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REVIEW

# Effects of micronutrients on DNA repair

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**Abstract**
*Background* DNA repair is an essential cellular function, which, by removing DNA damage before it can cause mutations, contributes crucially to the prevention of cancer. Interest in the influence of micronutrients on DNA repair activity is prompted by the possibility that the protective effects of fruits and vegetables might thus be explained. Two approaches to measuring repair—monitoring cellular removal of DNA damage and incubating cell extract with specifically damaged DNA in an in vitro assay—have been applied in cell culture, whole animal studies, and human trials. In addition, there are numerous investigations at the level of expression of DNA repair–related genes.
*Results* Depending on the pathway studied and the phytochemical or food tested, there are varied reports of stimulation, inhibition or no effect on DNA repair. The clearest findings are from human supplementation trials in which lymphocytes are assessed for their repair capacity ex vivo. Studying cellular repair of strand breaks is complicated by the fact that lymphocytes appear to repair them very slowly. Applying the in vitro repair assay to human lymphocytes has revealed stimulatory effects on repair of oxidised bases by various micronutrients or a fruit- and vegetable-rich diet, while other studies have failed to demonstrate effects.
*Conclusions* Despite varied results from different studies, it seems clear that micronutrients can influence DNA repair, usually but not always enhancing activity. Different modes of DNA repair are likely to be subject to different regulatory mechanisms. Measures of gene expression tend to be a poor guide to repair activity, and there is no substitute for phenotypic assays.

**Keywords** DNA repair · Base excision repair · Nucleotide excision repair · Micronutrients · Antioxidants

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## Introduction

Epidemiological evidence points fairly strongly to an association between fruit and vegetable consumption and avoidance of various types of cancer [1], but there is still a debate and confusion over the mechanism(s) of the protective effect. For many years, the antioxidant hypothesis held sway: fruit and vegetables invariably contain antioxidants (vitamins C and E, carotenoids, flavonoids, etc.); free radical damage to DNA can result in mutations and thus eventually in cancer; antioxidants scavenge free radicals and prevent this damage; therefore, antioxidants must be responsible for the protection against cancer. There is a great deal of evidence that antioxidants, or antioxidant-rich foods, given to volunteers in intervention trials, do indeed decrease the level of endogenous DNA oxidation measured in lymphocytes and enhance the resistance of lymphocytes to oxidative damage ex vivo [2, 3]. But the level of DNA

base oxidation is now recognised to be far lower (by orders of magnitude) than was often supposed [4], and in any case, reactive oxygen species are involved in immune protection and in cell signalling pathways [5], so suppressing it too much might be detrimental. Our endogenous antioxidant defences (catalase and superoxide dismutase, glutathione and associated enzymes, and molecules such as albumin and uric acid with antioxidant properties) are evidently capable of keeping reactive oxygen species and their effects to a low, tolerable and perhaps even desirable level. At the same time, large-scale clinical trials with antioxidant supplements and death or disease as the endpoint have produced far from encouraging results, and meta-analyses show a positive association between antioxidant supplementation (at least with high doses) and overall mortality [6, 7]. So the antioxidant hypothesis is undergoing serious reappraisal.

The nutritional modulation of DNA damage levels, as a possible biomarker of carcinogenesis, has been extensively reviewed [8]. However, more attention should be paid to alternative biomarkers and chemopreventive mechanisms. Fruits and vegetables are not just vehicles for antioxidants; they contain innumerable micronutrients, many of which have antioxidant properties, but it is becoming clear that they play many different roles in metabolism. Micronutrients have effects on phase I and phase II biotransformation/detoxification pathways, on cell signalling pathways and on endogenous antioxidant systems. In many studies, micronutrients have been identified as significant modulators of gene expression. Here, we concentrate on an attribute of micronutrients that, until 10 years ago, was hardly considered: the ability to modulate DNA repair pathways. For this review, we searched PubMed, using search terms including DNA damage/strand breaks, DNA repair/DNA damage removal [alternatively base excision repair, nucleotide excision repair], micronutrients/phytochemicals and individual micronutrients such as vitamin C/lycopene/$\beta$-carotene.

We discuss first the reported effects of various micronutrients on DNA repair in cell culture model systems and then the modulation of DNA repair in animals, either by means of a diet deficient in a particular micronutrient or by means of supplementation. Finally, we survey the investigations of DNA repair and its regulation in humans. It should be appreciated that the human studies are too diverse in terms of study design, type of nutrient interaction and repair assay methodology to attempt a formal meta-analysis. The effects of macronutrients, or of dietary restriction, are not covered in this review.

Several distinct repair pathways exist—single- and double-strand break (SB) rejoining, base excision repair (BER) of oxidised or alkylated bases, nucleotide excision repair (NER) of bulky adducts and helix-distorting intra-strand cross-links, mismatch repair, inter-strand cross-link repair—and each is likely to have different modes of regulation.

There are various approaches to measuring repair and its inhibition or enhancement by nutritional and other environmental factors. Investigations can be carried out in cell culture, in animals or in human intervention trials. Levels of expression of DNA repair-associated genes can be measured by microarray assays or RT-PCR. Phenotype—that is, repair enzyme activity—is assessed either by a 'challenge' assay, treating cells with damaging agent and monitoring the removal of the damage over time, or by an in vitro approach, incubating a cell-free extract with a substrate of DNA containing specific damage.

It is debatable what is the best measure of repair capacity to be derived from the 'challenge' assay [9]. If time points at close intervals after the start of incubation are studied, an initial rate of removal of damage can be estimated, but this will probably depend on the level of incident damage, which is likely to vary between individuals (for example, as a function of antioxidant status). A parameter that takes this into account is the $t_{1/2}$ of damage removal, again requiring measurement of damage at several time points to be sure of accurately estimating the halfway point. What is clearly not very informative (but is often employed) is a single measurement of residual damage at a certain time after the incident damage—usually a time when most of the damage has been repaired.

The comet assay (a simple method for measuring DNA SBs, based on the relaxation of DNA supercoiling by breaks and subsequent extension of DNA to form a 'tail' under electrophoresis [10]) is most often used to monitor rejoining of SBs by cells; it is also applied to the repair of oxidised bases, by incorporating a digestion of DNA with either formamidopyrimidine DNA glycosylase (FPG) or endonuclease III to convert oxidised purines or pyrimidines, respectively, into SBs [11].

The alternative to the challenge assay, that is, the in vitro repair assay, takes various forms; the substrate can be an oligonucleotide or plasmid engineered with a specific altered base in the sequence, repair activity being indicated by either nicking of the DNA or incorporation of labelled nucleotide into a repair patch [12–15]. Alternatively, the comet assay can be used, in which case the substrate is in the form of agarose-embedded nucleoids derived by lysis of cells treated with a specific DNA-damaging agent [16–19].

In Table 1, we summarise the various repair pathways and processes that have been examined in the papers reviewed here and give an indication of the biological relevance.

**Table 1** Different DNA repair–related processes that have been studied in micronutrient investigations and their biological significance

| Repair pathways, regulatory processes, assays | Endpoint: significance |
|---|---|
| SB rejoining | Ligation of breaks indicates completion of repair process |
| BER; challenge assay (removal of oxidised or alkylated bases) | Removal of damage (and restoration of intact DNA) indicates completion of repair process |
| NER; challenge assay (removal of pyrimidine dimers or bulky adducts) | Removal of damage (and restoration of intact DNA) indicates completion of repair process |
| BER; in vitro assay: | Removal of damaged base (+AP site) creating alkali-labile site (SB) represents incision activity; initial stage of repair |
| nicking of oligonucleotide, plasmid or nucleoid substrate with specific base damage; detected as SBs | [NB: It is not clear to what extent this repair activity resembles the coordinated action of BER in vivo] |
| can be expanded by studying incorporation of nucleotides at the nicks | Nucleotide incorporation represents overall repair from incision through repair synthesis |
| NER; in vitro assay: | Removal of lesions creating SB represents incision activity; initial stage of repair |
| nicking of oligonucleotide, plasmid or nucleoid substrate with pyrimidine dimers or bulky adducts; detected as SBs | [NB: It is not clear to what extent this repair activity resembles the coordinated action of NER in vivo] |
| can be expanded by studying incorporation of nucleotides at the nicks | Nucleotide incorporation represents overall repair from incision through repair synthesis |
| Poly ADP-ribose polymerase (PARP) activity (ADP-ribose polymerisation by incorporation of radioactive labelled NAD in nuclear protein) | Enzyme involved in creating the SB recognition signal |
| DNA ligase activity (measuring conversion of radioactive pyrophosphate) | Enzyme sealing the final phosphodiester bond to fully repair the DNA |
| DNA methylation (CpG islands) on specific genes, especially the promoter regions | Regulation of gene expression, which can alter the amount of proteins |
| Gene expression (mRNA levels) | Can potentially lead to alteration in amount of protein |
| Concentrations of repair enzymes and related proteins | Can potentially lead to alteration in repair activity |

## Modulation of DNA repair by micronutrients in cultured cells

The effect of carotenoids on SB repair has been studied in cultured cells. Astley et al. [20] incubated Molt-17 cells (a human lymphocyte cell line) with 8 μM β-carotene, lutein or β-cryptoxanthin and induced DNA damage with $H_2O_2$; they found that the carotenoid-supplemented cells showed a decrease in SBs over a 2-h incubation, while control cells did not. This paper also reports that an in vitro assay based on incorporation of labelled nucleotide into a repair patch showed no detectable stimulation by carotenoids. A similar approach—monitoring the rejoining of SBs—was applied by Glei et al. [21], using human lymphocytes incubated with 2 μM β-carotene or lycopene. The carotenoids were taken up into the cells, but had no effect on the rate of rejoining. We monitored both the rejoining of SBs and the removal of oxidised bases (8-oxoguanine introduced by treatment with photosensitiser and visible light) by human cell lines HeLa and Caco-2, incubated with β-cryptoxanthin [22]. Both kinds of repair were enhanced—at concentrations (1 and 4 μM) not much higher than are found in human plasma. In addition, we found an enhancement of in vitro repair on a nucleoid DNA substrate containing 8-oxoguanine, indicating higher 8-oxoguanine DNA glycosylase (hOGG1) activity. There was no detectable effect on concentrations of repair proteins (by Western blotting).

Human Caco-2 and HepG2 cells and the Chinese hamster fibroblast line V79 were preincubated with the common flavonoids, quercetin, rutin or myricetin, at 50 μM before treatment with $H_2O_2$ to induce SBs; the rate of rejoining was unchanged by any of the flavonoids [23] (50 μM is a high concentration and has been shown both to induce DNA damage and to protect against $H_2O_2$-induced breakage [24]). In contrast, Min and Ebeler [25] reported an increased expression of *hOGG1* mRNA after $H_2O_2$ treatment, when Caco-2 cells were preincubated with 100 μM quercetin. Caco-2 cells were also the subject of studies by Ramos et al. [26, 27] with extracts of *Salvia* species and their major phenolic constituents, rosmarinic acid and luteolin, and the triterpenoid ursolic acid. The extent of SB rejoining after $H_2O_2$ treatment was increased by preincubation with *Salvia* extracts, ursolic acid or luteolin while the in vitro repair of 8-oxoguanine was marginally enhanced. In HepG2 cells, 20 μM quercetin—and also sulforaphane at 10 μM—had no effect on the rate of removal of adducts caused by PhIP (a heterocyclic amine) [28].

The phenolic antioxidants curcumin (10–100 μM) and silymarin (10–40 μM) increased the activity of $O^6$-methylguanine-DNA methyltransferase (MGMT) in human lymphocytes and tumour cell lines, and also raised the level of *MGMT* mRNA [29]. MGMT is a so-called 'suicide enzyme' that removes the methyl group from $O^6$-methylguanine, becoming alkylated and inactive in the process; it was previously thought to be constitutively produced and non-inducible.

Resveratrol (at 5–30 μM) inhibits DSB repair—both homologous recombination (HR) and non-homologous end-joining (NHEJ)—via the ATM/ATR and p53 signalling pathway. ATM is believed to minimise error-prone repair, and activation by resveratrol might therefore play a role in genome stabilisation and chemoprevention [30].

Rather than adding a phytochemical, Williams and Jacobson [31] cultured HaCaT cells (immortalised human keratinocytes) in a state of folate deficiency and found—in addition to the expected increase in uracil misincorporation—a diminished capacity to repair SBs induced by solar simulated light or $H_2O_2$.

Finally, total oligomeric flavonoids (TOF, obtained by water/acetone extraction and chloroform-mediated precipitation) and organic solvent extracts from the traditional medicinal plant *Acacia salicina* (rich in micronutrients such as tannins, flavonoids, coumarins) induced expression of genes involved in BER and NER, as well as antioxidant defence pathways, in K562 human lymphoblastoid cells [32].

It is important to recognise that many cell culture studies employ concentrations of phytochemicals that are far higher than could be reached in vivo, or at least in the blood (around 1 μM, as a rough guide). Higher concentrations can, however, be relevant to possible exposure of the epithelial cells of the gastrointestinal tract.

With such a mixed bag of DNA-damaging agents and micronutrients tested, repair pathways studied, practical approaches and concentrations, it is impossible to draw any general conclusions. It is important to recognise that apparently negative results might simply reflect failure of the test compound to enter the cells (or to remain active within the cells). It is therefore advisable, if possible, to check for the presence of the compound within the cells. However, where effects are seen, they can be very convincing; Fig. 1 shows the effects of β-cryptoxanthin on both SB rejoining and removal of oxidised bases, as well as an enhancement of in vitro repair, in human tumour cells.

## Animal studies of the effect of micronutrients on DNA repair

Animal studies looking at the modulation of DNA repair by micronutrients are listed in Table 2, which summarises reports on the effects of micronutrient-deficient diets as well as supplemented diets.

Several groups have studied the effect of disrupting 1-carbon metabolism on DNA stability. Folate, choline and methionine interact in this pathway. Bagnyukova et al. [33]

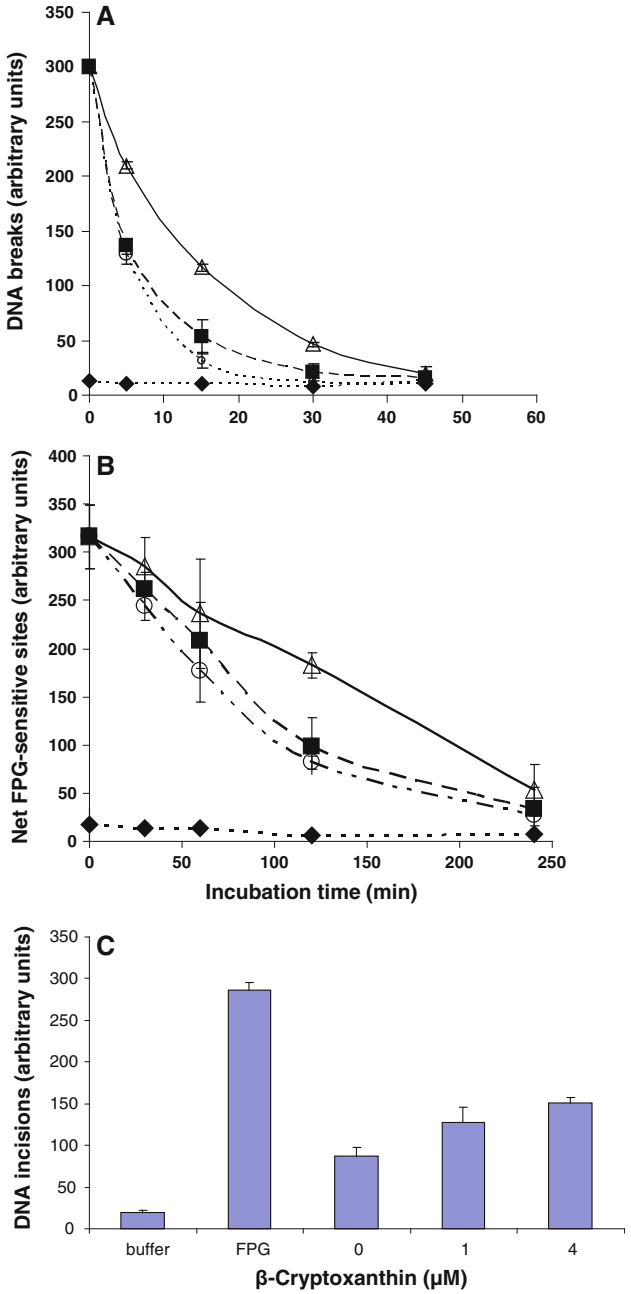


**Fig. 1** Enhancement of HeLa cell DNA repair by *β*-cryptoxanthin. **a** Strand break repair. Cells were treated with $H_2O_2$ (30 μM) for 5 min on ice and incubated without (*triangles*) or with *β*-cryptoxanthin at 1 μM (*squares*) or 4 μM (*circles*). Untreated cells were also incubated (*diamonds*). **b** BER. Cells were treated with photosensitiser Ro 19-8022 plus light to induce 8-oxoguanine in DNA and incubated with or without *β*-cryptoxanthin. (Symbols as for **a**.) **c** In vitro BER. Extract from cells preincubated for 2 h with 0, 1 or 4 μM *β*-cryptoxanthin was incubated for 10 min with agarose-embedded nucleoid DNA containing 8-oxoguanine (from cells treated with Ro 19-8022 plus light). Negative and positive controls (incubations with buffer or FPG) are also shown. From [22], by permission of Oxford University Press

fed rats a folate-/methyl-deficient diet; 1-carbon metabolism was disrupted, and oxidative damage was found in DNA in the brain. Compared with control rats, there was an increase in DNA SBs and base oxidation, and probably as a consequence, expression of DNA repair genes coding for AP endonuclease 1 (*Ape1*) and DNA polymerase *β* (*Polb*) was increased at the same time. Similarly, Duthie et al. [34] observed folate deficiency in rats to increase levels of 8-oxoguanine in lymphocytes as well as to enhance the amounts of OGG1 and MGMT proteins in the liver, but not in the colon. In an early report by Klaude and von der Decken [35], a methionine-/cysteine-deficient diet was shown to decrease MGMT protein levels significantly in mouse liver, but not in kidney, lung, testis and brain. These observations indicate that distinct tissues can respond differently to micronutrient deficiencies or elevated DNA damage.

Often a combination of choline/folate deficiency with a low methionine diet is adopted in animal studies. In an early paper, Henning et al. [36] found an increase in the activity of poly ADP-ribose polymerase (PARP) in the liver of rats on such a diet. Polymerisation of ADP-ribose is an early response to DNA damage, apparently stabilising SBs and recruiting other repair-related proteins to the site [37]. James et al. [38] confirmed the increase in PARP activity and also reported a substantial increase in the ratio of dUTP/dTTP in the DNA precursor pool, consistent with a block in folate-dependent de novo biosynthesis of thymidylate leading to misincorporation of uracil. Uracil DNA glycosylase removes the misincorporated base, but a 'futile repair cycle' might ensue with re-incorporation of uracil, and a consequent increase in repair-related SBs (as previously shown to occur under these conditions [39]).

Poly ADP-ribose is synthesised from ADP-ribose donated by NAD (nicotinamide adenine dinucleotide), and the precursor for NAD is vitamin B3 (niacin—nicotinic acid—or nicotinamide). Zhang et al. [40] found chronic niacin deficiency hard to maintain, with a variable effect on PARP activity. Rawling et al. [41] reported a decrease in liver poly (ADP-ribose) concentrations associated with mild niacin deficiency. However, after chronic exposure to a low methionine, folate- and choline-deficient diet combined with niacin deficiency, rats showed decreased NAD concentration and reduced PARP activity in liver, and a high incidence of hepatocarcinomas [42].

Song et al. [43] were interested in the effect of marginal zinc deficiency (common in humans) and found that severe or marginal zinc deficiency in rats increased oxidative stress, increased DNA SBs in white blood cells and increased OGG1 protein levels. Surprisingly, though the PARP protein has two zinc fingers, its concentration was not affected. In a subsequent study, both mRNA and protein levels of PARP were increased in the prostate of rats

**Table 2** Summary of micronutrient interventions in animal studies with DNA repair as an endpoint

| Intervention | Study design | Animal model | Tissue | Assay | Results | References |
|---|---|---|---|---|---|---|
| *Administering a deficient diet* | | | | | | |
| Methyl deficiency | Low methionine (0.18%) diet, lacking in choline and folic acid, or a control diet (methyl-adequate) supplemented with 0.4% methionine, 0.3% choline, and 2 mg/kg folic acid for 18–36 weeks | 4-week-old male Fischer 344 rats ($n = 4$/group) | Brain | Gene expression; Taqman gene expression arrays<br>SBs by random oligonucleotide-primed synthesis (ROPS)<br>Levels of 8-oxoguanine using ELISA kit | ↑ expression *APE1* and *POLB*<br>↑ DNA SBs and base oxidation | [33] |
| Folate deficiency | Folate-sufficient (5 mg/kg) or folate-free for 24 weeks | 6–7-week-old male hooded-Lister rats ($n = 24$/group) | Lymphocytes, liver, colon | Comet assay + FPG for 8-oxoguanine levels<br>$^{32}$P-labelled oligonucleotide cleavage | ↑ 8-oxoguanine levels in lymphocytes<br>↑ OGG1 and MGMT proteins in liver | [34] |
| Low methionine–cysteine | Fed diet low in methionine–cysteine (10% of the control diet) for 6 days | White male outbred NMRI mice<br>60 days old ($n = 7$–10) | Liver, kidney, testis, brain, lung | Radioactive protein assay based on transfer of the methyl group from O$^6$me-guanine to MGMT | MGMT protein levels ↓ in liver<br>No changes in other tissues | [35] |
| Low methionine–choline | Low methionine, choline-devoid diet for 3 weeks | Young male Sprague–Dawley rats ($n = 7$/group) | Liver | Polymer-based radioactive [$^3$H]NAD incorporation assay | ↑ PARP activity | [36] |
| Folate/methyl deficiency | Diet low in methionine (0.18%) and lacking in choline and folic acid, or control diet (0.4% methionine, 0.3% choline and 2 mg/kg folic acid) for 4 weeks | 4-week-old male Fischer 344 rats kept for 2, 5, 7 days, 3, and 9 weeks | Liver | Polymer-based radioactive [$^3$H]NAD incorporation assay<br>HPLC for dNTP levels | ↑ PARP activity<br>↑ dUTP/dTTP ratios | [38] |
| Niacin deficiency | Low niacin diet<br>Experiment 1: 1.5 mg/kg; Experiment 2: 1.5 mg/kg for 40 days and then decreased to 0.5 mg/kg | Weaning male Sprague–Dawley rats ($n = 4$–6/group) killed at 34 or 60 days | Liver, splenic lymphocytes | Polymer-based radioactive [$^3$H]NAD incorporation assay with liver nuclei extract<br>DNA SBs detected after hypoxanthine–xanthine oxidase exposure, by alkaline unwinding | Experiment 1:<br>↓ PARP activity at 34 and 60 days in liver<br>↑ DNA SBs at 34 days in splenic lymphocytes<br>Experiment 2 (in liver):<br>↑ PARP activity at 34 days<br>↑ PARP activity at 60 days<br>↑ DNA SBs at 60 days | [40] |
| Niacin deficiency | Diets containing 0.9 g/kg or 1.1 g/kg tryptophan and no added niacin, for 3 weeks | Weaning male Fischer 344 rats, killed at 3 weeks ($n = 10$–15) | Liver | ADP-ribose polymerisation assay using HPLC for final quantification | ↓ Poly ADP-ribose for both niacin-deficient diets | [41] |
| Methyl-deficient diet + niacin deficiency | Fed 12% casein methionine-/choline-/folate-deficient diet for 2 months, followed by 6% casein, 6% gelatin diet deficient in choline, folate and niacin, up to 17 months | Young male Fischer 344 rats ($n = 3$–4/group) | Liver | Polymer-based radioactive [$^3$H]NAD incorporation assay | ↓ PARP activity at 6 and 17 months<br>↑ number & size of liver tumours | [42] |

**Table 2** continued

| Intervention | Study design | Animal model | Tissue | Assay | Results | References |
|---|---|---|---|---|---|---|
| Zinc deficiency | Zinc-adequate diet (30 mg Zn/kg), mild zinc-deficient diet (6 mg Zn/kg) for 42 days, or severe zinc-deficient diet (1 mg Zn/kg), for 21 days | 3–5-week-old male Sprague–Dawley rats ($n$ = 10–12/group) | Liver, blood cells | DNA damage analysis via comet assay,<br>Protein levels by Western blot analysis,<br>P53 binding activity via electrophoretic mobility shift assay | Mild zinc deficiency:<br>↑ DNA damage,<br>↑ OGG1 levels,<br>no change in PARP & p53<br>Severe zinc deficiency:<br>↑ DNA damage,<br>↑ OGG1 & p53 levels,<br>no change in PARP or in p53 binding activity | [43] |
| Zinc deficiency + chronic exercise | Zinc-adequate diet (30 mg Zn/kg), or marginally zinc-deficient diet (5–6 mg Zn/kg), with or without voluntary wheel-running, for 6 weeks | 5-week-old male Sprague–Dawley rats<br>($n$ = 12/group) | Prostate | DNA damage analysis via comet assay,<br>Levels of 8-oxodeoxyguanosine using ELISA kit,<br>Protein levels by Western blot analysis,<br>Gene expression analysis | Without exercise:<br>DNA damage constant,<br>↑ PARP & p53 gene expression and protein levels<br>With exercise:<br>↑ base oxidation,<br>no further change in PARP & p53 gene expression | [44] |
| Vitamin A deficiency | Control diet (50 IU/day vitamin A), or vitamin A deficient diet for 9 weeks,<br>followed by vitamin A supplementation for 2–3 weeks | Weanling male Wistar rats<br>($n$ = 8/group for controls)<br>($n$ = 4/group for supplementation) | Liver | SBs were analysed via alkaline sucrose gradient centrifugation<br>Radioactive [$C^{14}$]NAD, [$^{32}$P]dATP, and [$^{32}$P]pyrophosphate protein assays to detect PARP, DNA polymerase $\beta$, and DNA ligase activity, respectively | Vit A deficiency:<br>enhanced effect of hepatocarcinogens;<br>↑ DNA damage,<br>↑ PARP, DNA polymerase $\beta$ and DNA ligase activity<br>Vit A supplementation:<br>protects against carcinogenesis; DNA damage and enzyme activities back to normal | [45] |
| Copper deficiency | Control diet (0.3 mg/100 g) or copper deficient diet for 11 weeks, followed by copper supplementation for 2 weeks | Weanling male Wistar rats<br>($n$ = 8/group for controls)<br>($n$ = 4/group for supplementation) | Liver | Radioactive [$C^{14}$]NAD, [$^{32}$P]dATP, and [$^{32}$P]pyrophosphate protein assays to detect PARP, DNA polymerase $\beta$, and DNA ligase activity, respectively | Cu deficiency:<br>enhanced effect of aflatoxin B1;<br>↑ PARP, DNA polymerase $\beta$ and DNA ligase activity<br>Cu supplementation:<br>prevents effects of aflatoxin B1; DNA enzyme activities return to normal | [46] |
| *Dietary supplementation* | | | | | | |
| Nicotinamide, zinc, and carotenoids | Supplementation of nicotinamide, zinc, and carotenoids for 2–8 weeks | Female W/Fu rats ($n$ = 3–6/group) | Spleen | Analysis of DNA damage removal (single and double SBs) using alkaline and neutral elution | ↑ DNA repair of both single and double SBs induced by 12 Gy whole-body irradiation | [47] |

**Table 2** continued

| Intervention | Study design | Animal model | Tissue | Assay | Results | References |
|---|---|---|---|---|---|---|
| Rutin | Supplement of rutin was mixed in the diet (1 and 10 mg/100 g) fed for 2 weeks | Weanling male Wistar rats ($n = 8$/group) | Liver | DNA single SBs and repair measured by alkaline sucrose gradient centrifugation<br>Radioactive [$C^{14}$]NAD, [$^{32}$P]dATP, and [$^{32}$P]pyrophosphate protein assays to detect PARP, DNA polymerase $\beta$, and DNA ligase activity, respectively | Rutin supplementation:<br>prevents adverse effects of aflatoxin B1 and *N*-nitrosodimethylamine;<br>↓ DNA damage, and elevated DNA repair enzyme activities return to normal | [48] |
| Ellagic acid | Control diet or diet supplemented with ellagic acid (400 ppm) and dehydrated berries (5% w/w) with varying ellagic acid contents—blueberry (low), strawberry (medium) and raspberry (high), for 3 weeks | 8-week-old female CD-1 mice ($n = 6$/group) | Liver | DNA oxidation by $^{32}$P-post-labelling/TLC<br>Gene expression analysis | Blueberries and strawberries reduced DNA adducts by 25%<br>Ellagic acid (400 ppm) and raspberries;<br>↓ DNA adducts by 48–59%,<br>↑ *XPA, ERCC5* and *DNA ligase III* expression | [49] |
| Proanthocyanidin | Supplementation with grape seed proanthocyanidins for 40 weeks | 6–7-week-old female C3H/HeN mice ($n = 20$/group) | UVB-exposed skin | Gene expression | ↑ expression of *XPA, XPC, DDB2,* and *RPA1* | [50] |
| Fruit | Fed control diet or a diet with 8% peach or nectarine extract, for 14 weeks | 18–19-month-old male C57Bl/6J mice ($n = 6$–7/group) | Liver | Oligonucleotide assay to detect enzyme activity<br>Gene expression via Illumina microarrays | ↑ OGG1 incision activity in nuclear liver extracts, while mitochondrial liver incision activity only ↑ with nectarine extract,<br>↓ *XPA, TDG* expression<br>↑ *NTHL1* expression | [51] |
| Antioxidants | Control or supplemented diet (taurine, carnitine, tocopherol acetate, flaxseed and rapeseed providing α-linolenic and linoleic acids, and linden flowers as source of flavonoids and other antioxidants), from 80 days of pregnancy to 14–28 days of lactation | Pregnant sows (*Sus scrofa domesticus*, $n = 6$/group), looking in offspring ($n = 3$–9/group) | Colon, hippocampus | 8-oxoguanine via HPLC-ECD<br>Comet assay–based in vitro assay for BER and NER | Colon:<br>↓ 8-oxoguanine levels,<br>supplementation diminishes oxidative stress induced ↓in NER<br>Hippocampus:<br>↓ 8-oxoguanine levels,<br>higher BER activity immediately after birth | [52, 53] |

subjected to a marginal zinc deficiency, and in combination with chronic exercise, there was an increase in DNA oxidation [44].

Activities of PARP as well as DNA polymerase $\beta$ and DNA ligase, three enzymes induced in rat liver by aflatoxin B1-treament were higher in rats deficient in vitamin A [45] or copper [46] and were brought down by supplementation.

Turning from micronutrient-deficient diets to supplementation of the normal diet, in experiments by Sheng et al. [47], rats were supplemented for several weeks with a mixture of nicotinamide, zinc and carotenoids, subjected to whole-body ionising irradiation, and 3 h later, splenocyte DNA was analysed for residual DNA damage (single and double SBs) using alkaline and neutral elution; the repair of both single and double SBs was enhanced, in comparison with control animals. Webster et al. [48] found that, in rats supplemented with the flavonoid glycoside rutin, the yield of DNA breaks induced by aflatoxin B1 or *N*-nitrosodimethylamine in the liver was decreased, and the carcinogen-induced increases in activity of PARP, DNA polymerase $\beta$ and DNA ligase activities were reversed—an early indication of the effect of non-essential micronutrients on DNA damage levels, though whether this involves a direct effect on repair remains unclear.

More recently, the emphasis has been on the effect of supplementation with micronutrients on the expression of genes coding for repair-related proteins. Aiyer et al. [49] fed mice a diet containing either ellagic acid (a polyphenol found in many berries), or dehydrated blueberry, strawberry or raspberry. Ellagic acid and raspberry had particularly strong effects, increasing mRNA levels of *Xpa*, *Ercc5* and *Dnl3* (DNA ligase III) by three- to eightfold. Grape seed proanthocyanidins given to mice increased the expression of *Xpa*, *Xpc*, *Ddb2* and *Rpa1*—genes for proteins involved in NER [50]. C57Bl/6J mice fed a diet with 8% peach or nectarine extract (rich in vitamins C and A, niacin, potassium and polyphenolic compounds) for 14 weeks showed significantly increased OGG1 incision activity in nuclear extracts from liver, compared with control mice, while liver mitochondrial extract showed significantly increased incision activity only after nectarine extract supplementation [51]. In the same study, nectarine and peach extract supplementation resulted in significantly downregulated expression of *Xpa* and *Tdg*, while *Nthl1* (the gene responsible for the removal of Fapy DNA adducts) was upregulated in mouse liver.

Langie et al. [52] measured NER activity with the in vitro comet-based assay in colonic tissue extracts from piglets. The effect of a maternal diet rich in antioxidants and other micronutrients (e.g. taurine, carnitine, tocopherol acetate and flavonoids) on the response of the piglets to an oxidative stress–inducing injection of iron was tested. The stressed animals showed a decrease in NER activity compared with controls, and this was partially reversed by the micronutrient supplementation. More recent observations by Langie et al. [53], using the same animal model but looking at the hippocampus, showed that the supplementation protected the piglets against the effects of acute oxidative stress caused by birth. Levels of 8-oxoguanine were significantly lower, and initial BER activity was higher in supplemented animals compared with controls.

Overall, the experimental modulation of DNA repair in animals seems to be quite informative. Deficient diets generally tend to increase DNA damage and possibly as a result lead to increased DNA repair activity. However, sometimes decreased repair activity is seen, which might then be the cause rather than the consequence of increased DNA damage. Supplemented diets mostly improve DNA repair and result in decreased DNA damage levels. The beauty of animal studies is that diets are simply altered or supplemented with micronutrient, so that the animals absorb the micronutrients in a natural way, increasing the value of the in vivo observations. In addition, the advantage of an animal model is that organs of interest can be studied in great detail, rather than having access only to blood cells as a surrogate tissue. However, there are major issues when extrapolating from animal data to humans. Although DNA repair genes are highly conserved among eukaryotes, there are clear differences in organisation and regulation of repair between species [54], and of course, there are also substantial differences in absorption and metabolism of xenobiotics, including micronutrients, among the mammals.

## Evidence from human intervention trials of the influence of micronutrients on DNA repair

Table 3 summarises published reports of DNA repair in humans, studied through intervention trials with individual micronutrients, single fruit or vegetable supplements, or whole diets, and with single doses or supplementation periods of one to several weeks. Blood samples are taken—and lymphocytes isolated—before and after supplementation, and repair assays performed ex vivo. Often the trial includes a parallel control group receiving no supplement, or a placebo; an alternative design is the crossover trial, in which all subjects receive the same supplements, doses and/or placebo, preferably in different order, which minimises the chance of 'period effects' (changes with time that are not related to the intervention, but to some other uncontrolled factor) and provides for each subject to act as his/her own control (see Fig. 2).

The simplest form of DNA repair measured in these trials is the rejoining of SBs induced by $H_2O_2$. This is generally found to be very slow, taking up to 24 h to

**Table 3** A summary of human intervention trials with DNA repair as endpoint

| Supplementation (daily dose) | Study design | Subjects | Assay | Results (effect of supplementation) | References |
|---|---|---|---|---|---|
| Vitamin C (1 g) | Single dose; samples taken before and 2 h after | 8 volunteers | Repair of SBs and oxidised pyrimidines after $H_2O_2$ (comet assay) | Slow repair in most cases, apparently enhanced by vitamin C in a few | [56] |
| Nicotinamide, Zn, carotenoids | Weekly samples during 5-week baseline and 7-week supplementation | 4 volunteers | Cellular SB rejoining after $H_2O_2$ (alkaline elution) | Fewer breaks remaining at 60 min | [47] |
| Lutein (15 mg); $\beta$-carotene (15 mg); lycopene (15 mg) | 1-week intervention period with each, with 3-week washout; samples before and after | 8 young volunteers | Cellular SB rejoining after $H_2O_2$ (comet assay) | Rejoining of SBs slow; apparently enhanced by $\beta$-carotene and lycopene but not lutein | [57] |
| Carotenes (12 mg) + $\alpha$-tocopherol (1.75 mg); vitamin C (60 mg); mandarin oranges (300 g); carrots (200 g); placebo | 3-week intervention trial; samples taken before and after | Healthy non-smokers, $\sim$10 per group | Cellular SB rejoining after $H_2O_2$ (comet assay)<br>In vitro assays for SB rejoining and oxidised base repair | No rejoining over 4 h except in carrots group<br>Carrots group; increase in both assays. 10-fold inter-individual variation | [58] |
| Se (100 μg), retinol (300 μg), $\beta$-carotene (150 μg), vit C (90 mg), vit E (30 mg) | 6-week controlled intervention trial; samples taken before and after | Young, healthy non-smokers; 32 in supplemented group, 8 controls | BER (in vitro comet assay, 8-oxoguanine substrate) | No significant effect. Wide inter-individual variation (40×) | [64] |
| Folate (1.2 mg) | 12-week placebo-controlled intervention trial | Healthy non-smokers; 30 suppl., 31 placebo | BER (in vitro comet assay, 8-oxoguanine substrate) | Decrease in BER (OGG) in lowest quartile of initial folate status | [65] |
| Coenzyme $Q_{10}$ | 1-week intervention trial | Non-smokers: 3 male, 3 female | BER (in vitro comet assay, 8-oxoguanine substrate) | Increase in BER (OGG) | [59] |
| Coenzyme $Q_{10}$ (2 × 100 mg), riboflavin (2 × 10 mg), niacin (2 × 50 mg) | 45- and 90-day interventions; samples taken before and after | 42 Controls; 84 breast cancer patients, untreated; 84 tamoxifen-treated for >1 year, coenzyme $Q_{10}$-supplemented | PARP protein concentration (PARP binds to SBs; early response) | Increase in PARP after supplementation in tamoxifen-treated patients | [70] |
| 182 mg vitamin E, and 0.5 g vitamin C (conventional or slow-release capsules); placebo | 4-week placebo-controlled trial | Male smokers; 3 groups (18, 18, 10) | BER (in vitro comet assay, 8-oxoguanine substrate) | Increase in BER after slow-release capsules | [60] |
| Kiwifruit (1, 2 or 3) | Crossover trial; 3-week interventions with washout periods | Healthy, young adults; 14, in 3 groups | BER (in vitro comet assay, 8-oxoguanine substrate)<br>Gene expression: *OGG1, APE-1* | Increase in BER (OGG)<br>No effect on gene expression | [61] |
| Control (normal diet); antioxidant-rich fruits and vegetables; 3 kiwifruits | 8-week controlled intervention study, samples taken before and after | Healthy men (but with risk factors for heart disease) BER: $\sim$23/group; NER: $\sim$12/group | In vitro comet assay; BER (8-oxoguanine substrate), NER (UV-damaged substrate) | Increase in BER, but decrease in NER | [62] |
| Broccoli (250 g) | Crossover trial, 10 days with 20-day washout | Young healthy smokers; 11 assayed for BER, 17 for gene expression | BER (in vitro comet assay, 8-oxoguanine substrate)<br>Gene expression: *OGG1, HO-1, NUDT1* | No change<br>No change | [63] |

**Table 3** continued

| Supplementation (daily dose) | Study design | Subjects | Assay | Results (effect of supplementation) | References |
|---|---|---|---|---|---|
| Blueberry and apple juice | 4-week intervention study; samples taken before and after | 36 healthy volunteers (male and female) | In vitro comet assay for NER with B(a)P-diol epoxide-damaged substrate | No effect overall, but an enhancement in subjects carrying multiple low-activity alleles of repair genes | [66] |
| Flavonoid-rich diet | 1-month intervention, samples taken before and after | Healthy male smokers (9) | *Gene expression: APEX, ERCC1, ERCC2, ERCC4, MGMT, OGG1, XPA, XPC, XRCC1, XRCC3, AHR, CYP1A1* | Decrease in *AHR* mRNA, increase in others (significant for *XRCC3*) | [67] |
| Fruit and vegetable depletion (control); plus fruit and vegetables; plus vitamins and minerals | 24-day controlled intervention study | Healthy non-smokers; groups of ~ 13 for repair phenotype: groups of ~ 9–16 for gene expression | Gene expression: *OGG1* and *ERCC1*. BER (in vitro comet assay, 8-oxoguanine substrate) | No effect on either BER activity or gene expression | [60, 69] |
| Control (normal diet); antioxidant-rich fruits and vegetables; 3 kiwifruits | 8-week controlled intervention study, samples taken before and after | Healthy men (but with risk factors for heart disease); 3 groups of ~ 10 | Gene expression; Gene set enrichment analysis | 'DNA and repair' gene sets most prominently upregulated in both intervention groups | [68] |

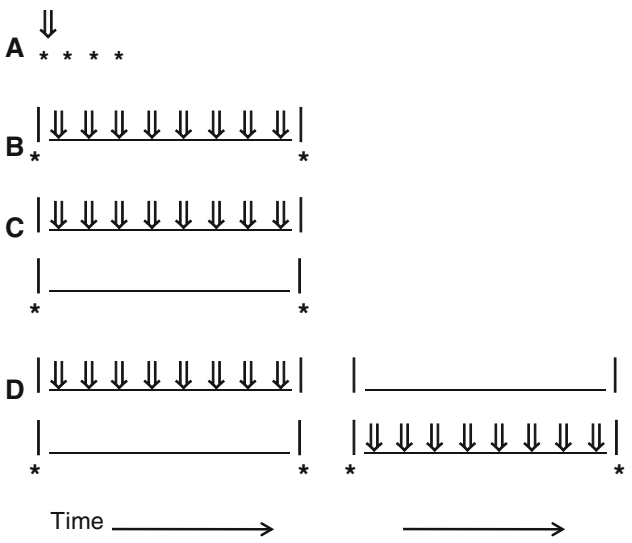


**Fig. 2** Different designs for human nutritional intervention studies. Supplementation is indicated by *arrows* and sampling by *asterisks*. **a** Single dose with sampling at intervals. **b** Uncontrolled intervention, with multiple doses (normally daily), and sampling at beginning and end of intervention period. **c** Placebo-controlled intervention trial; like **b**, but with a parallel study group taking no supplement, preferably taking a placebo. **d** Controlled crossover study, with each subject taking both placebo and supplement, but in different orders. More complicated designs could involve, for instance, testing with several doses of a nutritional factor

complete, compared with the rapid rejoining seen in most cultured cells [11, 55]. Supplementation with vitamin C [56], $\beta$-carotene, lycopene (but not lutein) [57] or carrots [58] apparently accelerated the rejoining process. However, the interpretation of these results is complicated by the fact that freshly isolated lymphocytes suffer oxidative stress, and subsequent DNA damage, from the sudden exposure to atmospheric oxygen. We reported an increase in SBs in lymphocytes, without $H_2O_2$ treatment, lasting a few hours after isolation; and this transient increase was less pronounced in lymphocytes taken after carotenoid supplementation [57] (Fig. 3). It follows from this that the apparent slow rejoining of $H_2O_2$-induced SBs might be a conflation of the repair of induced damage and the introduction of further damage by oxygen exposure—and that the apparent effect of carotenoids might be, in fact, simply an antioxidant protection against this further damage rather than an enhancement of repair.

BER has been studied in vitro with a DNA substrate containing 8-oxoguanine in a number of human trials, with varied results. Increases in OGG1 activity were reported after coenzyme $Q_{10}$ [59], slow-release vitamin C (but not conventional vitamin C capsules) [60], carrots (but not carotenes, vitamin C or mandarin oranges) [58], kiwifruit [61] and a high fruit and vegetable diet [62]. There was no effect of broccoli [63], or a cocktail of Se, retinol, $\beta$-carotene, vitamin C and vitamin E [64]. After folate

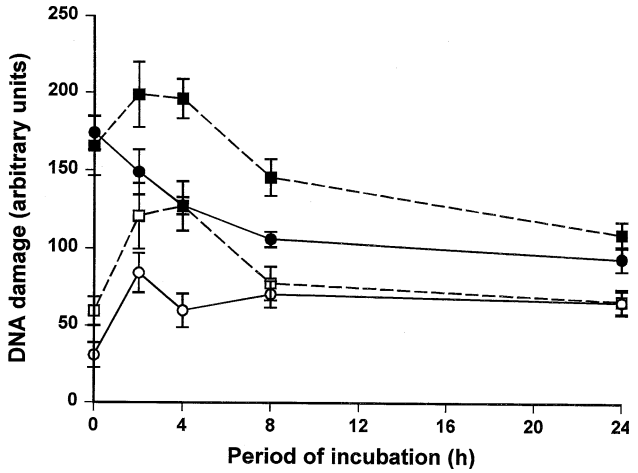


**Fig. 3** Kinetics of SB rejoining in lymphocytes. Volunteers took 15 mg of lycopene per day for 1 week. Lymphocytes were isolated from blood samples from five subjects showing an increase in plasma lycopene during the supplementation period. *Squares* represent samples taken before supplementation, and *circles* represent samples after supplementation. *Solid symbols* lymphocytes treated with 100 μM $H_2O_2$, and *open symbols* untreated lymphocytes, incubated at 37 °C. DNA breaks were measured with the comet assay. *Bars* indicate SEM. From [57], with permission from Springer Medizin

supplementation, surprisingly, a decrease was seen, but only in individuals who were relatively folate-deficient [65].

NER is less often measured than BER. Langie et al. [66], supplementing with blueberry and apple juice, reported no effect on NER (with a substrate containing bulky adducts), except that individuals carrying 1 or more low-activity (variant) alleles for *XPC*-K939Q and/or *RAD23B*-A249V showed an increase upon the intervention. Brevik et al. [62] (measuring NER on a UV-damaged substrate) found a decrease in subjects taking a fruit- and vegetable-rich diet or a kiwifruit supplement.

In several studies, expression of a variety of DNA repair-related genes has been examined. Guarrera et al. [67] report a general tendency for increased expression after a flavonoid-rich diet, and 'DNA and repair' gene sets were upregulated after a high fruit and vegetable diet or kiwifruit supplementation [68]; whereas Møller et al. [69], Riso et al. [63] and Collins et al. [61] found no effect on expression of *hOGG1* and other repair genes. Premkumar et al. [70] studied protein concentrations and found, in breast cancer patients receiving tamoxifen, an increase in the amount of PARP after supplementation with coenzyme $Q_{10}$, riboflavin and niacin. However, it is worth pointing out that measuring mRNA or protein levels is not necessarily a good guide to enzyme activity. A high level of mRNA does not necessarily imply more protein production, and more protein does not always result in higher enzyme activity, since post-translation modifications (among other modifiers of protein structure) might affect enzyme activity. In fact, in a careful study, Paz-Elizur et al. [12] measured both *hOGG1* gene expression and enzyme activity in a group of human subjects, and found only a poor correlation between them.

Until fairly recently, there was little interest in the regulation of human DNA repair by exogenous factors, and it was generally assumed that functions as important as DNA repair were unlikely to be readily affected by nutrition. However, the various modes of repair show quite extensive inter-individual variation (Fig. 4), and this is unlikely to be solely due to genetic polymorphisms. Different levels of exposure to DNA-damaging agents in the environment might be responsible for some of the variation, if DNA repair enzymes are induced by their substrate; but as discussed here, differences in nutrition might also contribute.

While certain studies show similar observations in animals and humans [47], others show contrasting data (effect of folate deficiency; compare [34] and [65]) or effects that are only seen in certain subpopulations with specific

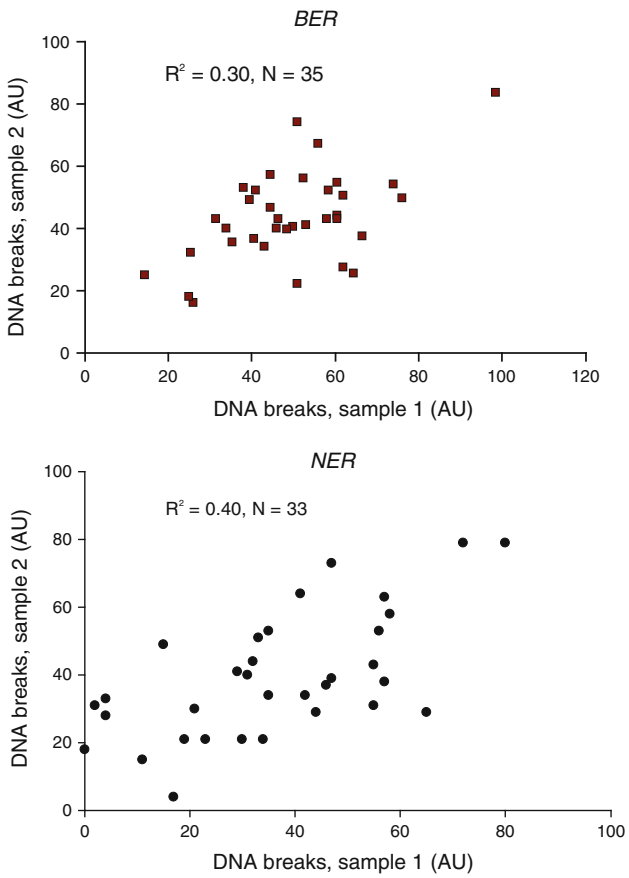


**Fig. 4** Repair activities (arbitrary units, AU) in extracts from human lymphocyte samples taken on occasions approximately 2 months apart. *Upper panel* BER measured on a substrate containing 8-oxoguanine. *Lower panel* NER measured on a substrate from cells irradiated with UV(C). From [18] with permission from Springer Science + Business Media

genetic background (such as the effect of blueberries; compare [49] and [66]). Moreover, while laboratory animals are generally inbred, humans are diverse assortments of several million genetic polymorphisms, which adds to the difficulty of discerning possible effects of micronutrients on DNA repair.

A further limitation of human studies is that generally only white blood cells are available for study, and the assumption that these are a reliable surrogate for true cancer target tissues is convenient but not well substantiated. Studies are needed where repair rates are measured in cancer patients, looking at tumour and normal tissue, and at lymphocytes. We are currently developing appropriate in vitro repair assays for application to tissue biopsies.

## The involvement of epigenetics in the modulation of DNA repair

Epidemiological studies provide compelling evidence that diet, including micronutrient supplementation, can have profound effects on the level of DNA damage and its removal. Although the cellular and molecular routes by which (micro)nutrients affect DNA repair gene expression or enzyme activity are still not fully understood, epigenetic processes (i.e. changes in gene expression and phenotype without a corresponding alteration in DNA sequence) have emerged as a plausible mechanism [71]. DNA methylation is currently the most widely studied epigenetic modification, occurring mainly as 5-methylcytosine in CpG dinucleotides (CpGs) [72]. CpG-rich sequences—CpG islands (CGI)—are found mainly in gene promoter regions and are normally unmethylated in expressed genes [73]. Hypermethylation of CGIs has been linked with transcriptional silencing of the associated genes, while hypomethylation is generally believed to enhance gene expression. While some epigenetic marks are established during embryonic and foetal development and remain relatively stable during adulthood, there is growing evidence that environmental exposures (e.g. diet, lifestyle, air pollution, heavy metals) can change the methylation status of gene promoters, modify their expression and ultimately contribute to cellular dysfunction (reviewed in [71, 74]). There are few studies of possible direct links between changes in DNA methylation patterns of DNA repair genes caused by micronutrients and changes in gene expression. Fang et al. [75] reported that the green tea polyphenol epigallocatechin 3-gallate (EGCG) reverses *MGMT* and *MLH1* gene promoter hypermethylation in human oesophageal cancer cells, accompanied by a re-expression of those genes. The same group reported that the isoflavone genistein has a similar effect [76].

As mentioned above, studying gene expression does not necessarily give any indication of enzyme activity. Therefore, Langie et al. [77] recently studied methylation changes in BER-related genes and their effect on BER incision activity as assessed by the comet assay–based in vitro repair assay. This is the first report of significantly increased methylation of the *Ogg1* gene promoter with ageing in mice brain and, as a possible consequence, a significant decrease in *Ogg1* expression in the oldest mice, as well as significantly reduced BER activity. In addition, concerning the modulating effect of micronutrients, Langie et al. recently observed maternal antioxidant supplementation to modulate the effect of early life oxidative stress on the *APE1* gene promoter methylation and expression as well as on BER activity in the hippocampus of piglets [53]. Currently, Langie et al. are studying other tissues in parallel to the brain, since DNA repair might be affected differently in post-mitotic and mitotic tissues by ageing as well as nutrition (unpublished data). Nonetheless, environmental exposures, including micronutrient supplementation, have been shown to change the methylation status of genes encoding DNA repair enzymes, altering their expression and modulating DNA repair capacity and susceptibility to oxidative DNA damage.

## Discussion

What influences DNA repair capacity? It seems that an individual has a characteristic, intrinsic repair capacity, at least as indicated by the correlation seen between measurements carried out several weeks apart (Fig. 4). We do not know the extent of intra-individual variation over a longer time-scale. Part of the inter-individual variation is likely to be genetically determined. A value of $R^2$ of 0.4 (Fig. 4) might imply that 40% of the variation from person to person is explained by an intrinsic repair capacity (i.e. a characteristic repair rate in each person, representing a genetic predisposition, together with other influences such as lifestyle, smoking habits, etc.). The remaining variation is due to day-to-day variation in environmental exposure, health and lifestyle (including diet and exercise), plus potentially measurable experimental variation. There is increasing evidence, from cell culture, animal and human studies, that DNA repair can indeed be modulated by nutritional factors, specifically micronutrients (Table 4).

Individual differences in intrinsic repair capacity are regarded as probable indicators of susceptibility to cancer, since those with a high repair capacity should repair damage more quickly and so decrease the chance of unrepaired damage being present when a cell replicates its DNA, resulting in possible mutation. But it is possible, on the other hand, that a high repair capacity reflects a high

**Table 4** Overview of the effects of micronutrients on DNA repair

| Class of compound | Bioactive substance | Model | Effect | References |
|---|---|---|---|---|
| Carotenoids | $\beta$-carotene | Cultured cells | Enhanced rejoining of $H_2O_2$-induced SBs | [20] |
| | | Human | Enhanced rejoining of $H_2O_2$-induced SBs | [57] |
| | Mixed carotenes | Human | Enhanced rejoining of $H_2O_2$-induced SBs | [58] |
| | Lutein | Cultured cells | Enhanced rejoining of $H_2O_2$-induced SBs | [20] |
| | $\beta$-cryptoxanthin | Cultured cells | Enhanced SB rejoining & 8-oxoguanince removal, increased hOGG1 activity | [22] |
| | Lycopene | Human | Enhanced rejoining of $H_2O_2$-induced SBs | [57] |
| Vitamins | B vitamins (folic acid, choline, methionine, niacin) | Cultured cells | Deficiency caused ↓ repair of SBs | [31] |
| | | Animal | Deficiency led to ↑ SB and base oxidation and ↑ *APE1* and *POLB* expression, | [33–36, 38] |
| | | | ↑ OGG1, MGMT and PARP activity/protein expression, but ↓ MGMT protein levels | |
| | | | In contrast: | |
| | | | Deficiency also showed ↓ PARP activity and poly ADP-ribose | [40–42] |
| | | Human | Folate led to ↓ BER (OGG1) activity in lowest quartile of initial folate status | [65] |
| | Vitamin A | Animal | Supplementation reversed aflatoxin B1- or *N*-nitrosodimethylamine-induced ↑ of PARP, DNA polymerase $\beta$ and DNA ligase activity | [45] |
| | Vitamin C | Human | Apparent enhanced repair of $H_2O_2$-induced SBs and oxidised bases in certain subjects | [56] |
| | Vitamin E + C | Human | ↑ BER (OGG1) activity after slow release | [60] |
| Flavonoid polyphenolics | Quercetin | Cultured cells | ↑ Expression of *hOGG1* after $H_2O_2$ exposure | [25] |
| | Luteolin | Cultured cells | ↑ SB rejoining after $H_2O_2$ exposure | [27] |
| | Silymarin | Cultured cells | ↑ MGMT activity & gene expression | [29] |
| | Rutin | Animal | Supplementation reversed aflatoxin B1- or *N*-nitrosodimethylamine -induced ↑ of PARP, DNA polymerase $\beta$ and DNA ligase activity, and ↓ induced DNA breaks | [48] |
| | Proanthocyanidins | Animal | ↑ expression of *XPA, XPC, DDB2* & *RPA1* | [50] |
| | Flavonoid-rich diet (either ± controlled supplementation with green tea, bilberry juice and soya products) | Human | ↑ expression of several BER and NER genes, ↓ expression of *AHR* | [67] |
| Triterpenoid | Ursolic acid | Cultured cells | ↑ SB rejoining after $H_2O_2$ exposure | [27] |
| Phenolic acid | Ellagic acid | Animal | ↑ expression of *XPA, ERCC5, DNA ligase III* | [49] |

**Table 4** continued

| Class of compound | Bioactive substance | Model | Effect | References |
|---|---|---|---|---|
| Other non-flavonoid phenolics | Curcumin | Cultured cells | ↑ MGMT activity & gene expression | [29] |
| | Resveratrol | Cultured cells | Activated the ATM/ATR-dependent DNA damage response and enhanced the fidelity of double SB repair | [30] |
| Quinone | Coenzyme $Q_{10}$ | Human | ↑ BER (OGG1) activity | [59] |
| Trace minerals | Zinc | Animal | Deficiency led to ↑ SBs and base oxidation, ↑ OGG1 protein levels, ↑ PARP protein levels & gene expression | [43, 44] |
| | Copper | Animal | Supplementation reversed aflatoxin B1-induced ↑ of PARP, DNA polymerase $\beta$ and DNA ligase activity | [46] |
| Plant extracts | *Salvia species* | Cultured cells | ↑ SB rejoining after $H_2O_2$ exposure | [26] |
| | *A. salicina* | Cultured cells | ↑ expression of various DNA repair genes | [32] |
| Fruit | Raspberry extract | Animal | ↑ expression of *XPA, ERCC5, DNA ligase III* | [49] |
| | Nectarine or peach | Animal | ↑ OGG incision activity, ↑ expression of *NTHL1*, ↓ *XPA & TDG* | [51] |
| | Kiwifruit | Human | ↑ BER (OGG1) activity, ↓ NER (UV-induced) activity, Upregulation of DNA repair gene set | [61, 62, 68] |
| | Blueberry–apple juice | Human | Enhanced repair in subjects carrying variant/low-activity genes for XPC/Rad23B | [66] |
| | Antioxidant-rich fruits | Human | Upregulation of DNA repair gene set | [68] |
| Vegetables | Carrots | Human | ↑ SB rejoining and oxidised base repair | [58] |
| Mix of micronutrients | Niacin + Zinc + carotenoids | Animal | ↑ repair of single and double SBs | [47] |
| | | Human | ↑ rejoining of $H_2O_2$-induced SBs | [47] |
| | Blend of antioxidant and other micronutrients (e.g. taurine, carnitine, tocopherol acetate and flavonoids) | Animal | Supplementation protected against oxidative stress induced ↓ NER activity in colon, ↓ levels of 8-oxoguanine after birth and ↑ BER activity right after birth in brain | [52, 53] |
| | Coenzyme $Q_{10}$ + riboflavin + niacin | Human | ↑ PARP activity in tamoxifen-treated cancer patients | [70] |
| | Diet enriched with antioxidant-rich foods (juices, fruit, vegetables, nuts, herbs) | Human | ↑ BER (OGG1) activity, but ↓ NER (UV-induced) activity | [62] |

level of exposure to DNA-damaging agents, if DNA repair is inducible by its substrate. The evidence for this is inconsistent: occupational, or lifestyle exposure to genotoxins is sometimes associated with increased repair capacity, sometimes with a decrease, and sometimes there is no effect (reviewed in [9]). Generally, in the reports we have reviewed here, if a change in repair activity is seen, it is an increase; but this is not invariably the case. In short, at present we can probably safely say that an observed increase in repair activity, whether as an induced response to the presence of DNA damage or as a consequence of micronutrient stimulation, for example, is likely to be beneficial to the organism. However, whether DNA repair capacity can be regarded as a marker of individual cancer risk is, in our view, doubtful.

Different repair pathways are likely to have different modes of regulation. BER is essential to deal with the inevitable presence of oxidation damage caused by reactive oxygen species in the body, and the enzymes are assumed to be constitutive. In contrast, NER deals with damage caused by agents that are not invariably present, such as UV in sunlight or food mutagens, and so this is more likely to be an inducible (facultative) process. Circumstantial evidence for this comes from the apparently much greater inter-individual variability in NER of UV damage (about sevenfold between highest and lowest) compared with BER of 8-oxoguanine (about threefold), measured in lymphocytes with the comet in vitro repair assay [18] (Fig. 4). It might afford an explanation of the contrasting effects of the high fruit and vegetable diet on BER and NER that we found in the Oslo antioxidant trial described above [62]; we can speculate that this diet might decreases the exposure to bulky adducting chemicals to such an extent—by accelerating their metabolism and excretion, for example—that the NER pathway becomes downregulated.

Most, though not all, of the human supplementation trials described here were carried out in healthy individuals. Various diseases are associated with inflammation and oxidative stress and, in consequence, show elevated levels of oxidative damage to DNA [78, 79]. There is recent evidence that a disease state can affect the capacity for DNA repair [80], and it is likely that the response to nutritional factors might be altered. Undoubtedly, it is simpler to study healthy individuals, avoiding the extra level of complexity that disease represents; but knowledge of an individual's level of DNA repair and responsiveness to nutritional factors might well play an important role in defining treatment regimes, particularly, of course, in the case of radio- and chemotherapy of cancer, where DNA damage to cancer cells is the prime mode of action.

While they are not necessarily unhealthy, individuals with a 'low antioxidant status'—i.e. relatively low plasma levels of the dietary antioxidants, as a result of a diet poor in fruits and vegetables—might be expected to respond more strongly to antioxidant supplements, whether the endpoint examined is oxidative damage to biomolecules such as DNA or inducible processes including DNA repair. The idea of studying subjects suffering from oxidative stress in order to maximise the effect of supplementation has been discussed [2], but not rigorously examined. It is the rationale behind the design of, for example, the Oslo Antioxidant Trial, with subjects at risk of cardiovascular disease through a generally poor lifestyle [62].

In animal or human studies, it is unusual to find attempts to establish whether dose–effect relationships exist. It is slightly more common in cell culture. The report by Lorenzo et al. [22] is one of the few to show a clear dose–response with different experimental endpoints, and at doses close to those attainable in vivo. On the other hand, different 'doses' of kiwifruit showed a clear effect on BER, but no sign of a dose–response relationship.

The lack of correspondence between DNA repair gene expression and enzyme activity is not surprising and is not unique to the DNA repair field. The amount of a particular protein present in the cell depends not only on the rate of transcription and translation, but also on the rate of turnover; and an enzyme's activity is not simply related to the amount of protein, but is affected also by the presence of various co-factors. Measurement of mRNA levels can of course give valuable information; but to compare phenotypes, there is no substitute for direct measurement of enzyme activities.

In conclusion, and bearing in mind a possible publication bias against reporting null effects, there are now sufficient studies showing positive or negative effects of micronutrients (or fruits/vegetables) on DNA repair to be confident that nutritional modulation of this process is real and of biological significance. While we do not fully understand the implications of, for example, opposite effects on BER and NER in the same lymphocyte samples, it is reasonable to speculate that the regulation of repair can be added to the list of biological processes that are influenced by what we eat—and, specifically, that this might constitute part of the explanation for the cancer-preventive effects of many plant-based foods. Future investigations in this field should focus on elucidating the underlying mechanisms of the nutritional modulation of DNA repair, studying redox as well as epigenetic regulation of DNA repair pathways in addition to changes in gene expression.

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## References


1. WCRF/AICR (2007) Food, nutrition, physical activity, and the prevention of cancer. American Institute for Cancer Research, Washington, pp 1–517
2. Møller P, Loft S (2006) Dietary antioxidants and beneficial effect on oxidatively damaged DNA. Free Rad Biol Med 41:388–415
3. Hoelzl C, Knasmüller S, Misik M, Collins A, Dusinska M, Nersesyan A (2009) Use of single cell gel electrophoresis assays for the detection of DNA-protective effects of dietary factors in humans: recent results and trends. Mutat Res 681:68–79
4. ESCODD, 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
5. D'Autréaux B, Toledano MB (2007) ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. Natl Rev Mol Cell Biol 8:813–824
6. Miller ER III, Pastor-Barriuso R, Dalal D, Riemersma RA, Appel LJ, Guallar E (2005) Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. Ann Intern Med 142:37–46
7. Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C (2007) Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. JAMA 297:842–857
8. Hwang ES, Bowen PE (2007) DNA damage, a biomarker of carcinogenesis: its measurement and modulation by diet and environment. Crit Rev Food Sci Nutr 47:27–50
9. Collins AR, Azqueta A (2012) DNA repair as a biomarker in human biomonitoring studies; further applications of the comet assay. Mutat Res. doi:10.1016/j.mrfmmm.2011.03.005
10. Collins AR (2004) The comet assay for DNA damage and repair. Mol Biotech 26:249–261
11. Collins AR, Horvathova E (2001) Oxidative DNA damage, antioxidants and DNA repair; applications of the comet assay. Biochem Soc Trans 29:337–341
12. Paz-Elizur T, Elinger D, Leitner-Dagan Y, Blumenstein S, Krupsky M, Berrebi A, Schechtman E, Livneh Z (2007) Development of an enzymatic DNA repair assay for molecular epidemiology studies: distribution of OGG activity in healthy individuals. DNA Repair 6:45–60
13. Elliott RM, Astley S, Southon S, Archer DB (2000) Measurement of cellular repair activities for oxidative DNA damage. Free Rad Biol Med 28:1438–1446
14. Sauvaigo S, Guerniou V, Rapin D, Gasparutto D, Caillat S, Favier A (2004) An oligonucleotide microarray for the monitoring of repair enzyme activity toward different DNA base damage. Analyt Biochem 333:182–192
15. Millau JF, Raffin AL, Caillat S, Claudet C, Arras G, Ugolin N, Douki T, Ravanat JL, Breton J, Oddos T, Dumontet C, Sarasin A, Chevillard S, Favier A, Sauvaigo S (2008) A microarray to measure repair of damaged plasmids by cell lysates. Lab Chip 8:1713–1722
16. Collins AR, Dusinska M, Horvathova E, Munro E, Savio M, Stetina R (2001) Inter-individual differences in DNA base excision repair activity measured in vitro with the comet assay. Mutagenesis 16:297–301
17. Langie SAS, Knaapen AM, Brauers KJJ, van Berlo D, van Schooten F-J, Godschalk RWL (2006) Development and validation of a modified comet assay to phenotypically assess nucleotide excision repair. Mutagenesis 21:153–158
18. Gaivão I, Piasek A, Brevik A, Shaposhnikov S, Collins AR (2009) Comet assay-based methods for measuring DNA repair in vitro; estimates of inter- and intra-individual variation. Cell Biol Toxicol 25:45–52
19. Langie SA, Cameron KM, Waldron KJ, Fletcher KP, von Zglinicki T, Mathers JC (2011) Measuring DNA repair incision activity of mouse tissue extracts towards singlet oxygen-induced DNA damage: a comet-based in vitro repair assay. Mutagenesis 3:461–471
20. Astley SB, Elliott RM, Archer DB, Southon S (2002) Increased cellular carotenoid levels reduce the persistence of DNA single-strand breaks after oxidative challenge. Nutr Cancer 43:202–213
21. Glei M, Liegibel UM, Ebert MN, Böhm V, Pool-Zobel BL (2002) Beta-carotene reduces bleomycin-induced genetic damage in human lymphocytes. Toxicol Appl Pharm 179:65–73
22. Lorenzo Y, Azqueta A, Luna L, Bonilla F, Dominguez G, Collins AR (2009) The carotenoid $\beta$-cryptoxanthin stimulates the repair of DNA oxidation damage in addition to acting as an antioxidant in human cells. Carcinogenesis 30:308–314
23. Aherne SA, O'Brien NM (2000) Lack of effect of the flavonoids, myricetin, quercetin, and rutin, on repair of $H_2O_2$-induced DNA single-strand breaks in Caco-2, HepG2 and V79 cells. Nutr Cancer 38:106–115
24. Duthie SJ, Collins AR, Duthie GG, Dobson VL (1997) Quercetin and myricetin protect against hydrogen peroxide-induced DNA damage (strand breaks and oxidised pyrimidines) in human lymphocytes. Mutat Res 393:223–231
25. Min K, Ebeler SE (2009) Quercetin inhibits hydrogen peroxide-induced DNA damage and enhances DNA repair in Caco-2 cells. Food Chem Toxicol 47:2716–2722
26. Ramos AA, Azqueta A, Pereira-Wilson C, Collins AR (2010) Polyphenolic compounds from Salvia species protect cellular DNA from oxidation and stimulate DNA repair in cultured human cells. J Agric Food Chem 58:7465–7471
27. Ramos AA, Pereira-Wilson C, Collins AR (2010) Protective effects of ursolic acid and luteolin against oxidative DNA damage include enhancement of DNA repair in Caco-2 cells. Mutat Res 692:6–11
28. Bacon JR, Williamson G, Garner RC, Lappin G, Langouët S, Bao Y (2003) Sulforaphane and quercetin modulate PhIP-DNA adduct formation in human HepG2 cells and hepatocytes. Carcinogenesis 24:1903–1911
29. Niture SK, Velu CS, Smith QR, Bhat GJ, Srivenugopal KS (2006) Increased expression of the MGMT repair protein mediated by cysteine prodrugs and chemopreventative natural products in human lymphocytes and tumor cell lines. Carcinogenesis 28:378–389
30. Gatz SA, Keimling M, Baumann C, Dörk T, Debatin KM, Fulda S, Wiesmüller L (2008) Resveratrol modulates DNA double-strand break repair pathways in an ATM/ATR-p53- and -Nbs1-dependent manner. Carcinogenesis 29:519–527
31. Williams JD, Jacobson MK (2010) Photobiological implications of folate depletion and repletion in cultured human keratinocytes. J Photochem Photobiol B Biol 99:49–61
32. Bouhlel I, Valenti K, Kilani S, Skandrani I, Ben Sghaier M, Mariotte AM, Dijoux-Franca MG, Ghedira K, Hininger-Favier I, Laporte F, Chekir-Ghedira L (2008) Antimutagenic, antigenotoxic and antioxidant activities of *Acacia salicina* extracts (ASE) and modulation of cell gene expression by $H_2O_2$ and ASE treatment. Toxicol In Vitro 22:1264–1272
33. Bagnyukova TV, Powell CL, Pavliv O, Tryndyak VP, Pogribny IP (2008) Induction of oxidative stress and DNA damage in rat brain by a folate/methyl-deficient diet. Brain Res 1237:44–51

34. Duthie SJ, Grant G, Pirie LP, Watson AJ, Margison GP (2010) Folate deficiency alters hepatic and colon MGMT and OGG-1 DNA repair protein expression in rats but has no effect on genome-wide DNA methylation. Cancer Prev Res 3:92–100
35. Klaude M, von der Decken A (1988) $O^6$-methylguanine-DNA methyltransferase levels in tissues of methionine-cysteine deficient subadult and adult mice. Comp Biochem Physiol C 91:603–606
36. Henning SM, Mckee RW, Swendseid ME (1989) Hepatic poly(ADP ribose) polymerase activity in methyl donor-deficient rats. J Nutr 119:1528–1531
37. Althaus FR, Kleczowska HE, Malanga M, Müntener CR, Pleschke JM, Ebner M, Auer B (1999) Poly ADP-ribosylation: a DNA break signal mechanism. Mol Cell Biochem 193:5–11
38. James SJ, Miller BJ, Basnakian AG, Pogribny IP, Pogribna M, Muskhelishvili L (1997) Apoptosis and proliferation under conditions of deoxynucleotide pool imbalance in liver of folate/methyl deficient rats. Carcinogenesis 18:287–293
39. Pogribny IP, Basnakian AG, Miller BJ, Lopatina NG, Poirier LA, James SJ (1995) Breaks in genomic DNA and within the p53 gene are associated with hypomethylation in livers of folate/methyl-deficient rats. Cancer Res 55:1894–1901 (Erratum in: Cancer Res 1995, 55:2711)
40. Zhang JZ, Henning SM, Swendseid ME (1993) Poly(ADP-Ribose) polymerase activity and DNA strand breaks are affected in tissues of niacin-deficient rats. J Nutr 123:1349–1355
41. Rawling JM, Jackson TM, Driscoll ER, Kirkland JB (1994) Dietary niacin deficiency lowers tissue poly(ADP-ribose) and NAD+ concentrations in Fischer-344 rats. J Nutr 124:1597–1603
42. Henning SM, Swendseid ME, Coulson WF (1997) Male rats fed methyl- and folate-deficient diets with or without niacin develop hepatic carcinomas associated with decreased tissue NAD concentrations and altered poly(ADP-ribose) polymerase activity. J Nutr 127:30–36
43. Song Y, Leonard SW, Traber MG, Ho E (2009) Zinc deficiency affects DNA damage, oxidative stress, antioxidant defenses, and DNA repair in rats. J Nutr 139:1626–1631
44. Song Y, Elias V, Loban A, Scrimgeour AG, Ho E (2010) Marginal zinc deficiency increases oxidative DNA damage in the prostate after chronic exercise. Free Rad Biol Med 48:82–88
45. Webster RP, Gawde MD, Bhattacharya RK (1996) Effect of different vitamin A status on carcinogen-induced DNA damage and repair enzymes in rats. In Vivo 10:113–118
46. Webster RP, Gawde MD, Bhattacharya RK (1996) Modulation by dietary copper of aflatoxin B1-induced activity of DNA repair enzymes poly (ADP-ribose) polymerase, DNA polymerase beta and DNA ligase. In Vivo 10:533–536
47. Sheng Y, Pero RW, Olsson AR, Bryngelsson C, Hua J (1998) DNA repair enhancement by a combined supplement of carotenoids, nicotinamide, and zinc. Cancer Det Prev 22:284–292
48. Webster RP, Gawde MD, Bhattacharya RK (1996) Protective effect of rutin, a flavonol glycoside, on the carcinogen induced DNA damage and repair enzymes in rats. Cancer Lett 109:185–191
49. Aiyer HS, Vadhanam MV, Stoyanova R, Caprio GD, Clapper ML, Gupta RC (2008) Dietary berries and ellagic acid prevent oxidative DNA damage and modulate expression of DNA repair genes. Int J Mol Sci 9:327–341
50. Vaid M, Sharma SD, Katiyar SK (2010) Proanthocyanidins inhibit photocarcinogenesis through enhancement of DNA repair and xeroderma pigmentosum group A-dependent mechanism. Cancer Prev Res 3:1621–1629
51. Croteau DL, de Souza-Pinto NC, Harboe C, Keijzers G, Zhang Y, Becker K, Sheng S, Bohr VA (2010) DNA repair and the accumulation of oxidatively damaged DNA are affected by fruit intake in mice. J Gerontol A Biol Sci Med Sci 65:1300–1311
52. Langie SA, Kowalczyk P, Tudek B, Zabielski R, Dziaman T, Olinski R, van Schooten FJ, Godschalk RW (2010) The effect of oxidative stress on nucleotide-excision repair in colon tissue of newborn piglets. Mutat Res 1–2:75–80
53. Langie SAS, Kowalczyk P, Tomaszewski B, Maas LM, Moonen EJ, Godschalk RWL, Tudek B, van Schooten FJ, Zabielski R, Mathers JC (2012) Redox regulation and epigenetic regulation of the APE1 gene in the brain of young piglets: the effect of early life exposures. Abstracts from the 9th International Comet Assay Workshop held in Kusadasi, Turkey; 13–16 Sep 2011. Mutagenesis (in press)
54. Taylor EM, Lehmann AR (1998) Conservation of eukaryotic DNA repair mechanisms. Int J Radiat Biol 74:277–286
55. Collins AR, Ma A, Duthie SJ (1995) The kinetics of repair of oxidative DNA damage (strand breaks and oxidised pyrimidines) in human cells. Mutat Res 336:69–77
56. Collins AR, Duthie SJ, Fillion L, Gedik CM, Vaughan N, Wood SG (1997) Oxidative DNA damage in human cells: the influence of antioxidants and DNA repair. Biochem Soc Trans 25:326–331
57. Torbergsen AC, Collins AR (2000) Recovery of human lymphocytes from oxidative DNA damage; the apparent enhancement of DNA repair by carotenoids is probably simply an antioxidant effect. Eur J Nutr 39:80–85
58. Astley S, Elliott RM, Archer DB, Southon S (2004) Evidence that dietary supplementation with carotenoids and carotenoid-rich foods modulates the DNA damage: repair balance in human lymphocytes. Br J Nutr 91:63–72
59. Tomasetti M, Alleva R, Borghi B, Collins AR (2001) In vivo supplementation with coenzyme Q10 enhances the recovery of human lymphocytes from oxidative DNA damage. FASEB J 15:1425–1427
60. Guarnieri S, Loft S, Riso P, Porrini M, Risom L, Poulsen H, Dragsted LO, Møller P (2008) DNA repair phenotype and dietary antioxidant supplementation. Br J Nutr 99:1018–1024
61. Collins AR, Harrington V, Drew J, Melvin R (2003) Nutritional modulation of DNA repair in a human intervention study. Carcinogenesis 24:511–515
62. Brevik A, Karlsen A, Azqueta A, Estaban AT, Blomhoff R, Collins A (2011) Both base excision repair and nucleotide excision repair in humans are influenced by nutritional factors. Cell Biochem Funct 29:36–42
63. Riso P, Martini D, Møller P, Loft S, Bonacina G, Moro M, Porrini M (2010) DNA damage and repair activity after broccoli intake in young healthy smokers. Mutagenesis 25:595–602
64. Caple F, Williams EA, Spiers A, Tyson J, Burtle B, Daly AK, Mathers JC, Hesketh JE (2010) Inter-individual variation in DNA damage and base excision repair in young, healthy non-smokers: effects of dietary supplementation and genotype. Br J Nutr 103:1585–1593
65. Basten GP, Duthie SJ, Pirie L, Vaughan N, Hill MH, Powers HJ (2006) Sensitivity of markers of DNA stability and DNA repair activity to folate supplementation in healthy volunteers. Br J Cancer 94:1942–1947
66. Langie SAS, Wilms LC, Hämäläinen S, Kleinjans JCS, Godschalk RWL, van Schooten FJ (2010) Modulation of nucleotide excision repair in human lymphocytes by genetic and dietary factors. Br J Nutr 103:490–501
67. Guarrera S, Sacerdote C, Fiorini L, Marsala R, Polidoro S, Gamberini S, Saletta F, Malaveille C, Talaska G, Vineis P, Matullo G (2007) Expression of DNA repair and metabolic genes in response to a flavonoid-rich diet. Br J Nutr 98:525–534
68. Bøhn SK, Myhrstad MC, Thoresen M, Holden M, Karlsen A, Tunheim SH, Erlund I, Svendsen M, Seljeflot I, Moskaug JØ, Duttaroy AK, Laake P, Arnesen H, Tonstad S, Collins A, Drevon CA, Blomhoff R (2010) Blood cell gene expression associated with cellular stress defense is modulated by antioxidant-rich food

in a randomised controlled clinical trial of male smokers. BMC Med. http://www.biomedcentral.com/1741-7015/8/54
69. Møller P, Vogel U, Pedersen A, Dragsted LO, Sandström B, Loft S (2003) No effect of 600 grams fruit and vegetables per day on oxidative DNA damage and repair in healthy nonsmokers. Cancer Epi Biomark Prev 12:1016–1022
70. Premkumar VG, Yuvaraj S, Shanthi P, Sachdanandam P (2008) Co-enzyme Q10, riboflavin and niacin supplementation on alteration of DNA repair enzyme and DNA methylation in breast cancer patients undergoing tamoxifen therapy. Br J Nutr 100:1179–1182
71. Mathers JC, Strathdee G, Relton CL (2010) Induction of epigenetic alterations by dietary and other environmental factors. In: Herceg Z, Ushijima T (eds) Advances in genetics, vol 71. Academic Press, Burlington, pp 1–39
72. Bird A (2002) DNA methylation patterns and epigenetic memory. Genes Dev 1:6–21
73. Jones PA, Takai D (2001) The role of DNA methylation in mammalian epigenetics. Science 293:1068–1070
74. Zawia NH, Lahiri DK, Cardozo-Pelaez F (2009) Epigenetics, oxidative stress, and Alzheimer disease. Free Radic Biol Med 9:1241–1249
75. Fang MZ, Wang Y, Ai N, Hou Z, Sun Y, Lu H, Welsh W, Yang CS (2003) Tea polyphenol (2)-epigallocatechin-3-gallate inhibits DNA methyltransferase and reactivates methylation-silenced genes in cancer cell lines. Cancer Res 63:7563–7570
76. Fang MZ, Chen D, Sun Y, Jin Z, Christman JK, Yang CS (2005) Reversal of hypermethylation and reactivation of p16INK4a, RARbeta, andMGMT genes by genistein and other isoflavones from soy. Clin Cancer Res 11:7033–7041
77. Langie SA, Tomaszewski B, Cameron KM, Fletcher KP, Lisanti S, Godschalk RW, van Schooten FJ, von Zglinicki T, Mathers JC (2011) The ageing brain: effects on DNA repair and DNA methylation in mice. In: Abstracts of UKEMS/Dutch EMS-sponsored workshop on biomarkers of exposure and oxidative DNA damage and 7th GUM $^{32}$P-Postlabelling Workshop, Münster, Germany; March 28–29, 2011. Mutagenesis 26:706
78. Halliwell B (1994) Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? Lancet 344:721–724
79. Dusinska M, Lietava J, Olmedilla B, Raslova K, Southon S, Collins AR (1999) Indicators of oxidative stress, antioxidants and human health. In: Basu TK, Temple NJ, Garg ML (eds) Antioxidants in human health. CAB International, Wallingford, pp 411–422
80. Obtulowicz T, Swoboda M, Speina E, Gackowski D, Rozalski R, Siomek A, Janik J, Janowska B, Ciesla JM, Jawien A, Banaszkiewicz Z, Guz J, Dziaman T, Szpila A, Olinski R, Tudek B (2010) Oxidative stress and 8-oxoguanine repair are enhanced in colon adenoma and carcinoma patients. Mutagenesis 25:463–471