SHORT COMMUNICATION

Oxidative DNA damage in humans: comparison between high and low habitual fruit and vegetable consumption

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We investigated whether men with a habitual high consumption of vegetables and fruit have a lower excretion of 8-oxo-7,8-dihydro-2′-deoxyguanosine(8-oxodG), a urinary marker for oxidative DNA damage, than men with a low consumption of vegetables and fruit. Ten pairs of healthy non-smoking males aged between 28 and 59 years, matched for age (<10 years) and body-mass index (<2 kg m^{-2}) were selected from a dietary validation study. Habitual food intake was estimated with 12 monthly 24-h recalls. Men in the high vegetable and fruit group consumed an average of 224 g day^{-1} (range 101–330 g day^{-1}) more vegetables and fruit than men in the low vegetable and fruit group. Excretion of 8-oxodG was 95 pmol kg^{-1} day^{-1} (95% CI –29, 219) higher in the high vegetable and fruit group than in the low vegetable and fruit group (paired *t*-test, *P* = 0.11). Excretion of 8-oxodG was not correlated with intake of vitamins, energy, fat, nor with blood concentrations of antioxidant (pro)vitamins, but it was inversely correlated with age. The present findings do not suggest that humans with a habitual high fruit and vegetables consumption have less oxidative DNA damage as measured by 8-oxodG excretion than men with low consumption of fruit and vegetables.

Keywords: *fruit, vegetables, antioxidants, 8-oxodG, oxidative stress.*

Introduction

There is considerable epidemiological evidence that populations with a high intake of fruit and vegetables have a lower rate of incidence and mortality from various forms of cancer, but especially from cancers of the digestive and respiratory tract (Steinmetz and Potter 1991a). Fruit and vegetables contain a large number of natural components, e.g. vitamins, dietary fibre, minerals, flavonoids and glucosinolates, that could, by a variety of mechanisms, explain the cancer protective effects of these foods (Steinmetz and Potter 1991b). Most of these components are strong antioxidants, and oxidative DNA damage is believed to play an important role in tumour development (Ames *et al.* 1995). The reduced risk of cancer for humans with a high intake of vegetables and fruit may therefore be caused by a reduction in DNA damage by antioxidants typically present in these foods. We therefore investigated the hypothesis whether humans with habitual high consumption of vegetables and fruit have less oxidative DNA damage than those with a low consumption of vegetables and fruit. The most common and potentially mutagenic damage of DNA caused by reactive oxygen species is that arising by 8-hydroxylation of the guanine base (Ames *et al.* 1993). This DNA-adduct is presumably repaired by nucleases and the resulting product, 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), is excreted, independently of DNA from dietary sources such as meat, into the urine (Shigenaga *et al.* 1989). The rate of excretion of 8-oxodG may therefore serve as a useful biomarker of oxidative DNA damage in the whole body.

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METHODS

We selected from a validation study for a new food frequency questionnaire (Ocké *et al.* 1997a), 10 pairs of healthy, non-smoking men (age range 28–59 years) with a high and with a low consumption of vegetables and fruit, respectively. Men using analgesics were excluded from the analysis. Habitual food intake was estimated using the average of 12 monthly 24-h dietary recalls and nutrient intakes were calculated using a computerized version of The Netherlands food composition table. Pairs were matched according to age (<10 years) and body-mass index (<2 kg m^{-2}). The difference between pairs with respect to their consumption of fruit and vegetables was chosen to be at least 100 g daily. Subjects collected a 24-h urine sample (self-reported) for the determination of levels of 8-oxodG. The concentration of 8-oxodG was measured by high performance liquid chromatography and electrochemical detection as described by Loft *et al.* (1992), with a slight modification of the separation technique (Verhagen *et al.* 1997). Quantification was carried out with three-point calibration curves which were established for each sample by addition of the genuine compound. Parallel to urine collection, non-fasting blood samples were drawn in which serum α-tocopherol and carotenoids were determined as described previously (Ocké *et al.* 1997b).

Results

The mean (±SD) difference between the highest and lowest pairs in relation to their consumption of fruit and vegetables was 224±82 g daily (Table 1). Energy intake, fat intake, and vitamin E intake did not differ between pairs with high and low consumption of fruit and vegetables, whereas vitamin C and β-carotene intake was significantl

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	High (n = 10) Mean ± SD	Low (n = 10) Mean ± SD	Mean differences high–low (95% CI)	Paired t-test P value
Selection variables				
Vegetables (g day^{-1})	246±88	147±36	99 (39, 158)	—
Fruit (g day^{-1})	202±108	77±46	126 (61, 191)	—
Vegetables and fruit (g day^{-1})	448±111	224±53	224 (166, 283)	—
Matching variables				
Age (years)	46±10	45±10	1 (–2, 3)	—
Body-mass index (kg m^{-2})	25.8±2.4	26.0±2.0	–0.1 (–0.9, 0.6)	—
Descriptives				
Energy intake (MJ day^{-1})	11.3±1.7	10.4±1.7	0.9 (–1.0, 2.7)	0.313
Fat intake (g day^{-1})	101.6±22.9	101.4±31.9	0.2 (–32.0, 32.3)	0.990
Vitamin C intake (mg day^{-1})	113.2±24.0	70.9±19.2	42.2 (16.0, 68.5)	0.005
Vitamin E intake (mg day^{-1})	16.5±5.4	15.9±6.0	0.7 (–4.2, 5.6)	0.762
β-carotene intake (mg day^{-1})	2.10±0.93	1.36±0.51	0.73 (–0.00, 1.47)	0.050
Serum α-tocopherol (μmol l$^{1)}$	36.9±6.9	45.4±12.4	–8.5 (–18.9, 1.9)	0.096
Serum β-carotene (μmol l^{1})	0.34±0.16	0.28±0.14	0.06 (–0.08, 0.20)	0.366
Serum a-carotene (μmol l^{1})	0.07±0.05	0.06±0.05	0.02 (–0.04, 0.07)	0.549
Serum lycopene (μmol l^{1})	0.40±0.26	0.44±0.25	–0.05 (–0.33, 0.24)	0.714

Table 1. Characteristics of 10 pairs of men with high and low consumption of fruit and vegetables.

vegetable and fruit group. Blood levels of carotenoids did not differ between the low and the high fruit and vegetable group. Serum α-tocopherol was lower in the group with a high vegetables and fruit consumption than in the group who consumed little vegetables and fruit, although this difference was not statistically significant (paired t-test, P = 0.10) (Table 1).

Mean urinary excretion of 8-oxodG of all participants was 354±137 pmol kg^{-1} day^{-1}. Excretion of 8-oxodG could not be determined in one participant because of an interfering peak in the chromatogram, leaving a total of nine pairs for the analysis. 8-OxodG excretion was not lower in men with a high fruit and vegetables consumption, but, rather, tended to be higher in this group (95 pmol kg^{-1} day^{-1} 95% CI-29, 219) although this difference did not reach statistical significance (Table 2). In the 19 participants, 8-oxodG excretion was not correlated with the consumption of vegetables and fruit, any specific vegetables or fruit (e.g. cruciferous vegetables), intake of energy, fat and vitamins, serum levels of antioxidant (pro)vitamins, nor with body-mass index (all Spearman rank correlation coefficients <0.25). Age (range 28–59 years) was inversely correlated to 8-oxodG excretion (r = –0.50, P = 0.03). We also calculated the vegetable and fruit consumption of the participants using only the dietary 24-h recall prior to urine collection. Participants were divided into a high and low vegetable and fruit group using the same selection and matching criteria as in the original analysis. Men in the high vegetable and fruit group consumed an average of 187±92 g more vegetables and fruit than men in the low vegetable and fruit group and 8-oxodG excretion was 12 pmol kg^{-1} day^{-1} (95% CI –147, 171, paired t-test P = 0.866) higher in the high vegetable and fruit group.

Discussion

Our results do not suggest that non-smoking men with a high habitual consumption of vegetables and fruit have less oxidative DNA damage, as measured by excretion of the DNA repair product 8-oxodG, than men with a low habitual consumption of vegetables and fruit. Although the power of our study is too limited to exclude such an association, it seems more likely that habitual vegetable and fruit consumption is not an important determinant of 8-oxodG excretion. Antioxidant vitamins in the diet and blood levels of antioxidant (pro)vitamins also did not predict 8-oxodG excretion, whereas age was inversely related to 8-oxodG excretion.

Loft *et al.* (1992) reported earlier that 8-oxodG excretion was not associated with intake of antioxidant vitamins in 83 men and women. In that study only smoking, body-mass index and gender were identified as strong predictors of 8-oxodG excretion. We observed previously in a dietary intervention study that 8-oxodG excretion was low

Pair	Difference in vegetable and fruit consumption (g day^{-1})	Levels of 8-oxodG in urine (pmol kg^{-1} day^{-1}) High	Low	High–Low
1	+330	510	523	–13
2	+323	255	—[a]	—
3	+317	146	300	–154
4	+280	406	441	–35
5	+217	310	202	108
6	+188	399	82	317
7	+179	482	195	287
8	+156	475	440	35
9	+154	458	203	255
10	+101	479	422	57
Mean ±SD	224±82	392±119[b]	312±150[b]	95±161[b]
(95% CI)				(–29, 219)
Paired *t*-test				$P = 0.114$

Table 2. Differences in 8-oxodG excretion in 10 pairs of non-smoking men with a high and low consumption of vegetables and fruit. Pairs were matched for age and body-mass index.

[a] Not determined because of interfering peaks.

[b] Figures do not add up because mean difference is calculated excluding pair no. 2.

males after daily consumption of 300 g of Brussels sprouts in comparison with non-cruciferous vegetables (Verhagen *et al.* 1995). In another intervention study, these results were recently confirmed in men, but not in women (Verhagen *et al.* 1997). In the present study however, we did not observe any association between habitual consumption of cruciferous vegetables and 8-oxodG excretion. A decrease in vitamin C intake from 250 mg to 5 mg daily has been found to be associated with increased 8-oxodG production in human seminal DNA, whereas repletion resulted in a decrease in 8-oxodG production (Fraga *et al.* 1991). On the other hand, supplementation with β-carotene did not affect excretion of 8-oxodG in smoking males (van Poppel *et al.* 1995).

It is possible that long-term average intake of vegetables and fruit is less predictive for oxidative DNA damage, but the diet 24 h prior to 8-oxodG measurement was also not associated with oxidative DNA damage. Observational studies, conducted within one country (e.g. The Netherlands) with uniform dietary habits may lack sufficient contrast in vegetable and fruit consumption, which is needed to detect an association. This may explain why an effect was observed on 8-oxodG excretion in intervention studies in which a large contrast was achieved through supplementation. The need for large contrasts could also be explained by limitations in estimating individual food intake by dietary assessment methods. Although we used 12 repeated 24-hour recalls, a method considered to provide reasonably valid estimates of habitual food intake, we cannot exclude misclassification of exposure. This is also suggested by the poor correlation between intake and blood levels of carotenoids. This issue is extensively discussed by Ocké *et al.* (1997b). On the other hand, the blood levels of β-carotene, α-carotene and lycopene themselves, which reflect vegetable and fruit consumption, but do not suffer from these limitations, were also not associated with 8-oxodG excretion. Also, serum α-tocopherol, which is considered to be the major lipophilic antioxidant in the human body, was not associated with 8-oxodG excretion.

Ageing has been associated with a lower DNA repair activity and in our study age was indeed inversely related to 8-oxodG excretion and it was the only factor predicting 8-oxodG excretion. Fraga *et al.* (1991) observed that oxidative DNA damage in rats, determined in various organs, increased with age, but that urinary 8-oxodG excretion in rats decreased with age, possibly due to a decrease in DNA repair activity. It seems therefore that urinary excretion of 8-oxodG is an integrated biomarker, reflecting both oxidation DNA *damage* and DNA *repair capacity*, rather than being merely an indicator of damage caused by oxidants to DNA. Our study population consisted only of non-smoking men, who are much less exposed to oxidative stress than smokers, and we cannot exclude that in smokers, 8-oxodG excretion is more strongly governed by oxidative DNA damage caused for instance by cigarette smoke and by protection from dietary antioxidants. Alternatively, the origin of released 8-oxodG has been questioned by Lindahl (1993). It cannot be ruled out that 8-oxodG could be caused by degradation of DNA from dead cells by unspecific nucleases and oxidation could have occurred during passage through the kidney.

Clearly, more studies on markers of oxidative DNA damage and repair, including urinary 8-oxodG excretion, and their determinants are needed, Age and smoking are two important confounding factors that have to be taken into account in such studies.

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