Inter-individual variation in nucleotide excision repair in young adults: effects of age, adiposity, micronutrient supplementation and genotype

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Nucleotide excision repair (NER) is responsible for repairing bulky helix-distorting DNA lesions and is essential for the maintenance of genomic integrity. Severe hereditary impairment of NER leads to cancers such as those in xeroderma pigmentosum, and more moderate reductions in NER capacity have been associated with an increased cancer risk. Diet is a proven modifier of cancer risk but few studies have investigated the potential relationships between diet and NER. In the present study, the plasmid-based host cell reactivation assay was used to measure the NER capacity in peripheral blood mononuclear cells from fifty-seven volunteers aged 18–30 years before and after 6 weeks of supplementation with micronutrients (selenium and vitamins A, C and E). As a control, nine individuals remained unsupplemented over the same period. Volunteers were genotyped for the following polymorphisms in NER genes: ERCC5 Asp1104His (rs17655); XPC Lys939Gln (rs2228001); ERCC2 Lys751Gnl (rs13181); XPC PAT (an 83 bp poly A/T insertion–deletion polymorphism in the XPC gene). NER capacity varied 11-fold between individuals and was inversely associated with age and endogenous DNA strand breaks. For the first time, we observed an inverse association between adiposity and NER. No single polymorphism was associated with the NER capacity, although significant gene–gene interactions were observed between XPC Lys939Gln and ERCC5 Asp1104His and XPC Lys939Gln and ERCC2 Lys751Gnl. While there was no detectable effect of micronutrient supplementation on NER capacity, there was evidence that the effect of fruit intake on the NER capacity may be modulated by the ERCC2 Lys751Gnl single nucleotide polymorphism.

Nucleotide excision repair: Dietary intervention: Ageing: Polymorphisms: Adiposity

Nucleotide excision repair (NER) is one of the five evolutionarily conserved DNA repair systems present in humans, and is responsible for the repair of numerous mutagenic lesions, which either distort the DNA helix or block transcription1,2. Severe impairment of NER leads to disease, as seen in sufferers of the autosomal recessive disease xeroderma pigmentosum, who are deficient in NER and display 1000 times increased risk of skin cancer and 10-fold increased risk of internal neoplasia3. At the population level, among healthy volunteers after supplementation with kiwi fruit healthy volunteers after supplementation with kiwi fruit (10) found that people in the lowest tertile of folate intake had 18% lower NER capacity (P>0.001) than those in the highest tertile (100). Base excision repair (BER) capacity increased significantly in lymphocytes from healthy volunteers after supplementation with kiwi fruit (111). Similarly, supplementation with the antioxidant coenzyme Q10 (CoQ10) enhanced BER activity in human subjects (112), while supplementation with cooked carrots increased repair

Abbreviations: BER, base excision repair; DART, Dietary Antioxidant Repair Trial; HCR, host cell reactivation; NER, nucleotide excision repair; PBMC, peripheral blood mononuclear cells; SNP, single nucleotide polymorphism; Tris, 2-amino-2-hydroxymethyl-propane-1,3-diol.

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of H$_2$O$_2$-damaged plasmid DNA\textsuperscript{(13)}. However, we are not aware of any intervention studies in human subjects that have reported the effects of micronutrient supplementation on the NER capacity. Cooke et al.\textsuperscript{(14)} have investigated the effects of supplementation with 400 mg vitamin C/D for 16 weeks on cellular macromolecular damage including the formation of cytosine–glyoxal adducts in DNA. These authors speculated that such adducts might be repaired by NER, but did not make any direct measurements of the NER activity\textsuperscript{(14), (15)}.

Given the lack of information on factors influencing inter-individual variation in the NER capacity, the aims of the present study were to (i) quantify the extent of inter-individual variation in the NER capacity in young adults; (ii) determine the effect of micronutrient supplementation on the NER capacity; and (iii) investigate the influence genotype on the NER capacity.

Methods

Participants and study design

A total of sixty-six volunteers (twenty-five males and forty-one females) aged between 18 and 30 years were recruited from within and around Newcastle University as part of the Dietary Antioxidant Repair Trial (DART) study. The study was approved by the Newcastle and North Tyneside Local Research Ethics Committee. Smokers and those currently taking nutritional supplements were excluded.

Of the sixty-six recruits, fifty-seven were asked to take a commercially available micronutrient supplement containing selenium (100 μg) and vitamins A (450 μg), C (90 μg) and E (30 mg) daily for 6 weeks (Wassen International, Surrey, UK). The remaining nine subjects were unsupplemented for 25 ml whole blood using buoyant density centrifugation. The isolated PBMC were frozen slowly to −80°C and thawed quickly at 37°C until the last trace of ice remained, transferred immediately to pre-warmed growth medium (80% RPMI 1640 containing 10% heat-inactivated fetal calf serum), were added to a transfection mix and centrifuged at 1500 rpm for 10 min to form a cell pellet. The cell pellet was resuspended in 20 ml growth media, counted using a haemocytometer and transferred to an upright T20 tissue culture flask. PBMC were treated with 50 μg/ml of the mitogen phytohaemagglutinin-P (Sigma) and incubated at 37°C in 5% CO$_2$ for 72 h. After the 72 h incubation, the cells were counted, pelleted by centrifugation and washed twice with 2-amino-2-hydroxymethyl-propane-1,3-diol (Tris)-buffered saline and centrifuged at 1500 rpm for 10 min to form a cell pellet. The cell pellet was resuspended in 20 ml growth media, counted using a haemocytometer and transferred to an upright T20 tissue culture flask. PBMC were treated with 50 μg/ml of the mitogen phytohaemagglutinin-P (Sigma) and incubated at 37°C in 5% CO$_2$ for 72 h. After the 72 h incubation, the cells were counted, pelleted by centrifugation and washed twice with 2-amino-2-hydroxymethyl-propane-1,3-diol (Tris)-buffered saline (pH 7-3). Then the cells were divided into eight aliquots of 1 × 10⁶ cells, half of which were transfected with the damaged plasmid (400 J/m² UV light, 253 nm) and half with the equivalent undamaged plasmid using the diethylamino ethanol (DEAE)–dextran transfection procedure\textsuperscript{(17)}. In 12 × 75 mm tubes (Sterilin, Melbourne, Australia), 1 × 10⁶ cells, suspended in 200 μl Tris-buffered saline, were added to a transfection mix containing 50 ng plasmid DNA (stock concentration 10 ng/ml), 12.5 μl DEAE–dextran in Tris-buffered saline (2 mg/ml) and 32.5 μl Tris-buffered saline (pH 7-3) and incubated at room temperature for 15 min. The transfection mix was then washed from the cells with fresh growth media and the cells were resuspended in 2 ml fresh growth media. The tubes were capped loosely and incubated for 40 h (37°C, 5% CO$_2$) to allow DNA repair and the expression of the plasmid to take place. After 40 h, the cells were lysed and luciferase activity was measured on a luminometer (Turner Biosystems, Sunnyvale, CA, USA) using the Promega luciferase assay system, according to the manufacturer’s protocol. NER capacity was calculated as the ratio of mean reporter gene expression
Genotyping

The subjects were genotyped for four polymorphisms in three NER genes: ERCC5 Asp1104His (rs17655); XPC Lys939Gln (rs2228001); ERCC2 Lys751Gln (rs13181); XPC PAT (an 83 bp poly A/T insertion–deletion polymorphism in the XPC gene) using PCR and restriction fragment length polymorphism. Genotyping procedures, primers and reaction conditions were as described previously: ERCC5 Asp1104His (rs17655)(18); XPC Lys939Gln (rs2228001)(19); ERCC2 Lys751Gln (rs13181)(20); XPC PAT(21).

Statistical analysis

All statistical analyses were carried out using SPSS v 11.0 software (SPSS Inc., Chicago, IL, USA). Means with their standard errors are presented, and \( P < 0.05 \) was considered statistically significant. Effect of intervention on repair capacity was examined using the analysis of covariance with pre-supplementation values as covariate. Correlation analysis was performed using linear regression analysis. Factors individually associated with the NER capacity were analysed as covariates in a univariate ANOVA model. The corrected \( R^2 \) value and significance of the model gave an estimate of the combined contribution of these factors to total variance in the NER capacity. For the analysis of single genotypes, one-way ANOVA was performed. For the analysis of two-gene and diet–gene interactions, individuals were dichotomised as either homozygotes for the common allele or carriers of the uncommon allele.

Results

Study subjects

In total, twenty-five males and forty-one females aged between 18 and 30 years (mean age 22 years) were recruited to the DART study. Mean BMI was 23 ± 0.47 kg/m², with the following adiposity distribution: forty-nine normal weight (18–25 kg/m²); thirteen overweight (> 25–30 kg/m²); three obese (> 30 kg/m²); one underweight (< 18 kg/m²). Two of the overweight individuals were in the unsupplemented group. There were no significant differences between the supplemented and unsupplemented groups for age, height or BMI, nor were there significant differences for any dietary characteristics.

Nucleotide excision repair capacity assay validation

There was an inverse log-linear relationship between the NER capacity (percentage of expression from damaged/undamaged plasmid) and the plasmid UV damage in primary PBMC, consistent with previous use of the HCR assay(17). As a negative control, immortalised cells, GM02253 (Coriell Cell Repositories) derived from a sufferer of xeroderma pigmentosum group D, were subject to the HCR assay and displayed less than 0.1 % repair capacity. Across all runs of the HCR assay in primary PBMC, there were no associations between the NER capacity and (1) the number of cells extracted from an individual’s blood sample; (2) the luciferase expression achieved from the undamaged plasmid post-transfection; or (3) sample storage time. On three occasions, approximately 1 week apart, 20 ml whole blood was taken from three individuals, not participating in the intervention, in 2 £ 10 ml EDTA vacutainers (no sample was taken from subject 2 on the third occasion) and the HCR assay was performed in duplicate on the cells from each sample. Fig. 1 (a) shows the close agreement between duplicate measurements of the NER capacity in the samples taken on the same day. There was much greater between-day variation in the NER capacity, repair capacity appearing to increase across the three sampling occasions for individuals 1 and 3 (Fig. 1 (b)).

Inter-individual variation in nucleotide excision repair capacity

NER capacity at baseline (pre-supplementation) was measured in sixty-three individuals and varied approximately 11-fold,
ranging from 2.3 to 25.9% (mean 10 (sd 5.6%); Fig. 2 (a)). There was no significant difference between males and females for mean NER capacity (P=0.3). At baseline, despite the narrow age range of participants, the NER capacity decreased significantly with increasing age ($R^2$ 0.14, $P=0.001$; Fig. 3 (a)). Similarly, the NER capacity at baseline decreased significantly with increasing BMI ($R^2$ 0.08, $P=0.042$; Fig. 3 (b)). NER capacity was also inversely associated with endogenous DNA strand breaks, measured using the comet assay and reported elsewhere, in the cells obtained from the same volunteers on the same occasions, ($R^2$ 0.17, $P=0.005$; Fig. 2 (c)). Age and BMI were not correlated, and while endogenous damage was not correlated with BMI, it was associated inversely with age ($R^2$ 0.14, $P=0.012$). Analysis using a general linear regression model showed that age, BMI and endogenous DNA damage accounted for 26% of the total variance in the NER capacity ($R^2$ 0.26, $P=0.004$).

![Fig. 2.](image1)

Inter-individual variation in nucleotide excision repair (NER) capacity (%) among study subjects' pre-supplementation and (b) correlation of NER capacity in all individuals' pre- and post-supplementation ($R^2$ 0.4, $P=0.001$; (●) supplemented, (○) unsupplemented).

![Fig. 3.](image2)

Fig. 3. Associations between nucleotide excision repair (NER) capacity at baseline and (a) age ($R^2$ 0.14, $P<0.001$), (b) BMI ($R^2$ 0.08, $P=0.02$) and (c) endogenous oxidative DNA damage ($R^2$ 0.17, $P=0.005$).
Effect of genotype on nucleotide excision repair capacity

The subjects were genotyped for four polymorphisms that have previously shown associations with either cancer risk or NER capacity itself. Observed genotype frequencies and associated NER capacity at baseline are presented in Table 1, but no significant effect of individual genotype on NER capacity could be detected. To assess the contribution of combined genotypes on NER capacity, individuals were dichotomised as either homozygotes for the common allele or carriers of the uncommon allele and the data were examined by univariate ANOVA. Two significant genotype interactions were found between pairs of polymorphisms and NER capacity. There was evidence of an interaction between the XPC Lys939Gln single nucleotide polymorphism (SNP) and both the ERCC5 Asp1104His and the ERCC2 Lys751Gln SNP in respect of the NER capacity (P=0.01 and 0.03, respectively; Fig. 4). NER capacity in those homozygous for the ERCC Asp allele was apparently unaffected by XPC Lys939Gln genotype. However, in those carrying one or two copies of the ERCC5 His allele, NER capacity was decreased from 11.5 in those who were XPC Lys/Lys homozygotes, to 3.6 in those who carried one or more of the XPC Gln allele (Fig. 4 (a)). Similarly, XPC Lys939Gln genotype did not appear to change the NER capacity of those who carried the ERCC2 Gln allele [Lys/Gln heterozygotes or Gln/Gln homozygotes]. However, in ERCC2 Lys/Lys homozygotes, the NER capacity decreased from 11.0 in those who were also XPC Lys homozygotes, to 3.1 in those who carried one or two copies of the XPC Gln allele (Fig. 4 (b)).

Effect of diet

Pre- and post-supplementation measurements of the NER capacity were highly correlated (R² 0.4, P<0.001; Fig. 2 (b)). Analysis of covariance of post-supplementation NER capacity, with pre-supplementation NER capacity as the covariate, showed no significant effect of supplementation (P=0.42; Table 2). Estimates of habitual intakes of major food groups over the preceding year were obtained via a food frequency questionnaire and the contributions of the macronutrients, fat, protein, carbohydrate and alcohol to total energy intake were calculated. NER capacity was not significantly associated with any of the derived dietary variables. However, there was evidence of a diet–gene interaction (P=0.041) in determining the NER capacity in this group of subjects (Fig. 5). Among Lys/Lys homozygotes of the ERCC2 Lys751Gln SNP, mean NER capacity (mean of pre- and post-supplementation measurements) increased with increasing fruit intake (expressed in tertiles of servings per day). By contrast, in those carrying one or more of the Gln alleles, there was little or no relationship between fruit intake and NER capacity.

Discussion

This is the first study to investigate the determinants of inter-individual variation in the NER capacity in young adults, and to test the hypothesis that micronutrient supplementation can enhance NER capacity. We observed an 11-fold inter-individual variation in the NER capacity, which is similar to that observed previously in respect of the expression levels of DNA repair genes (10-fold) and NER capacity (4-7/10-fold). Of the factors investigated, we observed that 26% of the observed variation in the NER capacity was explained by age, BMI and endogenous DNA strand breaks. Furthermore, significant gene–gene interactions and preliminary evidence of potential diet–gene interactions were observed. Our measurements of the NER capacity were made using the established and validated HCR and were reproducible; duplicate measurements of repair capacity in separate PBMC samples were highly correlated (R² > 0.9). Interestingly, among three individuals, who were not part of the intervention study, the NER capacity appeared to increase over a 3-week period (measurement taken weekly). This parallels the findings reported recently in which both NER and BER capacities increased over a 6-month period in Norwegian subjects, and suggests that repair capacity can be a dynamic and may be influenced by external factors over relatively short periods of time. The same upward trend in NER capacity was not observed among those participating in the DART study, where mean NER capacity was similar pre- and post-supplementation and values for individuals were correlated.

To our knowledge, the DART study is one of the only two studies in which measurements of both BER (see Caple et al. for details) and NER capacities have been made in the same individuals on the same occasions. Gaião et al. measured NER and BER in lymphocytes from thirty-three individuals.

Table 1. Genotype distribution and mean nucleotide excision repair (NER) capacity among genotypes at baseline

| Polymorphism        | Genotype       | Frequency | NER capacity (%) | | |
|---------------------|----------------|-----------|------------------|---|
|                     |                | n          | Mean          | SEM    | P*   |
| ERCC5 Asp1104His (n 64) rs17655 | Asp/Asp           | 37         | 9.2           | 1.0    | 0.04 |
|                     | Asp/His         | 24         | 9.6           | 1.2    | 0.03 |
|                     | His/His         | 3          | 7.6           | 3.3    | 0.61 |
| XPC insertion/deletion (n 62) | +/+              | 56         | 10.2          | 0.8    | 0.42 |
|                     | +/-             | 4          | 5.6           | 2.7    | 0.25 |
|                     | –/–             | 2          | 8.2           | 3.8    | 0.25 |
| XPC Lys939Gln (n 62) rs2228001 | Lys/Lys           | 28         | 9.9           | 1.0    | 0.01 |
|                     | Lys/Gln         | 28         | 9.3           | 1.0    | 0.03 |
|                     | Gln/Gln         | 6          | 6.5           | 2.1    | 0.27 |
| ERCC2 Lys751Gln (n 59) rs13181 | Lys/Lys           | 23         | 11.2          | 1.2    | 0.01 |
|                     | Lys/Gln         | 32         | 10.1          | 1.0    | 0.03 |
|                     | Gln/Gln         | 2          | 8.6           | 4.9    | 0.73 |

* P value from one-way ANOVA of NER capacity v. genotype.
individuals using modified comet assays and reported no correlation between NER and BER capacities. In the larger study reported here, and using assays that are specific for each form of DNA repair, we found no evidence that capacities for these two DNA repair systems are correlated. Given that there is little overlap between the proteins responsible for the two DNA repair systems (27), the independence of these capacities is not unexpected.

Surprisingly, given the youth of the study group (18–30 years), NER capacity decreased significantly with increasing age. Previous studies have reported that NER capacity, measured using the HCR assay, was significantly and inversely associated with age among 117 (28) and 135 (29) healthy control subjects aged 20–60 years. Interestingly, Goukassin et al. (30) have reported significant reductions across the lifespan in the removal of thymine dimers following UV-induced damage in dermal fibroblasts derived from newborns and adults aged 21–34 and 63–88 years. There is a well-established positive association between acquisition of somatic mutations and age (31), and this is supported by the present study where the levels of endogenous DNA strand breaks increased with age. In addition, we observed a significant inverse association between endogenous strand breaks and NER capacity. Strand breaks, although not repaired by the NER system, may be indicative of the overall cellular DNA damage and repair balance. As with other aspects of ageing, distinguishing causes from consequences is challenging. However, recent data suggest that the age-related decrease in DNA repair capacity is a direct cause of ageing rather than a consequence of accumulated DNA damage since mutations in the ERCC2 gene, essential in NER, cause accelerated ageing syndromes in human subjects and the pattern of gene expression changes in ERCC2-deficient mice parallels those seen in aged animals (32).

In the present study, we report, for the first time, a significant inverse relationship between BMI and NER capacity in young adults. Mechanistic links between adiposity and DNA repair are unknown but may involve damage to cellular macromolecules, including DNA, leading to altered gene expression, which could arise as a result of the chronic low-level inflammation associated with obesity (33). Several studies have demonstrated that energy restriction in rodents (which reduces adiposity) has a positive effect on DNA repair in a

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**Fig. 4.** Interactions between the XPC Lys939Gln (rs2228001) and (a) ERCC5 Asp1104His (rs17655) and (b) ERCC2 Lys751Gln (rs13181) genotypes on nucleotide excision repair (NER) capacity. (−−−−−−−−−), ERCC5 Asp/Asp, ERCC5 Asp/His or His/His. (−−−−−−−−−), ERCC2 Lys/Lys, ERCC2 Lys/Gln or Gln/Gln.

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**Table 2.** Nucleotide excision repair (NER) capacity in supplemented and unsupplemented groups pre- and post-micronutrient supplementation

<table>
<thead>
<tr>
<th>Supplemented</th>
<th>Time point</th>
<th>n</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean (SEM)</th>
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<tr>
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<td>Post</td>
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<td>1.5</td>
<td>32.3</td>
<td>11.6 (0.9)</td>
</tr>
<tr>
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<td>Pre</td>
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<td>4.3</td>
<td>25.1</td>
<td>10.0 (2.2)</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>8</td>
<td>2.6</td>
<td>19.9</td>
<td>9.3 (2.0)</td>
</tr>
</tbody>
</table>

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**Fig. 5.** Interaction between tertiles of fruit intake (■, low; □, medium; ●, high) and ERCC2 Lys751Gln (rs13181) genotype on nucleotide excision repair (NER) capacity. NER capacity is the mean (SEM) of measurements pre- and post-micronutrient supplementation. Individuals were dichotomised for genotype based on the presence or absence of the uncommon (Gln) allele of the Lys751Gln genotype. Fruit intake was divided into tertiles based on portions per day. $P=0.041$ for interaction.
range of cell types\textsuperscript{(34)} and, importantly, Gedik \textit{et al.}\textsuperscript{(35)} demonstrated that rats fed an energy-restricted diet did not show the same age-related decline in BER capacity seen in rats fed \textit{ad libitum}.

SNP in a number of NER genes have been associated with NER capacity in human subjects\textsuperscript{(23,31)}. Although no single polymorphism was associated with NER capacity in the present study, when pairs of SNP were analysed, two significant gene–gene interactions were observed. In both, volunteers with the lowest capacity for NER carried the uncommon Gln allele of the XPC Lys939Gln, suggesting that this allele may limit DNA repair when in the presence of other polymorphisms, in this case the ERCC2 Lys751Gln or ERCC5 Asp1104His SNP. The ERCC2 gene product, XPD, forms part of the TFIH complex, essential for both transcription and NER, and there are strong functional interactions between the TFIH complex and the protein products of ERCC5, XPG and XPC\textsuperscript{(36)}. Therefore, disruption of these protein–protein interactions in certain allelic combinations may explain the observed gene–gene interactions. However, it should be noted that the present study was designed primarily to investigate the effect of a nutritional intervention rather than to investigate genotypic effects. As a consequence, given the relatively small size of the present study, it is underpowered to investigate either genotype–phenotype associations or gene–gene interactions and the apparent interactions observed here will need to be tested in larger cohorts.

NER capacity among supplemented individuals increased slightly post-intervention, but there was no significant difference in post-supplement NER capacity when compared with the unsupplemented controls. Analysis of habitual dietary intake showed none of the derived dietary variables to be associated significantly with NER capacity, although we found preliminary evidence of an interaction between fruit intake and the ERCC2 Lys715Gln polymorphism on NER capacity. In those carrying the Gln allele, NER capacity was unaffected by fruit intake but repair capacity increased with increasing fruit intake in ERCC2 Lys/Lys homozygotes. This observation is consistent with the report by Shen \textit{et al.}\textsuperscript{(37)} that breast cancer risk was reduced by 33\% in carriers of the XRCC2 194Trp allele only when in combination with high fruit intake. The apparent interaction we have observed must be treated with caution until repeated in independent, larger groups of subjects, but suggests that both genotypic and environmental effects need to be considered in the investigations of inter-individual differences in NER capacity.

In the present study, we have observed an 11-fold variation in NER capacity among young non-smoking adults. Furthermore, we have demonstrated that age, BMI, endogenous DNA strand breaks, diet–gene interactions and gene–gene interactions are all associated with NER capacity, although other genotypic and lifestyle determinants remain to be investigated. A reduced capacity to perform NER throughout the life course may predispose to disease and premature ageing, so achieving maximal repair capacity is likely to reduce disease risk. Since dietary and other lifestyle factors have significant impact on the ageing phenotype, BMI and endogenous levels of DNA damage, these lifestyle factors will be important targets for future research on manoeuvres through which NER capacity may be maximised.

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References

frequency questionnaire and identification of extreme nutrient


