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Age-related increases in DNA repair and antioxidant protection: A comparison of the Boyd Orr Cohort of elderly subjects with a younger population sample

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Abstract

Background One commonly held theory of ageing is that it is caused by oxidative damage to critical molecules in the body, including proteins, lipids and nucleic acids. Accumulation of oxidative DNA damage with age will occur if there is an increase in reactive oxygen species in the body, or a decline in antioxidant defences, or a reduced efficiency of DNA repair.
Subjects and Methods Using the comet assay, we have measured DNA breaks and oxidised purines in lymphocytes from subjects of different age groups: 20–35 ($n = 40$), 63–70 ($n = 35$), and 75–82 ($n = 22$). We also measured the resistance of lymphocyte DNA to H_2O_2-induced oxidative damage, and the repair activity of cell-free lymphocyte extracts on a substrate containing 8-oxoguanine.
Results We found an increase in oxidative base damage in old age, but this apparently does not result from deterioration of either antioxidant defence or DNA repair. In fact, both of these tend to increase with age. There were few age-related differences in plasma levels of dietary antioxidants: tocopherols and retinol were higher in the older subjects, while lycopene was highest in the youngest age group.
Conclusions It is possible, that in old age, antioxidant defences and DNA repair are induced, in response to a higher level of oxidative damage, as mitochondria become more leaky and release more reactive oxygen. It is equally possible that older people, as survivors, had relatively high levels of antioxidant defences and DNA repair earlier in their lives, compared with those who did not survive to such an age.

Keywords: *DNA damage, DNA repair, antioxidants, Boyd Orr cohort, comet assay, elderly*

Introduction

It is commonly proposed that ageing results, at least in part, from an accumulation of oxidative damage in critical biomolecules including DNA, leading to an eventual breakdown of cellular and organ function. Oxidation occurs because reactive oxygen species are released from the mitochondria during respiration, as well as from cells involved in the inflammatory response; in addition, exogenous agents, such as tobacco smoke, introduce free radicals to the body. The extent of oxidative damage is moderated by antioxidant defences (catalase, superoxide dismutase, glutathione and its associated enzymes). Dietary antioxidants including vitamin

C, vitamin E, carotenoids and flavonoids may also play a role. Damage to DNA is removed by efficient cellular repair pathways. As a result, in spite of a constant and substantial influx of damage, a dynamic steady state is normally maintained at a relatively low level of damage. As we age, there may be an increase in the rate at which damage occurs, as mitochondria lose membrane integrity and leak more superoxide radicals, leading to a greater general exposure to reactive oxygen damage. The problem will be compounded by age-related declines in antioxidant defences and in DNA repair, resulting in an increase in the steady state level. We here set out to test whether such changes do occur with age.

Relatively few reports exist on levels of oxidative DNA damage in animals or humans of different ages. Kaneko *et al.* [**1**] measured 8-oxodGuo (a marker of DNA base oxidation) in various rat organs using high pressure liquid chromatography (HPLC) with electrochemical detection and found a steady level from 2 to 24 months, doubling at 30 months, while Helbock *et al.* [**2**] reported a 2.75-fold increase in 8-oxodGuo in rat liver at 24–26 months, compared with 3–6 months. In contrast, Hirano *et al.* [**3**] found no difference in various organs between 5 and 30 months, though levels of 8-oxodGuo in these adult rats tended to be higher than in 3-week-old rats. We recently reported modest but significant increases in 8-oxodGuo in brain and liver of 17-month-old compared with 1-month-old rats [**4**]. An alternative approach to the measurement of oxidised bases in DNA is based on the use of enzymes that convert the oxidised bases to strand breaks. Using the alkaline comet assay (single cell gel electrophoresis) to measure these breaks, we found no significant change in oxidised pyrimidines (sites sensitive to endonuclease III) in rat lymphocytes from 3 to 17 months [**4**]. Similarly, in mouse hepatocytes, there was no increase in oxidised purines (sites sensitive to formamidopyrimidine DNA glycosylase, FPG), measured using alkaline elution, from 2 to 27 months [**5**].

Evidence for an increase in DNA oxidation in humans is also inconclusive. Barnett and King [**6**] used an alkaline unwinding assay combined with ELISA detection of single-stranded DNA to measure strand breaks in lymphocyte DNA, and found a higher level in subjects aged 65–69 years, than in a group aged 35–39 years; but the same method applied to 75–80 year olds showed similar levels of damage to those found in 35–39 year olds [**7**]. Nonagenarians had similar levels of strand breaks and oxidised bases (measured with the comet assay) as middle-aged subjects (40–60 years) [**8**], and yet Mutlu-Tűrkoğlu *et al.* [**9**] reported a doubling in DNA strand breaks comparing 61–85 year olds with 21–40 year old subjects.

Inter-laboratory validation studies have shown that assays for oxidative DNA damage in human leukocyte samples based on HPLC or GC-MS are unreliable, as they suffer a major problem of oxidation during sample preparation, which it has not been possible to eliminate by improvements in technique [**10, 11**]. Methods based on the use of FPG, including the comet assay, show less experimental variation and are less prone to spurious oxidation than the chromatographic methods. We therefore chose the comet assay to measure oxidative DNA damage, antioxidant status, and capacity for DNA repair, in subjects of three age groups: 20–35, 63–70, and 75–82 years. The older age groups belong to the Boyd Orr cohort (BOC), set up in the 1930s as a survey of family nutrition and health in the UK. Surviving children from these families were located, recalled, and asked to participate in a follow-up study to relate present-day health status with childhood nutrition. The youngest age group was specially recruited for this study.

Subjects and methods: see Appendix 5 in the supplementary data on the Journal website http://www.ageing.oxfordjournals.org/.

Results

DNA damage

The comet assay measures DNA strand breaks. The background level of strand breaks was significantly lower in the youngest group (20–35) than in both older groups (P ANOVA<0.001) (Figure 1, top).

Damaged bases (specifically 8-oxoGua, FaPy-Gua and FaPy-Ade) were measured by incubating agarose-embedded nucleoids (lysed cells) with FPG before electrophoresis; the enzyme converts these bases to breaks, and the increase in comet tail intensity indicates the frequency of oxidised purines. Figure 1 (lower panel) shows that group 1 (20–35) and Group 2 (63–70) have the same mean levels of damage, while FPG-sites in Group 3 (75–82) are significantly higher ($P<0.001$).

We also measured DNA breaks induced by treating cells with H_2O_2. Resistance to this *in vitro* oxidative challenge gives an indication of the antioxidant status of the cells (as is indicated by the pronounced decrease in the yield of breaks when lymphocytes from antioxidant-supplemented subjects are treated with H_2O_2: see, for example, [**12**]). It is clear from Figure 2 that lymphocytes from Group 3 sustained fewer H_2O_2-induced breaks compared with the two younger age-groups in terms of mean or median values (P ANOVA<0.001). Differences in mean values are highly significant. (The data shown in this figure are net H_2O_2-induced breaks, i.e. after subtracting the background strand breaks from the breaks measured in the presence of H_2O_2. Essentially the same pattern is seen if total breaks are compared.)

DNA repair

We estimated the activity of OGG1 (8-oxoGua DNA glycosylase) with an *in vitro* assay. Extracts prepared from lymphocytes were incubated with agarose-embedded nucleoids from cells treated with the photosensitiser Ro 19–8022 plus light, a treatment that induces 8-oxoGua—the OGG1 substrate—with little other base damage [**13**]. Breaks introduced by the extract were measured by subsequent alkaline electrophoresis as in the standard comet assay.

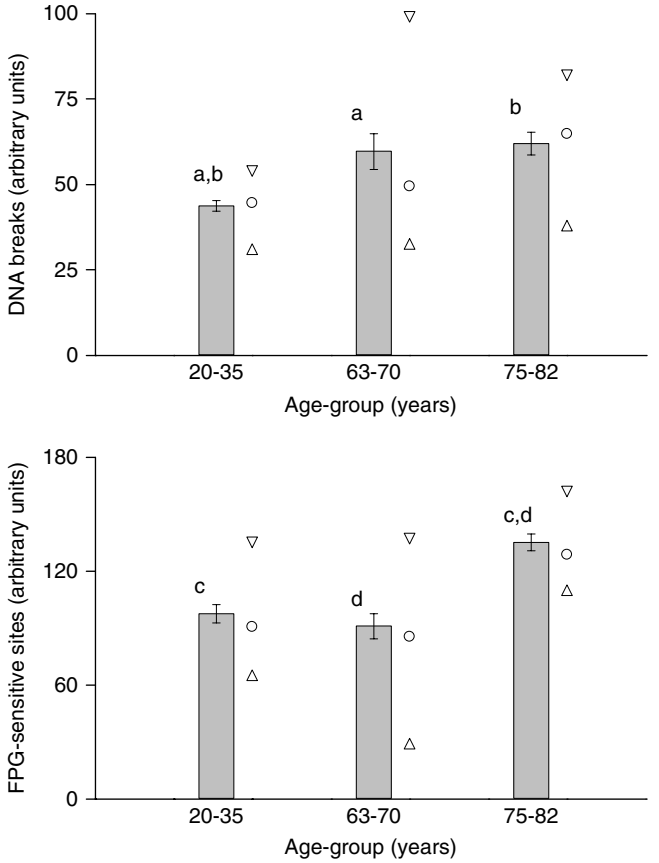

Figure 1. Endogenous DNA damage in lymphocytes, measured with the comet assay. Top, strand breaks; bottom, oxidised purines (FPG-sensitive sites). Mean values are shown with SEM, as well as the median (◦) and range (10–90 percentiles). Significant differences: a, $P = 0.003$; b, c, d, $P<0.001$; P ANOVA (each dataset) <0.001.

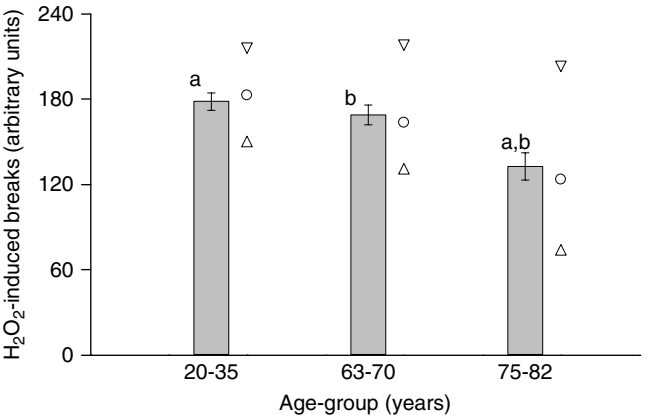

Figure 2. Antioxidant resistance; DNA strand breaks induced in lymphocyte DNA by H_2O_2 *in vitro*. Mean values are shown with SEM, as well as the median (◦) and range (10–90 percentiles). Significant differences: a, $P<0.001$; b, $P = 0.003$. P ANOVA< 0.001.

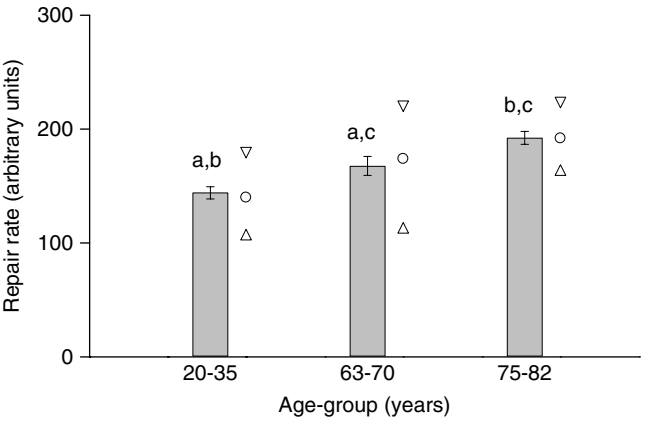

Figure 3. DNA repair (OGG1) capacity of lymphocyte extract. Mean values are shown with SEM, as well as the median (o) and range of values (10–90 percentiles). Significant differences: a, $P = 0.02$; b, $P<0.001$; c, $P = 0.04$. P ANOVA< 0.001.

There was an increase in mean incision rate with age, significant differences being seen between all groups (P ANOVA<0.001) (Figure 3).

Results were similar when the analysis excluded smokers and vitamin supplement takers in the oldest age group.

Plasma antioxidants

Concentrations of vitamin C, carotenoids and tocopherols were measured in plasma by HPLC (see Appendix 2 in the supplementary data on the Journal website http://www.ageing.oxfordjournals.org/). There were no significant differences between the two BOC groups, but they differ from the youngest group. α-Tocopherol and retinol occur at higher concentrations in Groups 2 and 3 than in the youngest age group. γ-Tocopherol is higher in Group 2 than in Group 1, but this association should be interpreted cautiously because the P-value (0.02) is greater than the Bonferroni-corrected threshold P-value of 0.004. In contrast, lycopene is present at twice as high a level in Group 1 as in the older groups.

Correlations between markers of genetic stability and age

Table 3 (see Appendix 3 in the supplementary data on the Journal website http://www.ageing.oxfordjournals.org/) shows correlations between biomarkers of DNA damage and repair and plasma antioxidant levels, and between these parameters and age, for the two BOC groups combined. There are positive correlations of age with FPG-sites ($r = 0.49, P<0.001$) and repair rate ($r = 0.25, P = 0.06$), and a negative correlation between age and H_2O_2-induced breaks ($r = -0.39, P<0.01$), consistent with differences in mean values for the two age groups within the cohort. The only noteworthy correlations with antioxidant levels are the negative correlations between repair rate and lutein/zeaxanthin ($r = -0.31, P<0.05$) and the marginally significant correlation between repair rate and retinol ($r = 0.25, P = 0.06$).

In the oldest age group, which included participants who used vitamin and mineral supplements, supplement use had no significant effect on basal DNA damage (strand breaks or oxidised bases), DNA repair or resistance to H_2O_2-induced damage. Nor did it affect plasma levels of α-tocopherol. Further, these markers of DNA stability did not vary by sex, alcohol intake and levels of exercise.

Discussion

In a recent investigation of occupational exposure to mineral fibres, the comet assay was used with FPG to measure oxidative damage in human lymphocyte DNA. Samples were collected in Slovakia from 239 workers exposed to asbestos or other mineral fibres and 149 unexposed controls [**14**]. Subjects' ages ranged from 21 to 88 years, with most in the middle of the range. As levels of DNA base damage were apparently not affected by exposure, the groups were combined for statistical analysis. There was a significant correlation between FPG-sensitive sites and age ($r = 0.17, P = 0.001$). We have now used the same technique to measure background levels of base oxidation in lymphocyte DNA from subjects living in Scotland, in three distinct age groups: 20–35, 63–70, and 75–82 years. (For details of subjects, see Appendix 1, and for precise age distribution, see Appendix 4, supplementary data, on the Journal website http://www.ageing.oxfordjournals.org/.)

While the two younger groups had similar levels of FPG-sites, damage was significantly higher in the oldest age group. Background DNA strand breaks (also higher in older subjects) are difficult to interpret, as they can arise from various forms of DNA damage, represent alkali-labile apurinic/apyrimidinic (AP) sites as well as frank breaks, and include breaks present as intermediates during active cellular DNA repair. In lymphocytes, they may also result from genetic rearrangements occurring in connection with stimulation of the immune system.

The comet assay without FPG was used to measure DNA breaks induced by *in vitro* treatment of lymphocytes with H_2O_2, reflecting antioxidant status. H_2O_2-induced breaks were similar for 20–35-year-old and 63–70-year-old subjects, but significantly lower in the oldest age group. In our recent rat study [**4**], we found a continuous and dramatic decrease in H_2O_2-induced breaks over the period of bimonthly sampling from 3 to 17 months. Other reports from human studies give a mixed picture. King *et al.* [**7**] found no change in H_2O_2-resistance with age. Mutlu-Tűrkoğlu *et al.* [**9**] found *higher* levels of H_2O_2-induced breaks in their elderly group (61–85 years).

Antioxidant status can be assessed in plasma samples using a simple assay for ferric reducing capacity (FRAP). FRAP values were lower in elderly people [**9**], consistent with the higher levels of H_2O_2 damage. But elevated FRAP values were reported in nonagenarians [**8**].

Some studies have shown that dietary antioxidant levels decline in the elderly, as their ability for absorption decreases and lifestyle factors lead to reduced consumption of healthier foods and overall intake of food [**15, 16**]. Plasma carotenoid levels are generally reported to be lower in the elderly (reviewed in [**17**]), although plasma levels of vitamin A are maintained in old age [**18**]. Studies of plasma α-tocopherol have indicated a decrease, increase or no change with age [**19, 20**]. We found a general tendency for plasma concentrations of tocopherols and retinol to be elevated in the older age groups. The striking difference in lycopene concentrations (higher in the youngest age group by at least two-fold) reflects what would be expected in terms of lifestyle and diet, younger people consuming more products containing processed tomatoes (the main source of lycopene). We found similar low lycopene levels in another cohort of healthy old people [**21**]. Elderly subjects are reported to show impaired absorption and utilisation of vitamin C and plasma levels lower than those in younger individuals [**22**]. However, the vitamin C levels measured by us are similar across the age range (and considerably higher than the levels in our other ageing cohort [**21**]).

Most published reports on DNA repair as a function of age in humans deal with rejoining of strand breaks induced in lymphocytes by ionising radiation or treatment with H_2O_2. Turner *et al.* [**23**] reported no difference in repair kinetics over an age range of 0–89. Harris *et al.* [**24**] found certain aged subjects to be deficient in rejoining, compared with younger subjects. Barnett and King [**6**] reported slower repair of strand breaks in a 65–69-year-old group than in a group aged 35–39 years. While strand breaks are repaired relatively quickly by ligation, damaged bases are repaired by slower base excision repair. The critical step in this pathway is the detection and removal of the damaged base by a specific glycosylase—in the case of 8-oxoGua, OGG1. This glycosylase tends to stay associated with the baseless sugar (i.e. its product); APE1, an AP-endonuclease, enhances OGG1 turnover by competing for the AP site [**25**]. Using the comet-based *in vitro* assay for OGG1, we found a significant increase in enzyme activity with age in the Slovak population [**14**]. In the present study of selected age groups, highest OGG1 activity was seen in the oldest age group.

Although we restricted selection in Groups 1 and 2 to healthy subjects, not taking supplements, we were not able to impose these limitations on Group 3 without greatly reducing sample size/power. Inevitably, in old age, disease will affect a substantial proportion of the population, and with the limited number of BOC survivors available, we had to include some with a history of cardiovascular disease, asthma or cancer. Several subjects in Group 3 also took dietary supplements, in most cases fish oil. Supplement use had no significant effect on basal DNA damage, DNA repair or H_2O_2-resistance. Nor did it affect plasma levels of α-tocopherol. The population groups differed in sex distribution, levels of alcohol consumed and exercise taken, but none of these factors affected significantly the markers of genetic instability.

The hypothesis underlying this study is that genetic instability (specifically, instability reflecting increased oxidative stress) increases with age, and that we should therefore

expect to see higher levels of DNA damage, lower levels of antioxidant protection, and/or lower capacity for DNA repair in ageing human populations. One prediction was fulfilled, since we found significantly more oxidised bases in lymphocyte DNA in the oldest age group compared with the two younger age groups. Presumably, this results from an increase in reactive oxygen species as mitochondria age. Paradoxically, however, resistance to *in vitro* oxidative damage, and repair of 8-oxoguanine, were actually higher in the oldest age group.

There are two plausible explanations for these findings. First, it is possible that in old age there is a stimulation of antioxidant defences and DNA repair, in response to a higher level of oxidative stress (leakage of reactive oxygen from aged mitochondria), which in turn leads to the increase in endogenous DNA damage. This runs counter to the general notion of old age as a period when defences are likely to be deficient rather than up-regulated. While extrapolations from animals to man with regard to ageing should be treated circumspectly, it is worth pointing out that we found increases in most antioxidant enzyme activities in various organs of 17-month-old compared with 1-month-old rats [**4**]. A second possible explanation is that the members of the oldest age group, representing the survivors of the cohort, had relatively high levels of antioxidant defences and DNA repair earlier in their lives, and that their resistant phenotype contributed to their survival. This hypothesis of survivor bias should be tested in a prospective study.

Key points

- In a comparison of subjects aged 75–82, 63–70 and 20–35, oxidative damage to lymphocyte DNA was highest in the oldest age group.
- The oldest age group showed the greatest degree of resistance to oxidative damage *in vitro*, i.e. they had a higher level of antioxidant defence.
- An *in vitro* assay for DNA repair (with oxidised guanine as substrate) showed an increase in activity with age, across all three age groups.

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Conflicts of interest

None declared.

Supplementary data

Supplementary data for this article are available online at http://ageing.oxfordjournals.org.

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PLEASE NOTE: The very long list of references supporting this article has meant that only the most important are listed here and are represented by bold type throughout the text. The full list of references is available on the journal website http://www.ageing.oxfordjournals.org/ as appendix 6.

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