain reproducible and comparable microarray data. Cross-analysis of hybridization signals from independent experiments performed in triplicate ensures a high reliability of results. For routine applications, e.g. GeneSpring™ provides a user-friendly solution for the computational analysis of raw data allowing hierarchical clustering of data sets. However, evaluation of whole-genome microarrays requires particular efforts with regard to the management of data. Changes in the transcriptome indicated by microarray assays depend finally on the confirmation of data by quantitative RT-PCR which is the most sensitive method for the detection of rarely expressed transcripts[456]. The incorporation of SYBR Green dye or the disruption of the Taqman probe exhibit fluorescence during RT-PCR, and are frequently using techniques for the quantitative analysis of mRNA expression. Together, cDNA or oligonucleotide microarrays are regarded as powerful tools for the profiling of the transcriptome, and being greatly versatile in gene throughput by customization and applicability in various experimental approaches.

Results of microarray studies concerning the transcription of genes by oxidative stress. Several reviews on the impact of oxidative stress on gene regulation have been published[80,457–459], probably the most comprehensive overview is the one by Allen & Tressin[80]. However, most of the data contained in these surveys are derived from experiments with conventional methods (including RT-PCR). The present paper is confined to newer findings obtained with arrays which are increasingly used in the last years.

The investigations can be grouped into three categories namely *in vitro* experiments with stable cell lines or primary cells; animal studies and gene expression analyses with humans. Table 4 gives an overview of groups of genes which were measured in a number of studies.

In vitro studies: An interesting and promising approach has been published by Scherf and coworkers[460] who assessed gene expression profiles in 60 human derived cancer cell lines for a drug discovery screen. They used relatively large arrays (8000 genes) and tested approximately 70 000 compounds; subsequently the results were clustered according to different parameters including known mechanisms of action of the different compounds. The database which emerged from this large study is available from NCI (http//dtp.nci.nih.gov) and provides a valuable source of information. It was employed for example in a study by Efferth & Oesch[461] to characterize the molecular mechanisms of the toxicity of two antimalarial drugs; the authors selected from the database 170 genes involved in oxidative defence and metabolism which they used to design a custom made array and tested the acute toxic effects of the compounds in the individual cell lines; on the basis of the patterns they found distinct differences in the mode of action of the two drugs.

All other studies with human derived cells comprised a lower number of cell lines and chemicals. Murray *et al.* [462] compared

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Table 4. Examples for genes which are transcriptionally regulated by oxidative stress

Gene name [Gene bank ID]	Protein function and occurrence
GPX1 [NM_000581], GPX2 [NM_002083], GPX3 [NM_002084], GPX4 [NM_002085], GPX5 [NM_001509], GPX6 [NM_182701], GPX7 [NM_015696]	Different forms of glutathione peroxidase which detoxify H_2O_2 *OS**: GPX2 mainly in the GI tract and liver, GPX4 – testes, GPX5 – epidymis, GPX6 – embryonal form *LO*†: GPX1, GPX2 cytoplasm, GPX3, GPX5, GPX6 – secreted protein GPX4 – mitochondria, cytoplasm
GSTZ1 [NM_145870]	Maleylacetoacetate isomerase: bifunctional enzyme catalyzing glutathione-conjugation with specific substances – has also GPx activity *OS*: primary in liver and kidney *LO*: cytoplasm
PRDX1 [NM_002574], PRDX2 [NM_005809], PRDX3 [NM_006793], PRDX4 [NM_006406], PRDX5 [NM_012094], PRDX6 [NM_004905]	Peroxiredoxins reduce peroxides, PRDX1, PRDX2 may be involved in cell signalling cascades of growth factors and TNFα by regulating the intracellular concentrations of H_2O_2. PRDX3 and PRDX4 are involved in MAP3K13 which triggers the regulation of NFκB, PRDX6 is involved in the detoxification of H_2O_2, fatty acids, and phospholipids *LO*: PRDX1, PRDX2, PRDX4 – cytoplasm, PRDX3 – mitochondria, PRDX5 – mitochondria, cytoplasm, peroxisomes, PRDX6 – cytoplasm, lysosome
CAT [NM_001752]	Catalase: detoxification of H_2O_2, one of the most important detoxifying enzymes found in many eukaryotic and prokaryotic cells *LO*: Peroxisomes
MGST3 [NM_004528]	Microsomal glutathione *S*-transferase 3: functions as a glutathione peroxidase *OS*: heart, skeletal muscle *LO*: microsome
SOD1 [NM_000454], SOD2 [NM_000636], SOD3 [NM_003102]	Superoxide dismutase which detoxifies $O_2^{\cdot -}$ *LO*: SOD1 is found intracellulary (cytoplasm), SOD3 (Cu–Zn dependent) is found in extracellular fluids (plasma, plasma, lymph and synovial fluid). SOD2 (Mn – form) is found in mitochondria
TXNRD1 [NM_003330], TXNRD2 [NM_006440]	Thioredoxin reductase, contributes to oxidative stress resistance, maintains thioredoxin in a reduced state *OS*: TXNRD2 is highly expressed in the prostate, ovary, liver, testes, uterus, colon and small intestine *LO*: TXNRD1 – cytoplasm, TXNRD2 – mitochondria
MT3 [NM_005954]	Metallothionein-3: binds heavy metals and is known to be also involved in oxidative defence *LO*: Abundant in a subset of astrocytes in the normal human brain
NOS2A [NM_000625]	Nitric oxide synthase (inducible) produces $NO^{\cdot}$ which is a messenger molecule with diverse functions *OS*: expressed in liver, retina, bone cells and epithelial cells of the lung, not expressed in platelets
AOX1 [NM_001159]	Aldehyde oxidase, catalyses the formation of carboxylic acids from aldehydes (aldehyde $+ H_2O + O_2 =$ carboxylic acid $+ H_2O_2$) *LO*: abundant in liver and muscle
EPHX2 [NM_001979]	Epoxide hydrolase 2 – substrates are hydroxyl perepoxides such as alkene oxides, oxiranes, catalyses also the detoxification of toxic xenobiotics *LO*: cytoplasm, peroxisomes
FOXM1 [NM_202002]	Forkhead box protein M1 transcriptional activation factor: may play a role in the control of cell proliferation *OS*: Expressed in thymus, testis, small intestine, colon, followed by ovary; appears to be expressed only in adult organs containing proliferating/cycling cells or in response to growth factors *LO*: nucleus
GLRX2 [NM_016066]	Glutaredoxin-2 (mitochondrial form): glutathione dependent oxidoreductase that maintains mitochondrial redox homeostasis upon induction of apoptosis by oxidative stress *OS*: Widely expressed in different organs (brain, heart, skeletal muscle, colon, etc.) *LO*: Isoform 1: mitochondria. Isoform 2: nuclei
FDX1 [NM_004109]	Ferredoxin-1: participates in the synthesis of thyroid hormones; electron transport intermediate of cytochrome P450 *LO*: mitochondria, mitochondrial matrix
CCNA2 [NM_001237]	Cyclin-A2 – cell cycle regulation *LO*: In contrast to cyclin A1, which is present only in germ cells, this cyclin is expressed in a broad variety of tissues
JUND [NM_005354]	Transcription factor jun-D: binds to AP-1 site and upon cotransfection stimulates the activity of a promoter that bears an AP-1 site, binds DNA as a dimer *LO*: nucleus

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Table 4. *Continued*

Gene name [Gene bank ID]	Protein function and occurrence
HMOX1 [NM_002133]	Heme oxygenase 1 − cleaves the heme ring at the alpha methene bridge to form biliverdin, which is subsequently converted to bilirubin by biliverdin reductase. Under physiological conditions, the activity of heme oxygenase is highest in the spleen, where senescent erythrocytes are sequestrated and destroyed *LO*: microsomes
HSPA1A [NM_005345], HSPA1B [NM_005346]	In cooperation with other chaperones, Hsp70s stabilize preexistent proteins against aggregation and mediates the folding of newly translated polypeptides in the cytosol as well as within organelles. These chaperones participate in these processes through their ability to recognize nonnative conformations of other proteins
GCLM [NM_002061]	8-Glutamyl-cysteinyl ligase − ATP + L-glutamate + L-cysteine = ADP + phosphate + gamma-L-glutamyl-L-cysteine *LO*: in all tissues examined; highest levels in skeletal muscles
TOP2A [NM_001067], TOP2B [NM_001068]	DNA topoisiomerase: control of the topological states of DNA by transient breakage and subsequent rejoining of DNA strands; topoisomerase II causes double-strand breaks *LO*: TOP2A: generally located in the nucleoplasm, TOP2B: in the cytoplasm
DDIT3 [NM_004083]	DNA damage-inducible transcript 3: inhibits the DNA-binding activity of C/EBP and LAP by forming heterodimers that can not bind DNA *LO*: nucleus

*OS, Organ specificity.
†LO, Location.

the effects of different types of stress (heat shock, ESR stress, oxidative stress and crowding) in human fibroblasts and HeLa cells and found clear differences in the responses. On the contrary, similar transcription patterns were identified by Chuang *et al.* [463] who analysed the effects of three ROS generating chemicals namely H_2O_2, 4-HNE and tBOH in human retinal cells. Other papers with human derived cells were published by Yoneda *et al.* [464], who studied the effects of H_2O_2 and tobacco smoke in bronchial epithelial cells and Morgan and coworkers[465] who used the human derived liver cell line HepG2 to investigate the effects of a variety of ROS generating chemicals. Except in the later study, relative large arrays (>1000 genes) were used in these investigations. In some experiments, ionising radiation was used to induce stress responses; for example Amundson *et al.* [466] analysed the expression of genes caused by γ-radiation in twelve different human cell lines.

Another topic which was addressed in *in vitro* gene expression studies concerns the impact of specific cell functions (transcription factors, enzymes involved in signalling pathways) which are regulated by ROS. Investigations which fall into this category are for example analyses of the impact of PI3K in tBOH induced gene expression in IMR3 cells[467], experiments on the role of the BRCA1 gene in breast cancer cells[468], as well as comparative studies with cell lines differing in their p53 status, Nrf2 functions and SOD activity[469–471].

Animal experiments: Only a few studies have been carried out in which the effects of ROS were investigated in laboratory rodents have been published so far. Examples for experiments with different oxidants are described in the articles of McMillian *et al.* [472,473] who analysed gene expression patterns of oxidants in rat livers and attempted to establish compound specific expression signatures. Examples for the use of knockout animals are the investigations of Yoshihara *et al.* [474] and Thimmulappa and coworkers[475] who studied comparatively gene expression patterns in normal, SOD and Nrf2 deficient animals.

Another topic which has been addressed in a number of *in vivo* array studies concerns the impact of ageing on gene transcription and oxidant responses. As mentioned above, it is assumed that several age related diseases are due to increased oxidative damage and it was shown by Lee *et al.* [476–478] that transcription patterns in muscles, brain and heart of mice are age dependent. Edwards *et al.* [479] found subsequently, that paraquat induced expression patterns differ significantly depending on the age of the animals.

Human studies: Only few articles have appeared in the last years. An example for an occupational study is the one published by Wang *et al.* [480] who investigated the responses of blood cells in metal fume exposed workers. It is assumed that ROS are at least partly involved in the toxic effects of metals and indeed the authors found that oxidative damage responsive genes were altered in their expression. Microarrays were also used in a number of studies with patients who suffered from ROS related diseases such as rheumatoid arthritis, inflammatory bowel disease, arterial fibrillation and sickle cell anaemia[481–483].

The results of all these investigations aimed at identifying genes which are transcriptionally regulated by ROS depend largely on the experimental model used and the most important parameters are the origin of the cells, the time schedule as well as the mechanisms by which oxidative damage is induced; also the design and size of array technique plays an important role.

The results of the *in vitro* experiments described above show that the patterns of gene alterations caused by oxidants depends primarily on the type of indicator cells used and to a lesser extent on the chemicals[462,484]. On the basis of the current state of knowledge one may expect that genes which

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are predominately altered in their transcription are those which are regulated by ROS dependent transcription factors. Indeed, it was found in some studies that Nrf2/ARE, apoptosis and p53 regulated genes are affected[467,468,472,473]. However, in some investigations only little evidence for the up regulation of such genes was detected[468,485,486] and often genes were identified which are involved in cell cycle regulation, cellular communication, biosynthesis and metabolism. Some of them are controlled by ROS dependent signalling pathways and transcription factors but the patterns seen in the different studies are highly divergent and it is not possible to identify specific marker genes[467,468,484,487].

What are the reasons for the strong inconsistencies? As mentioned above, one of the important parameters is the origin of the cells. For example, Murray *et al.* [462] compared the effects of ROS generating chemicals in human fibroblasts and HeLa cells under identical experimental conditions and found the former cells by far more responsive. The reasons for these discrepancies may be due to the fact that signalling pathways and transcription factors are partly tissue specific and may be impaired in cancer cells lines which are frequently used in array studies. Nrf2 and p53 are found in a broad variety of different organs[488], while NFκB is lacking in a number of tissues[80]. It is known that p53 is frequently mutated in tumours in many organs[489–491], and it was shown in a comparative study with p53 + and p53 − cell lines that loss of this function has a substantial impact on oxidative stress induced gene transcription[469]. In addition, it is so that xenobiotic drug metabolising as well as antioxidant enzymes, which are controlled by the ARE element are highly organ specific (see Table 3). It is also well documented that stable cell lines used routinely in toxicological studies lack the activities of phase I, phase II and antioxidant enzymes which are regulated by ARE via Nrf2[299], therefore strong efforts have been made to establish cell lines which have retained the activities of these enzymes in an inducible form. Also other factors may account for the inconsistency of the results[462] found strong differences between the results of array studies conducted *in vitro* and in rodents, and the authors hypothesized that the fact that substantially more genes were transcriptionally regulated *in vivo* may be due to the inadequate representation of cell communication in cultures.

Another important parameter which has been often neglected is the time dependency of AO responses[462,464,487]. For example, three distinct phases could be discriminated in experiments with human bronchial epithelial cells. The first was characterised by upregulation of apoptosis related genes and MAPKs, the second by activation of proteins involved in the turnover of damaged proteins and only in the third (10 h after the challenge) activation of genes was observed which are involved in the detoxification of ROS[464,487].

Despite these inconsistencies, it is possible to define a number of genes which are typical markers of oxidative stress under experimental conditions (Table 3) and it was possible to establish distinct signatures of gene expression for different types of oxidative tress in specific experimental models such as macrophage activation, peroxisomal proliferation and ROS releasing chemicals in rat hepatocytes *in vivo* [472,473]. Also in *in vitro* studies with a human derived liver cell line a clear difference was observed between oxidative and non-oxidative stress responses[465].

It is known for other areas of genomic research that studies interrogating gene transcription in similar contexts often resulted in poor overlapping lists of regulated genes (for a general review see[492]); are a typical example are the strongly divergent results obtained in investigations aimed at identifying retinoic acid responsive genes[493]. These inconsistencies have lead to strong efforts to elucidate the methodological reasons for these variations and it became clear that correlations are improved when low abundant genes are excluded[494]. Different platforms were established which attempted to standardise the techniques of array based approaches. The outcome of these efforts have been published in a number of recent articles[495–498].

Results of microarray studies with dietary antioxidants. In total, results from approximately 60 studies are available at present in which the impact of dietary antioxidants on gene expression patterns was investigated. The number of publication has almost doubled in the last two years and it is also interesting that many of the newer studies were conducted with laboratory rodents; nevertheless *in vitro* experiments with stable cell lines are still dominating. It is also notable, that research focused predominantly on specific types of compounds such as resveratrol, a phenolic compound from red wine, epigallocatechingallate (EGCG), the main catechin in green tea, cucurmin contained in the spice tumeric, sulphoraphane the breakdown product of glucobrassicin (a glucosinolate found in cruciferous vegetables) and the phytoestrogen genistein which is contained in soy beans. Several reviews have been published which contain results of microarray studies with phytochemicals. The most comprehensive one by Narayanan[31] describes data obtained in twenty studies (3 *in vivo*, 17 *in vitro*) with various dietary components, the findings with EGCG are summarised in the paper of Mariappan *et al.* [499], results with carotenoids can be found in the overview of Elliott[500].

Table 5 summarises the results of newer studies which have not been evaluated in the aforementioned articles. The first part describes results of *in vitro* experiments with cells lines, the second contains *in vivo* experiments with laboratory rodents.

Apart from the *in vitro* and animal studies, also a few human intervention trials have been carried out with antioxidants. For example Majewitz *et al.* [501] conducted a study with normal and apoE smokers (which carry a specific mutation in apolipoprotein E and are at increased risk for coronary heart disease). Before and after vitamin C supplementation (60 mg/P/4 weeks) they analysed monocytes by use of a small array (225 genes). After the intervention, the expression of 22 of the genes was altered in normal smokers, 71 were altered in apoE individuals. The most interesting observation was the down regulation of TNF-β and its receptor, also genes encoding for the neutrophil growth factor receptor and the monocyte chemoattractant protein receptor were affected. These genes are involved in inflammatory responses and it is known that TNF-β is regulated by NFκB. Another human study was published by Hoffmann and coworkers[502] who analysed the effects of consumption of a polyphenolics mix in lymphocytes of healthy individuals by use of a small targeted array (containing 96 transcripts related to drug metabolism). Fifty six genes were found expressed in the target cells and seven of them were altered after the intervention, three of them encoded for cytochrome P450 isozymes

Table 5. Examples for results obtained with dietary antioxidants in microarray experiments

Treatment/aim	Cells/AS*	Results†	References
In vitro investigations			
Quercetin *Aim*: study the impact on growth inhibition of prostate cancer cells	PK-3, NLCap, DU-145 human prostate cancer cells lines; BG-9 normal human fibroblast cells line *AS*: number of genes not specified	In prostate cancer cell lines ↓ of the expression of cell cycle related genes (e.g. E- and D-type cycline genes) was observed as well as ↓ of tumour suppressor genes (e.g. CBP, PTEN, MSH2, TGFβR1, ALK-5) and ↓ of oncogenes (p53, CDK2)	(615)
Quercetin *Aim*: study the antitumour activity of Q	Human CO-115 colon adenocarcinoma cells *AS*: whole genome microarray	5000–7000 genes were affected, specific effects on genes related to cell cycle arrest (CDKN-group) and modulation of the expression of apoptosis related genes (e.g. p53-related genes), only one antioxidant gene (LOXL3) was altered	(616)
Genistein *Aim*: study the impact on prostate cancer development	Human prostate cancer cell lines LNCaP and PC-3 *AS*: 557 genes related to cancer	11 genes were strongly altered; ↑ glutathione peroxidase 1, aldolase A, quiescin Q6, ras homolog gene family, member D; ↓ of apoptosis inhibitor (survivin), MAPK6, fibronectin 1, topoisomerase IIα etc.	(617)
Epigallocatechin gallate *Aim*: study effects on cell division	SH-SY5Y human neuroblastoma cell lines *AS*: 25 genes	↓ of expression of apoptotic genes (Bax and Bcl-2) and of the cell cycle inhibitor Gadd45 as well as caspase 6	(618,619)
Epigallocatechin gallate *Aim*: investigation of the protective role of EGCG on breast cancer development	Dimethyl benzanthracene transformed breast cancer cell line D3-1 *AS*: 7500 genes	EGCG treatment caused changes in transformed cells that promote a more "normal" phenotype. ↓ of AhR (a transcription factor involved in the biological responses to polycyclic aromatic hydrocarbons, e.g. CYP1A1, CYP1A2 and CYP1B1). Also changes involved in nucleo-cytoplasmatic transport (SCS-1) were affected ↑ of dehydrocarbonases (AKR)	(620)
Epigallocatechin gallate *Aim*: study impact of gene regulation of glucose metabolism	Rat H4IIE hepatoma cells *AS*: size not specified	↓ of number of genes involved in fatty acid synthesis, oxidation and activation as well as in triacethylglycerol synthesis ↑ of genes involved in glycolysis and glucose transport	(621)
Diallyl disulfide *Aim*: investigation on cell division and overall tumour behaviour	HCT-15 human colon tumour cell line *AS*: size of the array not specified	↓ of cell cycle regulated genes (e.g. Cdk6) as well as oncogenes, tumour suppressors and extracellular matrix and communication genes ↑ cell cycle proteins (cdk2, 3, 4, etc.) as well as different growth factors, microfilaments, protein turnover related genes ↑ DNA damage related genes (e.g. XP-related genes)	(622)
Diallyl trisulfide *Aim*: investigation of lipid-lowering properties	Human derived hepatoma cells (HepG2) *AS*: 452 genes	Only three genes were altered: ↑ PPAR-α (peroxisome activated receptor alfa) and HNF-4α (hepatocyte nuclear factor 4 alfa), ↓ CYP7A1 (involved in the oxidation of xenobiotics)	(623)
Gallic acid *Aim*: investigation of antioxidant effects	Human chronic myogenous leukaemia cell K562 *AS*: 82 genes (antioxidant enzymes and DNA repair)	Several antioxidant genes ↓, i.e. GPX, thioredoxin (TXN), thioredoxin peroxidase (AOE372), several DNA polymerase genes (POLD1 and 2), X-ray repair genes (XRCC5), DNA-3-methyladenine glycosidase	(32)
Vanillin and cinnamaldehyde *Aim*: investigation of prevention of formation of spontaneous mutations	Mismatch-repair deficient (MMR^-) human colon cancer cell line HCT116 *AS*: 14 500 genes	Eight genes affected which play a role in DNA-damage and oxidative and stress response, e.g. ↑ HMOX1 (heme oxygenase), HSPA1B (heat shock protein) ↓ of 14 genes involved in cell growth and differentiation: (MAPK2, FGFR2, TGFB111, etc.)	(624)
Resveratrol *Aim*: general chemoprotective properties	Human ovarian cancer cell line PA-1 *AS*: 7448 genes	118 genes were altered ↑ of antioxidant enzymes (NQO 1, NAD(P)H quinone oxireductase 1, thioredoxin reductase 1), apoptosis related genes (p21), investigation of time course of gene regulation	(625)

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Table 5. *Continued*

Treatment/aim	Cells/AS*	Results†	References
Lycopene *Aim*: investigation of the effects on breast cancer genes	Human breast cancer cells lines (MCF7, MDA-MB-231) and the fibroblastic cell line MCF-10a *AS*: 202 genes	Changes in genes related to apoptosis, cell cycle and signalling. Apoptosis related genes: p53, caspase 8, TNF (all ↓), cell cycle genes: cyclin E, etc. ↑. Also various DNA-repair genes ↑	(626)
Vitamin E *Aim*: investigations on gene expression related to prevention of DNA-adduct formation by lipid peroxidation products	Differentiated and undifferentiated human colonocytes (with/without oxidative stress) *AS*: global gene expression	In total 118 genes were affected which concern the cell cycle (cyclin D1, p27, p21, MAPKs, CDK-2); DNA-repair (p21, RAD54 homologous recombination) and also connective tissue related genes	(627)
Apple flavonoids *Aim*: study patterns of gene expression (not specified)	HT29 human colon cancer cells *AS*: 96 genes of drug metabolism	↑ of glutathione tranferases (GSTP-1, GSTP-2, MGST2) and cytochromes CYCP4F3, CHST5 genes ↓ of EPHX1	(628)
Resveratrol *Aim*: investigations on the role of macrophage inhibitory cytokine-1 (MIC-1) in resveratrol-induced growth inhibition of human pancreatic cancer cell lines	CD18 and S2-013 human pancreatic cell lines *AS*: global gene expression	↑ of expression of MIC-1 in both cell lines ↑ of expression of growth differentiation factor 15 (GDF15), sensescence-associated epithelial membrane protein (SEMP), major histocompatibility complex, class I, F (HLA-F) and 17 other genes in S2-013 cell line	(629)
Genistein (G) *Aim*: investigations on the effect of G on global gene expression patterns in androgen-responsive human prostate cancer cell line	LNCaP androgen-responsive human prostate cancer cell line *AS*: global gene expression	28 genes were affected 19 androgen up-regulated genes were ↓ by G (TNF-induced protein, prostatic kallikrein 2, prostate specific antigen kallikrein 3 and others) 4 androgen down-regulated genes ↑ by G (dopa decarboxylase, BRCA-1-associated RING domain 1, butyrylcholinesterase, phosphoinositide-3-kinase) 5 genes ↑ by both androgen and G (stearoyl-CoA desaturase, UDP-glucose dehydrogenase)	(630)
Phenethylisothiocyanate (PEITC) *Aim*: to further clarify the molecular effects of PEITC in causing death of human colon adenocarcinoma cells	HCT-116 human colon adenocarcinoma cell line *AS*: small cluster of apoptosis-related genes	↑ of expression of GADD45	(631)
Quercetin *Aim*: to elucidate possible mechanisms involved in inhibition of proliferation of tumour cells	Caco-2 human colon cancer cell line *AS*: expression of 4000 human genes	↓ expression of cell cycle genes (for example CDC6, CDK4 and cyclin D1), ↓ cell proliferation and induced cell cycle arrest ↑ of expression of several tumour suppressor genes. In addition, genes involved in signal transduction pathways like beta catenin/TCF signalling and MAPK signal transduction were influenced by quercetin	(632)
Vitamin E *Aim*: effects on global gene expression – time dependency	♂ Fisher 344 rats fed with vitamin E deficient diet or Vit E supplemented diet (60 mg/kg) *AS*: 7000 genes *t.o.* testes and liver Several time points were monitored	Five genes and seven sequence taqs were altered at least at three time points (gamma glutamylcysteinyl synthetase, GSH synthetase, 5-α-steroid reductase, factor IX, scavenger receptor CD36), furthermore, subunit of 8-GCS, coagulation factor IX, steroid reductase type 1, etc.	(633,634)
Turmeric supplements *Aim*: mechanism of action of turmeric in the treatment of arthritis	♀ Lewis rats were treated i.p. with 25 μg rhamnose/g bw and with various doses of turmeric extract i.p. (0·5–1·0 μl/g) *AS*: 31 000 genes *t.o.* arthritic joints	↑ of a variety of chemokines and cytokines (GRO/KC, MCP-1, MIP-1α, MIP3α, CXC chemokine LIX) and adhesion factors that facilitate inflammatory cell recruitment to the joint for another group of target genes. COX2 was ↓ significantly, gene expression controlled by transcriptional factors TIL-2, TNFα was ↑	(505)

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Table 5. *Continued*

Treatment/aim	Cells/AS*	Results†	References
Epigallocatechin gallate *Aim*: comparison of the impact of Nrf2 on expression profiles	♂ C57BL/6J (Nrft/t) and ♂ C57BL/6J/Nrf (−/−) knockout mice EGCG: 200 mg/kg bw oral single dose *AS*: 34 000 genes *t.o.* liver and colon	EGCG regulated 671 Nrf2 dependent genes and 256-independent genes in the liver; in the colon 228 Nrf2 regulated genes and 98 independent genes were identified. Genes fell into the functional categories: proteolysis, detoxification, transport, cell growth and apoptosis, cell adhesion and transcription factors	(635)
Red wine polyphenolics (RWP) *Aim*: investigate the effect on the prevention of carcinogenesis in the intestinal mucosa	♂ Fisher 344 rats fed with high fat diet RWP were fed in the diet (50 mg/kg) *AS*: 5677 genes *t.o.* colon mucosa	20 genes ↑, 366 ↓, among the ↓ genes 41 were related to immune- and anti-inflammatory response. In addition also genes were affected which are involved in steroid metabolism, ↓ of genes involved in energy metabolism pathways (COX6-8, etc.). ↑ of cholesterol 7α-hydroxylase (CYP7A1)	(636)
Resveratrol (R) *Aim*: investigation on stress response in rat liver	♂ and ♀ CD rats *t.o.* liver Treatment: different doses of R (0·3–3·0 gm/kg/day), oral administration, 28 days *AS*: 140 stress related genes	Dose dependent effects ↑ of CYP-450 isoenzymes as well as CYP-reductase induction of AO genes such as SOD2 and thisulfate sulfurtransferase (TST) at the highest dose, but decrease of these genes at lower doses. Other genes affected were MAPK, p38, CAT, HO-1, as well as UGT-encoding genes. In ♀ the number of genes altered was ca. 2-fold higher than in ♂	(504)
Genistein (G) *Aim*: study impact of the isoflavone on fatty acid metabolism	♂ C57BL/6 mice fed low fat diet, a high fat diet or high fat diet supplemented with genistein (2 g/kg), 12 weeks *AS*: 6531 transcripts *t.o.* liver	97 genes were altered by high fat diet, 80 of them were normalized by G supplementation. Many of them were associated with cholesterol biosynthesis, but additionally also genes involved in detoxification and inflammatory processes were affected, e.g. metallothionein 1, GST, kallikrein B (plasma1), serine proteinase inhibitor (clade A, member 3G), serine protease inhibitor 1–5. Also apoptosis related genes were affected	(637)
Vitamin C (Vc) *Aim*: impact on lifespan and antioxidant defence mechanisms	C57BL/6 mice, sex not specified, maintained either at normal temperature (+22°C) or +7°C (increase of metabolism) and supplemented diet (180 mg/kg), lifespan feeding *AS*: 163 genes *t.o.* liver	After 6 months no gene alterations were found, after 22 months: 3 genes ↑ (COX2, p21, UDP–glucuronosyltransferase) 8 genes ↓: e.g. SOD, quinone NAD(P)H-dehydrogenase and different other genes not involved in AO defence	(506)
Caffeic acid phenetyl ester *Aim*: impact on atherosclerosis	Apolipoprotein E-deficient mice (Apoe − /−) and normal C57/B6, 30 mg/kg bw 12 weeks *AS*: 3758 genes expression *t.o.* aorta Analyses were conducted in untreated mice and mice which were under oxidative stress	Altered genes are not clearly specified; overall expression induced by oxidative stress was reverted by CAPE-treatment Authors mention that that basic transcription factors, growth factors and cytokines as well as cell adhesion genes are ↓ in apoe − /− mice after CAPE treatment	(638)

* AS, Arrays size (genes number); SD, Sprague–Dawley

† ↓, downregulation; ↑, upregulation; ↔, no changes; CDKN, cyclin-dependent kinase inhibitor; COX, cyclooxygenase; FGFR2, fibroblast growth factor receptor 2; GAPDH, glyceraldehyde-3-phosphatase dehydrogenase; LOXL, lysyl oxidase-like genes; MAPK2, mitogen-activated kinase 2; MCP-1, monocyte chemoattractant protein 1; MPK6, mitogen-activated protein kinase 6; NAD(P)H, nicotinamide adenine dinucleotide phosphate; NQO1, NAD(P)H dehydrogenase, quinone 1; TNF, tumour necrosis factor; TGFB, transforming growth factor beta; TF, transcription factor; SOD, superoxide dismutase; n.s., not statistically significant.

(2F1, 3A4 and 3A5), two for them for sulfotransferases (SULT 2A1 and 1C2) the others were associated with microsomal GST, nicotinamide–*N*-methyltransferase and the ATP binding cassette.

All of the compounds listed in the table are potent antioxidants and it is apparent that many of the transcriptional changes which were detected concern genes which encode for AO defence, inflammatory responses, apoptosis and cell signalling and division. Nevertheless, it is not possible, to define a general signature of AO effects. The lack of a common pattern may be due to the fact that the chemical structures of the antioxidants as well as their mode of action and tissue specificity differs substantially. For example vitamins C and E differ strongly in their organ distribution and it their ability to inactive ROS species[503]. Furthermore, also the experimental design of the studies plays an important role. As described above, the expression patterns seen with resveratrol were highly sex specific, in female mice the number of genes which were transcriptionally altered in hepatic tissue was approximately 3 times higher in females as compared to males[504]. Also organ differences have an impact on the results of array studies, for example EGCG affected in the liver of mice three times more genes as compared to the colon[505]. Another important parameter affecting the outcome is the time dependency of the expression patterns and also the dose dependency of the responses (see Table 5).

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The results of microarray analyses do at present not allow to draw firm conclusions if a compound elicits ROS protective effects. For example, findings obtained with moderate doses of vitamin C in mice[506] indicate that it rather induces inflammation (up regulation of COX 2) and reduces ROS protection (down regulation of SOD). Also the results obtained with diallyldisulfide in HepG2 cells were unspecific and not indicative for antioxidant properties which are well documented for this compound[507]. However, it may be possible that general antioxidant gene expression patterns can be defined on the basis of comparative standardised experiments with a variety of compounds which are lacking at present. The currently available results show that microarray studies nevertheless provide highly useful information concerning the molecular mechanisms of prevention of ROS related diseases. Typical examples are the results of studies with prostate cancer cell lines and compounds such as resveratrol and sulforaphane which showed that these compounds interact with the transcription of genes involved in cell cycle regulation and apoptosis; these findings provide a possible explanation for protective effects of these dietary components towards prostate cancer cell line LNCaP (for review see[31]); also the assumption of cancer protective effects of EGCG and retinoids could be enhardened by results of array studies[499,500].

Proteomics

Basic principles. Proteomics is a screening technology based on the separation, quantification and identification of proteins from biological samples and allows to directly identify proteins bearing oxidative modifications. Adaptive cell responses in order to cope with the cell damage imposed by oxidative stress may as well be investigated by transcriptomics. As proteins are translated by ribosomes based on the information provided by mRNA molecules, no protein can be synthesised without the appropriate RNA. On the other hand, the mere existence of mRNA does not mean that the corresponding protein gets translated. It only means that the protein might get translated upon demand. This defines one main difference between proteomics and transcriptomics. Transcriptomics shows which genes are expressed, but does not tell which proteins actually get translated. The picture gets even more complicated, when time is considered. Some proteins such as histones are almost exclusively translated during the S-phase of the cell cycle, but not in G0[508]. A poor correlation of proteomics to transcriptomics data may be the consequence, because these proteins may be abundant in G0, but no corresponding mRNA may be detectable at this time. On the other hand, several regulatory proteins such as p53 may be subject to continuous protein degradation by proteasomes. Here, protein amounts may accumulate to minute and undetectable amounts only, while the mRNA may be easily detectable. This explains why proteomics may give very different results when compared to transcriptomics.

In order to assign the identity of the modified molecules, separation of proteins may be applied before the detection of oxidative modification. 2D-PAGE is still the most important technique to separate proteins according to electric charge and molecular weight[509]. Protein spots on 2D gels can be identified by Western analysis or mass spectrometric analysis of tryptic digests and quantified by a variety of techniques including silver staining, fluorescence detection or autoradiography[510]. This approach is used to perform comparative analysis of protein fractions isolated from e.g. treated and untreated cells. Spot patterns can be compared in order to detect alterations in relative amounts or, quite relevant to oxidative stress, protein modifications accompanied by changes of the molecular charge of the protein. Application of immunological detection of carbonyl groups or nitro-tyrosine by Western analysis may subsequently identify the modified proteins. Thus, application of 2D-PAGE may enable the detection of upregulated and/or modified proteins consequent to oxidative stress. The main draw-back of 2D-PAGE is its limited resolution and sometimes poor reproducibility concealing many important effects[511]. The number of proteins accessible via 2D-PAGE is usually in the order of several hundreds and hardly exceeds thousand different proteins[509].

Another essential technique for proteome analysis is mass spectrometry. Upon tryptic digestion, peptides can be obtained from isolated proteins which can be forwarded to mass analysis. Determination of the molecular weight of the peptides by e.g. MALDI-TOF provides a fingerprint of peptide masses for each protein[512]. As tryptic peptides may be predicted from databases for all known proteins, a best-fit search can be performed resulting in candidates which show theoretical peptide masses comparable to those identified in the experiment. This technique is called fingerprint analysis and can be applied only to well-purified proteins as potentially obtained from 2D-PAGE. Minimal contamination of peptide preparations may render fingerprint data not accessible for meaningful interpretation and are a main drawback of this method.

Much more robust and powerful is the identification of peptide sequences obtained from peptide fragmentation analysis. Peptides can be fragmented specifically in a mass spectrometer resulting in the breakage of a single peptide bond per molecule[513]. Ordering of the resulting peptide fragments may generate mass differences according to single amino acids in the peptide. As a result, not only total masses of peptides are determined as in case of fingerprint analysis, but complete amino acid sequences are obtained, which allow identification of proteins with much higher confidence. Furthermore, amino acid modifications may be determined by fragmentation analysis. When applied to isolated spots from 2D-gels, proteins can be identified with very high confidence. Furthermore, specific modifications such as oxidation of methionine, cysteine, tryptophan or others can be determined[514]. On the other hand, the determination of amino acid sequences renders separation of proteins before tryptic digest unnecessary. Protein mixtures may be digested and then peptide mixtures separated by e.g. nano-flow chromatography[515]. Peptide sequences may be determined successively and peptides derived from one protein sorted after the experiment. This powerful technique is called shotgun analysis and enables the identification of more proteins as 2D-PAGE/MS[511]. As long as peptide modifications are detected, protein modification can also be assigned. The major drawback of this approach is the limited sequence coverage rendering a lot of information missing and the inapplicability of immunological detection methods[516].

As a consequence, the experimental design studies has to be carefully prepared for proteome. First of all, it should be clear what to look at. In case of assessment of the extent of damage in response to a defined challenge, a bulk

analysis summarizing the extent of e.g. carbonyl formation with chemical or immunological methods may be appropriate. When the identity of target proteins appears desirable, 2D-PAGE can be used to assign the identity to the modified proteins. Here, the poor access of e.g. membrane proteins by most proteome analysis techniques poses[517] a severe limitation. Mass analysis of the tryptic digest of an isolated 2D-spot may easily identify the corresponding protein because a few peptide fragmentation data may be sufficient for unequivocal protein identification. When the target amino acids of a modified protein are desired, full sequence coverage of the identified peptides is desired, but hard to realise. Peptides have to be ionised before they can be analysed by mass spectrometry. Ionisation is an unpredictable event and may be strongly dependent on chemical properties of the peptide. Consequently, only a subset of the peptides generated by proteolytic digests can usually be detected by mass spectrometry[518].

Results of proteomic studies concerning oxidative stress. In order to identify proteins related to oxidative stress, several strategies were employed. A direct consequence of ROS-mediated damage may be amino acid modifications such as oxidation or nitration. Such modifications are accessible via peptide fragmentation analysis by mass spectrometry. While this method is very exact providing direct proof of oxidation together with the identification of the target protein and target amino acid, it has several draw-backs. First, it is almost impossible to survey the total amino acid sequence of proteins. Consequently, a negative result is hardly a proof for the absence of modifications. Second, proteins are very sensitive to artifacts introduced by sample handling procedures. For the proteome analysis of cells, they have to be lysed to extract the proteins for further analyses. Upon cell lysis, organelles get ruptured, calcium is set free, enzymes may get activated and many biochemical reactions including oxidations may get out of control. Also separation of proteins by 2D-PAGE exposes proteins to oxygen. This can be directly observed by the detection of oxidized methionine in samples from the same cell lysis, which were processed by 2D-PAGE and shotgun analysis in parallel. The proteins separated by 2D-PAGE generally show more methionine oxidation compared to the same proteins analysed by shotgun proteomics[519]. Thus, it is essential to most accurately control all experimental conditions and run comparative experiments strictly in parallel. Actually, several amino acid modifications consequent to oxidative stress are detectable by mass spectrometry, including oxidation of cysteine[520,521], methionine and tryptophan[519], and nitration of tyrosine[522]. Beside direct amino acid modification, the formation of disulfide bridges by oxidation may form protein adducts[523] or protein glutathione adducts[524]. Amino-acid adducts can also be detected with antibodies specific for e.g. nitrotyrosine and carbonyl adducts[525,526]. As mentioned above, the assignment of the target protein identity might be a daunting task and the assignment of the modified amino acid within the sequence is impossible. On the other hand, modified peptides which may be missed by MS-based analysis might be easily detected with antibodies.

In contrast to the identification of proteins directly targeted by oxidative stress, an alternative approach is the investigation of the response of cells via up-regulation and down-regulation of regulatory proteins[82]. Here, rather indirect effects are observed, which may be consequent to transcriptional alterations of genes helping the cell to manage the stress situation and, on the other hand, proteasomal degradation of damaged proteins. As a consequence, the readout of such an experiment may become very complex, because we can see a lot of protein alterations, but we do not know where they are coming from. To reduce the complexity of the biological system, prokaryotes and yeast were used to investigate the basic responses to oxidative stress[527,528]. These works indicate that the expected up-regulation of antioxidant proteins and chaperones may be accompanied by substantial alterations in the metabolic state of the cells. Again, several approaches are feasible to investigate cellular responses. First, cells can be treated with sub-lethal concentrations of agents mediating oxidative stress such as H_2O_2. After a defined period of time, treated and untreated samples are forwarded to proteome analysis and the results compared in a quantitative fashion. Several studies with human monocytes and epithelial cells demonstrated that again, besides the obvious target proteins such as redox regulators and chaperones, a broad variety of other protein families seems to be involved in regulatory processes consequent to oxidative stress including cytoskeletal proteins and glycolytic proteins[529,530].

Second, cells which are very sensitive to oxidative stress may be compared to others which were made resistant e.g. by chronic exposure with increasing H_2O_2 concentrations[531]. Here, differentially expressed proteins may include those which actually mediate resistance. Obviously, proteins differentially expressed due to different differentiation or activation states of the investigated cells may repress the identification of the true players related to oxidative stress control. Rather multiple comparisons of various cell systems or additional functional assays may allow to identify the relevant proteins. For example, it was demonstrated that enzyme aldose reductase identified by comparative analysis indeed mediates protection in Chinese hamster fibroblasts[531].

Finally, the involvement of oxidative stress in human diseases has been investigated by analysis of clinical material and it was shown that proteomics may help to understand pathophysiological processes relevant to the disease state. The involvement and relevance of protein oxidation in brain tissue in Alzheimer's disease has been clearly demonstrated[532,533]. Specific oxidative modifications may also be involved in obesity[534], asthma[535], and various forms of inflammatory diseases[536].

Results of proteomic studies with dietary antioxidants. Although numerous position papers emphasized the importance of proteomics techniques in the investigations of health effects of dietary factors[537–546], the number of studies which contain data on the effects of food related compounds on protein patterns is strikingly low. One of the reasons may be that proteomics requires, unlike other techniques described in this article, a sound financial background to establish a proteomics facility. Most of the existing labs are specialised in the analysis of specific cells and tissues. Table 6 summarizes examples for results obtained in recent studies.

It is quite clear that in most *in vitro* studies more drastic effects were observed than in human trials. This discrepancy may be not only due to the differences in the effects of the compounds tested, but also to the use of high doses in the

Table 6. Examples for results obtained with dietary antioxidants in recent proteomics studies

Treatment/methods/aim*	Cells/species†	Results‡	Reference
In vitro			
Diallyl trisulfide (DATS) 25, 50 μM for 24, 48, 72 and 96 h 2DE, MALDI-TOF MS and LC–MS/MS *Aim*: responses of protein expression to DATS treatment of cells	BGC823, human gastric cancer cell line 41 spots identified	17 proteins ↓ and 27 proteins ↑ after DATS treatment ~50 % of these sensitive proteins are associated with *apoptotic pathways*, e.g. GST pi ↓, cytokeratin 8 ↓, VDAC-1 ↓, VDAC-2 ↓, annexin I ↑, enolase 1 ↑, ATP-synthase C ↑, etc.	(639)
Quercetin 150 μM for 72 h 2DE, MALDI-TOF MS *Aim*: identify cellular targets of putative colorectal cancer protective effect of flavonoids	HT-29, human colon cancer cells 28 spots identified	*Alterations of apoptosis involved proteins*: anti-apoptotic heat shock proteins hsp 70-1 and HSPBP1 ↓; GrpE ↓ (substrate of hsp-70), caspases ↑ (various cytoskeletal proteins: lamins, ninein), annexin I, II ↑, human DEAD box protein ↓, stathmin ↓, triosephosphate isomerase ↓ (all over-expressed in human cancers) *Alterations of metabolism*: Krebs-cycle enzymes	(405)
Resveratrol (RSV) 50, 100, 200 μM for 12 and 24 h 2DE, MALDI-TOF MS *Aim*: identify mechanisms of RSV triggered apoptosis	4 HCT116, human colon cancer cell lines (Bax +/−; −/−, p53 +/+; −/−) 4 spots identified	*RSV responsive events*: fragmentation of lamin A/C protein; caspase 6 activation (caspase protease ↑, accumulation of the active form); ribosomal protein p0 ↑, dUTPase ↓, stathmin 1 ↓; data suggest that caspase 6 and its cleavage of lamin A plays an important role in *apoptosis signalling* triggered by RSV	(640)
Resveratrol (RSV) 50 μM for 12, 24 and 48 h RT PCR 2DE-SDS-PAGE, MALDI MS *Aim*: study anti-carcinogenic mechanism of RSV against prostate cancer	LNCap, DU145, PC-3, human prostate cancer cell lines 1 spot identified	24 polypeptide spots were markedly ↑ or ↓ 1 of them was identified by MS as phosphoglycerate mutase B ↓ (essential for glucose metabolism, functional significance unclear); apoptosis ↑ by RSV (determined by DAPI and Annexin V staining); microarray analysis of cDNA showed differential expression of 48 genes (involved in cell cycle regulation, apoptosis and glycolysis)	(641)
Cocoa-derived pentameric procyanidin (pentamer) 100 μg/ml for 48 or 72 h p53 and pRb: PAGE Multiplayer dot blot, immunoblotting *Aim*: elucidate which mechanism the pentamer causes cell cycle arrest in breast cancer cells	Human breast cancer cells (MDA MB-231; -436; -438; SKBR-3, MCF-7) and B(a)p immortalized cells (184-A1N4, -B5) 45 spots	*G1-modulatory proteins*: specific dephosphorylation, ↔ protein expression (Cdc2, forkhead TF) ↔ p53 dephosphorylation, ↔ in protein expression *Retinoblastoma protein*: ↓ of expression; specific cytotoxic effects of the pentamer in breast cancer cells due to site specific dephosphorylation and ↓ of cell cycle regulatory proteins	(642)
L-Ascorbic acid (LAA) 0·5mM for 30 min 2DE, MALDI-TOF MS *Aim*: to identify the early protein targets of LAA induced toxicity in leukaemia cells	NB4 human leukemia cells 9 spots identified	9 proteins were sensitive to LAA treatment: e.g. subunit of protein disulfide isomerase protein ↓, Ig heavy chain binding protein (BiP = Hsp70 chaperone) ↓, tropomyosin ↓, etc. Change in intracellular thiol/disulfide conditions (GSSG ↑, modulation of disulfide bond formation); activation of oxidative stress-inducible apoptosis (measured by FACS analysis)	(643)
All-trans retinoic acid (vitamin A related compound) 1 μM for up to 7 days, 2DE, MALDI-TOF MS; MS peptide sequencing *Aim*: to analyse the molecular mechanism of Vit A induced growth inhibition in breast cancer cells	MCF-7, human breast cancer cells 18 spots identified	Alterations in clusters of proteins involved in the TGFβ signalling pathway and apoptosis (e.g. post-transcriptional and splicing factors: hnRNP A/B ↓, H1 ↑, H2 ↑, H3 ↓) *Energy production*: GTP ATP phopspho-transferase ↓; glutamate dehydrogenase ↓ *Cell proliferation/differentiation*: Rho GDP dissociation inhibitor ↑; nuclear protein Hcc-1 ↓	(644)
Flavone (a potent apoptosis inducer) 150 μM, 24 h 2DE, MALDI-TOF MS *Aim*: search for the molecular targets of flavone induced apoptosis in human colon cancer cells	HT-29, a human colon cancer cell line 20 spots identified	*Apoptosis associated proteins* (annexins ↓, cytoskeletal caspase substrates and heat shock proteins only detected in control) *Metabolic changes*: enzymes of the citric acid cycle ↓ (succinate dehydrogenase, isocitrate dehydrogenase, NADPH dehydrogenase, etc.) *AO-enzymes*: protein levels of catalase and thioredoxin peroxidase ↓ *Marker of colon cancer*: protein kinase C-β not detectable	(645)
Genistein (co-treatment with oxidised LDL) *v.* ox-LDL treatment alone 2·5 μM, 24 h (ox-LDL 5 μM) 2DE, MALDI-TOF MS *Aim*: mechanism of atherosclerosis preventive effects of genistein	EA. hy926, human endothelial cells 47 spots identified	47 spots were found to differ after ox-LDL treatment; combined treatment (ox-LDL + genistein) could reverse in the case of 29 proteins the alterations induced by the stressor e.g. cyto-skeletal proteins, annexins, *S*-adenosylhomocysteine hydrolase (↓ by ox-LDL, ↑ by genistein), methionine adenosyl-transferase subunit (↓ by ox-LDL, ↑ by genistein)	(646)

Table 6. *Continued*

Treatment/methods/aim*	Cells/species†	Results‡	Reference
Genistein (in combination with homocysteine or ox-LDL) 2·5 or 25 μM 2DE, MALDI-TOF MS *Aim*: search of molecular targets of anti-atherogenic effects of genistein	HUVEC, primary human endothelial cell line 19 spots identified	Genistein reversed stressor induced alterations (8 of 10 proteins altered by homocysteine and 2 of 9 altered by ox-LDL), e.g. homocysteine reduced proteins were reversed by genistein: annexin V (anti-thrombotic protein) ↑, lamin A (role in prevention of hypertension) ↑, apoptosis linked gene 2-protein ↑; ox-LDL induced proteins were reversed by genistein: ubiquitin conjugating enzyme 12 (foam cell formation) ↓, aldehyde dehydrogenase ↓	(544)
In vivo studies			
Control food vs AO rich diet: Vit C, Vit E, L-carnithine, α-lipoic acid (combined with a program of behavioural enrichment) for 2,8 years 2DE, MALDI-TOF MS *Aim*: effect of an AO-fortified diet on cognitive dysfunction and oxidative stress in a canine model of human ageing	Beagle dogs $n = 23$, sex n.i. 4 treatment groups 6 spots identified *t.o.* brain (parietal cortex)	↓ in expression levels of oxidative stress biomarkers: protein carbonyl levels of glutamate dehydrogenase ↓, GAPDH ↓, neurofilament triplet protein ↓, α-enolase ↓, GIT- and fascin actin bundling protein ↓, Cu/Zn SOD ↑, fructose bisphosphate aldolase C ↑, creatine kinase ↑, glutamate dehydrogenase ↑, GAPDH ↑ (increased enzyme activities: SOD, GST, hemeoxygenase)	(546)
Grape seed extract (rich in proanthocyanidins) *v.* control food 5 % in the diet for 6 weeks 2DE, MALDI-TOF *Aim*: analysis of molecular basis of AO activities of polyphenols, search for alterations in specific (protective) brain proteins	♀ SD rats $n = 10$, 2 groups 18 spots identified 7 of these by LC–MS/MS *t.o.* brain	18 altered proteins identified: e.g. heat shock proteins associated with protein folding and apoptosis ↑ (HSP-60, HSC 70 and 71), creatine kinase brain β chain ↑ (ATP-turnover), neurofilament triplet protein light and medium chain ↑ (NF-L, NF-M: cytoskeletal components important for neuronal maintenance) Glial fibrillary acidic protein ↓, vimentin ↓ (= cytoskeletal proteins, ↑ in Alzheimer's disease), enolase ↓ (glycolytic pathway)	(647)
Basal diet and 3 different vegetable diets: 10, 20 and 40 % vegetable mixtures (lyophilised cauliflower, carrots, peas and onions mixed with basal diet), 2DE, MALDI-TOF MS *Aim*: effect of increased vegetable intake on protein expression	♀ C57Bl6 mice $n = 28$, 4 groups Analysis of 6 spots identified *t.o.* colon (mucosa cells)	39 proteins displayed differential expression; 6 could be identified (with a role in the protection against colon cancer): myosin regulatory light chain 2 ↑ but n.s., carbonic anhydrase I ↑, high mobility group protein 1 ↓ but n.s., pancreatitis associated protein 3 ↑, GAPDH ↓, ATP synthase oligomycin sensitivity conferral protein ↓	(648)
Genistein (isoflavone in soy) 500 μg/g bw, s.c., day 16, 18 and 20 postpartum (prepubertal) 2DE, MALDI-TOF MS and immunobloting *Aim*: to elucidate mechanisms of suppression of chemically induced breast cancer and effect on mammary gland differentiation	♀ SD rats $n = 5$ rats/group Immunoblots: $n = 8–10$ rats/group *t.o.* breast (mammary gland) 6 spots identified	Proteins important for *cell differentiation* and *gland maturation* were found altered: GTP-cycohydrolase ↑, tyrosine hydroxylase ↑, vascular endothelial growth factor receptor 2 ↓, fertility protein SP22 ↑, γ-synuclein ↑, peroxiredoxin 1 ↓, ABC-transporter ↑	(649)
Diet supplemented with cruciferous (radish, sprouts, broccoli, cabbage… 436 g/d), allium (chives, garlic, onion, 190 g/d) or apiaceous vegetables (dill, celery, carrots, 270 g/d) *v.* control diet; cross-over study design, 7 days MALDI-TOF MS *Aim*: monitoring the impact of different vegetable diets on protein expression in plasma	Healthy volunteers $n = 38$, 2 groups 1 spot identified *t.o.* blood (analysis of plasma proteins)	The cruciferous diet yielded the largest impact on serum proteins, further classification showed alterations of 2 polypeptides in the broccoli-group: one could be identified as B-chain of α 2-HS glycoprotein ↓ (involved in insulin resistance and immune functions)	(650)
Human studies			
Brussels sprouts 300 g/P/d for 5 days; dietary inter vention study 2D-PAGE, MS/MS; protein amount: fluorescence detection; protein synthesis: autoradiography quantification (metabolic labelling with ^{35}S-methionine/cysteine) *Aim*: monitoring the impact of sprout consumption on protein expression	Healthy non-smoking volunteers $n = 5$, ♀ = 3, ♂ = 2 48 spots identified *t.o.* blood (primary white blood cells)	511 spots were detected, 48 of them identified 2 alterations after sprout consumption were found: ↑ of synthesis of Mn-SOD (1·56-fold); ↓ of synthesis of heat shock protein 70 kDa protein (2·27-fold)	(385)

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Table 6. *Continued*

Treatment/methods/aim*	Cells/species†	Results‡	Reference
Vitamin C (Vc) supplementation prospective randomised open label trial 250 mg, 3 × /week, oral for 2 months; CE and ESI-TOF-MS; relevant polypeptides: MALDI TOF/TOF *Aim*: investigate the effects of Vc on biomarkers of oxidative stress and inflammation in haemodialysis (HD) patients	Haemodialysis pts $n = 40$, ♀ + ♂; 2 groups +/− Vit C and control group, $n = 13$, ♀ + ♂ 1 CE fraction identified *t.o.* blood (plasma proteins)	30 polypeptides showed different levels in HD patients in comparison to individuals with normal renal function Levels of 15 polypeptides were altered after Vc supplementation in, only one of them could be identified and most likely represents β-2-microglobulin ↓ (predictor of renal insufficiency)	(651)
α-Tocopherol (AT) *v.* placebo, double blind, parallel design 134 or 268 mg (200 or 400 IU)/day for 14 or 28 days; 2DE, MALDI-MS *Aim*: examine effects of AT on protein expression	Healthy volunteers $n = 32$ (11 ♂, 21 ♀) 3 groups ($n = 10$) 1 spot identified *t.o.* blood (plasma proteins)	12 proteins ↑ after supplementation; one of them was identified: ↑ proapolipoprotein A1 (result confirmed by ELISA)	(652)

SwissProt Accession numbers: VDAC-1, P21796; VDAC-2, P45880; HSPBP1, Q9NZL4; GrpE, Q9HAV7; DEAD, Q92499; TGFβ, PO1137; Rho GDP, P52565; forkhead TF, Q08050.

* AO, antioxidant; bw, body weight; CE, capillary electrophoresis; 2 DE, two-dimensional electrophoresis; 2DE-SDS-PAGE, two-dimensional electrophoresis-sodium dodecylsulfate polyacrylamide gel electrophoresis; ESI, electro spray ionisation; h, hour; LC–MS/MS, liquid chromatography ion trap tandem mass spectrometry; MALDI-TOF MS, matrix assisted laser desorption ionization time-of-flight mass spectrometry; n.i., not indicated; ox-LDL, oxidised low density lipoproteins; s.c., subcutaneous.

† SD, Sprague–Dawley, pts, patients, t.o., target organ.

‡ ↓, downregulation; ↑, upregulation; ↔, no changes; TF, transcription factor; SOD, superoxide dismutase; GAPDH, glyceraldehyde-3-phosphatase dehydrogenase; n.s., not significant.

experiments with isolated cells. Unphysiological test conditions my lead to secondary effects which may be due to cell death and not caused by the test compound itself. Furthermore, in some of the studies high abundant proteins (α-2 HS glycoprotein) with high inter-individual variations were postulated to be biomarkers for dietary effects. Many of the compounds/diets altered the expression of proteins, which play a role in apoptosis, cytoskeleton structure and energy metabolism, while no substantial alterations of enzymes were seen which are involved in ROS defence, except SOD, which was found upregulated in some of the studies.

Metabolomics

Impact of the experimental design on the outcome of antioxidant studies. In the last chapters we described extensively the technical difficulties of AO studies and the limitations of the reliability of different methods. Also the experimental design has a strong impact on the outcome and on the predictive value of investigations concerning antioxidant properties of dietary compounds which are conducted to find out if beneficial effects can be expected in humans.

General problems of the experimental designs of AO studies. Fig. 4 summarises the advantages and disadvantages of different experimental models. At present around 90 % of AO experiments are conducted *in vitro* with stable cell lines or with subcelluar fractions.

The classical strategy used to identify dietary antioxidants is the screening of a large number of potential candidates in high throughput systems and *in vitro* by use of stable cell lines and subsequently investigate the most promising compounds in more reliable models (e.g. in animals studies and in human intervention trials). This approach is justified on the basis of the assumption that the key mode of action of dietary antioxidants is the direct (non-enzymatic) inactivation of ROS which can be easily detected in this simple systems. However, it became clear over the last years that the mechanisms by which dietary compounds protect against ROS are by far more complex and that indirect modes of action such as induction of enzymes and other defence mechanisms play an important role. For example, the coffee diterpeonids cawheol and kafestol do not protect human lymphocytes under *in vitro* conditions against ROS mediated DNA damage[282] while Huber *et al.*[394] found in experiments with rats that they are potent inducers of the enzyme gamma-glutamylcysteine synthetase (GCS) which catalyses the rate limiting step of the synthesis of glutathione which is a potent antioxidant[547]. It is conceivable that the increased GSH levels seen in the plasma of coffee drinkers[548] and the protection against oxidative DNA damage observed in a human intervention trial and in animal experiments[282,549] with coffee are due to this mechanism.

AO enzymes and also transcription factors which regulate cellular defence mechanisms may be not represented adequately in stable cancer cell lines which are used in AO studies. For example, it is known that that p53 controls the transcription of a variety of genes which encode for responses towards antioxidants[550]. Since > 50 % of human tumours are p53 deficient due to mutations[551], cell lines derived from them will not adequately reflect AO responses. Also the origin of the cells is important, while p53 and NrF2 are found in many organs, NFκB is not or only weakly expressed in certain tissues[80]; also the activities of AO enzymes show strong organ specificities (see Table 4). As mentioned above, the differences in the transcription pattern of genes in studies with oxidants and antioxidants support the assumption that the cellular responses depend largely on the type of indicator cells used.

Another important issue concerns the representation of phase I and phase II enzymes which metabolise dietary antioxidants and therefore may alter their protective properties;

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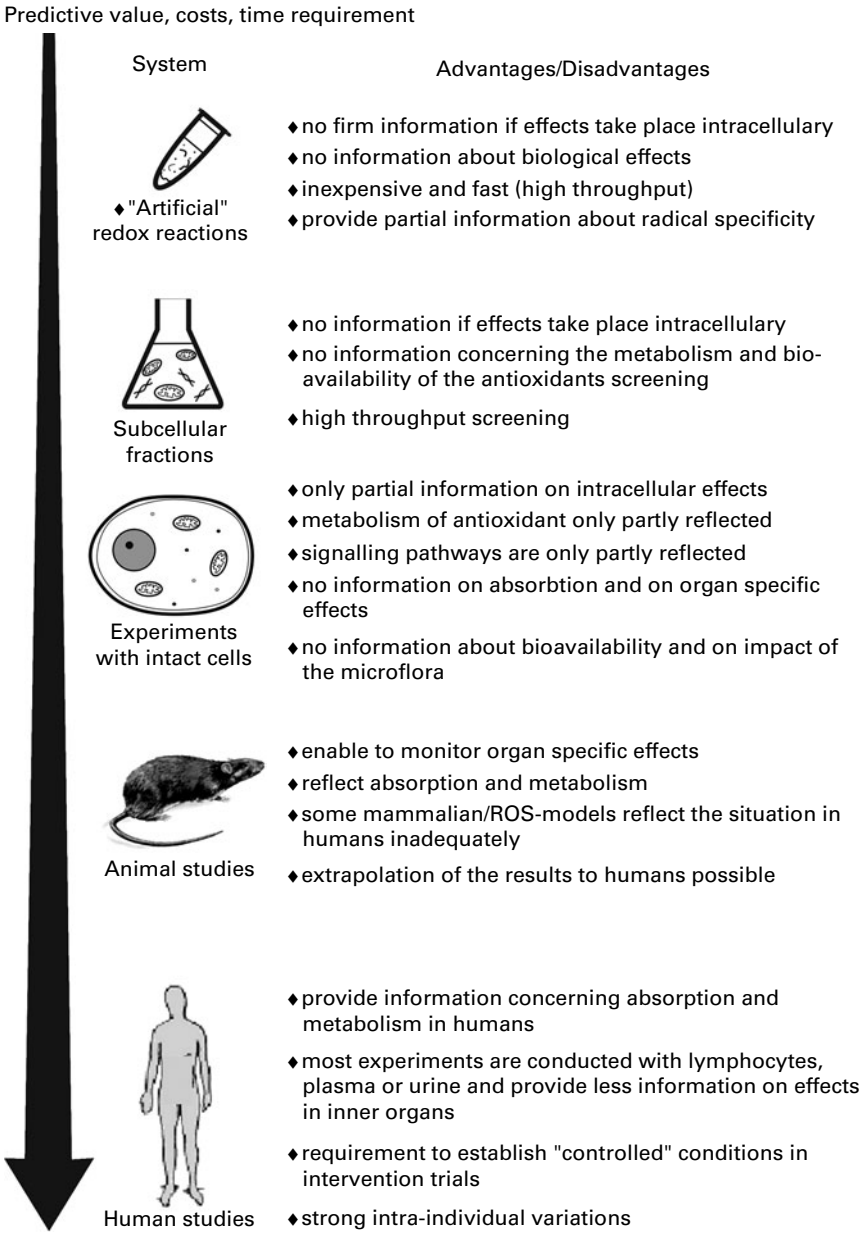

Fig. 4. Advantages and disadvantages of different experimental used to investigate ROS – protective effects of phytochemicals.

furthermore also reactions catalysed by the intestinal microflora are not reflected under *in vitro* conditions. Polyphenolics are extensively metabolised in the gut and in the body and non-conjugated metabolites most often account for a minor fraction of the circulating metabolites[552]. In the case of isoquercitrin it was shown that completely different conjugates are formed under *in vitro* conditions (i.e. after exposure to colon cell lines) as in the serum of rats[553,554]. Hydroxycinnamic acids which are potent oxidants in coffee are glucuronated in the body[555,556], but stable cell lines and peripheral lymphocytes which are used in *in vitro* experiments lack glucuronosyltransferase (UGT) activity which catalyses this reaction[557,558]. In most cases, it is not known if and to which extent the AO properties of conjugates differ from that of the parent compounds, but for glucuronides of isoflavones and epicatechin it has been shown that they provide no protection against oxidative stress[559,560] while the parent compounds are potent antioxidants. The inadequate representation of drug metabolising enzymes is a general problem which is also encountered in acute toxicity and genotoxicity experiments with stable cells lined and various attempts were made to solve it. For example, the cultivation conditions of primary hepatocytes which possess a broad spectrum of xenobiotic drug metabolising enzymes have been improved[561,562], other solutions may be the establishment of hepatoma cell lines which have retained the activities of several enzymes in an inducible form, (for review see[299]) and the construction of genetically engineered lines which express human phase I and phase II enzymes[563,564].

The cultivation conditions of the cells may have a strong impact on the outcome of antioxidant studies. It is known that the composition of the medium affects the sensitivity

of cells towards ROS. For example when lymphocytes are treated with ROS-generating chemicals in serum, they are up to five times less sensitive as compared to treatment in regular medium (L. Elbling, personal communication). When coffee or coffee specific compounds (chlorogenic and caffeic acid) were tested at high concentrations in genotoxicity tests with bacteria or human lymphocytes *in vitro,* strong effects were observed which were attributed to formation of H_2O_2, while with lower doses clear antioxidant effects were detectable and it was postulated by Aeschbacher *et al.*[565] that adverse effects do not take place under realistic *in vivo* conditions.

On the basis of the results of *in vitro* experiments it was possible to identify a number of highly potent antioxidants such as chlorophylls[566], curcumin[567], EGCG[568] and anthocyanins[569] which are currently sold as food supplements and/or used for the production of functional foods[570]. However, the results of experiments in which protection of oxidative DNA damage was monitored with foods containing these compounds are not promising, i.e. negative results were for example obtained with green vegetables, EGCG supplemented products[289,298,571] and anthocyan rich blueberries[348]. The reasons for these disappointing results are probably due to the fact that the active compounds which have a large molecular configuration are only poorly absorbed, therefore protective effects can be expected in the digestive tract but not in inner organs. In recent investigations, we found in a human intervention trial (SCGE experiments) with lymphocytes that gallic acid (GA), a small phenolic molecule, contained in specific plant foods reduces the formation of oxidised bases in peripheral lymphocytes with 30–40 fold higher efficiency as vitamins E and C while under *in vitro* conditions similar protective effects were observed[296,572]. One of the reasons for the strong antioxidant properties of GA may be its high absorption rate[573].

Overall, the predictive value of results obtained in *in vivo* experiments with rodents are higher as those of *in vitro* findings but one of the problems encountered in the interpretation of animal derived results is due to the fact that putative antioxidants were often administered at high doses which are irrelevant for humans.

The major problem of human studies concerns the fact that most experiments are carried out with peripheral blood cells and plasma and it remains unclear if and to which extent protection against ROS mediated damage can be expected in inner organs. In the case of GA we observed significant reduction of radical induced damage in a variety of inner organs in animal experiments[381,572], also for a number of other compounds such as lycopene and vitamins and E it is known that the reduction of damage seen in blood cells in humans is paralleled by protective effects in a variety of tissues[574,575].

An important question which has been neglected in human intervention trials concerns the impact of the overall antioxidant status of the participants on the outcome of protection studies. It can be expected that individuals which consume AO rich diets respond less sensitive. In the case of folic acid it has been shown that supplementation is only effective in regard to improvement of the DNA stability in individuals with low intake levels[576]. On the basis of these findings Fenech[577] developed the "genome health clinic and genome health nutrigenomics" concept which postulates individualised supplementation strategies on the basis of biochemical and DNA-stability measurements and can be also applied to antioxidants[578,579].

Justification of heath claims for antioxidant properties of dietary factors?

Growing consumer concerns about the health attributes of food products and supplements and the increased production of health foods have led to extensive discussions of the scientific evidence required for health claims[580]. Already in 2001, the global sales of functional foods were estimated USD 47,6 billion worldwide[581]. As a consequence, authorities such as the US Food and Drug Administration and the European Commission have issued new rules directed at inaccuracies, confusion and false information related to functional and dose related risk reduction claims[582–584]. The EU regulation on nutrition and health claims made on foods (No. 1924/2006) entered into force on July 1st, 2007 and it is clearly stated in article 6 that claims have to be "based on and substantiated by generally accepted scientific evidence".

The evaluation of the methods which are currently used to investigate the antioxidant properties of foods and food constituents show that not all approaches provide reliable results. Data from *in vitro* experiments cannot be extrapolated to the human situation in general, while the value of results obtained in animal and human studies depends largely on the experimental design and on the techniques employed. As mentioned above, frequently used methods such as TBARS measurements and the quantification of protein bound carbonyls and breath hydrocarbons do not provide firm evidence for AO effects, also some TAC measurements, which are conducted under unphyiological conditions (e.g. TOSC, CUPRAC), may not be reliable[171,257]. Despite the uncertainities concerning the reliability of TAC measurements, results of such experiments can support the overall evidence for ROS protective effects but claims should be not solely based on such studies. Probably more relevant information can be expected from HPLC based isoprostane measurements as a marker for lipid peroxidation and from results of experiments concerning prevention of oxidative DNA-damage (HPLC based determination of oxidised bases, SCGE experiments with restriction enzymes). In this context it is notable that there is strong evidence that a number of ROS related diseases (including cancer) are causally directly related to damage of the genetic material (see introduction section).

As mentioned above, measurements of the induction of antioxidant enzymes do not provide reliable information for protective effects as they can also be induced by ROS themselves and their up regulation may reflect prooxidant effects.

Although the results obtained with -omics techniques no not allow to establish antioxidant specific patterns of gene transcription and protein alterations they can provide evidence for protective effects of antioxidants in specific experimental settings.

For example, it was found in a few studies that the transcription of genes caused by oxidative stress or by ROS related

diseases can be normalised by dietary antioxidants which strongly supports the assumption of protective properties.

A promising strategy for the justification of health claims may be the combination of different methods to a test battery, but the selection of the individual approaches and techniques to be included will be a matter of intense debate.

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References

1. Blomhoff R (2005) Dietary antioxidants and cardiovascular disease. *Curr Opin Lipidol* **16**, 47–54.
2. Elahi MM & Matata BM (2006) Free radicals in blood: evolving concepts in the mechanism of ischemic heart disease. *Arch Biochem Biophys* **450**, 78–88.
3. Lefer DJ & Granger DN (2000) Oxidative stress and cardiac disease. *Am J Med* **109**, 315–323.
4. Agarwal A, Sharma RK, Nallella KP, Thomas AJ Jr, Alvarez JG & Sikka SC (2006) Reactive oxygen species as an independent marker of male factor infertility. *Fertil Steril* **86**, 878–885.
5. Pasqualotto FF, Sharma RK, Kobayashi H, Nelson DR, Thomas AJ Jr & Agarwal A (2001) Oxidative stress in normo spermic men undergoing infertility evaluation. *J Androl* **22**, 316–322.
6. Von Kobbe C, May A, Grandori C & Bohr VA (2004) Werner syndrome cells escape hydrogen peroxide-induced cell proliferation arrest. *FASEB J* **18**, 1970–1972.
7. Yan T, Li S, Jiang X & Oberley LW (1999) Altered levels of primary antioxidant enzymes in progeria skin fibroblasts. *Biochem Biophys Res Commun* **257**, 163–167.
8. Kohen R & Nyska A (2002) Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicol Pathol* **30**, 620–650.
9. Gracy RW, Talent JM, Kong Y & Conrad CC (1999) Reactive oxygen species: the unavoidable environmental insult? *Mutat Res* **428**, 17–22.
10. Calabrese V, Guagliano E, Sapienza M, Mancuso C, Butterfield DA & Stella AM (2006) Redox regulation of cellular stress response in neurodegenerative disorders. *Ital J Biochem* **55**, 263–282.
11. Zimmermann JS & Kimmig B (1998) Pharmacological management of acute radiation morbidity. *Strahlenther Onkol* **174**, Suppl. 3, 62–65.
12. Sarma L & Kesavan PC (1993) Protective effects of vitamins C and E against gamma-ray-induced chromosomal damage in mouse. *Int J Radiat Biol* **63**, 759–764.
13. Gebhart E (1974) Antimutagens. Data and problems. *Humangenetik* **24**, 1–32.
14. Li L, Tsao R, Yang R, Liu C, Zhu H & Young JC (2006) Polyphenolic profiles and antioxidant activities of heartnut (*Juglans ailanthifolia* Var. *cordiformis*) and Persian walnut (*Juglans regia* L.). *J Agric Food Chem* **54**, 8033–8040.
15. Anagnostopoulou MA, Kefalas P, Kokkalou E, Assimopoulou AN & Papageorgiou VP (2005) Analysis of antioxidant compounds in sweet orange peel by HPLC–diode array detection–electrospray ionization mass spectrometry. *Biomed Chromatogr* **19**, 138–148.
16. Minoggio M, Bramati L, Simonetti P, Gardana C, Iemoli L, Santangelo E, Mauri PL, Spigno P, Soressi GP & Pietta PG (2003) Polyphenol pattern and antioxidant activity of different tomato lines and cultivars. *Ann Nutr Metab* **47**, 64–69.
17. Erdman JW Jr, Balentine D, Arab L, Beecher G, Dwyer JT, Folts J, Harnly J, Hollman P, Keen CL, Mazza G, Messina M, Scalbert A, Vita J & Williamson G & Burrowes J (2007) Flavonoids and heart health: proceedings of the ILSI North America Flavonoids Workshop, May 31–June 1, 2005, Washington, DC. *J Nutr* **137**, 718S–737S.
18. Kim JM, Chang HJ, Kim WK, Chang N & Chun HS (2006) Structure–activity relationship of neuroprotective and reactive oxygen species scavenging activities for allium organosulfur compounds. *J Agric Food Chem* **54**, 6547–6553.
19. Iwai K, Kishimoto N, Kakino Y, Mochida K & Fujita T (2004) *In vitro* antioxidative effects and tyrosinase inhibitory activities of seven hydroxycinnamoyl derivatives in green coffee beans. *J Agric Food Chem* **52**, 4893–4898.
20. Fujioka K & Shibamoto T (2006) Quantitation of volatiles and nonvolatile acids in an extract from coffee beverages: correlation with antioxidant activity. *J Agric Food Chem* **54**, 6054–6058.
21. Lopez-Velez M, Martinez-Martinez F & Del Valle-Ribes C (2003) The study of phenolic compounds as natural antioxidants in wine. *Crit Rev Food Sci Nutr* **43**, 233–244.
22. Hidalgo FJ, Leon MM & Zamora R (2006) Antioxidative activity of amino phospholipids and phospholipid/amino acid mixtures in edible oils as determined by the Rancimat method. *J Agric Food Chem* **54**, 5461–5467.
23. Luximon-Ramma A, Neergheen VS, Bahorun T, Crozier A, Zbarsky V, Datla KP, Dexter DT & Aruoma OI (2006) Assessment of the polyphenolic composition of the organic extracts of Mauritian black teas: a potential contributor to their antioxidant functions. *Biofactors* **27**, 79–91.
24. Antonious GF, Kochhar TS, Jarret RL & Snyder JC (2006) Antioxidants in hot pepper: variation among accessions. *J Environ Sci Health B* **41**, 1237–1243.
25. Masuda Y, Kikuzaki H, Hisamoto M & Nakatani N (2004) Antioxidant properties of gingerol related compounds from ginger. *Biofactors* **21**, 293–296.
26. Somparn P, Phisalaphong C, Nakornchai S, Unchern S & Morales NP (2007) Comparative antioxidant activities of curcumin and its demethoxy and hydrogenated derivatives. *Biol Pharm Bull* **30**, 74–78.
27. Hsu CY, Yang CM, Chen CM, Chao PY & Hu SP (2005) Effects of chlorophyll-related compounds on hydrogen peroxide induced DNA damage within human lymphocytes. *J Agric Food Chem* **53**, 2746–2750.
28. Xiong S, Melton LD, Easteal AJ & Siew D (2006) Stability and antioxidant activity of black currant anthocyanins in solution and encapsulated in glucan gel. *J Agric Food Chem* **54**, 6201–6208.
29. Semba RD, Lauretani F & Ferrucci L (2007) Carotenoids as protection against sarcopenia in older adults. *Arch Biochem Biophys* **458**, 141–145.
30. Aruoma OI, Sun B, Fujii H, Neergheen VS, Bahorun T, Kang KS & Sung MK (2006) Low molecular proanthocyanidin dietary biofactor Oligonol: its modulation of oxidative stress, bioefficacy, neuroprotection, food application and chemoprevention potentials. *Biofactors* **27**, 245–265.
31. Narayanan BA (2006) Chemopreventive agents alters global gene expression pattern: predicting their mode of action and targets. *Curr Cancer Drug Targets* **6**, 711–727.
32. Abdelwahed A, Bouhlel I, Skandrani I, Valenti K, Kadri M, Guiraud P, Steiman R, Mariotte AM, Ghedira K, Laporte F, Dijoux-Franca MG & Chekir-Ghedira L (2007) Study of antimutagenic and antioxidant activities of gallic acid and 1,2,3,4,

6-pentagalloylglucose from *Pistacia lentiscus*. Confirmation by microarray expression profiling. *Chem Biol Interact* **165**, 1–13.
33. Scandalios JG (2002) Oxidative stress responses – what have genome-scale studies taught us? *Genome Biol* **3**, 1019.
34. Choe E & Min DB (2006) Chemistry and reactions of reactive oxygen species in foods. *Crit Rev Food Sci Nutr* **46**, 1–22.
35. Foksinski M, Gackowski D, Rozalski R, Siomek A, Guz J, Szpila A, Dziaman T & Olinski R (2007) Effects of basal level of antioxidants on oxidative DNA damage in humans. *Eur J Nutr* **46**, 174–180.
36. Karihtala P & Soini Y (2007) Reactive oxygen species and antioxidant mechanisms in human tissues and their relation to malignancies. *Apmis* **115**, 81–103.
37. Fridovich I (1986) Biological effects of the superoxide radical. *Arch Biochem Biophys* **247**, 1–11.
38. Halliwell B & Gutteridge JM (1999) *Free Radicals in Biology and Medicine*, 3rd ed. Avon, England: Oxford University Press.
39. Beckman JS & Koppenol WH (1996) Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and the ugly. *Am J Physiol* **271**, C1424–C1437.
40. Czapski G & Goldstein S (1995) The role of the reactions of NO with superoxide and oxygen in biological systems: a kinetic approach. *Free Radic Biol Med* **19**, 785–794.
41. Shadyro OI, Yurkova IL & Kisel MA (2002) Radiation-induced peroxidation and fragmentation of lipids in a model membrane. *Int J Radiat Biol* **78**, 211–217.
42. Pentland AP (1994) Active oxygen mechanisms of UV inflammation. *Adv Exp Med Biol* **366**, 87–97.
43. Koren HS (1995) Associations between criteria air pollutants and asthma. *Environ Health Perspect* **103**, Suppl. 6, 235–242.
44. Victorin K (1994) Review of the genotoxicity of nitrogen oxides. *Mutat Res* **317**, 43–55.
45. Yu TW & Anderson D (1997) Reactive oxygen species-induced DNA damage and its modification: a chemical investigation. *Mutat Res* **379**, 201–210.
46. Wiseman A, Ridgway T, Goldfarb PS & Woods L (2002) Are food and environmental toxicants 'overdetected' by bioassays? *Trends Biotechnol* **20**, 13–15.
47. Parke DV & Sapota A (1996) Chemical toxicity and reactive oxygen species. *Int J Occup Med Environ Health* **9**, 331–340.
48. Ames BN (1986) Food constituents as a source of mutagens, carcinogens, and anticarcinogens. *Prog Clin Biol Res* **206**, 3–32.
49. Kanner J & Lapidot T (2001) The stomach as a bioreactor: dietary lipid peroxidation in the gastric fluid and the effects of plant-derived antioxidants. *Free Radic Biol Med* **31**, 1388–1395.
50. Richter C, Gogvadze V, Laffranchi R, Schlapbach R, Schweizer M, Suter M, Walter P & Yaffee M (1995) Oxidants in mitochondria: from physiology to diseases. *Biochim Biophys Acta* **1271**, 67–74.
51. Fleury C, Mignotte B & Vayssiere JL (2002) Mitochondrial reactive oxygen species in cell death signaling. *Biochimie* **84**, 131–141.
52. Dhalla NS, Temsah RM & Netticadan T (2000) Role of oxidative stress in cardiovascular diseases. *J Hypertens* **18**, 655–673.
53. Forman HJ & Torres M (2001) Signaling by the respiratory burst in macrophages. *IUBMB Life* **51**, 365–371.
54. Hawkins CL, Brown BE & Davies MJ (2001) Hypochlorite- and hypobromite-mediated radical formation and its role in cell lysis. *Arch Biochem Biophys* **395**, 137–145.
55. Rodrigues MR, Rodriguez D, Henrique Catalani L, Russo M & Campa A (2003) Interferon-gamma independent oxidation of melatonin by macrophages. *J Pineal Res* **34**, 69–74.
56. Davies KJ (1987) Protein damage and degradation by oxygen radicals. I. General aspects. *J Biol Chem* **262**, 9895–9901.
57. Grune T, Reinheckel T & Davies KJ (1997) Degradation of oxidized proteins in mammalian cells. *FASEB J* **11**, 526–534.
58. Levine RL & Stadtman ER (2001) Oxidative modification of proteins during aging. *Exp Gerontol* **36**, 1495–1502.
59. Stadtman ER (2004) Role of oxidant species in aging. *Curr Med Chem* **11**, 1105–1112.
60. Kushnareva Y, Murphy AN & Andreyev A (2002) Complex I-mediated reactive oxygen species generation: modulation by cytochrome c and NAD(P)+ oxidation–reduction state. *Biochem J* **368**, 545–553.
61. Squadrito GL & Pryor WA (1998) Oxidative chemistry of nitric oxide: the roles of superoxide, peroxynitrite, and carbon dioxide. *Free Radic Biol Med* **25**, 392–403.
62. Younes M & Siegers CP (1984) Interrelation between lipid peroxidation and other hepatotoxic events. *Biochem Pharmacol* **33**, 2001–2003.
63. Horton AA & Fairhurst S (1987) Lipid peroxidation and mechanisms of toxicity. *Crit Rev Toxicol* **18**, 27–79.
64. Zock PL & Katan MB (1998) Diet, LDL oxidation, and coronary artery disease. *Am J Clin Nutr* **68**, 759–760.
65. Forrester JS & Shah PK (2006) Emerging strategies for increasing high-density lipoprotein. *Am J Cardiol* **98**, 1542–1549.
66. Moller P & Wallin H (1998) Adduct formation, mutagenesis and nucleotide excision repair of DNA damage produced by reactive oxygen species and lipid peroxidation product. *Mutat Res* **410**, 271–290.
67. Barzilai A & Yamamoto K (2004) DNA damage responses to oxidative stress. *DNA Repair (Amst)* **3**, 1109–1115.
68. Niles JC, Wishnok JS & Tannenbaum SR (2006) Peroxynitrite-induced oxidation and nitration products of guanine and 8-oxoguanine: structures and mechanisms of product formation. *Nitric Oxide* **14**, 109–121.
69. Tsutsui H, Ide T & Kinugawa S (2006) Mitochondrial oxidative stress, DNA damage, and heart failure. *Antioxid Redox Signal* **8**, 1737–1744.
70. Vaca CE, Wilhelm J & Harms-Ringdahl M (1988) Interaction of lipid peroxidation products with DNA. A review. *Mutat Res* **195**, 137–149.
71. Ehrenberg L, Osterman-Golkar S, Segerbck D, Svensson K & Calleman CJ (1977) Evaluation of genetic risks of alkylating agents. III. Alkylation of haemoglobin after metabolic conversion of ethene to ethene oxide *in vivo*. *Mutat Res* **45**, 175–184.
72. Segerback D (1983) Alkylation of DNA and hemoglobin in the mouse following exposure to ethene and ethene oxide. *Chem Biol Interact* **45**, 139–151.
73. Doublie S, Bandaru V, Bond JP & Wallace SS (2004) The crystal structure of human endonuclease VIII-like 1 (NEIL1) reveals a zincless finger motif required for glycosylase activity. *Proc Natl Acad Sci U S A* **101**, 10284–10289.
74. Sanderson RJ, Bennett SE, Sung JS & Mosbaugh DW (2001) Uracil-initiated base excision DNA repair synthesis fidelity in human colon adenocarcinoma LoVo and *Escherichia coli* cell extracts. *Prog Nucleic Acid Res Mol Biol* **68**, 165–188.
75. Krokan HE, Drablos F & Slupphaug G (2002) Uracil in DNA – occurrence, consequences and repair. *Oncogene* **21**, 8935–8948.
76. Cooke MS, Evans MD, Dizdaroglu M & Lunec J (2003) Oxidative DNA damage: mechanisms, mutation, and disease. *FASEB J* **17**, 1195–1214.
77. Kim KS, Chang YJ, Chung YJ, Park CU & Seo HY (2007) Enhanced expression of high-affinity iron transporters via H-ferritin production in yeast. *J Biochem Mol Biol* **40**, 82–87.
78. Valko M, Rhodes CJ, Moncol J, Izakovic M & Mazur M (2006) Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem Biol Interact* **160**, 1–40.
79. Dalton TP, Shertzer HG & Puga A (1999) Regulation of gene expression by reactive oxygen. *Annu Rev Pharmacol Toxicol* **39**, 67–101.

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80. Allen RG & Tresini M (2000) Oxidative stress and gene regulation. *Free Radic Biol Med* **28**, 463–499.
81. Martindale JL & Holbrook NJ (2002) Cellular response to oxidative stress: signaling for suicide and survival. *J Cell Physiol* **192**, 1–15.
82. Mathers J, Fraser JA, McMahon M, Saunders RD, Hayes JD & McLellan LI (2004) Antioxidant and cytoprotective responses to redox stress. *Biochem Soc Symp*, 157–176.
83. Owuor ED & Kong AN (2002) Antioxidants and oxidants regulated signal transduction pathways. *Biochem Pharmacol* **64**, 765–770.
84. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M & Telser J (2007) Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* **39**, 44–84.
85. Ma Q, Battelli L & Hubbs AF (2006) Multiorgan autoimmune inflammation, enhanced lymphoproliferation, and impaired homeostasis of reactive oxygen species in mice lacking the antioxidant-activated transcription factor Nrf2. *Am J Pathol* **168**, 1960–1974.
86. Poli G, Leonarduzzi G, Biasi F & Chiarpotto E (2004) Oxidative stress and cell signalling. *Curr Med Chem* **11**, 1163–1182.
87. Abe J & Berk BC (1999) Fyn and JAK2 mediate Ras activation by reactive oxygen species. *J Biol Chem* **274**, 21003–21010.
88. Esposito F, Chirico G, Montesano Gesualdi N, Posadas I, Ammendola R, Russo T, Cirino G & Cimino F (2003) Protein kinase B activation by reactive oxygen species is independent of tyrosine kinase receptor phosphorylation and requires SRC activity. *J Biol Chem* **278**, 20828–20834.
89. Gopalakrishna R & Jaken S (2000) Protein kinase C signaling and oxidative stress. *Free Radic Biol Med* **28**, 1349–1361.
90. Kyriakis JM & Avruch J (2001) Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. *Physiol Rev* **81**, 807–869.
91. Lopez-Ilasaca M, Crespo P, Pellici PG, Gutkind JS & Wetzker R (1997) Linkage of G protein-coupled receptors to the MAPK signaling pathway through PI 3-kinase gamma. *Science* **275**, 394–397.
92. Iles KE & Forman HJ (2002) Macrophage signaling and respiratory burst. *Immunol Res* **26**, 95–105.
93. Torres M & Forman HJ (1999) Activation of several MAP kinases upon stimulation of rat alveolar macrophages: role of the NADPH oxidase. *Arch Biochem Biophys* **366**, 231–239.
94. Rao A, Luo C & Hogan PG (1997) Transcription factors of the NFAT family: regulation and function. *Annu Rev Immunol* **15**, 707–747.
95. Pinkus R, Weiner LM & Daniel V (1996) Role of oxidants and antioxidants in the induction of AP-1, NF-kappaB, and glutathione *S*-transferase gene expression. *J Biol Chem* **271**, 13422–13429.
96. Amiri KI & Richmond A (2005) Role of nuclear factor-kappa B in melanoma. *Cancer Metastasis Rev* **24**, 301–313.
97. Hofseth LJ, Hussain SP & Harris CC (2004) P53: 25 years after its discovery. *Trends Pharmacol Sci* **25**, 177–181.
98. Jin HO, An S, Lee HC, Woo SH, Seo SK, Choe TB, Yoo DH, Lee SB, Um HD, Lee SJ, Park MJ, Kim JI, Hong SI, Rhee CH & Park IC (2007) Hypoxic condition- and high cell density-induced expression of Redd1 is regulated by activation of hypoxia-inducible factor-1alpha and Sp1 through the phosphatidylinositol 3-kinase/Akt signalling pathway. *Cell Signal* **19**, 1393–1403.
99. Li MH, Jang JH & Surh YJ (2005) Nitric oxide induces apoptosis via AP-1-driven upregulation of COX-2 in rat pheochromocytoma cells. *Free Radic Biol Med* **39**, 890–899.
100. Sablina AA, Budanov AV, Ilyinskaya GV, Agapova LS, Kravchenko JE & Chumakov PM (2005) The antioxidant function of the p53 tumor suppressor. *Nat Med* **11**, 1306–1313.
101. Ehrlich V, Hoelzl C, Nersesyan A, Winter H, Koller V, Ferk F, Fenech M, Dusinska M & Knasmüller S (2007) Wheat sprout consumption and DNA stability: results from an controlled intervention trial. In *Synthetic and Natural Compounds in Cancer Therapy and Prevention*, p. 15 [Z Ďuračková, D Slámenova and M Micksche *et al.*, editors]. Bratislava, Slovakia.
102. Jauliac S, Lopez-Rodriguez C, Shaw LM, Brown LF, Rao A & Toker A (2002) The role of NFAT transcription factors in integrin-mediated carcinoma invasion. *Nat Cell Biol* **4**, 540–544.
103. Semenza GL (2000) HIF-1: mediator of physiological and pathophysiological responses to hypoxia. *J Appl Physiol* **88**, 1474–1480.
104. Huang HC, Nguyen T & Pickett CB (2000) Regulation of the antioxidant response element by protein kinase C-mediated phosphorylation of NF-E2-related factor 2. *Proc Natl Acad Sci USA* **97**, 12475–12480.
105. Nguyen T, Yang CS & Pickett CB (2004) The pathways and molecular mechanisms regulating Nrf2 activation in response to chemical stress. *Free Radic Biol Med* **37**, 433–441.
106. Favreau LV & Pickett CB (1995) The rat quinone reductase antioxidant response element. Identification of the nucleotide sequence required for basal and inducible activity and detection of antioxidant response element-binding proteins in hepatoma and non-hepatoma cell lines. *J Biol Chem* **270**, 24468–24474.
107. Gupta M, Dobashi K, Greene EL, Orak JK & Singh I (1997) Studies on hepatic injury and antioxidant enzyme activities in rat subcellular organelles following *in vivo* ischemia and reperfusion. *Mol Cell Biochem* **176**, 337–347.
108. Klaunig JE & Kamendulis LM (2004) The role of oxidative stress in carcinogenesis. *Annu Rev Pharmacol Toxicol* **44**, 239–267.
109. Michiels C, Raes M, Toussaint O & Remacle J (1994) Importance of Se-glutathione peroxidase, catalase, and Cu/Zn-SOD for cell survival against oxidative stress. *Free Radic Biol Med* **17**, 235–248.
110. Fridovich I (1986) Superoxide dismutases. *Adv Enzymol Relat Areas Mol Biol* **58**, 61–97.
111. Kwak MK, Wakabayashi N, Itoh K, Motohashi H, Yamamoto M & Kensler TW (2003) Modulation of gene expression by cancer chemopreventive dithiolethiones through the Keap1-Nrf2 pathway. Identification of novel gene clusters for cell survival. *J Biol Chem* **278**, 8135–8145.
112. Thimmulappa RK, Mai KH, Srisuma S, Kensler TW, Yamamoto M & Biswal S (2002) Identification of Nrf2-regulated genes induced by the chemopreventive agent sulforaphane by oligonucleotide microarray. *Cancer Res* **62**, 5196–5203.
113. McMahon M, Itoh K, Yamamoto M & Hayes JD (2003) Keap1-dependent proteasomal degradation of transcription factor Nrf2 contributes to the negative regulation of antioxidant response element-driven gene expression. *J Biol Chem* **278**, 21592–21600.
114. Itoh K, Wakabayashi N, Katoh Y, Ishii T, Igarashi K, Engel JD & Yamamoto M (1999) Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. *Genes Dev* **13**, 76–86.
115. Kwak MK & Kensler TW (2006) Induction of 26S proteasome subunit PSMB5 by the bifunctional inducer 3-methylcholanthrene through the Nrf2-ARE, but not the AhR/Arnt-XRE, pathway. *Biochem Biophys Res Commun* **345**, 1350–1357.
116. He X, Lin GX, Chen MG, Zhang JX & Ma Q (2007) Protection against chromium (VI)-induced oxidative stress and apoptosis by Nrf2. Recruiting Nrf2 into the nucleus and disrupting the nuclear Nrf2/Keap1 association. *Toxicol Sci* **98**, 298–309.
117. He X, Chen MG, Lin GX & Ma Q (2006) Arsenic induces NAD(P)H-quinone oxidoreductase I by disrupting the Nrf2 ×

British Journal of Nutrition

Keap1 × Cul3 complex and recruiting Nrf2 × Maf to the antioxidant response element enhancer. *J Biol Chem* **281**, 23620–23631.
118. Mann GE, Niehueser-Saran J, Watson A, Gao L, Ishii T, de Winter P & Siow RC (2007) Nrf2/ARE regulated antioxidant gene expression in endothelial and smooth muscle cells in oxidative stress: implications for atherosclerosis and preeclampsia. *Sheng Li Xue Bao* **59**, 117–127.
119. Kundu JK & Surh YJ (2005) Breaking the relay in deregulated cellular signal transduction as a rationale for chemoprevention with anti-inflammatory phytochemicals. *Mutat Res* **591**, 123–146.
120. Chen C & Kong AN (2004) Dietary chemopreventive compounds and ARE/EpRE signaling. *Free Radic Biol Med* **36**, 1505–1516.
121. Surh YJ, Kundu JK, Na HK & Lee JS (2005) Redox-sensitive transcription factors as prime targets for chemoprevention with anti-inflammatory and antioxidative phytochemicals. *J Nutr* **135**, 2993S–3001S.
122. Stange EF (2006) Review Article: the effect of aminosalicylates and immunomodulation on cancer risk in inflammatory bowel disease. *Aliment Pharmacol Ther* **24**, Suppl. 3, 64–67.
123. Chia VM, Newcomb PA, Bigler J, Morimoto LM, Thibodeau SN & Potter JD (2006) Risk of microsatellite-unstable colorectal cancer is associated jointly with smoking and nonsteroidal anti-inflammatory drug use. *Cancer Res* **66**, 6877–6883.
124. Kong AN, Owuor E, Yu R, Hebbar V, Chen C, Hu R & Mandlekar S (2001) Induction of xenobiotic enzymes by the MAP kinase pathway and the antioxidant or electrophile response element (ARE/EpRE). *Drug Metab Rev* **33**, 255–271.
125. Nishinaka T, Ichijo Y, Ito M, Kimura M, Katsuyama M, Iwata K, Miura T, Terada T & Yabe-Nishimura C (2007) Curcumin activates human glutathione *S*-transferase P1 expression through antioxidant response element. *Toxicol Lett* **170**, 238–247.
126. Tanigawa S, Fujii M & Hou DX (2007) Action of Nrf2 and Keap1 in ARE-mediated NQO1 expression by quercetin. *Free Radic Biol Med* **42**, 1690–1703.
127. Gonzalez-Gallego J, Sanchez-Campos S & Tunon MJ (2007) Anti-inflammatory properties of dietary flavonoids. *Nutr Hosp* **22**, 287–293.
128. Hong F, Freeman ML & Liebler DC (2005) Identification of sensor cysteines in human Keap1 modified by the cancer chemopreventive agent sulforaphane. *Chem Res Toxicol* **18**, 1917–1926.
129. Hong F, Sekhar KR, Freeman ML & Liebler DC (2005) Specific patterns of electrophile adduction trigger Keap1 ubiquitination and Nrf2 activation. *J Biol Chem* **280**, 31768–31775.
130. Wakabayashi N, Dinkova-Kostova AT, Holtzclaw WD, Kang MI, Kobayashi A, Yamamoto M, Kensler TW & Talalay P (2004) Protection against electrophile and oxidant stress by induction of the phase 2 response: fate of cysteines of the Keap1 sensor modified by inducers. *Proc Natl Acad Sci U S A* **101**, 2040–2045.
131. Eggler AL, Liu G, Pezzuto JM, van Breemen RB & Mesecar AD (2005) Modifying specific cysteines of the electrophile-sensing human Keap1 protein is insufficient to disrupt binding to the Nrf2 domain Neh2. *Proc Natl Acad Sci U S A* **102**, 10070–10075.
132. Le SB, Hailer MK, Buhrow S, Wang Q, Flatten K, Pediaditakis P, Bible KC, Lewis LD, Sausville EA, Pang YP, Ames MM, Lemasters JJ, Holmuhamedov EL & Kaufmann SH (2007) Inhibition of mitochondrial respiration as a source of adaphostin-induced reactive oxygen species and cytotoxicity. *J Biol Chem aufmann* **282**, 8860–8872.
133. Wiseman A (2006) Oxygen-induced reperfusion-injury is caused by ROS: amelioration is possible by recombinant-DNA antioxidant enzymes and mimics in selected tissues. *Med Hypotheses* **66**, 329–331.
134. Criddle DN, Gillies S, Baumgartner-Wilson HK, Jaffar M, Chinje EC, Passmore S, Chvanov M, Barrow S, Gerasimenko OV, Tepikin AV, Sutton R & Petersen OH (2006) Menadione-induced reactive oxygen species generation via redox cycling promotes apoptosis of murine pancreatic acinar cells. *J Biol Chem* **281**, 40485–40492.
135. McCarthy S, Somayajulu M, Sikorska M, Borowy-Borowski H & Pandey S (2004) Paraquat induces oxidative stress and neuronal cell death; neuroprotection by water-soluble coenzyme Q10. *Toxicol Appl Pharmacol* **201**, 21–31.
136. Srinivas P, Gopinath G, Banerji A, Dinakar A & Srinivas G (2004) Plumbagin induces reactive oxygen species, which mediate apoptosis in human cervical cancer cells. *Mol Carcinog* **40**, 201–211.
137. Phillips BJ, Anderson D & Gangolli SD (1986) Influence of phagocyte-derived active oxygen species in tissue responses to tumour promoters and irritants. *Food Chem Toxicol* **24**, 681–683.
138. Minamiyama Y, Takemura S, Hai S, Suehiro S & Okada S (2006) Vitamin E deficiency accelerates nitrate tolerance via a decrease in cardiac P450 expression and increased oxidative stress. *Free Radic Biol Med* **40**, 808–816.
139. Isomura H, Fujie K, Shibata K, Inoue N, Iizuka T, Takebe G, Takahashi K, Nishihira J, Izumi H & Sakamoto W (2004) Bone metabolism and oxidative stress in postmenopausal rats with iron overload. *Toxicology* **197**, 93–100.
140. Gregorevic P, Lynch GS & Williams DA (2001) Hyperbaric oxygen modulates antioxidant enzyme activity in rat skeletal muscles. *Eur J Appl Physiol* **86**, 24–27.
141. He SX, Luo JY, Wang YP, Wang YL, Fu H, Xu JL, Zhao G & Liu EQ (2006) Effects of extract from Ginkgo biloba on carbon tetrachloride-induced liver injury in rats. *World J Gastroenterol* **12**, 3924–3928.
142. Bruck R, Ashkenazi M, Weiss S, Goldiner I, Shapiro H, Aeed H, Genina O, Helpern Z & Pines M (2007) Prevention of liver cirrhosis in rats by curcumin. *Liver Int* **27**, 373–383.
143. Wirtz S, Neufert C, Weigmann B & Neurath MF (2007) Chemically induced mouse models of intestinal inflammation. *Nat Protoc* **2**, 541–546.
144. Fujimoto K, Arakawa S, Shibaya Y, Miida H, Ando Y, Yasumo H, Hara A, Uchiyama M, Iwabuchi H, Takasaki W, Manabe S & Yamoto T (2006) Characterization of phenotypes in Gstm1-null mice by cytosolic and *in vivo* metabolic studies using 1,2-dichloro-4-nitrobenzene. *Drug Metab Dispos* **34**, 1495–1501.
145. Erker L, Schubert R, Elchuri S, Huang TT, Tarin D, Mueller K, Zielen S, Epstein CJ & Wynshaw-Boris A (2006) Effect of the reduction of superoxide dismutase 1 and 2 or treatment with alpha-tocopherol on tumorigenesis in Atm-deficient mice. *Free Radic Biol Med* **41**, 590–600.
146. Ashe KH (2006) Molecular basis of memory loss in the Tg2576 mouse model of Alzheimer's disease. *J Alzheimers Dis* **9**, 123–126.
147. Hamaue N, Ogata A, Terado M, Ohno K, Kikuchi S, Sasaki H, Tashiro K, Hirafuji M & Minami M (2006) Brain catecholamine alterations and pathological features with aging in Parkinson disease model rat induced by Japanese encephalitis virus. *Neurochem Res* **31**, 1451–1455.
148. Zha Y, Le VT, Higami Y, Shimokawa I, Taguchi T & Razzaque MS (2006) Life-long suppression of growth hormone-insulin-like growth factor I activity in genetically altered rats could prevent age-related renal damage. *Endocrinology* **147**, 5690–5698.
149. Larsen E, Kwon K, Coin F, Egly JM & Klungland A (2004) Transcription activities at 8-oxoG lesions in DNA. *DNA Repair (Amst)* **3**, 1457–1468.

British Journal of Nutrition

150. Xie Y, Yang H, Cunanan C, Okamoto K, Shibata D, Pan J, Barnes DE, Lindahl T, McIlhatton M, Fishel R & Miller JH (2004) Deficiencies in mouse Myh and Ogg1 result in tumor predisposition and G to T mutations in codon 12 of the K-ras oncogene in lung tumors. *Cancer Res* **64**, 3096–3102.
151. Glasziou PP & Sanders SL (2002) Investigating causes of heterogeneity in systematic reviews. *Stat Med* **21**, 1503–1511.
152. Hartmann A, Niess AM, Grunert-Fuchs M, Poch B & Speit G (1995) Vitamin E prevents exercise-induced DNA damage. *Mutat Res* **346**, 195–202.
153. Sacheck JM, Milbury PE, Cannon JG, Roubenoff R & Blumberg JB (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.
154. Dennog C, Radermacher P, Barnett YA & Speit G (1999) Antioxidant status in humans after exposure to hyperbaric oxygen. *Mutat Res* **428**, 83–89.
155. Astley S, Langrish-Smith A, Southon S & Sampson M (1999) Vitamin E supplementation and oxidative damage to DNA and plasma LDL in type 1 diabetes. *Diabetes Care* **22**, 1626–1631.
156. Lean ME, Noroozi M, Kelly I, Burns J, Talwar D, Sattar N & Crozier A (1999) Dietary flavonols protect diabetic human lymphocytes against oxidative damage to DNA. *Diabetes* **48**, 176–181.
157. Sampson MJ, Astley S, Richardson T, Willis G, Davies IR, Hughes DA & Southon S (2001) Increased DNA oxidative susceptibility without increased plasma LDL oxidizability in Type II diabetes: effects of alpha-tocopherol supplementation. *Clin Sci (Lond)* **101**, 235–241.
158. Jaruga P, Jaruga B, Gackowski D, Olczak A, Halota W, Pawlowska M & Olinski R (2002) Supplementation with antioxidant vitamins prevents oxidative modification of DNA in lymphocytes of HIV-infected patients. *Free Radic Biol Med* **32**, 414–420.
159. Soccio M, Toniato E, Evangelista V, Carluccio M & De Caterina R (2005) Oxidative stress and cardiovascular risk: the role of vascular NAD(P)H oxidase and its genetic variants. *Eur J Clin Invest* **35**, 305–314.
160. Hercberg S, Czernichow S & Galan P (2006) Antioxidant vitamins and minerals in prevention of cancers: lessons from the SU.VI.MAX study. *Br J Nutr* **96**, Suppl. 1, S28–S30.
161. Flora SJ (2007) Role of free radicals and antioxidants in health and disease. *Cell Mol Biol (Noisy-le-grand)* **53**, 1–2.
162. Kan E, Undeger U, Bali M & Basaran N (2002) Assessment of DNA strand breakage by the alkaline COMET assay in dialysis patients and the role of vitamin E supplementation. *Mutat Res* **520**, 151–159.
163. Evans MD, Cooke MS, Akil M, Samanta A & Lunec J (2000) Aberrant processing of oxidative DNA damage in systemic lupus erythematosus. *Biochem Biophys Res Commun* **273**, 894–898.
164. Berliner LJ, Khramtsov V, Fujii H & Clanton TL (2001) Unique *in vivo* applications of spin traps. *Free Radic Biol Med* **30**, 489–499.
165. Khan N, Wilmot CM, Rosen GM, Demidenko E, Sun J, Joseph J, O'Hara J, Kalyanaraman B & Swartz HM (2003) Spin traps: *in vitro* toxicity and stability of radical adducts. *Free Radic Biol Med* **34**, 1473–1481.
166. Bottle SE, Hanson GR & Micallef AS (2003) Application of the new EPR spin trap 1,1,3-trimethylisoindole *N*-oxide (TMINO) in trapping HO. and related biologically important radicals. *Org Biomol Chem* **1**, 2585–2589.
167. Karoui H, Clément J-L, Rockenbauer A, Siri D & Tordo P (2004) Synthesis and structure of 5,5-diethoxycarbonyl-1-pyrroline *N*-oxide (DECPO). Application to superoxide radical trapping. *Tetrahedron Lett* **45**, 149–152.
168. Stolze K, Udilova N, Rosenau T, Hofinger A & Nohl H (2003) Spin trapping of superoxide, alkyl- and lipid-derived radicals with derivatives of the spin trap EPPN. *Biochem Pharmacol* **66**, 1717–1726.
169. Zhao H, Joseph J, Zhang H, Karoui H & Kalyanaraman B (2001) Synthesis and biochemical applications of a solid cyclic nitrone spin trap: a relatively superior trap for detecting superoxide anions and glutathiyl radicals. *Free Radic Biol Med* **31**, 599–606.
170. Halliwell B & Whiteman M (2004) Measuring reactive species and oxidative damage *in vivo* and in cell culture: how should you do it and what do the results mean? *Br J Pharmacol* **142**, 231–255.
171. Aruoma OI (2003) Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods. *Mutat Res* **523–524**, 9–20.
172. Ghiselli A, Serafini M, Maiani G, Azzini E & Ferro-Luzzi A (1995) A fluorescence-based method for measuring total plasma antioxidant capability. *Free Radic Biol Med* **18**, 29–36.
173. Glazer AN (1990) Phycoerythrin fluorescence-based assay for reactive oxygen species. *Methods Enzymol* **186**, 161–168.
174. Cao G, Alessio HM & Cutler RG (1993) Oxygen-radical absorbance capacity assay for antioxidants. *Free Radic Biol Med* **14**, 303–311.
175. Prior RL, Wu X & Schaich K (2005) Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J Agric Food Chem* **53**, 4290–4302.
176. Huang D, Ou B, Hampsch-Woodill M, Flanagan JA & Deemer EK (2002) Development and validation of oxygen radical absorbance capacity assay for lipophilic antioxidants using randomly methylated β-cyclodextrin as the solubility enhancer. *J Agric Food Chem* **50**, 1815–1821.
177. Huang D, Ou B, Hampsch-Woodill M, Flanagan JA & Prior RL (2002) High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. *J Agric Food Chem* **50**, 4437–4444.
178. Lussignoli S, Fraccaroli M, Andrioli G, Brocco G & Bellavite P (1999) A microplate-based colorimetric assay of the total peroxyl radical trapping capability of human plasma. *Anal Biochem* **269**, 38–44.
179. Frankel EN & Meyer AS (2000) The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *J Sci Food Agric* **80**, 1925–1941.
180. Wayner DDM, Burton GW, Ingold KU & Locke S (1985) Quantitative measurement of the total, peroxyl radical-trapping antioxidant capability of human blood plasma by controlled peroxidation. The important contribution made by plasma proteins. *FEBS Lett* **187**, 33–37.
181. DeLange RJ & Glazer AN (1989) Phycoerythrin fluorescence-based assay for peroxy radicals: a screen for biologically relevant protective agents. *Anal Biochem* **177**, 300–306.
182. Bartosz G, Janaszewska A, Ertel D & Bartosz M (1998) Simple determination of peroxyl radical-trapping capacity. *Biochem Mol Biol Int* **46**, 519–528.
183. Rota C, Chignell CF & Mason RP (1999) Evidence for free radical formation during the oxidation of 2′-7′-dichlorofluorescin to the fluorescent dye 2′-7′-dichlorofluorescein by horseradish peroxidase: possible implications for oxidative stress measurements. *Free Radic Biol Med* **27**, 873–881.
184. Ghiselli A, Serafini M, Natella F & Scaccini C (2000) Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. *Free Radic Biol Med* **29**, 1106–1114.

British Journal of Nutrition

185. Winston GW, Regoli F, Dugas AJ Jr, Fong JH & Blanchard KA (1998) A rapid gas chromatographic assay for determining oxyradical scavenging capacity of antioxidants and biological fluids. *Free Radic Biol Med* **24**, 480–493.
186. Regoli F & Winston GW (1999) Quantification of total oxidant scavenging capacity of antioxidants for peroxynitrite, peroxyl radicals, and hydroxyl radicals. *Toxicol Appl Pharmacol* **156**, 96–105.
187. Lichtenthaler R & Marx F (2005) Total oxidant scavenging capacities of common European fruit and vegetable juices. *J Agric Food Chem* **53**, 103–110.
188. Lundqvist H, Kricka LJ, Stott RA, Thorpe GH & Dahlgren C (1995) Influence of different luminols on the characteristics of the chemiluminescence reaction in human neutrophils. *J Biolumin Chemilumin* **10**, 353–359.
189. Whitehead TP, Thorpe GHG & Maxwell SRJ (1992) Enhanced chemiluminescent assay for antioxidant capacity in biological fluids. *Analytica Chimica Acta* **266**, 265–277.
190. Witko-Sarsat V, Nguyen AT, Knight J & Descamps-Latscha B (1992) Pholasin®: a new chemiluminescent probe for the detection of chloramines derived from human phagocytes. *Free Radic Biol Med* **13**, 83–88.
191. Swindle EJ, Hunt JA & Coleman JW (2002) A comparison of reactive oxygen species generation by rat peritoneal macrophages and mast cells using the highly sensitive real-time chemiluminescent probe pholasin: inhibition of antigen-induced mast cell degranulation by macrophage-derived hydrogen peroxide. *J Immunol* **169**, 5866–5873.
192. Glebska J & Koppenol WH (2005) Chemiluminescence of Pholasin caused by peroxynitrite. *Free Radic Biol Med* **38**, 1014–1022.
193. Popov I, Völker H & Lewin G (2001) Photochemiluminescent detection of antiradical activity. V. Application in combination with the hydrogen peroxide-initiated chemiluminescence of blood plasma proteins to evaluate antioxidant homeostasis in humans. *Redox Report* **6**, 43–48.
194. Esterbauer H, Jurgens G, Quehenberger O & Koller E (1987) Autoxidation of human low density lipoprotein: loss of polyunsaturated fatty acids and vitamin E and generation of aldehydes. *J Lipid Res* **28**, 495–509.
195. Quehenberger O, Jürgens G, Zadravec S & Esterbauer H (1988) Oxidation of human low density lipoprotein initiated by copper (II) chloride. *Basic Life Sci* **49**, 387–390.
196. Rice-Evans C, Leake D, Bruckdorfer R & Diplock AT (1996) Practical approaches to low density lipoprotein oxidation: whys, wherefores and pitfalls. *Free Radic Res* **25**, 285–311.
197. Wagner K-H, Tomasch R & Elmadfa I (2001) Impact of diets containing corn oil or olive/sunflower oil mixture on the human plasma and lipoprotein lipid metabolism. *Eur J Nutr* **40**, 161–167.
198. Holvoet P, Donck J, Landeloos M, Brouwers E, Luijtens K, Arnout J, Lesaffre E, Vanrenterghem Y & Collen D (1996) Correlation between oxidized low density lipoproteins and von Willebrand factor renal failure. *Thromb Haemost* **76**, 663–669.
199. Frankel EN, German JB & Davis PA (1992) Headspace gas chromatography to determine human low density lipoprotein oxidation. *Lipids* **27**, 1047–1051.
200. Bors W, Michel C & Saran M (1984) Inhibition of the bleaching of the carotenoid crocin. A rapid test for quantifying antioxidant activity. *Biochim Biophys Acta* **796**, 312–319.
201. Tubaro F, Ghiselli A, Rapuzzi P, Maiorino M & Ursini F (1998) Analysis of plasma antioxidant capacity by competition kinetics. *Free Radic Biol Med* **24**, 1228–1234.
202. Miller NJ, Rice-Evans C, Davies MJ, Gopinathan V & Milner A (1993) A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clin Sci* **84**, 407–412.
203. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M & Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* **26**, 1231–1237.
204. Van Den Berg R, Haenen G, Van Den Berg H & Bast A (1999) Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. *Food Chem* **66**, 511–517.
205. Miller NJ, Sampson J, Candeias LP, Bramley PM & Rice-Evans CA (1996) Antioxidant activities of carotenes and xanthophylls. *FEBS Lett* **384**, 240–242.
206. Benzie IFF & Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': the FRAP assay. *Anal Biochem* **239**, 70–76.
207. Benzie IFF & Szeto YT (1999) Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay. *J Agric Food Chem* **47**, 633–636.
208. Ou B, Huang D, Hampsch-Woodill M, Flanagan JA & Deemer EK (2002) Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: a comparative study. *J Agric Food Chem* **50**, 3122–3128.
209. Pulido R, Bravo L & Saura-Calixto F (2000) Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *J Agric Food Chem* **48**, 3396–3402.
210. Apak R, Guclu K, Ozyurek M & Karademir SE (2004) Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *J Agric Food Chem* **52**, 7970–7981.
211. Brand-Williams W, Cuvelier ME & Berset C (1995) Use of a free radical method to evaluate antioxidant activity. *Lebensm Wiss Technol* **28**, 25–30.
212. Bondet V, Brand-Williams W & Berset C (1997) Kinetics and mechanisms of antioxidant activity using the DPPH$^{\bullet}$ free radical method. *Food Science and Technology* **30**, 609–615.
213. Nomura T, Kikuchi M, Kubodera A & Kawakami Y (1997) Proton-donative antioxidant activity of fucoxanthin with 1,1-diphenyl-2-picrylhydrazyl (DPPH). *Biochem Mol Biol Int* **42**, 361–370.
214. Huang D, Ou B & Prior RL (2005) The chemistry behind antioxidant capacity assays. *J Agric Food Chem* **53**, 1841–1856.
215. Spiteller G (2006) Peroxyl radicals: inductors of neurodegenerative and other inflammatory diseases. Their origin and how they transform cholesterol, phospholipids, plasmalogens, polyunsaturated fatty acids, sugars, and proteins into deleterious products. *Free Radic Biol Med* **41**, 362–387.
216. Yagi K (1976) A simple fluorometric assay for lipoperoxide in blood plasma. *Biochem Med* **15**, 212–216.
217. Gutteridge JM (1986) Aspects to consider when detecting and measuring lipid peroxidation. *Free Radic Res Commun* **1**, 173–184.
218. Meagher EA & Fitzgerald GA (2000) Indices of lipid peroxidation *in vivo*: strengths and limitations. *Free Radic Biol Med* **28**, 1745–1750.
219. Wong SHY, Knight JA, Hopfer SM, Zaharia O, Leach CN Jr & Sunderman FW Jr (1987) Lipoperoxides in plasma as measured by liquid-chromatographic separation of malondialdehyde-thiobarbituric acid adduct. *Clin Chem* **33**, 214–220.
220. Karatas F, Karatepe M & Baysar A (2002) Determination of free malondialdehyde in human serum by high-performance liquid chroma tography. *Anal Biochem* **311**, 76–79.

British Journal of Nutrition

221. Wilson DW, Metz HN, Graver LM & Rao PS (1997) Direct method for quantification of free malondialdehyde with high-performance capillary electrophoresis in biological samples. *Clin Chem* **43**, 1982–1984.
222. Sim AS, Salonikas C, Naidoo D & Wilcken DEL (2003) Improved method for plasma malondialdehyde measurement by high-performance liquid chromatography using methyl malondialdehyde as an internal standard. *J Chromatogr B Analyt Technol Biomed Life Sci* **785**, 337–344.
223. Steghens J-P, Van Kappel AL, Denis I & Collombel C (2001) Diaminonaphtalene, a new highly specific regent for HPLC–UV measurement of total and free malondialdehyde in human plasma or serum. *Free Radic Biol Med* **31**, 242–249.
224. Cighetti G, Allevi P, Anastasia L, Bortone L & Paroni R (2002) Use of methyl malondialdehyde as an internal standard for malondialdehyde detection: validation by isotope-dilution gas chromatography–mass spectrometry. *Clin Chem* **48**, 2266–2269.
225. Stalikas CD & Konidari CN (2001) Analysis of malondialdehyde in biological matrices by capillary gas chromatography with electron-capture detection and mass spectrometry. *Anal Biochem* **290**, 108–115.
226. Del Rio D, Stewart AJ & Pellegrini N (2005) A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. *Nutr Metab Cardiovasc Dis* **15**, 316–328.
227. Ramel A, Wagner K-H & Elmadfa I (2004) Plasma antioxidants and lipid oxidation after submaximal resistance exercise in men. *Eur J Nutr* **43**, 2–6.
228. Konig D, Wagner KH, Elmadfa I & Berg A (2001) Exercise and oxidative stress: significance of antioxidants with reference to inflammatory, muscular, and systemic stress. *Exerc Immunol Rev* **7**, 108–133.
229. Morrow JD, Hill KE, Burk RF, Nammour TM, Badr KF & Roberts LJ II (1990) A series of prostaglandin F2-like compounds are produced *in vivo* in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. *Proc Natl Acad Sci U S A* **87**, 9383–9387.
230. Milne GL, Musiek ES & Morrow JD (2005) F2-isoprostanes as markers of oxidative stress *in vivo*: an overview. *Biomarkers* **10**, Suppl. 1, S10–S23.
231. Roberts LJ II & Morrow JD (2000) Measurement of F2-isoprostanes as an index of oxidative stress *in vivo*. *Free Radic Biol Med* **28**, 505–513.
232. Young IS (2005) Oxidative stress and vascular disease: insights from isoprostane measurement. *Clin Chem* **51**, 14–15.
233. Schwedhelm E & Boger RH (2003) Application of gas chromatography–mass spectrometry for analysis of isoprostanes: their role in cardiovascular disease. *Clin Chem Lab Med* **41**, 1552–1561.
234. Frank H, Hintze T, Bimboes D & Remmer H (1980) Monitoring lipid peroxidation by breath analysis: endogenous hydrocarbons and their metabolic elimination. *Toxicol Appl Pharmacol* **56**, 337–344.
235. Romero FJ, Bosch-Morell F, Romero MJ, Jareno EJ, Romero B, Marin N & Roma J (1998) Lipid peroxidation products and antioxidants in human disease. *Environ Health Perspect* **106**, Suppl. 5, 1229–1234.
236. Risby TH & Sehnert SS (1999) Clinical application of breath biomarkers of oxidative stress status. *Free Radic Biol Med* **27**, 1182–1192.
237. Esterbauer H, Schaur RJ & Zollner H (1991) Chemistry and Biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radic Biol Med* **11**, 81–128.
238. Benedetti A, Comporti M & Esterbauer H (1980) Identification of 4-hydroxynonenal as a cytotoxic product originating from the peroxidation of liver microsomal lipids. *Biochim Biophys Acta* **620**, 281–296.
239. Cahuana GM, Tejedo JR, Jimenez J, Ramirez R, Sobrino F & Bedoya FJ (2003) Involvement of advanced lipooxidation end products (ALEs) and protein oxidation in the apoptotic actions of nitric oxide in insulin secreting RINm5F cells. *Biochem Pharmacol* **66**, 1963–1971.
240. Holley AE, Walker MK, Cheeseman KH & Slater TF (1993) Measurement of *n*-alkanals and hydroxyalkenals in biological samples. *Free Radic Biol Med* **15**, 281–289.
241. Sharma R, Brown D, Awasthi S, Yang Y, Sharma A, Patrick B, Saini MK, Singh SP, Zimniak P, Singh SV & Awasthi YC (2004) Transfection with 4-hydroxynonenal-metabolizing glutathione *S*-transferase isozymes leads to phenotypic transformation and immortalization of adherent cells. *Eur J Biochem* **271**, 1690–1701.
242. Agarwal R (2004) Chronic kidney disease is associated with oxidative stress independent of hypertension. *Clin Nephrol* **61**, 377–383.
243. Winterbourn CC & Buss IH (1999) Protein carbonyl measurement by enzyme-linked immunosorbent assay. *Methods Enzymol* **300**, 106–111.
244. Levine RL, Wehr N, Williams JA, Stadtman ER & Shacter E (2000) Determination of carbonyl groups in oxidized proteins. *Methods Mol Biol* **99**, 15–24.
245. Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz A-G, Ahn B-W, Shaltiel S & Stadtman ER (1990) Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol* **186**, 464–478.
246. Alamdari DH, Kostidou E, Paletas K, Sarigianni M, Konstas AGP, Karapiperidou A & Koliakos G (2005) High sensitivity enzyme-linked immunosorbent assay (ELISA) method for measuring protein carbonyl in samples with low amounts of protein. *Free Radic Biol Med* **39**, 1362–1367.
247. Witko-Sarsat V, Friedlander M, Capeillere-Blandin C, Nguyen-Khoa T, Nguyen AT, Zingraff J, Jungers P & Descamps-Latscha B (1996) Advanced oxidation protein products as a novel marker of oxidative stress in uremia. *Kidney Int* **49**, 1304–1313.
248. Capeillere-Blandin C, Gausson V, Descamps-Latscha B & Witko-Sarsat V (2004) Biochemical and spectrophotometric significance of advanced oxidized protein products. *Biochim Biophys Acta* **1689**, 91–102.
249. Witko-Sarsat V, Friedlander M, Khoa TN, Capeillére-Blandin C, Nguyen AT, Canteloup S, Dayer J-M, Jungers P, Drüeke T & Descamps-Latscha B (1998) Advanced oxidation protein products as novel mediators of inflammation and monocyte activation in chronic renal failure. *J Immunol* **161**, 2524–2532.
250. Kaneda H, Taguchi J, Ogasawara K, Aizawa T & Ohno M (2002) Increased level of advanced oxidation protein products in patients with coronary artery disease. *Atherosclerosis* **162**, 221–225.
251. Kalousova M, Zima T, Tesar V, Dusilova-Sulkova S & Skrha J (2005) Advanced glycoxidation end products in chronic diseases – clinical chemistry and genetic background. *Mutat Res* **579**, 37–46.
252. Kilbey BJ, Legator M, Nichols W & Ramel C (1984) *Handbook of Mutagenicity Test Procedures*. Amsterdam: Elsevier Science Publisher.
253. Venitt SP & Parry JM (1984) *Mutagenicity Testing: A Practical Approach*. Oxford: IRL-Press.
254. Fahrig R (1993) *Mutationsforschung und genetische Toxikologie*. Darmstadt: Wiss Buchges.
255. Knasmüller S, Steinkellner H, Majer BJ, Nobis EC, Scharf G & Kassie F (2002) Search for dietary antimutagens and anticarcinogens: methodological aspects and extrapolation problems. *Food Chem Toxicol* **40**, 1051–1062.

British Journal of Nutrition

256. Knasmüller S, Majer BJ & Buchmann C (2004) Identifying antimutagenic constituents of food. In *Functional Foods, Ageing and Degenerative Disease*, pp. 581–614 [C Remacle and B Reusens, editors]. Cambridge, England/Boca Raton, Boston, New York, Washington, DC: CRC Press.
257. Hoelzl C, Bichler J, Ferk F, Simic T, Nersesyan A, Elbling L, Ehrlich V, Chakraborty A & 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.
258. Fenech M (1990) The cytokinesis-block micronucleus assay in nucleated cells. *Prog Clin Biol Res* **340B**, 195–206.
259. Ames BN (1972) A bacterial system for detecting mutagens and carcinogens. In *Mutagenic Effects of Environmental Contaminants*, pp. 57–66 [HE Sutton and MI Harris, editors]. New York: Academic Press.
260. Levin DE, Hollstein M, Christman MF, Schwiers EA & Ames BN (1982) A new Salmonella tester strain (TA102) with A X T base pairs at the site of mutation detects oxidative mutagens. *Proc Natl Acad Sci U S A* **79**, 7445–7449.
261. OECD (1997) Bacterial Reverse Mutation Test. *OECD Guidelines for the Testing of Chemicals. Test Guideline No. 471*
262. Rutten B & Gocke E (1988) The 'antimutagenic' effect of cinnamaldehyde is due to a transient growth inhibition. *Mutat Res* **201**, 97–105.
263. Wurgler FE, Friederich U, Furer E & Ganss M (1990) Salmonella/mammalian microsome assay with tetranitromethane and 3-nitro-L-tyrosine. *Mutat Res* **244**, 7–14.
264. Ostling O & Johanson KJ (1984) Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells. *Biochem Biophys Res Commun* **123**, 291–298.
265. Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, Miyamae Y, Rojas E, Ryu JC & Sasaki YF (2000) Single cell gel/comet assay: guidelines for *in vitro* and *in vivo* genetic toxicology testing. *Environ Mol Mutagen* **35**, 206–221.
266. Singh NP, McCoy MT, Tice RR & Schneider EL (1988) A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp Cell Res* **175**, 184–191.
267. Sasaki YF, Sekihashi K, Izumiyama F, Nishidate E, Saga A, Ishida K & Tsuda S (2000) The comet assay with multiple mouse organs: comparison of comet assay results and carcinogenicity with 208 chemicals selected from the IARC monographs and U.S. NTP Carcinogenicity Database. *Crit Rev Toxicol* **30**, 629–799.
268. Dhillon VS, Thomas P & Fenech M (2004) Comparison of DNA damage and repair following radiation challenge in buccal cells and lymphocytes using single-cell gel electrophoresis. *Int J Radiat Biol* **80**, 517–528.
269. Martino-Roth MG, Viegas J & Roth DM (2003) Occupational genotoxicity risk evaluation through the comet assay and the micronucleus test. *Genet Mol Res* **2**, 410–417.
270. Rojas E, Valverde M, Sordo M & Ostrosky-Wegman P (1996) DNA damage in exfoliated buccal cells of smokers assessed by the single cell gel electrophoresis assay. *Mutat Res* **370**, 115–120.
271. Szeto YT, Benzie IF, Collins AR, Choi SW, Cheng CY, Yow CM & Tse MM (2005) A buccal cell model comet assay: development and evaluation for human biomonitoring and nutritional studies. *Mutat Res* **578**, 371–381.
272. DeMarini DM (2006) Inhibition of fried meat-induced DNA-damage: use of cruciferous vegetables, yogurt and chlorophyllin in a dietary intervention study in humans. In *36th Annual Meeting of the European Environmental Mutagen Society. From Genes to Molecular Epidemiology*. Prague, Czech Republic.
273. Collins AR, Duthie SJ & Dobson VL (1993) Direct enzymic detection of endogenous oxidative base damage in human lymphocyte DNA. *Carcinogenesis* **14**, 1733–1735.
274. Collins AR & Horvathova E (2001) Oxidative DNA damage, antioxidants and DNA repair: applications of the comet assay. *Biochem Soc Trans* **29**, 337–341.
275. Collins AR (2004) The comet assay for DNA damage and repair: principles, applications, and limitations. *Mol Biotechnol* **26**, 249–261.
276. Chakraborty S, Roy M & Bhattacharya RK (2004) Prevention and repair of DNA damage by selected phytochemicals as measured by single cell gel electrophoresis. *J Environ Pathol Toxicol Oncol* **23**, 215–226.
277. Collins BH, Horska A, Hotten PM, Riddoch C & Collins AR (2001) Kiwifruit protects against oxidative DNA damage in human cells and *in vitro*. *Nutr Cancer* **39**, 148–153.
278. Hartmann A, Agurell E, Beevers C, Brendler-Schwaab S, Burlinson B, Clay P, Collins A, Smith A, Speit G, Thybaud V & Tice RR (2003) Recommendations for conducting the *in vivo* alkaline comet assay. *Mutagenesis* **18**, 45–51.
279. Burlinson B, Tice RR, Speit G, Agurell E, Brendler-Schwaab SY, Collins AR, Escobar P, Honma M, Kumaravel TS, Nakajima M, Sasaki YF, Thybaud V, Uno Y, Vasquez M & Hartmann A (2007) Fourth International Workgroup on Genotoxicity testing: results of the *in vivo* comet assay workgroup. *Mutat Res* **627**, 31–35.
280. Anderson D, Yu TW, Phillips BJ & Schmezer P (1994) The effect of various antioxidants and other modifying agents on oxygen-radical-generated DNA damage in human lymphocytes in the COMET assay. *Mutat Res* **307**, 261–271.
281. Anderson D, Basaran N, Dobrzynska MM, Basaran AA & Yu TW (1997) Modulating effects of flavonoids on food mutagens in human blood and sperm samples in the comet assay. *Teratog Carcinog Mutagen* **17**, 45–58.
282. Bichler J, Cavin C, Simic T, Chakraborty A, Ferk F, Hoelzl C, Schulte-Hermann R, Kundi M, Haidinger G, Angelis K & Knasmuller S (2007) Coffee consumption protects human lymphocytes against oxidative and 3-amino-1-methyl-5H-pyrido[4,3-b]indole acetate (Trp-P-2) induced DNA-damage: results of an experimental study with human volunteers. *Food Chem Toxicol* **45**, 1428–1436.
283. Elbling L, Weiss RM, Teufelhofer O, Uhl M, Knasmueller S, Schulte-Hermann R, Berger W & Micksche M (2005) Green tea extract and (−)-epigallocatechin-3-gallate, the major tea catechin, exert oxidant but lack antioxidant activities. *FASEB J* **19**, 807–809.
284. Kundu T, Bhattacharya RK, Siddiqi M & Roy M (2005) Correlation of apoptosis with comet formation induced by tea polyphenols in human leukemia cells. *J Environ Pathol Toxicol Oncol* **24**, 115–128.
285. Vanitha A, Murthy KN, Kumar V, Sakthivelu G, Veigas JM, Saibaba P & Ravishankar GA (2007) Effect of the carotenoid-producing alga, Dunaliella bardawil, on CCl_4-induced toxicity in rats. *Int J Toxicol* **26**, 159–167.
286. Kapiszewska M, Cierniak A, Papiez MA, Pietrzycka A, Stepniewski M & Lomnicki A (2007) Prolonged quercetin administration diminishes the etoposide-induced DNA damage in bone marrow cells of rats. *Drug Chem Toxicol* **30**, 67–81.
287. Gabbianelli R, Nasuti C, Falcioni G & Cantalamessa F (2004) Lymphocyte DNA damage in rats exposed to pyrethroids: effect of supplementation with vitamins E and C. *Toxicology* **203**, 17–26.
288. Liu HG & Xu LH (2007) Garlic oil prevents tributyltin-induced oxidative damage *in vivo* and *in vitro*. *J Food Prot* **70**, 716–721.

British Journal of Nutrition

289. Moller P & Loft S (2002) Oxidative DNA damage in human white blood cells in dietary antioxidant intervention studies. *Am J Clin Nutr* **76**, 303–310.
290. Moller P & Loft S (2004) Interventions with antioxidants and nutrients in relation to oxidative DNA damage and repair. *Mutat Res* **551**, 79–89.
291. Moller P & Loft S (2006) Dietary antioxidants and beneficial effect on oxidatively damaged DNA. *Free Radic Biol Med* **41**, 388–415.
292. Pool-Zobel BL, Bub A, Muller H, Wollowski I & Rechkemmer G (1997) Consumption of vegetables reduces genetic damage in humans: first results of a human intervention trial with carotenoid-rich foods. *Carcinogenesis* **18**, 1847–1850.
293. Riso P, Visioli F, Erba D, Testolin G & Porrini M (2004) Lycopene and vitamin C concentrations increase in plasma and lymphocytes after tomato intake. Effects on cellular antioxidant protection. *Eur J Clin Nutr* **58**, 1350–1358.
294. Gill CI, Haldar S, Porter S, Matthews S, Sullivan S, Coulter J, McGlynn H & Rowland I (2004) The effect of cruciferous and leguminous sprouts on genotoxicity, *in vitro* and *in vivo*. *Cancer Epidemiol Biomarkers Prev* **13**, 1199–1205.
295. Zhao X, Aldini G, Johnson EJ, Rasmussen H, Kraemer K, Woolf H, Musaeus N, Krinsky NI, Russell RM & Yeum KJ (2006) Modification of lymphocyte DNA damage by carotenoid supplementation in postmenopausal women. *Am J Clin Nutr* **83**, 163–169.
296. Ferk F, Knasmüller S, Dusinska M, Brantner A, Uhl M & Chakraborty A (2003) DNA-protective effects of sumach (*Rhus coriaria*) in human and human derived cells *in vitro* and identifications of its active principles. In *Eighth International Conference on Mechanisms of Antimutagenesis and Anticarcinogenesis*, p. 47 [G Bronzetti, LR Ferguson and S De Flora, editors]. Italy: Pisa.
297. Hoelzl C, Glatt H-R, Meinl W, Sontag G, Heidinger G, Kundi M, Simic T, Chakraborty A, Bichler J, Ferk F, Angelis K, Nersesyan A & Knasmüller S (2008) Consumption of Brussels sprouts protects peripheral human lymphocytes against 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and oxidative. *Mol Nutr Food Res* **53**, 330–341.
298. Moller P, Vogel U, Pedersen A, Dragsted LO, Sandstrom B & Loft S (2003) No effect of 600 grams fruit and vegetables per day on oxidative DNA damage and repair in healthy nonsmokers. *Cancer Epidemiol Biomarkers Prev* **12**, 1016–1022.
299. Knasmüller S, Mersch-Sundermann V, Kevekordes S, Darroudi F, Huber WW, Hoelzl C, Bichler J & Majer BJ (2004) Use of human-derived liver cell lines for the detection of environmental and dietary genotoxicants; current state of knowledge. *Toxicology* **198**, 315–328.
300. Knasmüller S, Parzefall W, Sanyal R, Ecker S, Schwab C, Uhl M, Mersch-Sundermann V, Williamson G, Hietsch G, Langer T, Darroudi F & Natarajan AT (1998) Use of metabolically competent human hepatoma cells for the detection of mutagens and antimutagens. *Mutat Res* **402**, 185–202.
301. Mersch-Sundermann V, Knasmüller S, Wu XJ, Darroudi F & Kassie F (2004) Use of a human-derived liver cell line for the detection of cytoprotective, antigenotoxic and cogenotoxic agents. *Toxicology* **198**, 329–340.
302. OECD (1997) Mammalian Erythrocyte Micronucleus Test. *OECD Guidelines for the Testing of Chemicals. Test Guideline No. 474*.
303. Hosseinimehr SJ, Azadbakht M, Mousavi SM, Mahmoudzadeh A & Akhlaghpoor S (2007) Radioprotective effects of hawthorn fruit extract against gamma irradiation in mouse bone marrow cells. *J Radiat Res (Tokyo)* **48**, 63–68.
304. Isbrucker RA, Bausch J, Edwards JA & Wolz E (2006) Safety studies on epigallocatechin gallate (EGCG) preparations. Part 1: genotoxicity. *Food Chem Toxicol* **44**, 626–635.
305. Misra S & Choudhury RC (2006) Vitamin C modulation of cisplatin-induced cytogenotoxicity in bone marrow, spermatogonia and its transmission in the male germline of Swiss mice. *J Chemother* **18**, 182–187.
306. Kitano M, Hosoe K, Fukutomi N, Hidaka T, Ohta R, Yamakage K & Hara T (2006) Evaluation of the mutagenic potential of ubidecarenone using three short-term assays. *Food Chem Toxicol* **44**, 364–370.
307. Subapriya R, Kumaraguruparan R, Abraham SK & Nagini S (2005) Protective effects of ethanolic neem leaf extract on DMBA-induced genotoxicity and oxidative stress in mice. *J Herb Pharmacother* **5**, 39–50.
308. Prahalathan C, Selvakumar E, Varalakshmi P, Kumarasamy P & Saravanan R (2006) Salubrious effects of lipoic acid against adriamycin-induced clastogenesis and apoptosis in Wistar rat bone marrow cells. *Toxicology* **222**, 225–232.
309. Fenech M & Morley AA (1985) Measurement of micronuclei in lymphocytes. *Mutat Res* **147**, 29–36.
310. Fenech M (2000) The *in vitro* micronucleus technique. *Mutat Res* **455**, 81–95.
311. Fenech M, Stockley C & Aitken C (1997) Moderate wine consumption protects against hydrogen peroxide-induced DNA damage. *Mutagenesis* **12**, 289–296.
312. Crott JW & Fenech M (1999) Effect of vitamin C supplementation on chromosome damage, apoptosis and necrosis *ex vivo*. *Carcinogenesis* **20**, 1035–1041.
313. Greenrod W, Stockley CS, Burcham P, Abbey M & Fenech M (2005) Moderate acute intake of de-alcoholized red wine, but not alcohol, is protective against radiation-induced DNA damage *ex vivo* – results of a comparative *in vivo* intervention study in younger men. *Mutat Res* **591**, 290–301.
314. Greenrod W & Fenech M (2003) The principal phenolic and alcoholic components of wine protect human lymphocytes against hydrogen peroxide- and ionizing radiation-induced DNA damage *in vitro*. *Mutagenesis* **18**, 119–126.
315. Kopjar N, Miocic S, Ramic S, Milic M & Viculin T (2006) Assessment of the radioprotective effects of amifostine and melatonin on human lymphocytes irradiated with gamma-rays *in vitro*. *Arh Hig Rada Toksikol* **57**, 155–163.
316. Dizdaroglu M (1994) Chemical determination of oxidative DNA damage by gas chromatography–mass spectrometry. *Methods Enzymol* **234**, 3–16.
317. Ames BN, Shigenaga MK & Hagen TM (1993) Oxidants, antioxidants, and the degenerative diseases of aging. *Proc Natl Acad Sci U S A* **90**, 7915–7922.
318. Chater S, Abdelmelek H, Douki T, Garrel C, Favier A, Sakly M & Ben Rhouma K (2006) Exposure to static magnetic field of pregnant rats induces hepatic GSH elevation but not oxidative DNA damage in liver and kidney. *Arch Med Res* **37**, 941–946.
319. Hays AM, Srinivasan D, Witten ML, Carter DE & Lantz RC (2006) Arsenic and cigarette smoke synergistically increase DNA oxidation in the lung. *Toxicol Pathol* **34**, 396–404.
320. Hofer T, Seo AY, Prudencio M & Leeuwenburgh C (2006) A method to determine RNA and DNA oxidation simultaneously by HPLC–ECD: greater RNA than DNA oxidation in rat liver after doxorubicin administration. *Biol Chem* **387**, 103–111.
321. Kim SY, Suzuki N, Laxmi YR, Umemoto A, Matsuda T & Shibutani S (2006) Antiestrogens and the formation of DNA damage in rats: a comparison. *Chem Res Toxicol* **19**, 852–858.
322. Shigenaga MK, Gimeno CJ & Ames BN (1989) Urinary 8-hydroxy-2′-deoxyguanosine as a biological marker of *in vivo* oxidative DNA damage. *Proc Natl Acad Sci USA* **86**, 9697–9701.
323. Dizdaroglu M (1990) Gas chromatography–mass spectrometry of free radical-induced products of pyrimidines and purines in DNA. *Methods Enzymol* **193**, 842–857.

British Journal of Nutrition

324. Gackowski D, Rozalski R, Roszkowski K, Jawien A, Foksinski M & Olinski R (2001) 8-Oxo-7,8-dihydroguanine and 8-oxo-7,8-dihydro-2′-deoxyguanosine levels in human urine do not depend on diet. *Free Radic Res* **35**, 825–832.
325. Cooke MS, Olinski R & Evans MD (2006) Does measurement of oxidative damage to DNA have clinical significance? *Clin Chim Acta* **365**, 30–49.
326. Kasai H (2003) A new automated method to analyze urinary 8-hydroxydeoxyguanosine by a high-performance liquid chromatography–electrochemical detector system. *J Radiat Res (Tokyo)* **44**, 185–189.
327. Loft S, Deng XS, Tuo J, Wellejus A, Sorensen M & Poulsen HE (1998) Experimental study of oxidative DNA damage. *Free Radic Res* **29**, 525–539.
328. Shigenaga MK, Aboujaoude EN, Chen Q & Ames BN (1994) Assays of oxidative DNA damage biomarkers 8-oxo-2′-deoxyguanosine and 8-oxoguanine in nuclear DNA and biological fluids by high-performance liquid chromatography with electrochemical detection. *Methods Enzymol* **234**, 16–33.
329. Harman SM, Liang L, Tsitouras PD, Gucciardo F, Heward CB, Reaven PD, Ping W, Ahmed A & Cutler RG (2003) Urinary excretion of three nucleic acid oxidation adducts and isoprostane F(2)alpha measured by liquid chromatography–mass spectrometry in smokers, ex-smokers, and nonsmokers. *Free Radic Biol Med* **35**, 1301–1309.
330. Chiou CC, Chang PY, Chan EC, Wu TL, Tsao KC & Wu JT (2003) Urinary 8-hydroxydeoxyguanosine and its analogs as DNA marker of oxidative stress: development of an ELISA and measurement in both bladder and prostate cancers. *Clin Chim Acta* **334**, 87–94.
331. Hofer T & Moller L (1998) Reduction of oxidation during the preparation of DNA and analysis of 8-hydroxy-2′-deoxyguanosine. *Chem Res Toxicol* **11**, 882–887.
332. Wiseman H, Kaur H & Halliwell B (1995) DNA damage and cancer: measurement and mechanism. *Cancer Lett* **93**, 113–120.
333. Douki T, Delatour T, Bianchini F & Cadet J (1996) Observation and prevention of an artefactual formation of oxidized DNA bases and nucleosides in the GC–EIMS method. *Carcinogenesis* **17**, 347–353.
334. Poulsen HE, Weimann A & Loft S (1999) Methods to detect DNA damage by free radicals: relation to exercise. *Proc Nutr Soc* **58**, 1007–1014.
335. Ravanat JL, Turesky RJ, Gremaud E, Trudel LJ & Stadler RH (1995) Determination of 8-oxoguanine in DNA by gas chromatography–mass spectrometry and HPLC–electrochemical detection: overestimation of the background level of the oxidized base by the gas chromatography–mass spectrometry assay. *Chem Res Toxicol* **8**, 1039–1045.
336. Hu CW, Wang CJ, Chang LW & Chao MR (2006) Clinical-scale high-throughput analysis of urinary 8-oxo-7,8-dihydro-2′-deoxyguanosine by isotope-dilution liquid chromatography–tandem mass spectrometry with on-line solid-phase extraction. *Clin Chem* **52**, 1381–1388.
337. Beckman KB & Ames BN (1997) Oxidative decay of DNA. *J Biol Chem* **272**, 19633–19636.
338. Adachi S, Zeisig M & Moller L (1995) Improvements in the analytical method for 8-hydroxydeoxyguanosine in nuclear DNA. *Carcinogenesis* **16**, 253–258.
339. Finnegan MT, Herbert KE, Evans MD, Griffiths HR & Lunec J (1996) Evidence for sensitisation of DNA to oxidative damage during isolation. *Free Radic Biol Med* **20**, 93–98.
340. Dreher D & Junod AF (1996) Role of oxygen free radicals in cancer development. *Eur J Cancer* **32A**, 30–38.
341. Cooke MS, Evans MD, Herbert KE & Lunec J (2000) Urinary 8-oxo-2′-deoxyguanosine – source, significance and supplements. *Free Radic Res* **32**, 381–397.
342. Shimoi K, Kasai H, Yokota N, Toyokuni S & Kinae N (2002) Comparison between high-performance liquid chromatography and enzyme-linked immunosorbent assay for the determination of 8-hydroxy-2′-deoxyguanosine in human urine. *Cancer Epidemiol Biomarkers Prev* **11**, 767–770.
343. Yoshida R, Ogawa Y & Kasai H (2002) Urinary 8-oxo-7,8-dihydro-2′-deoxyguanosine values measured by an ELISA correlated well with measurements by high-performance liquid chromatography with electrochemical detection. *Cancer Epidemiol Biomarkers Prev* **11**, 1076–1081.
344. OECD (2003) Measurement of DNA oxidation in human cells by chromatographic and enzymic methods. *Free Radic Biol Med* **34**, 1089–1099.
345. OECD (2002) Comparative analysis of baseline 8-oxo-7,8-dihydroguanine in mammalian cell DNA, by different methods in different laboratories: an approach to consensus. *Carcinogenesis* **23**, 2129–2133.
346. Collins AR, Cadet J, Moller L, Poulsen HE & Vina J (2004) Are we sure we know how to measure 8-oxo-7,8-dihydroguanine in DNA from human cells? *Arch Biochem Biophys* **423**, 57–65.
347. Gedik CM & Collins A (2005) Establishing the background level of base oxidation in human lymphocyte DNA: results of an interlaboratory validation study. *FASEB J* **19**, 82–84.
348. Duthie SJ, Jenkinson AM, Crozier A, Mullen W, Pirie L, Kyle J, Yap LS, Christen P & Duthie GG (2006) The effects of cranberry juice consumption on antioxidant status and biomarkers relating to heart disease and cancer in healthy human volunteers. *Eur J Nutr* **45**, 113–122.
349. Tunstall RG, Sharma RA, Perkins S, Sale S, Singh R, Farmer PB, Steward WP & Gescher AJ (2006) Cyclooxygenase-2 expression and oxidative DNA adducts in murine intestinal adenomas: modification by dietary curcumin and implications for clinical trials. *Eur J Cancer* **42**, 415–421.
350. Jung KJ, Wallig MA & Singletary KW (2006) Purple grape juice inhibits 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced rat mammary tumorigenesis and *in vivo* DMBA–DNA adduct formation. *Cancer Lett* **233**, 279–288.
351. Hong JT, Ryu SR, Kim HJ, Lee JK, Lee SH, Kim DB, Yun YP, Ryu JH, Lee BM & Kim PY (2000) Neuroprotective effect of green tea extract in experimental ischemia-reperfusion brain injury. *Brain Res Bull* **53**, 743–749.
352. Kaneko T, Tahara S & Takabayashi F (2003) Protective effect of fluvastatin, an HMG-CoA reductase inhibitor, on the formation of 8-oxo-2′-deoxyguanosine in the nuclear DNA of hamster pancreas after a single administration of *N*-nitrosobis(2-oxopropyl)amine. *Biol Pharm Bull* **26**, 1245–1248.
353. van Zeeland AA, de Groot AJ, Hall J & Donato F (1999) 8-Hydroxydeoxyguanosine in DNA from leukocytes of healthy adults: relationship with cigarette smoking, environmental tobacco smoke, alcohol and coffee consumption. *Mutat Res* **439**, 249–257.
354. Schins RP, Schilderman PA & Borm PJ (1995) Oxidative DNA damage in peripheral blood lymphocytes of coal workers. *Int Arch Occup Environ Health* **67**, 153–157.
355. Loft S, Poulsen HE, Vistisen K & Knudsen LE (1999) Increased urinary excretion of 8-oxo-2′-deoxyguanosine, a biomarker of oxidative DNA damage, in urban bus drivers. *Mutat Res* **441**, 11–19.
356. Marczynski B, Rihs HP, Rossbach B, Holzer J, Angerer J, Scherenberg M, Hoffmann G, Bruning T & Wilhelm M (2002) Analysis of 8-oxo-7,8-dihydro-2′-deoxyguanosine and DNA strand breaks in white blood cells of occupationally exposed workers: comparison with ambient monitoring, urinary metabolites and enzyme polymorphisms. *Carcinogenesis* **23**, 273–281.
357. Yoshida R, Ogawa Y, Shioji I, Yu X, Shibata E, Mori I, Kubota H, Kishida A & Hisanaga N (2001) Urinary 8-oxo-7,

British Journal of Nutrition

8-dihydro-2′-deoxyguanosine and biopyrrins levels among construction workers with asbestos exposure history. *Ind Health* **39**, 186–188.
358. Loft S, Astrup A, Buemann B & Poulsen HE (1994) Oxidative DNA damage correlates with oxygen consumption in humans. *FASEB J* **8**, 534–537.
359. Thompson HJ, Heimendinger J, Diker A, O'Neill C, Haegele A, Meinecke B, Wolfe P, Sedlacek S, Zhu Z & Jiang W (2006) Dietary botanical diversity affects the reduction of oxidative biomarkers in women due to high vegetable and fruit intake. *J Nutr* **136**, 2207–2212.
360. Thompson HJ, Heimendinger J, Gillette C, Sedlacek SM, Haegele A, O'Neill C & Wolfe P (2005) *In vivo* investigation of changes in biomarkers of oxidative stress induced by plant food rich diets. *J Agric Food Chem* **53**, 6126–6132.
361. Machowetz A, Poulsen HE, Gruendel S, Weimann A, Fito M, Marrugat J, de la Torre R, Salonen JT, Nyyssonen K, Mursu J, Nascetti S, Gaddi A, Kiesewetter H, Baumler H, Selmi H, Kaikkonen J, Zunft HJ, Covas MI & Koebnick C (2007) Effect of olive oils on biomarkers of oxidative DNA stress in Northern and Southern Europeans. *FASEB J* **21**, 45–52.
362. Jenkinson AM, Collins AR, Duthie SJ, Wahle KW & Duthie GG (1999) The effect of increased intakes of polyunsaturated fatty acids and vitamin E on DNA damage in human lymphocytes. *FASEB J* **13**, 2138–2142.
363. Dotan Y, Lichtenberg D & Pinchuk I (2004) Lipid peroxidation cannot be used as a universal criterion of oxidative stress. *Prog Lipid Res* **43**, 200–227.
364. Srinivasan M, Sudheer AR, Pillai KR, Kumar PR, Sudhakaran PR & Menon VP (2007) Lycopene as a natural protector against gamma-radiation induced DNA damage, lipid peroxidation and antioxidant status in primary culture of isolated rat hepatocytes *in vitro*. *Biochim Biophys Acta* **1770**, 659–665.
365. Srinivasan M, Rajendra Prasad N & Menon VP (2006) Protective effect of curcumin on gamma-radiation induced DNA damage and lipid peroxidation in cultured human lymphocytes. *Mutat Res* **611**, 96–103.
366. Sudheer AR, Muthukumaran S, Devipriya N & Menon VP (2007) Ellagic acid, a natural polyphenol protects rat peripheral blood lymphocytes against nicotine-induced cellular and DNA damage *in vitro*: with the comparison of *N*-acetylcysteine. *Toxicology* **230**, 11–21.
367. Schaefer S, Baum M, Eisenbrand G & Janzowski C (2006) Modulation of oxidative cell damage by reconstituted mixtures of phenolic apple juice extracts in human colon cell lines. *Mol Nutr Food Res* **50**, 413–417.
368. Gandhi NM & Nair CK (2005) Protection of DNA and membrane from gamma radiation induced damage by gallic acid. *Mol Cell Biochem* **278**, 111–117.
369. Duthie SJ, Gardner PT, Morrice PC, Wood SG, Pirie L, Bestwick CC, Milne L & Duthie GG (2005) DNA stability and lipid peroxidation in vitamin E-deficient rats *in vivo* and colon cells *in vitro* – modulation by the dietary anthocyanin, cyanidin-3-glycoside. *Eur J Nutr* **44**, 195–203.
370. Weisel T, Baum M, Eisenbrand G, Dietrich H, Will F, Stockis JP, Kulling S, Rufer C, Johannes C & Janzowski C (2006) An anthocyanin/polyphenolic-rich fruit juice reduces oxidative DNA damage and increases glutathione level in healthy probands. *Biotechnol J* **1**, 388–397.
371. Bub A, Watzl B, Blockhaus M, Briviba K, Liegibel U, Muller H, Pool-Zobel BL & Rechkemmer G (2003) Fruit juice consumption modulates antioxidative status, immune status and DNA damage. *J Nutr Biochem* **14**, 90–98.
372. Janse van Rensburg C, Erasmus E, Loots DT, Oosthuizen W, Jerling JC, Kruger HS, Louw R, Brits M & van der Westhuizen FH (2005) *Rosa roxburghii* supplementation in a controlled feeding study increases plasma antioxidant capacity and glutathione redox state. *Eur J Nutr* **44**, 452–457.
373. Gedik CM, Boyle SP, Wood SG, Vaughan NJ & Collins AR (2002) Oxidative stress in humans: validation of biomarkers of DNA damage. *Carcinogenesis* **23**, 1441–1446.
374. Hofer T, Karlsson HL & Moller L (2006) DNA oxidative damage and strand breaks in young healthy individuals: a gender difference and the role of life style factors. *Free Radic Res* **40**, 707–714.
375. Domanski MJ (2007) Primary prevention of coronary artery disease. *N Engl J Med* **357**, 1543–1545.
376. Bonassi S, Znaor A, Ceppi M, Lando C, Chang WP, Holland N, Kirsch-Volders M, Zeiger E, Ban S, Barale R, Bigatti MP, Bolognesi C, Cebulska-Wasilewska A, Fabianova E, Fucic A, Hagmar L, Joksic G, Martelli A, Migliore L, Mirkova E, Scarfi MR, Zijno A, Norppa H & Fenech M (2007) An increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of cancer in humans. *Carcinogenesis* **28**, 625–631.
377. Norppa H, Bonassi S, Hansteen IL, Hagmar L, Stromberg U, Rossner P, Boffetta P, Lindholm C, Gundy S, Lazutka J, Cebulska-Wasilewska A, Fabianova E, Sram RJ, Knudsen LE, Barale R & Fucic A (2006) Chromosomal aberrations and SCEs as biomarkers of cancer risk. *Mutat Res* **600**, 37–45.
378. Knudsen LE & Hansen AM (2007) Biomarkers of intermediate endpoints in environmental and occupational health. *Int J Hyg Environ Health* **210**, 461–470.
379. Maluf SW & Erdtmann B (2001) Genomic instability in Down syndrome and Fanconi anemia assessed by micronucleus analysis and single-cell gel electrophoresis. *Cancer Genet Cytogenet* **124**, 71–75.
380. Vives-Bauza C, Starkov A & Garcia-Arumi E (2007) Measurements of the antioxidant enzyme activities of superoxide dismutase, catalase, and glutathione peroxidase. *Methods Cell Biol* **80**, 379–393.
381. Ferk F, Chakraborty A, Simic T, Kundi M & Knasmüller S (2006) Detection of a "super antioxidant" in sumac (*Rhus coriaria*), a common spice and identification of gallic acid as its active principle. In *COST 925/927 "Molecular and Physiological Effects of Bioactive Food Components"*, p. 114 [J Gee and V Fogliano, editors]. Vienna, Austria.
382. Nelson SK, Bose SK, Grunwald GK, Myhill P & McCord JM (2006) The induction of human superoxide dismutase and catalase *in vivo*: a fundamentally new approach to antioxidant therapy. *Free Radic Biol Med* **40**, 341–347.
383. Kim HY, Kim OH & Sung MK (2003) Effects of phenol-depleted and phenol-rich diets on blood markers of oxidative stress, and urinary excretion of quercetin and kaempferol in healthy volunteers. *J Am Coll Nutr* **22**, 217–223.
384. Akturk O, Demirin H, Sutcu R, Yilmaz N, Koylu H & Altuntas I (2006) The effects of diazinon on lipid peroxidation and antioxidant enzymes in rat heart and ameliorating role of vitamin E and vitamin C. *Cell Biol Toxicol* **22**, 455–461.
385. Hoelzl C, Lorenz O, Haudek V, Gundacker N, Knasmuller S & Gerner C (2008) Proteome alterations induced in human white blood cells by consumption of Brussels sprouts: results of a pilot intervention study. *Proteomics Clin Appl* **2**, 108–117.
386. Pedraza-Chaverri J, Granados-Silvestre MD, Medina-Campos ON, Maldonado PD, Olivares-Corichi IM & Ibarra-Rubio ME (2001) Post-transcriptional control of catalase expression in garlic-treated rats. *Mol Cell Biochem* **216**, 9–19.
387. Yao P, Li K, Song F, Zhou S, Sun X, Zhang X, Nussler AK & Liu L (2007) Heme oxygenase-1 upregulated by Ginkgo biloba extract: potential protection against ethanol-induced oxidative liver damage. *Food Chem Toxicol* **45**, 1333–1342.
388. Prochaska HJ, Santamaria AB & Talalay P (1992) Rapid detection of inducers of enzymes that protect against carcinogens. *Proc Natl Acad Sci USA* **89**, 2394–2398.

British Journal of Nutrition

389. Prochaska HJ & Talalay P (1988) Regulatory mechanisms of monofunctional and bifunctional anticarcinogenic enzyme inducers in murine liver. *Cancer Res* **48**, 4776–4782.
390. Talalay P & Fahey JW (2001) Phytochemicals from cruciferous plants protect against cancer by modulating carcinogen metabolism. *J Nutr* **131**, 3027S–3033S.
391. Hintze KJ, Wald KA, Zeng H, Jeffery EH & Finley JW (2003) Thioredoxin reductase in human hepatoma cells is transcriptionally regulated by sulforaphane and other electrophiles via an antioxidant response element. *J Nutr* **133**, 2721–2727.
392. Wild AC & Mulcahy RT (2000) Regulation of gamma-glutamylcysteine synthetase subunit gene expression: insights into transcriptional control of antioxidant defenses. *Free Radic Res* **32**, 281–301.
393. Nardi G, Cipollaro M & Loguercio C (1990) Assay of gamma-glutamylcysteine synthetase and glutathione synthetase in erythrocytes by high-performance liquid chromatography with fluorimetric detection. *J Chromatogr* **530**, 122–128.
394. Huber WW, Scharf G, Rossmanith W, Prustomersky S, Grasl-Kraupp B, Peter B, Turesky RJ & Schulte-Hermann R (2002) The coffee components kahweol and cafestol induce gamma-glutamylcysteine synthetase, the rate limiting enzyme of chemoprotective glutathione synthesis, in several organs of the rat. *Arch Toxicol* **75**, 685–694.
395. Ip C (1984) Comparative effects of antioxidants on enzymes involved in glutathione metabolism. *Life Sci* **34**, 2501–2506.
396. Eaton DL & Hamel DM (1994) Increase in gamma-glutamylcysteine synthetase activity as a mechanism for butylated hydroxyanisole-mediated elevation of hepatic glutathione. *Toxicol Appl Pharmacol* **126**, 145–149.
397. Younes M (2004) Freie Radikale und reaktive Sauerstoffspezies. In *Lehrbuch der Toxikologie*, pp. 117–130 [H Marquart and S Schäfer, editors]. Stuttgart: Wissenschaftliche Verlagsgesellschaft mbH Stuttgart.
398. Boyne AF & Ellman GL (1972) A methodology for analysis of tissue sulfhydryl components. *Anal Biochem* **46**, 639–653.
399. Alvarez P, Alvarado C, Mathieu F, Jimenez L & De la Fuente M (2006) Diet supplementation for 5 weeks with polyphenol-rich cereals improves several functions and the redox state of mouse leucocytes. *Eur J Nutr* **45**, 428–438.
400. Rebrin I, Zicker S, Wedekind KJ, Paetau-Robinson I, Packer L & Sohal RS (2005) Effect of antioxidant-enriched diets on glutathione redox status in tissue homogenates and mitochondria of the senescence-accelerated mouse. *Free Radic Biol Med* **39**, 549–557.
401. Lii CK, Ko YJ, Chiang MT, Sung WC & Chen HW (1998) Effect of dietary vitamin E on antioxidant status and antioxidant enzyme activities in Sprague–Dawley rats. *Nutr Cancer* **32**, 95–100.
402. Landi S (2000) Mammalian class theta GST and differential susceptibility to carcinogens: a review. *Mutat Res* **463**, 247–283.
403. Tew KD & Ronai Z (1999) GST function in drug and stress response. *Drug Resist Updat* **2**, 143–147.
404. Soini Y, Kaarteenaho-Wiik R, Paakko P & Kinnula V (2003) Expression of antioxidant enzymes in bronchial metaplastic and dysplastic epithelium. *Lung Cancer* **39**, 15–22.
405. Wenzel U, Herzog A, Kuntz S & Daniel H (2004) Protein expression profiling identifies molecular targets of quercetin as a major dietary flavonoid in human colon cancer cells. *Proteomics* **4**, 2160–2174.
406. Surh YJ (2003) Cancer chemoprevention with dietary phytochemicals. *Nat Rev Cancer* **3**, 768–780.
407. Favata MF, Horiuchi KY, Manos EJ, Daulerio AJ, Stradley DA, Feeser WS, Van Dyk DE, Pitts WJ, Earl RA, Hobbs F, Copeland RA, Magolda RL, Scherle PA & Trzaskos JM (1998) Identification of a novel inhibitor of mitogen-activated protein kinase kinase. *J Biol Chem* **273**, 18623–18632.
408. Fukazawa H & Uehara Y (2000) U0126 reverses Ki-ras-mediated transformation by blocking both mitogen-activated protein kinase and p70 S6 kinase pathways. *Cancer Res* **60**, 2104–2107.
409. Bronstein I, Fortin J, Stanley PE, Stewart GS & Kricka LJ (1994) Chemiluminescent and bioluminescent reporter gene assays. *Anal Biochem* **219**, 169–181.
410. Mann DA (2002) The NFkappaB luciferase mouse: a new tool for real time measurement of NFkappaB activation in the whole animal. *Gut* **51**, 769–770.
411. Lai C, Jiang X & Li X (2006) Development of luciferase reporter-based cell assays. *Assay Drug Dev Technol* **4**, 307–315.
412. Sarkar FH, Adsule S, Li Y & Padhye S (2007) Back to the future: COX-2 inhibitors for chemoprevention and cancer therapy. *Mini Rev Med Chem* **7**, 599–608.
413. Kulmacz RJ & Lands WEM (1987). Cyclo-oxygenase: measurement, purification and properties. In *Prostaglandins and Related Substances: A Practical Approach*, pp. 209–227 [C Benedetto, RG McDonald-Gibson and S Nigam *et al.*, editors]. Washington, DC: IRL Press.
414. Pradelles P, Grassi J & Maclouf J (1990) Enzyme immunoassays of eicosanoids using acetylcholinesterase. *Methods Enzymol* **187**, 24–34.
415. Surh YJ, Chun KS, Cha HH, Han SS, Keum YS, Park KK & Lee SS (2001) Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: down-regulation of COX-2 and iNOS through suppression of NF-kappa B activation. *Mutat Res* **480–481**, 243–268.
416. Gaboriau F, Havouis R, Groussard K, Moulinoux JP & Lescoat G (2005) Measurement of ornithine decarboxylase activity in cell extracts using mass spectrometry detection of dansylated putrescine. *Anal Biochem* **341**, 385–387.
417. Pegg AE (2006) Regulation of ornithine decarboxylase. *J Biol Chem* **281**, 14529–14532.
418. Hunt NH & Fragonas JC (1992) Effects of anti-oxidants on ornithine decarboxylase in mitogenically-activated T lymphocytes. *Biochim Biophys Acta* **1133**, 261–267.
419. Friedman J & Cerutti P (1983) The induction of ornithine decarboxylase by phorbol 12-myristate 13-acetate or by serum is inhibited by antioxidants. *Carcinogenesis* **4**, 1425–1427.
420. Russell D & Snyder SH (1968) Amine synthesis in rapidly growing tissues: ornithine decarboxylase activity in regenerating rat liver, chick embryo, and various tumors. *Proc Natl Acad Sci U S A* **60**, 1420–1427.
421. Djurhuus R (1981) Ornithine decarboxylase (EC 4.1.1.1.17) assay based upon the retention of putrescine by a strong cation-exchange paper. *Anal Biochem* **113**, 352–355.
422. Seely JE, Poso H & Pegg AE (1983) Labeling and quantitation of ornithine decarboxylase protein by reaction with alpha-[5-14C]difluoromethylornithine. *Methods Enzymol* **94**, 206–209.
423. Seely JE & Pegg AE (1983) Changes in mouse kidney ornithine decarboxylase activity are brought about by changes in the amount of enzyme protein as measured by radioimmunoassay. *J Biol Chem* **258**, 2496–2500.
424. Wang Y & Bachrach U (2000) A luminescence-based test for determining ornithine decarboxylase activity. *Anal Biochem* **287**, 299–302.
425. Ordovas JM & Mooser V (2004) Nutrigenomics and nutrigenetics. *Curr Opin Lipidol* **15**, 101–108.
426. Ordovas JM & Corella D (2004) Nutritional genomics. *Annu Rev Genomics Hum Genet* **5**, 71–118.
427. Kussmann M, Affolter M & Fay LB (2005) Proteomics in nutrition and health. *Comb Chem High Throughput Screen* **8**, 679–696.
428. Gavaghan CL, Holmes E, Lenz E, Wilson ID & Nicholson JK (2000) An NMR-based metabonomic approach to investigate

British Journal of Nutrition

the biochemical consequences of genetic strain differences: application to the C57BL10J and Alpk:ApfCD mouse. *FEBS Lett* **484**, 169–174.
429. Glassbrook N & Ryals J (2001) A systematic approach to biochemical profiling. *Curr Opin Plant Biol* **4**, 186–190.
430. Scalbert A, Milenkovic D, Llorach R, Manach C & Leroux C (2007) Nutrigenomics: techniques and applications. In *Energy and protein metabolism and nutrition. EAAP publication No. 124*, pp. 259–276 [I Ortigues-Marty, editor]. Wageningen: Wageningen Academic Publishers.
431. Ovesná J, Slabý O, Toussaint O, Kodíček M, Maršík P, Pouchová V & Vaněk T (2008) High throughput 'omics' approaches to assess the effects of phytochemicals in human health studies. *Br J Nutr*. **99**, E-Suppl.1, ES127–ES134.
432. Stella C, Beckwith-Hall B, Cloarec O, Holmes E, Lindon JC, Powell J, van der Ouderaa F, Bingham S, Cross AJ & Nicholson JK (2006) Susceptibility of human metabolic phenotypes to dietary modulation. *J Proteome Res* **5**, 2780–2788.
433. Wang Y, Tang H, Nicholson JK, Hylands PJ, Sampson J & Holmes E (2005) A metabonomic strategy for the detection of the metabolic effects of chamomile (*Matricaria recutita* L.) ingestion. *J Agric Food Chem* **53**, 191–196.
434. Van Dorsten FA, Daykin CA, Mulder TP & Van Duynhoven JP (2006) Metabonomics approach to determine metabolic differences between green tea and black tea consumption. *J Agric Food Chem* **54**, 6929–6938.
435. Solanky KS, Bailey NJ, Beckwith-Hall BM, Davis A, Bingham S, Holmes E, Nicholson JK & Cassidy A (2003) Application of biofluid 1H nuclear magnetic resonance-based metabonomic techniques for the analysis of the biochemical effects of dietary isoflavones on human plasma profile. *Anal Biochem* **323**, 197–204.
436. Ito H, Gonthiera MP, Manach C, Morand C, Mennen L, Remesy C & Scalbert A (2004) High-throughput profiling of dietary polyphenols and their metabolites by HPLC–ESI–MS–MS in human urine. *Biofactors* **22**, 241–243.
437. Ito H, Gonthier MP, Manach C, Morand C, Mennen L, Remesy C & Scalbert A (2005) Polyphenol levels in human urine after intake of six different polyphenol-rich beverages. *Br J Nutr* **94**, 500–509.
438. Fardet A, Canlet C, Gottardi G, Lyan B, Llorach R, Remesy C, Mazur A, Paris A & Scalbert A (2007) Whole-grain and refined wheat flours show distinct metabolic profiles in rats as assessed by a 1H NMR-based metabonomic approach. *J Nutr* **137**, 923–929.
439. Mohn KL, Melby AE, Tewari DS, Laz TM & Taub R (1991) The gene encoding rat insulinlike growth factor-binding protein 1 is rapidly and highly induced in regenerating liver. *Mol Cell Biol* **11**, 1393–1401.
440. DeRisi J, Penland L, Brown PO, Bittner ML, Meltzer PS, Ray M, Chen Y, Su YA & Trent JM (1996) Use of a cDNA microarray to analyse gene expression patterns in human cancer. *Nat Genet* **14**, 457–460.
441. Rothstein JL, Johnson D, DeLoia JA, Skowronski J, Solter D & Knowles B (1992) Gene expression during preimplantation mouse development. *Genes Dev* **6**, 1190–1201.
442. Friedman J & Weissman I (1991) Two cytoplasmic candidates for immunophilin action are revealed by affinity for a new cyclophilin: one in the presence and one in the absence of CsA. *Cell* **66**, 799–806.
443. Liang P & Pardee AB (2003) Analysing differential gene expression in cancer. *Nat Rev Cancer* **3**, 869–876.
444. Lisitsyn N & Wigler M (1993) Cloning the differences between two complex genomes. *Science* **259**, 946–951.
445. Liang P & Pardee AB (1992) Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. *Science* **257**, 967–971.
446. Velculescu VE, Zhang L, Vogelstein B & Kinzler KW (1995) Serial analysis of gene expression. *Science* **270**, 484–487.
447. Brown PO & Botstein D (1999) Exploring the new world of the genome with DNA microarrays. *Nat Genet* **21**, 33–37.
448. Duggan DJ, Bittner M, Chen Y, Meltzer P & Trent JM (1999) Expression profiling using cDNA microarrays. *Nat Genet* **21**, 10–14.
449. Adams MD, Kelley JM, Gocayne JD, Dubnick M, Polymeropoulos MH, Xiao H, Merril CR, Wu A, Olde B, Moreno RF, *et al.* (1991) Complementary DNA sequencing: expressed sequence tags and human genome project. *Science* **252**, 1651–1656.
450. Greely HT (2001) Human genome diversity: what about the other human genome project? *Nat Rev Genet* **2**, 222–227.
451. Mikulits W, Pradet-Balade B, Habermann B, Beug H, Garcia-Sanz JA & Mullner EW (2000) Isolation of translationally controlled mRNAs by differential screening. *FASEB J* 14, 1641–1652.
452. Schena M, Heller RA, Theriault TP, Konrad K, Lachenmeier E & Davis RW (1998) Microarrays: biotechnology's discovery platform for functional genomics. *Trends Biotechnol* **16**, 301–306.
453. Pradet-Balade B, Boulme F, Beug H, Mullner EW & Garcia-Sanz JA (2001) Translation control: bridging the gap between genomics and proteomics? *Trends Biochem Sci* **26**, 225–229.
454. Waerner T, Alacakaptan M, Tamir I, Oberauer R, Gal A, Brabletz T, Schreiber M, Jechlinger M & Beug H (2006) ILEI: a cytokine essential for EMT, tumor formation, and late events in metastasis in epithelial cells. *Cancer Cell* **10**, 227–239.
455. Petz M, Kozina D, Huber H, Siwiec T, Seipelt J, Sommergruber W & Mikulits W (2007) The leader region of laminin B1 mRNA confers cap-independent translation. *Nucleic Acids Res* **35**, 2473–2482.
456. Bustin SA (2000) Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. *J Mol Endocrinol* **25**, 169–193.
457. Kunsch C & Medford RM (1999) Oxidative stress as a regulator of gene expression in the vasculature. *Circ Res* **85**, 753–766.
458. MacDonald J, Galley HF & Webster NR (2003) Oxidative stress and gene expression in sepsis. *Br J Anaesth* **90**, 221–232.
459. Finkel T & Holbrook NJ (2000) Oxidants, oxidative stress and the biology of ageing. *Nature* **408**, 239–247.
460. Scherf U, Ross DT, Waltham M, Smith LH, Lee JK, Tanabe L, Kohn KW, Reinhold WC, Myers TG, Andrews DT, Scudiero DA, Eisen MB, Sausville EA, Pommier Y, Botstein D, Brown PO & Weinstein JN (2000) A gene expression database for the molecular pharmacology of cancer. *Nat Genet* **24**, 236–244.
461. Efferth T & Oesch F (2004) Oxidative stress response of tumor cells: microarray-based comparison between artemisinins and anthracyclines. *Biochem Pharmacol* **68**, 3–10.
462. Murray JI, Whitfield ML, Trinklein ND, Myers RM, Brown PO & Botstein D (2004) Diverse and specific gene expression responses to stresses in cultured human cells. *Mol Biol Cell* **15**, 2361–2374.
463. Chuang YY, Chen Y, Gadisetti V, Chandramouli VR, Cook JA, Coffin D, Tsai MH, DeGraff W, Yan H, Zhao S, Russo A, Liu ET & Mitchell JB (2002) Gene expression after treatment with hydrogen peroxide, menadione, or *t*-butyl hydroperoxide in breast cancer cells. *Cancer Res* **62**, 6246–6254.
464. Yoneda K, Chang MM, Chmiel K, Chen Y & Wu R (2003) Application of high-density DNA microarray to study smoke- and hydrogen peroxide-induced injury and repair in human bronchial epithelial cells. *J Am Soc Nephrol* **14**, S284–S289.
465. Morgan KT, Ni H, Brown HR, Yoon L, Qualls CW Jr, Crosby LM, Reynolds R, Gaskill B, Anderson SP, Kepler TB,

British Journal of Nutrition

Brainard T, Liv N, Easton M, Merrill C, Creech D, Sprenger D, Conner G, Johnson PR, Fox T, Sartor M, Richard E, Kuruvilla S, Casey W & Benavides G (2002) Application of cDNA microarray technology to *in vitro* toxicology and the selection of genes for a real-time RT-PCR-based screen for oxidative stress in Hep-G2 cells. *Toxicol Pathol* **30**, 435–451.

466. Amundson SA, Bittner M, Chen Y, Trent J, Meltzer P & Fornace AJ Jr (1999) Fluorescent cDNA microarray hybridization reveals complexity and heterogeneity of cellular genotoxic stress responses. *Oncogene* **18**, 3666–3672.
467. Li J, Lee JM & Johnson JA (2002) Microarray analysis reveals an antioxidant responsive element-driven gene set involved in conferring protection from an oxidative stress-induced apoptosis in IMR-32 cells. *J Biol Chem* **277**, 388–394.
468. Bae I, Fan S, Meng Q, Rih JK, Kim HJ, Kang HJ, Xu J, Goldberg ID, Jaiswal AK & Rosen EM (2004) BRCA1 induces antioxidant gene expression and resistance to oxidative stress. *Cancer Res* **64**, 7893–7909.
469. Amundson SA, Do KT, Vinikoor L, Koch-Paiz CA, Bittner ML, Trent JM, Meltzer P & Fornace AJ Jr (2005) Stress-specific signatures: expression profiling of p53 wild-type and -null human cells. *Oncogene* **24**, 4572–4579.
470. Li Z, Khaletskiy A, Wang J, Wong JY, Oberley LW & Li JJ (2001) Genes regulated in human breast cancer cells overexpressing manganese-containing superoxide dismutase. *Free Radic Biol Med* **30**, 260–267.
471. Kirby J, Menzies FM, Cookson MR, Bushby K & Shaw PJ (2002) Differential gene expression in a cell culture model of SOD1-related familial motor neurone disease. *Hum Mol Genet* **11**, 2061–2075.
472. McMillian M, Nie A, Parker JB, Leone A, Kemmerer M, Bryant S, Herlich J, Yieh L, Bittner A, Liu X, Wan J, Johnson MD & Lord P (2005) Drug-induced oxidative stress in rat liver from a toxicogenomics perspective. *Toxicol Appl Pharmacol* **207**, 171–178.
473. McMillian M, Nie AY, Parker JB, Leone A, Bryant S, Kemmerer M, Herlich J, Liu Y, Yieh L, Bittner A, Liu X, Wan J & Johnson MD (2004) A gene expression signature for oxidant stress/reactive metabolites in rat liver. *Biochem Pharmacol* **68**, 2249–2261.
474. Yoshihara T, Ishigaki S, Yamamoto M, Liang Y, Niwa J, Takeuchi H, Doyu M & Sobue G (2002) Differential expression of inflammation- and apoptosis-related genes in spinal cords of a mutant SOD1 transgenic mouse model of familial amyotrophic lateral sclerosis. *J Neurochem* **80**, 158–167.
475. Thimmulappa RK, Scollick C, Traore K, Yates M, Trush MA, Liby KT, Sporn MB, Yamamoto M, Kensler TW & Biswal S (2006) Nrf2-dependent protection from LPS induced inflammatory response and mortality by CDDO-imidazolide. *Biochem Biophys Res Commun* **351**, 883–889.
476. Lee CK, Allison DB, Brand J, Weindruch R & Prolla TA (2002) Transcriptional profiles associated with aging and middle age-onset caloric restriction in mouse hearts. *Proc Natl Acad Sci U S A* **99**, 14988–14993.
477. Lee CK, Weindruch R & Prolla TA (2000) Gene-expression profile of the ageing brain in mice. *Nat Genet* **25**, 294–297.
478. Lee CK, Klopp RG, Weindruch R & Prolla TA (1999) Gene expression profile of aging and its retardation by caloric restriction. *Science* **285**, 1390–1393.
479. Edwards MG, Sarkar D, Klopp R, Morrow JD, Weindruch R & Prolla TA (2003) Age-related impairment of the transcriptional responses to oxidative stress in the mouse heart. *Physiol Genomics* **13**, 119–127.
480. Wang Z, Neuburg D, Li C, Su L, Kim JY, Chen JC & Christiani DC (2005) Global gene expression profiling in whole-blood samples from individuals exposed to metal fumes. *Environ Health Perspect* **113**, 233–241.
481. Kim YH, Lim DS, Lee JH, Shim WJ, Ro YM, Park GH, Becker KG, Cho-Chung YS & Kim MK (2003) Gene expression profiling of oxidative stress on atrial fibrillation in humans. *Exp Mol Med* **35**, 336–349.
482. Heller RA, Schena M, Chai A, Shalon D, Bedilion T, Gilmore J, Woolley DE & Davis RW (1997) Discovery and analysis of inflammatory disease-related genes using cDNA microarrays. *Proc Natl Acad Sci U S A* **94**, 2150–2155.
483. Jison ML, Munson PJ, Barb JJ, Suffredini AF, Talwar S, Logun C, Raghavachari N, Beigel JH, Shelhamer JH, Danner RL & Gladwin MT (2004) Blood mononuclear cell gene expression profiles characterize the oxidant, hemolytic, and inflammatory stress of sickle cell disease. *Blood* **104**, 270–280.
484. Weigel AL, Handa JT & Hjelmeland LM (2002) Microarray analysis of H_2O_2-, HNE-, or *t*BH-treated ARPE-19 cells. *Free Radic Biol Med* **33**, 1419–1432.
485. Suzuki T, Spitz DR, Gandhi P, Lin HY & Crawford DR (2002) Mammalian resistance to oxidative stress: a comparative analysis. *Gene Expr* **10**, 179–191.
486. Weindruch R, Kayo T, Lee CK & Prolla TA (2002) Effects of caloric restriction on gene expression. In *Nutrition and Ageing: Nestle Nutr Workshop Ser Clin Perform Programme*, pp. 17–32 [I Rosenberg and A Sastre, editors]. Basel/Vevey: Nestec LTD/S. Karger AG.
487. Yoneda K, Peck K, Chang MM, Chmiel K, Sher YP, Chen J, Yang PC, Chen Y & Wu R (2001) Development of high-density DNA microarray membrane for profiling smoke- and hydrogen peroxide-induced genes in a human bronchial epithelial cell line. *Am J Respir Crit Care Med* **164**, S85–S89.
488. Lee JM, Li J, Johnson DA, Stein TD, Kraft AD, Calkins MJ, Jakel RJ & Johnson JA (2005) Nrf2, a multi-organ protector? *FASEB J* **19**, 1061–1066.
489. Ivyna Bong PN, Patricia L, Pauline B, Edmund SU & Zubaidah Z (2006) Quantitative analysis of the expression of p53 gene in colorectal carcinoma by using real-time PCR. *Trop Biomed* **23**, 53–59.
490. De Flora S, Izzotti A, D'Agostini F, Bennicelli C, You M, Lubet RA & Balansky RM (2005) Induction and modulation of lung tumors: genomic and transcriptional alterations in cigarette smoke-exposed mice. *Exp Lung Res* **31**, 19–35.
491. Stewart SL, Querec TD, Ochman AR, Gruver BN, Bao R, Babb JS, Wong TS, Koutroukides T, Pinnola AD, Klein-Szanto A, Hamilton TC & Patriotis C (2004) Characterization of a carcinogenesis rat model of ovarian preneoplasia and neoplasia. *Cancer Res* **64**, 8177–8183.
492. Jarvinen AK, Hautaniemi S, Edgren H, Auvinen P, Saarela J, Kallioniemi OP & Monni O (2004) Are data from different gene expression microarray platforms comparable? *Genomics* **83**, 1164–1168.
493. van der Spek PJ, Kremer A, Murry L & Walker MG (2003) Are gene expression microarray analyses reliable? A review of studies of retinoic acid responsive genes. *Genomics Proteomics Bioinformatics* **1**, 9–14.
494. Shippy R, Sendera TJ, Lockner R, Palaniappan C, Kaysser-Kranich T, Watts G & Alsobrook J (2004) Performance evaluation of commercial short-oligonucleotide microarrays and the impact of noise in making cross-platform correlations. *BMC Genomics* **5**, 61.
495. Brazma A, Hingamp P, Quackenbush J, Sherlock G, Spellman P, Stoeckert C, Aach J, Ansorge W, Ball CA, Causton HC, Gaasterland T, Glenisson P, Holstege FC, Kim IF, Markowitz V, Matese JC, Parkinson H, Robinson A, Sarkans U, Schulze-Kremer S, Stewart J, Taylor R, Vilo J & Vingron M (2001) Minimum information about a microarray experiment

British Journal of Nutrition

(MIAME)-toward standards for microarray data. *Nat Genet* **29**, 365–371.
496. Irizarry RA, Warren D, Spencer F, Kim IF, Biswal S, Frank BC, Gabrielson E, Garcia JG, Geoghegan J, Germino G, Griffin C, Hilmer SC, Hoffman E, Jedlicka AE, Kawasaki E, Martinez-Murillo F, Morsberger L, Lee H, Petersen D, Quackenbush J, Scott A, Wilson M, Yang Y, Ye SQ & Yu W (2005) Multiple-laboratory comparison of microarray platforms. *Nat Methods* **2**, 345–350.
497. Larkin JE, Frank BC, Gavras H, Sultana R & Quackenbush J (2005) Independence and reproducibility across microarray platforms. *Nat Methods* **2**, 337–344.
498. Bammler T, Beyer RP, Bhattacharya S, Boorman GA, Boyles A, Bradford BU, Bumgarner RE, Bushel PR, Chaturvedi K, Choi D, Cunningham ML, Deng S, Dressman HK, Fannin RD, Farin FM, Freedman JH, Fry RC, Harper A, Humble MC, Hurban P, Kavanagh TJ, Kaufmann WK, Kerr KF, Jing L, Lapidus JA, Lasarev MR, Li J, Li YJ, Lobenhofer EK, Lu X, Malek RL, Milton S, Nagalla SR, O'Malley JP, Palmer VS, Pattee P, Paules RS, Perou CM, Phillips K, Qin LX, Qiu Y, Quigley SD, Rodland M, Rusyn I, Samson LD, Schwartz DA, Shi Y, Shin JL, Sieber SO, Slifer S, Speer MC, Spencer PS, Sproles DI, Swenberg JA, Suk WA, Sullivan RC, Tian R, Tennant RW, Todd SA, Tucker CJ, Van Houten B, Weis BK, Xuan S & Zarbl H (2005) Standardizing global gene expression analysis between laboratories and across platforms. *Nat Methods* **2**, 351–356.
499. Mariappan D, Winkler J, Parthiban V, Doss MX, Hescheler J & Sachinidis A (2006) Dietary small molecules and large-scale gene expression studies: an experimental approach for understanding their beneficial effects on the development of malignant and non-malignant proliferative diseases. *Curr Med Chem* **13**, 1481–1489.
500. Elliott R (2005) Mechanisms of genomic and non-genomic actions of carotenoids. *Biochim Biophys Acta* **1740**, 147–154.
501. Majewicz J, Rimbach G, Proteggente AR, Lodge JK, Kraemer K & Minihane AM (2005) Dietary vitamin C down-regulates inflammatory gene expression in apoE4 smokers. *Biochem Biophys Res Commun* **338**, 951–955.
502. Hofmann T, Liegibel U, Winterhalter P, Bub A, Rechkemmer G & Pool-Zobel BL (2006) Intervention with polyphenol-rich fruit juices results in an elevation of glutathione *S*-transferase P1 (hGSTP1) protein expression in human leucocytes of healthy volunteers. *Mol Nutr Food Res* **50**, 1191–1200.
503. Cotgreave IA, Moldeus P & Orrenius S (1988) Host biochemical defense mechanisms against prooxidants. *Annu Rev Pharmacol Toxicol* **28**, 189–212.
504. Hebbar V, Shen G, Hu R, Kim BR, Chen C, Korytko PJ, Crowell JA, Levine BS & Kong AN (2005) Toxicogenomics of resveratrol in rat liver. *Life Sci* **76**, 2299–2314.
505. Funk JL, Frye JB, Oyarzo JN, Kuscuoglu N, Wilson J, McCaffrey G, Stafford G, Chen G, Lantz RC, Jolad SD, Solyom AM, Kiela PR & Timmermann BN (2006) Efficacy and mechanism of action of turmeric supplements in the treatment of experimental arthritis. *Arthritis Rheum* **54**, 3452–3464.
506. Selman C, McLaren JS, Meyer C, Duncan JS, Redman P, Collins AR, Duthie GG & Speakman JR (2006) Life-long vitamin C supplementation in combination with cold exposure does not affect oxidative damage or lifespan in mice, but decreases expression of antioxidant protection genes. *Mech Ageing Dev* **127**, 897–904.
507. Belloir C, Singh V, Daurat C, Siess MH & Le Bon AM (2006) Protective effects of garlic sulfur compounds against DNA damage induced by direct- and indirect-acting genotoxic agents in HepG2 cells. *Food Chem Toxicol* **44**, 827–834.
508. Schumperli D (1988) Multilevel regulation of replication-dependent histone genes. *Trends Genet* **4**, 187–191.
509. Nordhoff E, Egelhofer V, Giavalisco P, Eickhoff H, Horn M, Przewieslik T, Theiss D, Schneider U, Lehrach H & Gobom J (2001) Large-gel two-dimensional electrophoresis-matrix assisted laser desorption/ionization-time of flight-mass spectrometry: an analytical challenge for studying complex protein mixtures. *Electrophoresis* **22**, 2844–2855.
510. Gerner C, Vejda S, Gelbmann D, Bayer E, Gotzmann J & Schulte-Hermann R & Mikulits W (2002) Concomitant determination of absolute values of cellular protein amounts, synthesis rates, and turnover rates by quantitative proteome profiling. *Mol Cell Proteomics* **1**, 528–537.
511. Rabilloud T (2002) Two-dimensional gel electrophoresis in proteomics: old, old fashioned, but it still climbs up the mountains. *Proteomics* **2**, 3–10.
512. Gras R, Muller M, Gasteiger E, Gay S, Binz PA, Bienvenut W, Hoogland C, Sanchez JC, Bairoch A, Hochstrasser DF & Appel RD (1999) Improving protein identification from peptide mass fingerprinting through a parameterized multi-level scoring algorithm and an optimized peak detection. *Electrophoresis* **20**, 3535–3550.
513. Fountain ST, Lee H & Lubman DM (1994) Ion fragmentation activated by matrix-assisted laser desorption/ionization in an ion-trap/reflectron time-of-flight device. *Rapid Commun Mass Spectrom* **8**, 407–416.
514. Spickett CM, Pitt AR, Morrice N & Kolch W (2006) Proteomic analysis of phosphorylation, oxidation and nitrosylation in signal transduction. *Biochim Biophys Acta* **1764**, 1823–1841.
515. Wolters DA, Washburn MP & Yates JR 3rd (2001) An automated multidimensional protein identification technology for shotgun proteomics. *Anal Chem* **73**, 5683–5690.
516. Bodnar WM, Blackburn RK, Krise JM & Moseley MA (2003) Exploiting the complementary nature of LC/MALDI/MS/MS and LC/ESI/MS/MS for increased proteome coverage. *J Am Soc Mass Spectrom* **14**, 971–979.
517. Watarai H, Inagaki Y, Kubota N, Fuju K, Nagafune J, Yamaguchi Y & Kadoya T (2000) Proteomic approach to the identification of cell membrane proteins. *Electrophoresis* **21**, 460–464.
518. Zhu K, Kim J, Yoo C, Miller FR & Lubman DM (2003) High sequence coverage of proteins isolated from liquid separations of breast cancer cells using capillary electrophoresis-time-of-flight MS and MALDI-TOF MS mapping. *Anal Chem* **75**, 6209–6217.
519. Manzanares D, Rodriguez-Capote K, Liu S, Haines T, Ramos Y, Zhao L, Doherty-Kirby A, Lajoie G & Possmayer F (2007) Modification of tryptophan and methionine residues is implicated in the oxidative inactivation of surfactant protein B. *Biochemistry* **46**, 5604–5615.
520. Paron I, D'Elia A, D'Ambrosio C, Scaloni A, D'Aurizio F, Prescott A, Damante G & Tell G (2004) A proteomic approach to identify early molecular targets of oxidative stress in human epithelial lens cells. *Biochem J* **378**, 929–937.
521. Rabilloud T, Heller M, Gasnier F, Luche S, Rey C, Aebersold R, Benahmed M, Louisot P & Lunardi J (2002) Proteomics analysis of cellular response to oxidative stress. Evidence for *in vivo* overoxidation of peroxiredoxins at their active site. *J Biol Chem* **277**, 19396–19401.
522. Koeck T, Fu X, Hazen SL, Crabb JW, Stuehr DJ & Aulak KS (2004) Rapid and selective oxygen-regulated protein tyrosine denitration and nitration in mitochondria. *J Biol Chem* **279**, 27257–27262.
523. Brennan JP, Wait R, Begum S, Bell JR, Dunn MJ & Eaton P (2004) Detection and mapping of widespread intermolecular protein disulfide formation during cardiac oxidative stress

British Journal of Nutrition

using proteomics with diagonal electrophoresis. *J Biol Chem* **279**, 41352–41360.
524. Fratelli M, Demol H, Puype M, Casagrande S, Eberini I, Salmona M, Bonetto V, Mengozzi M, Duffieux F, Miclet E, Bachi A, Vandekerckhove J, Gianazza E & Ghezzi P (2002) Identification by redox proteomics of glutathionylated proteins in oxidatively stressed human T lymphocytes. *Proc Natl Acad Sci U S A* **99**, 3505–3510.
525. Pocernich CB, Poon HF, Boyd-Kimball D, Lynn BC, Nath A, Klein JB & Butterfield DA (2005) Proteomic analysis of oxidatively modified proteins induced by the mitochondrial toxin 3-nitropropionic acid in human astrocytes expressing the HIV protein tat. *Brain Res Mol Brain Res* **133**, 299–306.
526. Stagsted J, Bendixen E & Andersen HJ (2004) Identification of specific oxidatively modified proteins in chicken muscles using a combined immunologic and proteomic approach. *J Agric Food Chem* **52**, 3967–3974.
527. Godon C, Lagniel G, Lee J, Buhler JM, Kieffer S, Perrot M, Boucherie H, Toledano MB & Labarre J (1998) The H_2O_2 stimulon in *Saccharomyces cerevisiae*. *J Biol Chem* **273**, 22480–22489.
528. Mostertz J, Scharf C, Hecker M & Homuth G (2004) Transcriptome and proteome analysis of *Bacillus subtilis* gene expression in response to superoxide and peroxide stress. *Microbiology* **150**, 497–512.
529. Chan HL, Gharbi S, Gaffney PR, Cramer R, Waterfield MD & Timms JF (2005) Proteomic analysis of redox- and ErbB2-dependent changes in mammary luminal epithelial cells using cysteine- and lysine-labelling two-dimensional difference gel electrophoresis. *Proteomics* **5**, 2908–2926.
530. Seong JK, Kim DK, Choi KH, Oh SH, Kim KS, Lee SS & Um HD (2002) Proteomic analysis of the cellular proteins induced by adaptive concentrations of hydrogen peroxide in human U937 cells. *Exp Mol Med* **34**, 374–378.
531. Keightley JA, Shang L & Kinter M (2004) Proteomic analysis of oxidative stress-resistant cells: a specific role for aldose reductase overexpression in cytoprotection. *Mol Cell Proteomics* **3**, 167–175.
532. Ding Q, Dimayuga E & Keller JN (2007) Oxidative damage, protein synthesis, and protein degradation in Alzheimer's disease. *Curr Alzheimer Res* **4**, 73–79.
533. Sultana R, Perluigi M & Butterfield DA (2006) Protein oxidation and lipid peroxidation in brain of subjects with Alzheimer's disease: insights into mechanism of neurodegeneration from redox proteomics. *Antioxid Redox Signal* **8**, 2021–2037.
534. Grimsrud PA, Picklo MJ Sr, Griffin TJ & Bernlohr DA (2007) Carbonylation of adipose proteins in obesity and insulin resistance: identification of adipocyte fatty acid-binding protein as a cellular target of 4-hydroxynonenal. *Mol Cell Proteomics* **6**, 624–637.
535. Ghosh S, Janocha AJ, Aronica MA, Swaidani S, Comhair SA, Xu W, Zheng L, Kaveti S, Kinter M, Hazen SL & Erzurum SC (2006) Nitrotyrosine proteome survey in asthma identifies oxidative mechanism of catalase inactivation. *J Immunol* **176**, 5587–5597.
536. Dalle-Donne I, Scaloni A, Giustarini D, Cavarra E, Tell G, Lungarella G, Colombo R, Rossi R & Milzani A (2005) Proteins as biomarkers of oxidative/nitrosative stress in diseases: the contribution of redox proteomics. *Mass Spectrom Rev* **24**, 55–99.
537. Go VL, Butrum RR & Wong DA (2003) Diet, nutrition, and cancer prevention: the postgenomic era. *J Nutr* **133**, 3830S–3836S.
538. Davis CD & Milner J (2004) Frontiers in nutrigenomics, proteomics, metabolomics and cancer prevention. *Mutat Res* **551**, 51–64.
539. Guengerich FP (2001) Functional genomics and proteomics applied to the study of nutritional metabolism. *Nutr Rev* **59**, 259–263.
540. Wang J, Li D, Dangott LJ & Wu G (2006) Proteomics and its role in nutrition research. *J Nutr* **136**, 1759–1762.
541. Trujillo E, Davis C & Milner J (2006) Nutrigenomics, proteomics, metabolomics, and the practice of dietetics. *J Am Diet Assoc* **106**, 403–413.
542. Keusch GT (2006) What do -omics mean for the science and policy of the nutritional sciences? *Am J Clin Nutr* **83**, 520S–522S.
543. Kim H (2005) New nutrition, proteomics, and how both can enhance studies in cancer prevention and therapy. *J Nutr* **135**, 2715–2718.
544. Fuchs D, Winkelmann I, Johnson IT, Mariman E, Wenzel U & Daniel H (2005) Proteomics in nutrition research: principles, technologies and applications. *Br J Nutr* **94**, 302–314.
545. Kussmann M & Affolter M (2006) Proteomic methods in nutrition. *Curr Opin Clin Nutr Metab Care* **9**, 575–583.
546. Opii WO, Joshi G, Head E, Milgram NW, Muggenburg BA, Klein JB, Pierce WM, Cotman CW & Butterfield DA (2008) Proteomic identification of brain proteins in the canine model of human aging following a long-term treatment with antioxidants and a program of behavioral enrichment: relevance to Alzheimer's disease. *Neurobiol Aging* **29**, 51–70.
547. Seifried HE, Anderson DE, Fisher EI & Milner JA (2007) A review of the interaction among dietary antioxidants and reactive oxygen species. *J Nutr Biochem* **18**, 567–579.
548. Grubben MJ, Nagengast FM, Katan MB & Peters WH (2001) The glutathione biotransformation system and colorectal cancer risk in humans. *Scand J Gastroenterol Suppl*, 68–76.
549. Hoelzl C, Bichler J, Ferk F, Ehrlich V, Cavin C, Simic T, Edelbauer L, Grasl-Kraupp B & Knasmüller S (2007) Antimutagenic and anticarcinogenic properties of coffee. In *The Activity of Natural Compounds in Diseses and Therapy*, pp. 127–138 [Z Ďuračková and S Knasmüller, editors]. Bratislava: Slovak Academic Press.
550. Li Y & Prives C (2007) Are interactions with p63 and p73 involved in mutant p53 gain of oncogenic function? *Oncogene* **26**, 2220–2225.
551. Levesque AA & Eastman A (2007) p53-based cancer therapies: is defective p53 the Achilles heel of the tumor? *Carcinogenesis* **28**, 13–20.
552. Manach C, Williamson G, Morand C, Scalbert A & Remesy C (2005) Bioavailability and bioefficacy of polyphenols in humans I. Review of 97 bioavailability studies. *Am J Clin Nutr* **81**, 230S–242S.
553. Depeint F, Gee JM, Williamson G & Johnson IT (2002) Evidence for consistent patterns between flavonoid structures and cellular activities. *Proc Nutr Soc* **61**, 97–103.
554. Depeint F (2003) Dietary phytochemicals and their effect on colon cell proliferation. PhD thesis, Institute of Food Research.
555. Nardini M, Cirillo E, Natella F & Scaccini C (2002) Absorption of phenolic acids in humans after coffee consumption. *J Agric Food Chem* **50**, 5735–5741.
556. Clifford MN (2004) Diet-derived phenols in plasma and tissues and their implications for health. *Planta Med* **70**, 1103–1114.
557. Mitoma J & Fukuda M (2006) Expression of specific carbohydrates by transfection with carbohydrate modifying enzymes. *Methods Enzymol* **416**, 293–304.
558. Hu Z & Wells PG (2004) Human interindividual variation in lymphocyte UDP-glucuronosyltransferases as a determinant of *in vitro* benzo[*a*]pyrene covalent binding and cytotoxicity. *Toxicol Sci* **78**, 32–40.
559. Zhang Y, Song TT, Cunnick JE, Murphy PA & Hendrich S (1999) Daidzein and genistein glucuronides *in vitro* are

British Journal of Nutrition

weakly estrogenic and activate human natural killer cells at nutritionally relevant concentrations. *J Nutr* **129**, 399–405.
560. Spencer JP, Schroeter H, Crossthwaithe AJ, Kuhnle G, Williams RJ & Rice-Evans C (2001) Contrasting influences of glucuronidation and *O*-methylation of epicatechin on hydrogen peroxide-induced cell death in neurons and fibroblasts. *Free Radic Biol Med* **31**, 1139–1146.
561. Eckl PM & Raffelsberger I (1997) The primary rat hepatocyte micronucleus assay: general features. *Mutat Res* **392**, 117–124.
562. Fahrig R, Rupp M, Steinkamp-Zucht A & Bader A (1998) Use of primary rat and human hepatocyte sandwich cultures for activation of indirect carcinogens: monitoring of DNA strand breaks and gene mutations in co-cultured cells. *Toxicol In Vitro* **12**, 431–444.
563. Dogra S, Doehmer J, Glatt H, Molders H, Siegert P, Friedberg T, Seidel A & Oesch F (1990) Stable expression of rat cytochrome P-450IA1 cDNA in V79 Chinese hamster cells and their use in mutagenicity testing. *Mol Pharmacol* **37**, 608–613.
564. Muckel E, Frandsen H & Glatt HR (2002) Heterologous expression of human *N*-acetyltransferases 1 and 2 and sulfotransferase 1A1 in *Salmonella typhimurium* for mutagenicity testing of heterocyclic amines. *Food Chem Toxicol* **40**, 1063–1068.
565. Aeschbacher HU, Wolleb U, Loliger J, Spadone JC & Liardon R (1989) Contribution of coffee aroma constituents to the mutagenicity of coffee. *Food Chem Toxicol* **27**, 227–232.
566. Kumar SS, Shankar B & Sainis KB (2004) Effect of chlorophyllin against oxidative stress in splenic lymphocytes *in vitro* and *in vivo*. *Biochim Biophys Acta* **1672**, 100–111.
567. Su CC, Chen GW, Lin JG, Wu LT & Chung JG (2006) Curcumin inhibits cell migration of human colon cancer colo 205 cells through the inhibition of nuclear factor kappa B/p65 and down-regulates cyclooxygenase-2 and matrix metalloproteinase-2 expressions. *Anticancer Res* **26**, 1281–1288.
568. Ogasawara M, Matsunaga T & Suzuki H (2007) Differential effects of antioxidants on the *in vitro* invasion, growth and lung metastasis of murine colon cancer cells. *Biol Pharm Bull* **30**, 200–204.
569. Heinonen M (2007) Antioxidant activity and antimicrobial effect of berry phenolics – a Finnish perspective. *Mol Nutr Food Res* **51**, 684–691.
570. Shahidi F (2004) Food phenolics and cancer chmoprevention. In *Funktional Foods, Aging and Degenerative Disesase*, pp. 669–680 [C Remacle and B Reusens, editors]. Cambridge, England: Woodhead Publishing Limited.
571. Watzl B & Leitzmann C (2005) *Bioaktive Substanzen in Lebensmitteln*. Stuttgart: Hippokrates Verlag.
572. Ferk F, Chakraborty A, Simic T, Kundi M & Knasmüller S (2006) Antioxidant and free radical scavenging activities of sumac (*Rhus coriaria*) and identification of gallic acid as its active principle. In *12th Scientific Symposium of the Austrian Pharmacological Society (APHAR). Joint Meeting with the Austrian Society for Toxicology (ASTOX)*, p. 11 [R Schulte-Herman, editor]. Vienna, Austria.
573. Shahrzad S, Aoyagi K, Winter A, Koyama A & Bitsch I (2001) Pharmacokinetics of gallic acid and its relative bioavailability from tea in healthy humans. *J Nutr* **131**, 1207–1210.
574. Ellinger S, Ellinger J & Stehle P (2006) Tomatoes, tomato products and lycopene in the prevention and treatment of prostate cancer: do we have the evidence from intervention studies? *Curr Opin Clin Nutr Metab Care* **9**, 722–727.
575. Domenici FA, Vannucchi MT, Jordao AA Jr, Meirelles MS & Vannucchi H (2005) DNA oxidative damage in patients with dialysis treatment. *Ren Fail* **27**, 689–694.
576. Beetstra S, Thomas P, Salisbury C, Turner J & Fenech M (2005) Folic acid deficiency increases chromosomal instability, chromosome 21 aneuploidy and sensitivity to radiation-induced micronuclei. *Mutat Res* **578**, 317–326.
577. Fenech M (2005) The Genome Health Clinic and Genome Health Nutrigenomics concepts: diagnosis and nutritional treatment of genome and epigenome damage on an individual basis. *Mutagenesis* **20**, 255–269.
578. Kazimirova A, Barancokova M, Krajcovicova-Kudlackova M, Volkovova K, Staruchova M, Valachovicova M, Paukova V, Blazicek P, Wsolova L & Dusinska M (2006) The relationship between micronuclei in human lymphocytes and selected micronutrients in vegetarians and non-vegetarians. *Mutat Res* **611**, 64–70.
579. Fenech M (2001) Recommended dietary allowances (RDAs) for genomic stability. *Mutat Res* **480-481**, 51–54.
580. Katan MB & de Roos NM (2003) Public health. Toward evidence-based health claims for foods. *Science* **299**, 206–207.
581. Sloan AE (2002) The natural and organic foods marketplace. *Food Technol* **56**, 32.
582. Derby BM & Levy AS (2005) Working Paper: Effects of Strength of Science Disclaimers on the Communication Impact of Health Claims. DoSS US Food and Drug Administration, editor
583. Regulation (2006) (EC) No. 1924/2006 of the European Parliament and the Council of 20 December 2006 on Nutrition and Health Claims made on Foods. *Official J Eur Union* **404**, L12/13–L12/17.
584. Nickelson L (2005) Legal context of consumer research on health claims and other food. Public Meeting: Assessing Consumer Perceptions of Health Claims, Colleage Park, Maryland http://www.cfsan.fda.gov ~ dms/qhctran.html#legal
585. Zhan CD, Sindhu RK, Pang J, Ehdaie A & Vaziri ND (2004) Superoxide dismutase, catalase and glutathione peroxidase in the spontaneously hypertensive rat kidney: effect of antioxidant-rich diet. *J Hypertens* **22**, 2025–2033.
586. McCord JM & Fridovich I (1969) Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). *J Biol Chem* **244**, 6049–6055.
587. Woolliams JA, Wiener G, Anderson PH & McMurray CH (1983) Variation in the activities of glutathione peroxidase and superoxide dismutase and in the concentration of copper in the blood in various breed crosses of sheep. *Res Vet Sci* **34**, 253–256.
588. Prohaska JR (1983) Changes in tissue growth, concentrations of copper, iron, cytochrome oxidase and superoxide dismutase subsequent to dietary or genetic copper deficiency in mice. *J Nutr* **113**, 2048–2058.
589. Marklund S & Marklund G (1974) Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem* **47**, 469–474.
590. DiSilvestro RA, Goodman J, Dy E & Lavalle G (2005) Soy isoflavone supplementation elevates erythrocyte superoxide dismutase, but not plasma ceruloplasmin in postmenopausal breast cancer survivors. *Breast Cancer Res Treat* **89**, 251–255.
591. Claiborne A (1985) Catalase activity. In *Handbook of Methods for Oxygen Free Radical Research*, pp. 283–284 [RA Greenwald, editor]. Boca Raton: CRC Press.
592. Beers RF Jr & Sizer IW (1952) A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J Biol Chem* **195**, 133–140.
593. Aebi H (1984) Catalase *in vitro*. *Methods Enzymol* **105**, 121–126.
594. Price VE, Sterling WR, Tarantola VA, Hartley RW Jr & Rechcigl M Jr (1962) The kinetics of catalase synthesis and destruction *in vivo*. *J Biol Chem* **237**, 3468–3475.
595. Paglia DE & Valentine WN (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* **70**, 158–169.

British Journal of Nutrition

596. Lawrence RA & Burk RF (1976) Glutathione peroxidase activity in selenium-deficient rat liver. *Biochem Biophys Res Commun* **71**, 952–958.
597. Sazuka Y, Tanizawa H & Takino Y (1989) Effect of adriamycin on the activities of superoxide dismutase, glutathione peroxidase and catalase in tissues of mice. *Jpn J Cancer Res* **80**, 89–94.
598. Chow S, Patel H & Hedley DW (2001) Measurement of MAP kinase activation by flow cytometry using phospho-specific antibodies to MEK and ERK: potential for pharmacodynamic monitoring of signal transduction inhibitors. *Cytometry* **46**, 72–78.
599. Yao P, Nussler A, Liu L, Hao L, Song F, Schirmeier A & Nussler N (2007) Quercetin protects human hepatocytes from ethanol-derived oxidative stress by inducing heme oxygenase-1 via the MAPK/Nrf2 pathways. *J Hepatol* **47**, 253–261.
600. Woo J, Iyer S, Cornejo MC, Mori N, Gao L, Sipos I, Maines M & Buelow R (1998) Stress protein-induced immunosuppression: inhibition of cellular immune effector functions following overexpression of haem oxygenase (HSP 32). *Transpl Immunol* **6**, 84–93.
601. Motterlini R, Foresti R, Bassi R & Green CJ (2000) Curcumin, an antioxidant and anti-inflammatory agent, induces heme oxygenase-1 and protects endothelial cells against oxidative stress. *Free Radic Biol Med* **28**, 1303–1312.
602. Benson AM, Hunkeler MJ & Talalay P (1980) Increase of NAD(P)H:quinone reductase by dietary antioxidants: possible role in protection against carcinogenesis and toxicity. *Proc Natl Acad Sci USA* **77**, 5216–5220.
603. Nho CW & Jeffery E (2001) The synergistic upregulation of phase II detoxification enzymes by glucosinolate breakdown products in cruciferous vegetables. *Toxicol Appl Pharmacol* **174**, 146–152.
604. De Long MJ, Prochaska HJ & Talalay P (1986) Induction of NAD(P)H:quinone reductase in murine hepatoma cells by phenolic antioxidants, azo dyes, and other chemoprotectors: a model system for the study of anticarcinogens. *Proc Natl Acad Sci U S A* **83**, 787–791.
605. Talalay P, Dinkova-Kostova AT & Holtzclaw WD (2003) Importance of phase 2 gene regulation in protection against electrophile and reactive oxygen toxicity and carcinogenesis. *Adv Enzyme Regul* **43**, 121–134.
606. Fahey JW, Zhang Y & Talalay P (1997) Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. *Proc Natl Acad Sci U S A* **94**, 10367–10372.
607. Rafi MM & Shafaie Y (2007) Dietary lutein modulates inducible nitric oxide synthase (iNOS) gene and protein expression in mouse macrophage cells (RAW 264.7). *Mol Nutr Food Res* **51**, 333–340.
608. Calvisi DF, Ladu S, Hironaka K, Factor VM & Thorgeirsson SS (2004) Vitamin E down-modulates iNOS and NADPH oxidase in c-Myc/TGF-alpha transgenic mouse model of liver cancer. *J Hepatol* **41**, 815–822.
609. Hevel JM & Marletta MA (1994) Nitric-oxide synthase assays. *Methods Enzymol* **233**, 250–258.
610. Chan MM, Huang HI, Fenton MR & Fong D (1998) *In vivo* inhibition of nitric oxide synthase gene expression by curcumin, a cancer preventive natural product with anti-inflammatory properties. *Biochem Pharmacol* **55**, 1955–1962.
611. Keum YS, Han YH, Liew C, Kim JH, Xu C, Yuan X, Shakarjian MP, Chong S & Kong AN (2006) Induction of heme oxygenase-1 (HO-1) and NAD[P]H: quinone oxidoreductase 1 (NQO1) by a phenolic antioxidant, butylated hydroxyanisole (BHA) and its metabolite, *tert*-butylhydroquinone (*t*BHQ) in primary-cultured human and rat hepatocytes. *Pharm Res* **23**, 2586–2594.
612. Hou DX, Fukuda M, Fujii M & Fuke Y (2000) Transcriptional regulation of nicotinamide adenine dinucleotide phosphate: quinone oxidoreductase in murine hepatoma cells by 6-(methylsufinyl)hexyl isothiocyanate, an active principle of wasabi (Eutrema wasabi Maxim). *Cancer Lett* **161**, 195–200.
613. Hollander PM & Ernster L (1975) Studies on the reaction mechanism of DT diaphorase. Action of dead-end inhibitors and effects of phospholipids. *Arch Biochem Biophys* **169**, 560–567.
614. Singh SV, Pan SS, Srivastava SK, Xia H, Hu X, Zaren HA & Orchard JL (1998) Differential induction of NAD(P)H:quinone oxidoreductase by anti-carcinogenic organosulfides from garlic. *Biochem Biophys Res Commun* **244**, 917–920.
615. Nair HK, Rao KV, Aalinkeel R, Mahajan S, Chawda R & Schwartz SA (2004) Inhibition of prostate cancer cell colony formation by the flavonoid quercetin correlates with modulation of specific regulatory genes. *Clin Diagn Lab Immunol* **11**, 63–69.
616. Murtaza I, Marra G, Schlapbach R, Patrignani A, Kunzli M, Wagner U, Sabates J & Dutt A (2006) A preliminary investigation demonstrating the effect of quercetin on the expression of genes related to cell-cycle arrest, apoptosis and xenobiotic metabolism in human CO115 colon-adenocarcinoma cells using DNA microarray. *Biotechnol Appl Biochem* **45**, 29–36.
617. Suzuki K, Koike H, Matsui H, Ono Y, Hasumi M, Nakazato H, Okugi H, Sekine Y, Oki K, Ito K, Yamamoto T, Fukabori Y, Kurokawa K & Yamanaka H (2002) Genistein, a soy isoflavone, induces glutathione peroxidase in the human prostate cancer cell lines LNCaP and PC-3. *Int J Cancer* **99**, 846–852.
618. Weinreb O, Mandel S & Youdim MB (2003) cDNA gene expression profile homology of antioxidants and their antiapoptotic and proapoptotic activities in human neuroblastoma cells. *FASEB J* **17**, 935–937.
619. Weinreb O, Mandel S & Youdim MB (2003) Gene and protein expression profiles of anti- and pro-apoptotic actions of dopamine, R-apomorphine, green tea polyphenol (−)-epigallocatechine-3-gallate, and melatonin, *Ann N Y Acad Sci* **993**, 351–361; discussion 387–393.
620. Guo S, Yang S, Taylor C & Sonenshein GE (2005) Green tea polyphenol epigallocatechin-3 gallate (EGCG) affects gene expression of breast cancer cells transformed by the carcinogen 7, 12-dimethylbenz[*a*]anthracene. *J Nutr* **135**, 2978S–2986S.
621. Wolfram S, Raederstorff D, Preller M, Wang Y, Teixeira SR, Riegger C & Weber P (2006) Epigallocatechin gallate supplementation alleviates diabetes in rodents. *J Nutr* **136**, 2512–2518.
622. Knowles LM & Milner JA (2003) Diallyl disulfide induces ERK phosphorylation and alters gene expression profiles in human colon tumor cells. *J Nutr* **133**, 2901–2906.
623. Zhou Z, Tan HL, Xu BX, Ma ZC, Gao Y & Wang SQ (2005) Microarray analysis of altered gene expression in diallyl trisulfide-treated HepG2 cells. *Pharmacol Rep* **57**, 818–823.
624. King AA, Shaughnessy DT, Mure K, Leszczynska J, Ward WO, Umbach DM, Xu Z, Ducharme D, Taylor JA, DeMarini DM & Klein CB (2007) Antimutagenicity of cinnamaldehyde and vanillin in human cells: global gene expression and possible role of DNA damage and repair. *Mutat Res* **616**, 60–69.
625. Yang SH, Kim JS, Oh TJ, Kim MS, Lee SW, Woo SK, Cho HS, Choi YH, Kim YH, Rha SY, Chung HC & An SW (2003) Genome-scale analysis of resveratrol – induced gene expression profile in human ovarian cancer cells using a cDNA microarray. *Int J Oncol* **22**, 741–750.
626. Chalabi N, Delort L, Le Corre L, Satih S, Bignon YJ & Bernard-Gallon D (2006) Gene signature of breast cancer cell lines treated with lycopene. *Pharmacogenomics* **7**, 663–672.
627. Lunec J, Halligan E, Mistry N & Karakoula K (2004) Effect of vitamin E on gene expression changes in diet-related carcinogenesis. *Ann N Y Acad Sci* **1031**, 169–183.

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628. Veeriah S, Kautenburger T, Habermann N, Sauer J, Dietrich H, Will F & Pool-Zobel BL (2006) Apple flavonoids inhibit growth of HT29 human colon cancer cells and modulate expression of genes involved in the biotransformation of xenobiotics. *Mol Carcinog* **45**, 164–174.
629. Golkar L, Ding XZ, Ujiki MB, Salabat MR, Kelly DL, Scholtens D, Fought AJ, Bentrem DJ, Talamonti MS, Bell RH & Adrian TE (2007) Resveratrol inhibits pancreatic cancer cell proliferation through transcriptional induction of macrophage inhibitory cytokine-1. *J Surg Res* **138**, 163–169.
630. Takahashi Y, Lavigne JA, Hursting SD, Chandramouli GV, Perkins SN, Barrett JC & Wang TT (2004) Using DNA microarray analyses to elucidate the effects of genistein in androgen-responsive prostate cancer cells: identification of novel targets. *Mol Carcinog* **41**, 108–119.
631. Powolny A, Takahashi K, Hopkins RG & Loo G (2003) Induction of GADD gene expression by phenethylisothiocyanate in human colon adenocarcinoma cells. *J Cell Biochem* **90**, 1128–1139.
632. van Erk MJ, Roepman P, van der Lende TR, Stierum RH, Aarts JM, van Bladeren PJ & van Ommen B (2005) Integrated assessment by multiple gene expression analysis of quercetin bioactivity on anticancer-related mechanisms in colon cancer cells *in vitro*. *Eur J Nutr* **44**, 143–156.
633. Barella L, Muller PY, Schlachter M, Hunziker W, Stocklin E, Spitzer V, Meier N, de Pascual-Teresa S, Minihane AM & Rimbach G (2004) Identification of hepatic molecular mechanisms of action of alpha-tocopherol using global gene expression profile analysis in rats. *Biochim Biophys Acta* **1689**, 66–74.
634. Rota C, Barella L, Minihane AM, Stocklin E & Rimbach G (2004) Dietary alpha-tocopherol affects differential gene expression in rat testes. *IUBMB Life* **56**, 277–280.
635. Shen G, Xu C, Hu R, Jain MR, Nair S, Lin W, Yang CS, Chan JY & Kong AN (2005) Comparison of (−)-epigallocatechin-3-gallate elicited liver and small intestine gene expression profiles between C57BL/6J mice and C57BL/6J/Nrf2 (−/−) mice. *Pharm Res* **22**, 1805–1820.
636. Dolara P, Luceri C, Filippo CD, Femia AP, Giovannelli L, Caderni G, Cecchini C, Silvi S, Orpianesi C & Cresci A (2005) Red wine polyphenols influence carcinogenesis, intestinal microflora, oxidative damage and gene expression profiles of colonic mucosa in F344 rats. *Mutat Res* **591**, 237–246.
637. Kim S, Sohn I, Lee YS & Lee YS (2005) Hepatic gene expression profiles are altered by genistein supplementation in mice with diet-induced obesity. *J Nutr* **135**, 33–41.
638. Hishikawa K, Nakaki T & Fujita T (2005) Oral flavonoid supplementation attenuates atherosclerosis development in apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol* **25**, 442–446.
639. Li N, Guo R, Li W, Shao J, Li S, Zhao K, Chen X, Xu N, Liu S & Lu Y (2006) A proteomic investigation into a human gastric cancer cell line BGC823 treated with diallyl trisulfide. *Carcinogenesis* **27**, 1222–1231.
640. Lee SC, Chan J, Clement MV & Pervaiz S (2006) Functional proteomics of resveratrol-induced colon cancer cell apoptosis: caspase-6-mediated cleavage of lamin A is a major signaling loop. *Proteomics* **6**, 2386–2394.
641. Narayanan NK, Narayanan BA & Nixon DW (2004) Resveratrol-induced cell growth inhibition and apoptosis is associated with modulation of phosphoglycerate mutase B in human prostate cancer cells: two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis and mass spectrometry evaluation. *Cancer Detect Prev* **28**, 443–452.
642. Ramljak D, Romanczyk LJ, Metheny-Barlow LJ, Thompson N, Knezevic V, Galperin M, Ramesh A & Dickson RB (2005) Pentameric procyanidin from *Theobroma cacao* selectively inhibits growth of human breast cancer cells. *Mol Cancer Ther* **4**, 537–546.
643. Park S, Lee J & Yeom CH (2006) A proteomic approach to the identification of early molecular targets changed by L-ascorbic acid in NB4 human leukemia cells. *J Cell Biochem* **99**, 1628–1641.
644. Wang Y, He QY, Chen H & Chiu JF (2007) Synergistic effects of retinoic acid and tamoxifen on human breast cancer cells: proteomic characterization. *Exp Cell Res* **313**, 357–368.
645. Herzog A, Kindermann B, Doring F, Daniel H & Wenzel U (2004) Pleiotropic molecular effects of the pro-apoptotic dietary constituent flavone in human colon cancer cells identified by protein and mRNA expression profiling. *Proteomics* **4**, 2455–2464.
646. Fuchs D, de Pascual-Teresa S, Rimbach G, Virgili F, Ambra R, Turner R, Daniel H & Wenzel U (2005) Proteome analysis for identification of target proteins of genistein in primary human endothelial cells stressed with oxidized LDL or homocysteine. *Eur J Nutr* **44**, 95–104.
647. Deshane J, Chaves L, Sarikonda KV, Isbell S, Wilson L, Kirk M, Grubbs C, Barnes S, Meleth S & Kim H (2004) Proteomics analysis of rat brain protein modulations by grape seed extract. *J Agric Food Chem* **52**, 7872–7883.
648. Breikers G, van Breda SG, Bouwman FG, van Herwijnen MH, Renes J, Mariman EC, Kleinjans JC & van Delft JH (2006) Potential protein markers for nutritional health effects on colorectal cancer in the mouse as revealed by proteomics analysis. *Proteomics* **6**, 2844–2852.
649. Rowell C, Carpenter DM & Lamartiniere CA (2005) Chemoprevention of breast cancer, proteomic discovery of genistein action in the rat mammary gland. *J Nutr* **135**, 2953S–2959S.
650. Mitchell BL, Yasui Y, Lampe JW, Gafken PR & Lampe PD (2005) Evaluation of matrix-assisted laser desorption/ionization-time of flight mass spectrometry proteomic profiling: identification of alpha 2-HS glycoprotein B-chain as a biomarker of diet. *Proteomics* **5**, 2238–2246.
651. Weissinger EM, Nguyen-Khoa T, Fumeron C, Saltiel C, Walden M, Kaiser T, Mischak H, Drueke TB, Lacour B & Massy ZA (2006) Effects of oral vitamin C supplementation in hemodialysis patients: a proteomic assessment. *Proteomics* **6**, 993–1000.
652. Aldred S, Sozzi T, Mudway I, Grant MM, Neubert H, Kelly FJ & Griffiths HR (2006) Alpha tocopherol supplementation elevates plasma apolipoprotein A1 isoforms in normal healthy subjects. *Proteomics* **6**, 1695–1703.

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