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Extreme exercise and oxidative DNA modification

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Extreme exercise increases oxygen uptake with a potential for increased formation of reactive oxygen species. Damage to biomolecules may occur if such an increase exceeds the protective capacity of antioxidant defence mechanisms. Vigorous exercise amounting to ~10 h a day for 30 days increased the rate of oxidative DNA modification by 33% (95% confidence limits, 3–67%; $P < 0.02$) in 20 men owing to the urinary excretion of 8-oxo-7,8-dihydro-2'-deoxyguanosine, an oxidatively modified deoxynucleoside originating from nuclear DNA repair, oxidation of the nucleotide pool from mitochondrial DNA and/or from cell turnover. Oxidative stress to DNA points to a risk for the development of cancer and premature ageing from extreme exercise.

Keywords: Deoxyguanosine, DNA damage, extreme exercise, free oxygen radicals, oxidative stress.

Introduction

Across species, those with high specific oxygen consumption live shorter and age much earlier than those with a low oxygen consumption (Adelman *et al.*, 1988; Shigenaga *et al.*, 1989; Cutler, 1991; Loft *et al.*, 1993). Recently, we demonstrated a close relationship between individually measured oxygen consumption and urinary excretion of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG), further lending support to the notion that individual oxygen consumption is a predictor of the rate of oxidative modification of DNA (Loft *et al.*, 1994). Exercise increases oxygen consumption and the oxidative modification of proteins (Witt *et al.*, 1992). The exercise 'dose' relates to the extent of lipid peroxidation measured by serum malon aldehyde and pentane production (Kanter *et al.*, 1993). Exercise would be expected to increase the rate of oxidative DNA damage, if the capacity of the antioxidant defence mechanisms is exceeded. Endurance exercise has been reported to lead to an adaptive response in muscle and adipose tissue, however, without changes to vitamin E or ubiquinone (Gohil *et al.*, 1987). Immediately after running or swimming, Inoue *et al.* (1993) reported no change in the urinary excretion of 8-oxodG and that the levels of 8-oxodG in lymphocyte DNA were unchanged or slightly reduced. Nielsen *et al.* (1995) reported that a short, all-out bout of rowing did not change the urinary excretion of 8-oxodG. The effects of long-standing vigorous exercise in man, however, is unknown. We therefore hypothesized that long-standing vigorous exercise implies an increased amount of reactive oxygen formed in the cells. We tested this hypothesis by estimating the rate of oxidative DNA modification from the urinary excretion of the DNA repair product 8-oxodG before and after 30 days of extreme exercise.

Materials and methods

Twenty-three healthy males, 11 of whom were smokers, with an average (± S.D.) age of 22 ± 2 years participated in a 30 day programme of physical training with the Danish Army as part of their career advancement. The physical training programme consisted of 8–11 h of vigorous exercise per day, 6 days per week. The training programme contained mostly conventional indoor and outdoor sports disciplines, including long-distance running. The object of the programme was to select qualified instructors for basic training of drafted soldiers, and all subjects exercised on a regular basis before entering the programme. Smoking, diet and

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other habits were reported to be unchanged during the 30 day period by the subjects.

Spot urine samples were collected after informed consent as reported by Vistisen *et al.* (1992) before lunch at the same time of day (1) the day before and (2) the last day of the 30 day period. The urine samples were stored at –20°C until analysis for 8-oxodG by a tri-dimensional HPLC separation with electrochemical detection described in detail by Loft *et al.* (1993). The intra- and inter-day coefficients of variation for the analysis were 8 and 10%, respectively (Loft *et al.*, 1995). The concentrations of 8-oxodG are stable for more than 3 years in such conditions. Three samples could not be analysed due to interfering peaks in accordance with an earlier report (Loft *et al.*, 1992). The values were standardized by the urinary creatinine concentration, and a paired *t*-test was used to compare pre- and post-exercise values.

Results

The creatinine standardized excretion of 8-oxodG increased from 1.03 ± 0.59 (mean ± S.D.) to 1.25 ± 0.59 nmol mmol^{-1} creatine after the 30 day training period, corresponding to an average 33% increase (95% confidence limits, 3–67%; $P < 0.02$). In the non-smokers ($n = 9$), the exercise induced a significant 50% increase in oxidative DNA modification (95% confidence limits, 2–98%; $P < 0.05$), whereas the 25% increase seen in the smokers ($n = 11$) did not reach significance ($P > 0.05$). The means (bars) and individual data (lines) are shown in Fig. 1.

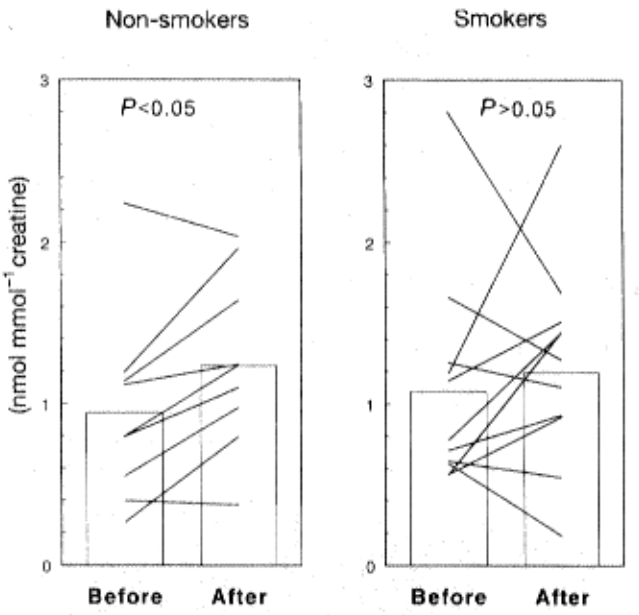

Figure 1 Urinary excretion of 8-oxo-7,8-dihydro-2'-deoxoguanosine (8-oxodG), in nmol mmol^{-1} creatine, in 9 non-smokers and 11 smokers before and after a 30 day programme of 8–11 h of vigorous exercise 6 days per week. In the group as a whole ($n = 20$), the excretion of 8-oxodG increased 33% (95% confidence limites, 3–67%) after the exercise programme ($P < 0.02$). The lines represent pre- and post-exercise values in each individual. The histograms show the mean values. The *P*-values for the smoking and the non-smoking groups are also given.

Discussion

The presence of free oxygen radicals and other reactive oxygen species formed during normal cellular respiration is believed to be the major cause of oxidative DNA modification. In the present study, we found that 30 days of intense exercise increased excretion of the oxidatively modified deoxynucleoside 8-oxodG. After 30 days, oxidation and repair of DNA can be assumed at a new steady-state in which urinary excretion is determined by the rate of oxidative DNA damage.

Immediately after acute exercise, increased repair has been suggested (Inoue *et al.*, 1993). Witt *et al.* (1992) reported no oxidative RNA damage from 90 min of sub-maximal exercise (65% $\dot{V}O_2$ max) in 11 individuals; however, such an exercise regimen may be too short and small to outbalance the quenching and repair of reactive oxygen species. By contrast, DNA damage was indicated by single cell gel electrophoresis in subjects asked to run as long as possible (Hartmann *et al.*, 1994).

Reactive oxygen species are formed from a minor four single electron transfer pathway metabolism of oxygen corresponding to a few percent of total oxygen consumption (Chance *et al.*, 1979). In the present study, we found a small increase only in the already high rate of oxidative DNA modification in the smokers as opposed to the non-smokers. Previously, we demonstrated 50% higher 8-oxodG excretion in smokers compared with non-smokers (Loft *et al.*, 1992, 1994). The apparently limited increase in 8-oxodG excretion in smokers may be explained by the smokers having a higher metabolic rate than the non-smokers before exercise commenced (Moffatt and Owens, 1991), presumably owing to an increased adrenergic drive or the high content of reactive oxygen species in the gas phase of tobacco smoke. Exercise also increases the adrenergic drive; however, this may be limited in smokers because of their higher basal drive. Another possible mechanism is psychological stress inflicted by the competitive nature of the 30 day programme, as demonstrated in rats (Adachi *et al.*, 1993).

Extensive repair is a necessity due to the high rate of oxidative modification of DNA. *In vivo* 8-oxodG repair occurs by two mechanisms, glycosylase removal of the oxidized base (yielding 8-oxoguanine) and nucleotide excision (yielding 8-oxodG) (Boiteux *et al.*, 1992; Klein *et al.*, 1992; Park *et al.*, 1992; Inoue *et al.*, 1993). The DNA repair response after exercise is not known.

The antioxidant defence mechanisms are reported to be elevated after exercise; for example, increased plasma levels of vitamin C (Garry and Appelzeller, 1983; Gleeson and Maughan, 1987) and vitamin E (Pincemail *et al.*, 1988). The exact mechanism of the increase is not known in detail, but redistribution from cellular compartments to plasma has been suggested. The notion is supported by the lowered levels of vitamin E in muscle seen after endurance training (Quintanilha, 1988). The role of antioxidant vitamins in the prevention of exercise-induced stress is still questioned (Gerster, 1989; Goldfarb, 1993). An acute bout of exercise does not affect hepatic or heart antioxidant enzyme activity, but some effects are seen in skeletal muscle, where glutathione peroxidase in particular shows an adaptive response (Li, 1993). The observed redistribution of antioxidant vitamins from cellular compartments to plasma without an adaptive antioxidant enzyme response is compatible with increased intracellular oxidative stress (i.e. compatible with oxidative DNA modification).

The relationship between oxidative DNA modification and the effects on health of long-standing and vigorous exercise is presently unknown but could imply risk for the development of cancer and premature ageing.

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