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Dietary and genetic modulation of DNA repair in healthy human adults

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The DNA in all cells of the human body is subject to damage continuously from exogenous
agents, internal cellular processes and spontaneous decomposition. Failure to repair such
damage is fundamental to the development of many diseases and to ageing. Fortunately, the
vast majority of DNA damage is detected and repaired by one of five complementary DNA
repair systems. However, recent studies have shown that even in healthy individuals there is a
wide inter-individual variation in DNA repair capacity. Part of this variation can be accounted
for by polymorphisms in the genes encoding DNA repair proteins. However, it is probable that
environmental factors, including dietary exposure as well as diet–gene interactions, are also
responsible for much of the difference in repair capacity between individuals. Whilst there is
some evidence from human studies that generalised malnutrition or low intakes of specific
nutrients may affect DNA repair, as yet there is limited understanding of the molecular
mechanisms through which nutrients can modulate this key cellular process.

DNA repair systems: Polymorphisms: Diet–gene interactions

DNA is constantly subject to damage arising either spontaneously or from a plethora of endogenous and exogenous agents. Such DNA damage, if unrepaired, leads to aberrant gene expression and is fundamental to the initiation and development of cancer, with additional implications for ageing and a wide range of diseases including diabetes, vascular disease and dementia. Although DNA damage arises frequently, with 2×10^5 damaging events occurring per cell per 24 h, mutation is rare, with only one in 10^{10} nucleotides becoming mutated per cell generation (Jackson & Loeb, 2001). This low incidence of mutation is largely a result of the ability of cells to perform an array of evolutionarily-conserved DNA repair mechanisms that maintain the integrity of the genome. Human cells have five complementary DNA repair systems, encoded by >150 genes and protein products. Each system detects and repairs specific types of DNA damage (see Table 1). The five human DNA repair systems are: direct reversal; mismatch repair; base excision repair (BER); double-strand break repair; nucleotide excision repair (NER).

DNA repair systems

Direct reversal

Direct reversal involves the direct removal of damage adducts from the DNA rather than entire damaged nucleotides. Although direct reversal systems are common in prokaryotic systems, in man only one such system exists. Alkylation of guanine at the O^6 position results in an O^6-alkylguanine lesion, which is capable of pairing with thymine and, if left unrepaired, results in a G→A transition mutation post replication. In human subjects O^6-alkylguanine can be repaired by O^6-methylguanine-DNA methyltransferase, which transfers the alkyl group to a cysteine residue within the protein, restoring the damaged guanine to its correct form.

Mismatch repair

Mismatch repair serves to repair a number of common mutagenic lesions. Misincorporation (mispairing) of bases

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Table 1. Summary of human DNA repair mechanisms

Repair pathway	Damage repaired	Sources of damage
Mismatch repair	Mismatched base pairs, small insertion loops	Replication errors, minor base modifications (oxidation, alkylation)
Base excision repair	Oxidised bases alkylation, abasic/AP sites, single-strand breaks	Reactive oxygen species, alkylating agents, spontaneous hydrolysis
Nucleotide excision repair	Bulky helix-distorting lesions	UV light, cigarette smoke, dietary factors (aflatoxin, PhiP, polyaromatic hydrocarbons(benzo[α]pyrine))
Double-strand break repair	Double-strand breaks, DNA cross-links	Ionising radiation, cross-linking agents (cisplatin), replication errors
Direct reversal	Alkylated bases: O^6-methyl-guanine	Alkylating agents, nitrosoureas, streptozotocin

PhiP, 2-amino-1-methyl-6-phenylimidazo [4,5-β] pyridine; AP, apurinic/apyrimidinic.

(e.g. T/C or A/G), caused by errors in DNA replication as well as alkyl adducts, and oxidatively-damaged bases are repaired by mismatch repair. One such mismatch lesion that is commonly found arises from the deamination of cytosines to uracil or methylated cytosines to thymine, which if unrepaired cause a C→T transversion post DNA replication. Mismatch repair also serves to repair small insertions and deletions in the DNA caused when the DNA polymerase 'slips', usually in repetitive microsatellite DNA sequences.

The process of mismatch repair involves twenty-six genes and proteins and their encoded proteins that act in three stages to remove and repair damage. First, the mismatched base is recognised by the MSH2/MSH6 heterodimer. The damaged strand around the lesion is unwound and removed, involving the MLH1/PMS2 heterodimer and other proteins. This stage leaves a single-stranded gap, which is repaired by specific polymerases and DNA ligase 1 (Jiricny & Nystrom-Lahti, 2000).

Double-strand break repair

Double-strand breaks are the most cytotoxic and potentially mutagenic lesions that can afflict DNA, as they can quickly lead to chromosomal breaks or exchanges and cell death. Double-strand breaks are induced by ionising radiation, mechanical stress and calastogens (agents causing visible chromosomal damage) such as the chemotherapeutic agent cisplatin (Pfeiffer et al. 2000). Repair of double-strand breaks may proceed through two distinct pathways: the error-free homologous recombination; the error-prone non-homologous end joining, which is crude in that it simply ligates the two ends of a double-strand break (Christmann et al. 2003). Homologous recombination uses the homology of the sister chromatid to facilitate high-fidelity repair of double-strand breaks. Homologous recombination begins with the digestion of one strand of DNA at the break site, resulting in a single-stranded 3′ overhang (Christmann et al. 2003). The RAD52 and RAD51 proteins promote the formation of nucleofilaments and facilitate interactions between the damaged strand and the undamaged DNA on the sister chromosome. Thereafter, strand exchange takes place, allowing the synthesis of new DNA across the break using the undamaged chromosome as a template.

Base excision repair

BER is responsible for the repair of numerous small mutagenic lesions that do not disrupt the DNA double helix. The most-frequently-repaired lesions are apurinic/apryimidinic sites, in which the base is missing from the DNA backbone (Seeberg et al. 1995). Base deamination, oxidation and alkylation, which can cause mispairing and lead to mutations post replication are also repaired through the BER pathway. BER can be divided into three stages: damage is recognised; damaged bases are removed creating an apurinic/apryimidinic site; apurinic/apryimidinic sites are filled with the synthesis of new DNA. In human subjects there are eleven glycosylases that are able to recognise damaged bases and cleave them from the sugar phosphate backbone, creating an apurinic/apryimidinic site (Hung et al. 2005). For repair to continue apurinic/apryimidinic sites must be incised to create a single-strand break, either by the glycosylase itself or by the apurinic/apryimidinic endonuclease enzyme. Such sites (created spontaneously, by glycosylases or by apurinic/apryimidinic endonuclease) are repaired in one of two ways: (1) short-patch repair, in which a single nucleotide in inserted into the apurinic/apryimidinic site by DNA polymerase β and DNA ligase; (2) long-patch repair, in which an additional two to thirteen nucleotides are removed and the gap repaired by polymerases and ligase (Christmann et al. 2003).

Nucleotide excision repair

NER is a complex DNA repair pathway involving over thirty genes and proteins (Friedberg, 2001) and is responsible for the repair of any DNA lesion that causes distortion to the DNA double helix. NER commonly repairs lesions induced by UV as well as numerous exogenous agents such as those derived from food and smoking, including 2-amino-1-methyl-6-phenylimidazo [4,5-β] pyridine and benzo[α]pyrene diol-epoxide.

NER can be divided into two distinct pathways: global genomic repair; transcription-coupled repair. Transcription-coupled repair repairs lesions that block the progression of RNA polymerase along actively-transcribed genes. Global genomic repair is independent of transcription and acts to repair lesions in non-transcribed regions of the genome as well as those in the non-transcribed strand of
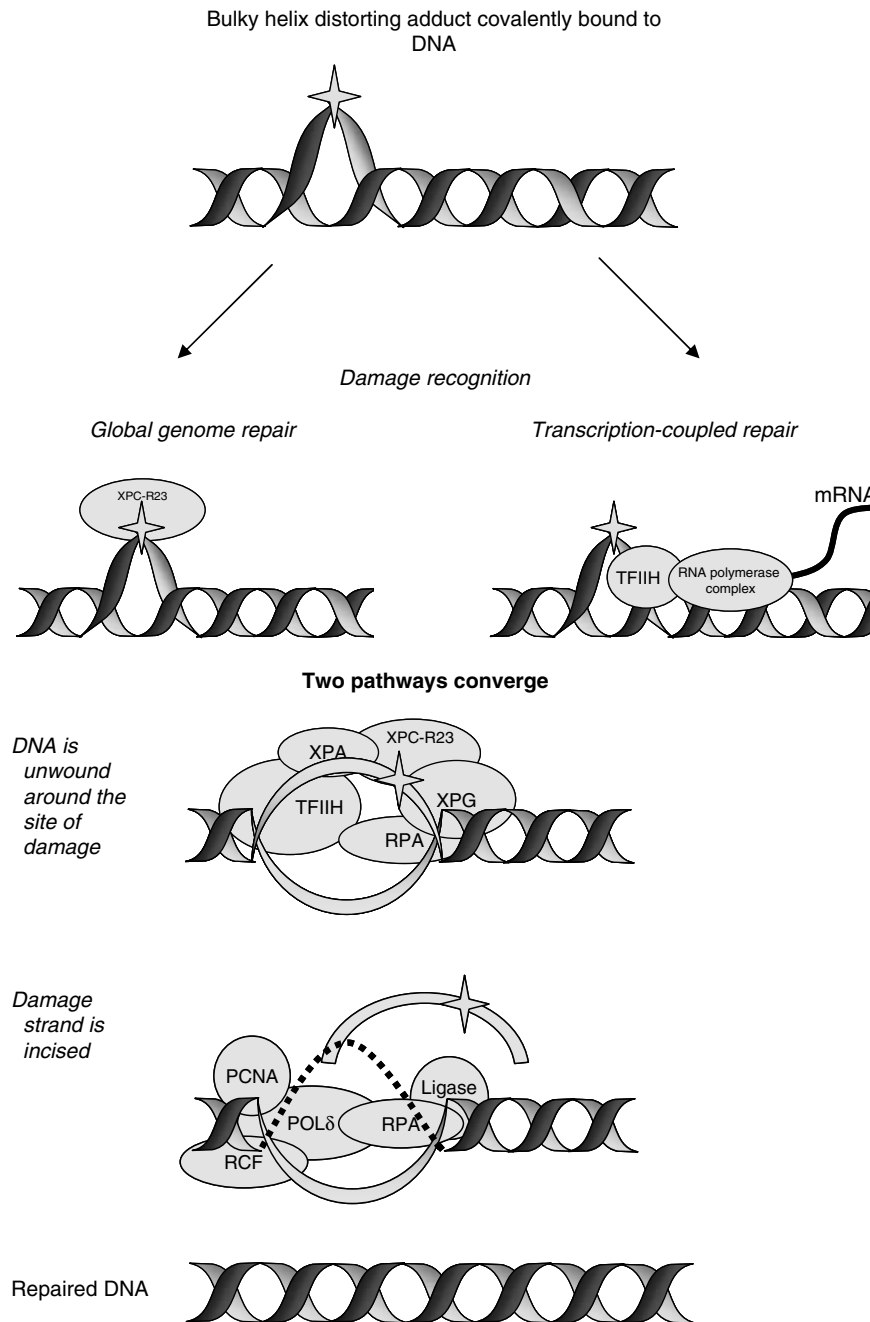
Fig. 1. Overview of nucleotide excision repair pathway. XPA, XPC, XPG, xeroderma pigmentosum complementation groups A, C and G; TFIIH, transcription factor 11H; RPA, replication protein A; PCNA, proliferating cell nuclear antigen; RCF, replication factor C; POLδ, DNA polymerase δ. (Adapted from Friedberg, 2001.)

active genes. These two pathways differ only in the way DNA damage is detected, with the subsequent repair steps being identical (see Fig. 1).

In transcription-coupled repair a helix-distorting lesion located in an actively-transcribed gene halts the progression of the RNA polymerase at the site of damage. The stalling of the polymerase initiates the removal and repair of the damage, allowing transcription to continue. In global genomic repair the xeroderma pigmentosum (XP) complementation group C (XPC) protein, in association

with UV excision repair protein RAD23 homologue B, recognises and binds to the helix distortion caused by the damage rather than to the lesion itself. The initiation of transcription-coupled repair is faster than the initiation of global genomic repair, presumably because of the implications of DNA damage during mRNA synthesis (Benhamou & Sarasin, 2000).

Once the site of damage has been recognised, DNA is unwound around the lesion by two helicase enzymes XPB (unwinding 3′ to 5′) and XPD (unwinding 5′ to 3′). Both

Table 2. Summary of genetic syndromes characterised by a defect in DNA repair

Disease	Type of genetic disorder	DNA repair system disrupted
Ataxia telangiectasia	Autosomal recessive	Strand break repair
Nijmegen breakage syndrome	Autosomal recessive	Strand break repair
Bloom's syndrome	Autosomal recessive	Strand break repair*
Werner syndrome	Autosomal recessive	Strand break repair
Rothman-Thomson syndrome	Autosomal recessive	Strand break repair*
Fanconi anaemia	Autosomal recessive	Strand break repair*
Hereditary non-polyposis colo-rectal cancer	Autosomal dominant	Mismatch repair
Xeroderma pigmentosa	Autosomal recessive	Nucleotide excision repair
Trichothiodystrophy	Autosomal recessive	Nucleotide excision repair
Cockayne syndrome	Autosomal recessive	Transcription-coupled nucleotide excision repair

*May be disruption to more than one repair pathway.

XPD and XPB are part of the basal transcription factor IIH complex, which is essential for the initiation of transcription by RNA polymerase II. This unwinding creates distinct junctions between double-stranded and single-stranded DNA, which are essential for the progression of repair (Friedberg, 2001). The damaged strand of DNA is incised at these two junctions, i.e. at the 3′ side of the damage by XPG protein and at the 5′ side by the XPF–excision repair cross-complementing rodent repair deficiency complementation group 1 (ERCC1) complex. This step results in cleavage and subsequent removal of approximately thirty nucleotides of single-stranded DNA containing the damage. The remaining single-stranded gap is filled by DNA polymerase δ or ε and the new strand is joined to the existing DNA by DNA ligase (de Laat et al. 1999).

Inter-individual variation in DNA repair capacity and disease

Loss, or severe impairment, of DNA repair processes results in a decrease in the ability to process DNA damage and ultimately leads to disease, most often cancer. A number of familial syndromes arise as a result of a disruption to DNA repair processes (summarised in Table 2). One such disorder is XP, which is a rare autosomal recessive syndrome characterised by a severe photosensitivity of sunlight-exposed areas of the skin from an early age. Patients with XP have inactivating mutations in any one of seven genes encoding proteins essential for NER, have ≤1000-fold increased risk of skin malignancies and are also ten to twenty times more likely to develop internal tumours at <20 years of age (de Boer & Hoeijmakers, 2000).

Fortunately, such heritable syndromes, resulting in a complete disruption to DNA repair processes, are rare and account for only a small proportion of cancer incidence. However, large inter-individual variations in DNA repair capacity have been observed in healthy populations. Qiao et al. (2002a) have found a 4·7-fold variation in NER capacity, measured using the host cell reactivation assay, in 102 healthy subjects (Qiao et al. 2002a). Also, DNA repair of UV-induced damage, measured using the unscheduled DNA synthesis assay varies over an approximately 8-fold range, again in healthy control subjects (Mohankumar et al. 1998). Amongst sixty-three young (mean age 21 years) healthy volunteers recruited to the Dietary Antioxidant Repair Trial there is a 10-fold inter-individual variation in NER capacity, measured using the host cell reactivation assay (see Fig. 2; Tyson et al. 2005).

Inter-individual variation in DNA repair capacity within the general population is believed to be associated with cancer risk. Case–control studies have shown that cancer patients have a repair capacity below that of healthy matched controls. For example, reduced NER capacity has been associated with increased risk of cancer of the skin, lung, head and neck, and breast (Ana et al. 2005). Additionally, there may be links between recombinational repair and sporadic breast cancer (Ralhan et al. 2006) and a reduced BER capacity may also increase risk of lung cancer (Paz-Elizur et al. 2003). In a review of studies carried out before1998 it has been concluded that there is a consistent positive correlation between reduced DNA repair capacity and cancer occurrence, with OR of between 1·4 and 75·3 (Berwick & Vineis, 2000). However, as yet no prospective studies have been conducted and a reduced DNA repair capacity could be a result of, rather than a cause of, cancer.

The possible reasons for the substantial inter-individual variation in DNA repair capacity may include genetic factors, such as polymorphisms in DNA repair genes, and variation in environmental exposure, including dietary and lifestyle factors. For example, variation in DNA repair is partly attributable to variations in age and BMI, both of which are inversely associated with NER capacity (Tyson et al. 2006).

Genetic polymorphisms, DNA repair and cancer risk

Common genetic polymorphisms in DNA repair genes have been associated with the inter-individual variation in DNA repair and also with cancer risk. The majority of genes encoding proteins important in DNA repair are polymorphic (Mohrenweiser et al. 2002). For example, there are a number of polymorphisms in the ERCC2 gene
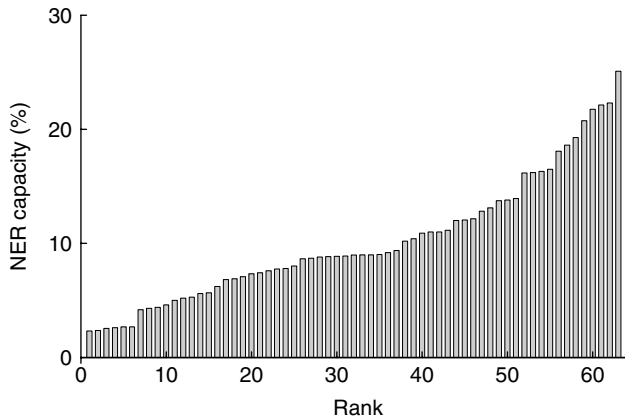
Fig. 2. Inter-individual variation in nucleotide excision repair capacity, measured using the host cell reactivation assay, in sixty-three healthy volunteers recruited to the Diet and Reinfarction Trial (Tyson *et al.* 2005).

that encodes for the XPD protein, a DNA helicase essential for NER. The *ERCC2* Asp312Asn single-nucleotide polymorphism (SNP) has a modulatory effect on both NER capacity and adduct levels, with carriers of one or more of the Asn alleles showing reduced repair capacity (Spitz *et al.* 2001) and increased adduct levels (Hou *et al.* 2002; Tang *et al.* 2002) compared with homozygotes of the Asp allele. In addition, the Gln allele of the *ERCC2* Lys751Gln SNP has been associated with both reduced NER capacity (Spitz *et al.* 2001; Qiao *et al.* 2002b) and increased prevalence of adducts (Palli *et al.* 2001; Hou *et al.* 2002; Tang *et al.* 2002). Similar effects are seen in other repair systems, with variants of the BER proteins 8-oxoguanine DNA glycosylase 1 and X-ray cross-complementing group 1 showing reduced activity (Pachkowski *et al.* 2006; Sokhansanj & Wilson, 2006).

The same polymorphisms that appear to modulate repair capacity have been associated with cancer risk. Again, polymorphisms of the *ERCC2* gene are implicated, with carriers of the uncommon alleles being found in greater frequencies amongst groups of cancer sufferers than amongst controls (Benhamou & Sarasin, 2005). Also, polymorphisms in BER genes are often found to convey increased cancer risk (Hung *et al.* 2005). A meta-analysis of BER polymorphisms and cancer risk (Hung *et al.* 2005) has shown that Cys/Cys homozygotes for the 8-oxoguanine DNA glycosylase 1 Ser326Cys polymorphism have an increased risk of lung cancer (OR 1·24) and the carriers of the Trp allele of the X-ray cross-complementing group 1 194Trp SNP are protected against tobacco-related cancers.

However, such genetic associations with repair capacity and with cancer risk are not observed consistently. For example, in contrast to the other findings the *ERCC2* Lys751Gln SNP has been reported to have no effect on NER capacity (Qiao *et al.* 2002b), adduct levels (Duell *et al.* 2000) or cancer risk (Benhamou & Sarasin, 2005). This lack of reproducibility in genetic-association studies is common and may arise for a number of reasons. Differential findings may be related to differences in study

size and statistical power, with large studies having greater power to detect smaller effects. Alternatively, the interactions between genetic and environmental factors could be important, with the effect of genotype being apparent only in certain environmental settings. Finally, polymorphisms believed to affect repair capacity and cancer may be in linkage disequilibrium with neighbouring polymorphisms in the same or adjacent genes.

The effect of multiple polymorphisms in NER genes on NER repair capacity has been investigated. In the Dietary Antioxidant Repair Trial (Tyson *et al.* 2006) NER capacity was measured in sixty-three subjects who had been genotyped for polymorphisms in key NER genes. Here, two significant gene–gene interactions were found when subjects were grouped according to genotype for each polymorphism based on the presence or absence of the uncommon allele. The *XPC* Lys939Gln SNP appears to interact with both the *ERCC5* Asp1104His and the *ERCC2* Lys751Gnl SNP ($P = 0·01$ and $P = 0·03$ respectively), with repair capacity being dependent on specific allelic combinations of the polymorphisms. In the case of *ERCC5* Asp1104His SNP repair capacity appears to be independent of the Asp allele, whereas carriers of the His allele show high repair in the absence of the Gln allele of the *XPC* Lys939Gln SNP and low repair in the presence of this SNP. In the case of the *ERCC2* Lys751Gnl SNP repair capacity is independent of the presence the Gln allele, but homozygotes of the Lys allele have a high repair capacity in the absence of the Gln allele of the Lys751Gnl SNP and low repair capacity in the presence of this SNP.

Nutritional modulation of DNA repair

There is a clear link between diet and cancer, with variation in diet accounting for over one-third of the variation in cancer incidence (Doll & Peto, 1981). Epidemiological evidence shows convincingly that diets rich in fruit and vegetables are associated with a decreased risk of cancer of the lung, mouth, pharynx, oesophagus, colon and rectum (World Cancer Research Fund/American Institute for Cancer Research, 1997). There are also probable risk reductions for laryngeal, pancreatic, breast and bladder cancers associated with such diets (World Cancer Research Fund/American Institute for Cancer Research, 1997). More recently, the European Prospective Investigation into Cancer and Nutrition Study has shown that diets low in fibre (Bingham *et al.* 2003) and/or high in red meat are associated with an increased risk of colo-rectal cancer (Gonzalez *et al.* 2002; Norat *et al.* 2005). Much effort has been put into defining the dietary constituents and the biological mechanisms underlying the protective effects of diet on cancer. Many studies (see Moller & Loft, 2004) have investigated the effects of dietary components on levels of DNA damage. However, DNA damage is only of consequence if it cannot be adequately repaired, so that the individual's ability to perform DNA repair may be at least as important as the damage they sustain.

To date a relatively small number of studies (summarised in Table 3) have investigated the influence of diet or nutrient status on DNA repair processes. Perhaps not

Table 3. Summary of studies investigating dietary modulation of DNA repair

Reference	Study design	DNA repair measurement	Subjects	Main outcomes
Moller & Loft (2004)	Blinded placebo-controlled intervention. Three treatment groups: (1) slow release vitamin C + vitamin E; (2) plain release vitamin C + vitamin E; (3) placebo. Treated for 4 weeks	OGG1 mRNA levels	Forty-eight male smokers (20–65 years)	No effect of intervention on OGG1 expression
Moller et al. (2003)	Intervention study. Parallel design with subjects treated for 24 d with: (1) 600 g fruit and vegetables; (2) tablets providing 'same' amount of antioxidants and minerals; (3) placebo	OGG1 and ERCC1 mRNA levels	Forty-three subjects (twenty-one female) aged 21–56 years	No effect of intervention on OGG1 or ERCC1 expression
Collins et al. (2003)	Randomised cross-over intervention study. Subjects supplemented with one, two and three kiwi fruit for 3 weeks with 2-week wash-out periods between treatments	BER capacity using a modified comet assay and OGG1 and APE1 mRNA levels	Fourteen (eight female) healthy non-smokers aged 26–54 years	Increase in BER capacity after supplementation, independent of no. of kiwi consumed. No change in OGG1 or APE1 expression
Tomasetti et al. (2001)	Intervention study. Subjects took 100 mg ubiquinine-10 for 1 week	BER capacity using a modified comet assay	Six healthy non-smokers (three female) aged 26–54 years	Significant ($P<0.05$) 2·7-fold increase in repair capacity after supplementation
Astley et al. (2004)	Intervention study. Subjects assigned to treatment group and asked to take one of the following for 3 weeks: (1) 200 g cooked minced carrots; (2) total of 11·9 mg α- and β-carotene + 1·75 mg α-tocopherol; (3) 298 g tinned mandarins, 60 mg vitamin C; (4) placebo capsules	The repair of single-strand breaks and oxidative lesions was measured using a patch synthesis assay	Sixty-four healthy male subjects aged 18–50 years	Repair capacity increased significantly ($P<0.05$) only after treatment 1 (supplementation with the cooked minced carrots). No effect of treatments 2, 3 or 4
Sheng et al. (1998)	Intervention study. Subjects supplemented with a combination of nicotinamide, Zn and carotenoids for 7 weeks	Followed repair of H_2O_2-induced DNA damage over time using the comet assay	Four healthy volunteers	Significant increase in repair seen ($P<0.01$) post supplementation
Chiricolo et al. (1993)	Intervention study. Children with Down syndrome (DS) and healthy controls were given 1 mg Zn/kg body weight for 4 months	Repair of γ-radiation followed using alkali elution assay	Fifteen children in each treatment group, mean age 9 years	'Abnormally' high repair rates in DS children pre-supplementation was returned to normal by Zn supplementation. No effect of supplementation in control group
Basten et al. (2006)	Blinded placebo-controlled intervention in human subjects supplemented with 1·2 mg folic acid or a placebo daily for 6 weeks	BER capacity using a modified comet assay	Sixty-one healthy subjects (20–60 years), thirty supplemented, thirty-one placebo control	No effect of folic acid on BER activity
Wei et al. (2003)	Retrospective observational study. Assessed dietary folate intake using FFQ	NER capacity using HCR assay	559 individuals with no malignancies, (mean age 61 years)	18% reduction ($P<0.01$) in repair capacity in those in the lowest tertile of folate intake compared with those in the highest tertile
Gonzalez et al. (2002)	Observational study of Mexican children. Three groups studied: (1) well-nourished non-infected; (2) well-nourished infected; (3) malnourished infected	Followed repair of H_2O_2-induced DNA damage over time using the comet assay	Nineteen children aged 6–26 months	The malnourished infected children had a significantly ($P<0.05$) lower repair capacity than the other two treatment groups

OGG1, 8-oxoguanine DNA glycosylase 1; ERCC1, excision repair cross-complementing rodent repair deficiency complementation group 1; BER, base excision repair; APE1, human apurinic endonuclease 1; NER, nucleotide excision repair; HCR, host cell reactivation.
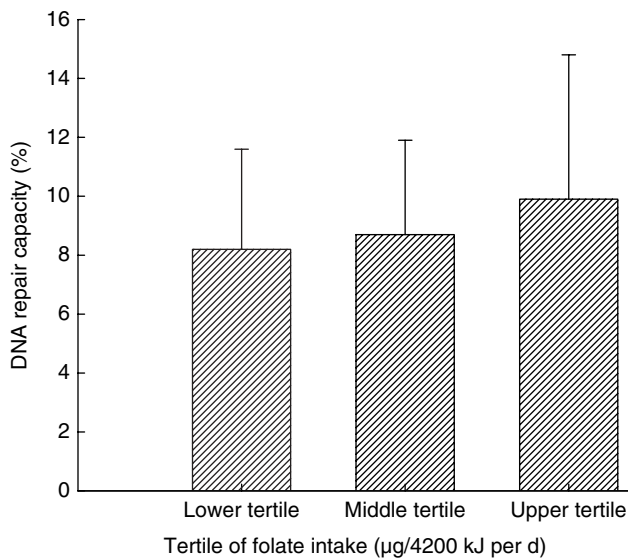
Fig. 3. Association between low dietary folate and DNA repair capacity (Wei *et al.* 2003). DNA repair capacity was measured using the host cell reactivation assay in 559 healthy individuals. Values are means with their standard errors represented by vertical bars. DNA repair capacity in the lowest tertile of folate intake was significantly lower than that in the highest tertile of folate intake ($P < 0.01$).

surprisingly, malnourished Mexican children carrying an infection have been found to have a lower capacity for repair of oxidative DNA damage when compared with uninfected well-nourished children (Gonzalez *et al.* 2002). Such findings indicate that undernutrition and associated ill health may impair the capacity for DNA repair, but they are not able to identify which factors, dietary or otherwise, are responsible. However, supplementation of healthy human volunteers with carrot extract increases repair of an H_2O_2-damaged plasmid DNA (Astley *et al.* 2004). Interestingly, supplementation with amounts of α- (3·7 mg) and β- (8·2 mg) carotene equivalent to that in the carrot extract have no effect on repair capacity. In the same study separate supplements of 298 g tinned mandarin oranges or 60 mg vitamin C were found to have no effect on repair capacity. In a further study (Collins *et al.* 2003) BER capacity, as measured using a modified comet assay, was found to be increased after supplementation of healthy human volunteers with one, two or three kiwi fruit per d, but there was no evidence of a dose–response relationship. Similarly, supplementation with the antioxidant ubiquinone-10 enhances BER activity in human subjects (Tomasetti *et al.* 2001). In a cohort of 559 healthy individuals those in the lowest tertile of folate intake were reported (Wei *et al.* 2003) to have an 18 % reduction in NER capacity compared with those in the highest tertile of intake (see Fig. 3). A significant ($P < 0.001$) inverse association was found between total dietary folate intake (adjusted for total energy intake) and repair capacity in non-users of supplemental folate. These findings are supported by *in vitro* experiments that have shown that folate depletion decreases the repair of peroxide-induced damage in human lymphocytes (Duthie

& Hawdon, 1998). In contrast, a recent placebo-controlled intervention study (Basten *et al.* 2006) has found no evidence that supplementation of healthy volunteers with 1·2 mg folic acid/d for 6 weeks affects BER activity (measured using a modified comet assay). However, the failure of the study to detect effects of supplementation does not prove that enhanced folate status is without effect on DNA repair capacity. The intervention study was of modest size (thirty subjects receiving supplement and thirty-one controls) and would therefore be able to detect only relatively large changes in repair. There was also a suggestion that those subjects with the lowest folate status initially showed a decrease in repair in response to supplementation (Basten *et al.* 2006). Additionally, it remains to be determined whether the dose of folic acid, which is well above the UK reference nutrient intake of 200 µg/d (Department of Health, 2002), is necessary to support optimal DNA repair.

Mechanisms through which diet may modulate DNA repair processes

As there has been limited research into the dietary modulation of DNA repair, there is little direct evidence for biological mechanisms through which DNA repair processes may be modified by diet. However, a number of possible mechanisms can be, and have been, proposed based on previous observations and knowledge of how diet can affect other cellular processes. Diet may modify DNA repair through changes at the level of transcription, although effects on mRNA stability translation, protein stability and protein trafficking should also be considered. Candidate mechanisms through which nutrients and other dietary components may influence gene expression have been reviewed recently (Mathers, 2006). The availability of any nutrient necessary for the proficient synthesis of DNA, RNA and proteins may impact on DNA repair processes. This factor may explain the decreased BER capacity of malnourished children reported by Gonzalez *et al.* (2002), although as yet there is no direct mechanistic evidence.

Some of the most convincing indirect mechanistic evidence in this area comes from *in vitro* work with Se, which (in the form of selenomethionine) increases NER capacity 2-fold in human fibroblast cells (Seo *et al.* 2002b). Follow-up work (Seo *et al.* 2002a) has found that this Se-induced increase in repair is dependent on both P53 and the protein redox factor-1, with Se reducing specific cytosine residues on P53. Furthermore, this Se-dependent reduction of P53 is lost in cells lacking redox factor-1, suggesting that redox factor-1 is an intermediary in the Se-dependent activation of P53. It has now been demonstrated (Fischer *et al.* 2006) that Se-induced activation of P53 is also dependent on the presence of the BRCA1 protein, which is believed to be important in recombinational repair and is frequently mutated in heritable breast cancer.

A further mechanism through which Se may influence DNA repair is through alterations to the binding activity of transcription factors and DNA repair proteins. Using mobility shift assays in an extracellular system it has been

shown (Handel *et al.* 1995) that Na_2SeO_3 (1 μM) inhibits binding of the transcription factor activator protein-1 to its consensus DNA sequence by 50%. Similar effects on consensus sequence binding of NF-κB, as well as transcription factors SP-1 and SP-3, have been reported (Youn *et al.* 2001). More specifically, reducible Se compounds inhibit the NER protein XPA and the BER protein formamidopyrimidine DNA glycosylase, both of which belong to a family of DNA-binding proteins (Blessing *et al.* 2004), known as Zn finger proteins, in which Zn atoms are complexed with cysteine and/or histidine residues, forming domains through which the protein can bind DNA. Reducible Se compounds lower the removal of oxidative damage by the formamidopyrimidine DNA glycosylase enzyme and binding of the XPA protein in a dose-dependent manner (Blessing *et al.* 2004). Zn atoms are released from both XPA and formamidopyrimidine DNA glycosylase on Se treatment, suggesting that the displacement of Zn from Zn fingers results in inactivation of the protein. Approximately 3% of all proteins contain Zn finger motifs, including P53 and several other important DNA repair proteins. Thus, disruption to their functional domains could exert wide-ranging effects on DNA repair and other cellular processes. The intake and bioavailability of Zn itself may be of importance, since adequate Zn status is required for optimal functioning of these proteins (Ho, 2004).

An additional important mechanism through which diet may influence DNA repair is via hypermethylation of CpG islands (short stretches of DNA with a higher frequency of the CG sequence) in the promoter regions of repair genes, leading to gene silencing. The O^6-methylguanine-DNA methyltransferase (which repairs alkylated guanine bases; Nakagawachi *et al.* 2003) and *MLH1* (a key component of mismatch repair; Feinberg & Tycko, 2004) genes are both silenced by promoter hypermethylation during tumourigenesis. Folate may alter DNA methylation via its role as a methyl donor and thus influence the expression of key DNA repair genes. Other dietary factors, including Zn, Se and vitamin C, may also alter DNA methylation (Friso & Choi, 2002).

Given its apparent benefit in extending longevity in rodents and some other animals, it is interesting to note the effects of energy restriction on DNA repair capacity, which to date has been investigated in four animal studies (Guo *et al.* 1998; Cabelof *et al.* 2003; Stuart *et al.* 2004; Gedik *et al.* 2005). Rats fed an energy-restriction diet do not show the age-related decline in either BER (Cabelof *et al.* 2003) or NER (Guo *et al.* 1998) seen in rats fed an unrestricted diet. A third study (Gedik *et al.* 2005) has found that energy restriction has no effect on the repair of peroxide-induced strand breaks in ageing rats. However, in the latter study the aged rats were sampled at 17 months as compared with 24 months in the first two studies. Furthermore, DNA repair was quantified by inducing strand breaks, not by following the repair of DNA adducts as in the previous two studies. A fourth study (Stuart *et al.* 2004) has found that the activity of specific BER enzymes in liver, but not kidney, tissue increases in mice fed an energy-restriction diet. Here, the effects of ageing were not considered, and it is yet to be established whether energy restriction can enhance DNA repair or simply prevent its age-related decline. The results of the Stuart *et al.* (2004) study also suggest that tissue-specific differences in repair should be considered in future observational and intervention studies.

One of the most obvious ways in which energy restriction could affect DNA repair is through altered gene expression. Animal studies have shown that energy restriction alters the expression of numerous genes, including those involved in DNA repair pathways (Lee *et al.* 1999; Weindruch *et al.* 2001). Energy restriction is known to reduce the production of harmful oxygen species, and it may also alter protein synthesis and immune function (Dirx *et al.* 2003). It is possible that one or more of these effects may contribute to the changes in DNA repair associated with reduced energy intake. Increased DNA repair capacity could contribute to the increased lifespan and reduced age-associated changes seen in organisms in which energy intake is restricted.

Concluding remarks

DNA damage if unrepaired leads to aberrant gene expression, which is fundamental to the initiation and progression of many diseases. Much research aimed at understanding the beneficial health effects of diet and dietary components has focused on DNA damage. However, DNA damage is only of consequence if left unrepaired. It is clear that inter-individual variation in DNA repair capacity in apparently-healthy populations is high and that there may be important implications for disease risk if the capacity for DNA repair is suboptimal. This inter-individual variation in DNA repair capacity is explained in part by polymorphisms in the genes that encode DNA repair proteins. However, to date it has not been shown that any single polymorphism can account consistently for variations in DNA repair capacity, and future studies should utilise high-throughput genomic technologies to assess the effects of multiple polymorphisms on repair capacity.

Although only a limited number of studies have investigated the effects of diet and dietary factors on DNA repair processes, the results support the hypothesis that nutrition may have an important influence on DNA repair. There is some evidence that whole diet and specific dietary components and nutrients can modify DNA repair processes. However, further investigations will be required to determine which components of the diet affect DNA repair and to establish intake levels that optimise repair capacity.

As with several other areas of human physiology, interactions between environmental exposure, especially dietary exposure, and genetic make up will be an important area of investigation. Future studies should address the hypothesis that dietary exposure may have different effects on health depending on the genetic background of the individual. Progress in this area will be helped by the development of high-throughput technologies for quantifying DNA repair capacity and by more objective and less-labour-intensive methods for assessing dietary exposure.

Acknowledgments

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