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Restricted pulmonary diffusion capacity after exercise is not an ARDS-like injury

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Pulmonary diffusion capacity (DL_{CO}) is reduced 2 h after various types of exercise, such as rowing, treadmill running, arm cranking and marathon running. The decrease in DL_{CO} may involve alterations in the alveolar-capillary membrane as well as depletion of the central blood volume. We hypothesized that the reduction in DL_{CO} might also be influenced by oxygen free radicals, acute phase proteins and endotoxin, which are also involved in the adult respiratory distress syndrome (ARDS). Ten competitive male oarsmen performed a 6 min 'all-out' ergometer row. Single breath DL_{CO} was determined before and 2 h after rowing and venous blood samples were also obtained during the row. Absolute DL_{CO} decreased by 11% (range 0–20%) 2 h after rowing, whereas the concentration of endotoxin did not change significantly and interleukin (IL)-1-α, IL-8 and tumour necrosis factor (TNF)-α were below the levels of detection before, during and 2 h after rowing. Oxygen free radicals were evaluated by oxidative modification of amino acids and DNA. Corrected for creatinine in urine voided 3 h post-exercise, the DNA repair product 8-oxo-7,8-dehydro-2-deoxyguanosine (8-oxodG) did not change significantly. The ratio of fluorescence due to dityrosine to that due to tryptophan in plasma proteins increased after exercise. This might reflect an effect of oxygen free radicals, but it might also indicate an altered relative composition of plasma proteins. These results suggest that the reduced pulmonary diffusion capacity following exercise is unrelated to factors typically associated with ARDS.

Keywords: Adult respiratory distress syndrome, cytokines, endotoxins, oxygen free radicals, rowing.

Introduction

Pulmonary diffusion capacity (DL_{CO}) decreases ~15% 2 h after various types of exercise, such as rowing (Rasmussen *et al.*, 1988; Rasmussen *et al.*, 1992; Hanel *et al.*, 1993), treadmill running (Rasmussen *et al.*, 1992), arm cranking (Rasmussen *et al.*, 1986; Rasmussen *et al.*, 1992) and marathon running (Miles *et al.*, 1983). The post-exercise restriction of DL_{CO} persists for at least 6 h, and the pre-exercise DL_{CO} is not re-established for 1–2 days (Rasmussen *et al.*, 1988; Rasmussen *et al.*, 1992). The persisting post-exercise reduction in DL_{CO} might indicate alterations of the alveolar-capillary membrane (Manier *et al.*, 1991), but administration of 10 mg furosemide (a diuretic drug) intravenously does not re-establish the pre-exercise DL_{CO} (Hanel *et al.*, 1994). On the contrary, the increase in thoracic electrical impedance (Rasmussen *et al.*, 1992; Hanel *et al.*, 1994) suggests that post-exercise central blood volume depletion may influence DL_{CO} by reducing the number of erythrocytes present in the capillaries of the exchanging alveoli.

We hypothesized that the reduction in DL_{CO} after exercise might also involve endotoxins, acute phase cytokines and oxygen free radicals. These factors are part of the mediators typically associated with the adult respiratory distress syndrome (ARDS) (Runcie and Ramsay, 1990), characterized by pulmonary insufficiency related to microvascular lung injury (Demling *et al.*, 1990). Thus, the plasma concentrations of lipopolysaccharides (LPS), interleukin (IL)-1-α, IL-8 and tumour necrosis factor (TNF)-α were determined before, during and after 'all-out' ergometer rowing. The

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generation of oxygen free radicals was evaluated indirectly by oxidative modification of DNA and amino acids.

Methods

Ten competitive male oarsmen completed the protocol after giving their informed consent. The study was approved by the Ethics Committee of Copenhagen (KF.V.92-158). Their median physical characteristics were as follows: age 23 (range 19–30) years, body mass 81 (71–92) kg, height 185 (181–196) cm, maximal oxygen uptake ($\dot{V}O_2$ max) 5.8 (4.9–6.6) $l\ min^{-1}$. None of the subjects had any disease or injury 3 weeks prior to the experiment, nor did they take any medication. Training was not allowed on the day prior to the experiment, and the subjects were not allowed to drink or eat after midnight on the day of the experiment, which began at 08:15 h.

Exercise was performed on a rowing ergometer (Concept II, Morrisville, VT, USA). After 10 min of an individually paced warm-up period commencing at 11:00 h, the subjects performed a maximal rowing protocol simulating actual race conditions in a 6 min all-out effort with which they all were familiar. The ergometer was connected to a computer (Concept II) with work rate stored in memory and a read-out of time for every '500 m'. Heart beats were recorded using a short-range radio telemetry system (Sport Tester PE 3000, Polar Electro, Kempele, Finland), and heart rate was stored in memory every 15 s.

Blood samples were obtained from a venous catheter inserted at ~09:45 h through an antecubital vein to the superior caval vein. Before and 2 h post-exercise, blood samples were taken after 15 min of supine rest. During rowing, 6 ml of blood for detection of cytokines were obtained during the last 2 min of exercise. The 10 ml of blood used for the determination of LPS and the 3 ml of blood used for total protein, dityrosine and tryptophan were obtained within 10 min of the cessation of exercise. Blood was collected in ice-cooled tubes with added aprotinin (Bayer, Leverkusen, Germany) and the samples for the detection of LPS were collected in tubes sterilized by radiation.

The DL_{CO} was assessed at 09:00 h using the single breath method (Krogh, 1914) after supine rest with the subjects seated. A single inspiration of a mixture of carbon monoxide (0.28%) and oxygen (21%) was performed and during a 10 s breath hold the rate of disappearance of CO from the alveolar gas was calculated. Inspiratory and expiratory concentrations of CO were measured with an infrared analyser (Morgan type 101, Rainham, UK). All measurements of DL_{CO} were made in duplicate and the means of the two determinations noted.

The blood samples obtained for determination of cytokines were centrifuged and plasma was frozen at −80° C. The IL-1-α, TNF-α and IL-8 were detected in plasma using ELISA-kits (Immunotech, Marseille, France; Biokine, Cambridge, MA, USA; R&D Systems, Minneapolis, MN, USA). The blood sample for determination of LPS was centrifuged within 10 min, and plasma was frozen immediately and stored at −20° C. The concentration of LPS in plasma was evaluated by rocket immunoelectrophoresis (Baek, 1983).

Chemically modified amino acids may be markers of oxidative stress. Dityrosine increases in response to H_2O_2 by oxidation of the amino acid tyrosine (Valoti *et al.*, 1992), whereas the hydroxyl radical (•OH) causes oxidation of tryptophan (Singh *et al.*, 1981). The •OH radicals may also react with DNA to give base-adducts (Halliwell and Dizdaroglu, 1992), repaired and excreted in the urine (Loft *et al.*, 1992).

The blood samples for determination of dityrosine and tryptophan were stored ice-cooled until centrifugation, which took place within 2 h. The total amount of protein in plasma was measured using the method of Lowry *et al.* (1951), and the determination of dityrosine and tryptophan in plasma by fluorescence was measured with excitation at 325 and 280 nm and emission at 415 and 345 nm, respectively (Valoti *et al.*, 1992; Singh *et al.*, 1981). The fluorescence of bovine serum albumin at concentrations of 50.0, 62.5, 87.5 and 100 mg ml^{-1} was determined in duplicate, measured at the same excitation and emission as dityrosine and tryptophan.

To obtain a baseline value for 8-oxodG urinary excretion, the subjects were instructed to collect urine voided between 11:00 and 14:00 h on the day before the study (urine sample 1), and the urine was stored at 5° C. On the test day, the subjects were told not to void urine until just prior to rowing (urine sample 2). Urine sample 3 was collected after rowing for 3 h, during which time the subjects were offered cold drinks *ad libitum*. Five millilitres of each urine sample were frozen at −5° C. The urinary concentration of 8-oxodG was measured using high-performance liquid chromatography (HPLC) with electrochemical detection (Loft *et al.*, 1992) and was related to the concentration of creatinine to correct for differences in urine volume.

The results are presented as medians (plus range). Friedman's test of variance was used to evaluate changes with time and Wilcoxon's test was used to locate differences. A P-value<0.05 was considered significant.

Results

Maximal heart rate was 189 (173–197) beats min^{-1} and work rate was 391 (329–445) W, indicating maximal

rowing. This work bout decreased DL_{CO} from 14.0 (12.0–17.2) mmol min^{-1} kPa^{-1} [105 (90–129) mmol min^{-1} $mmHg^{-1}$] to 12.6 (10.0–17.1) mmol min^{-1} kPa^{-1} [95 (75–129) mmol min^{-1} $mmHg^{-1}$] 2 h after exercise, corresponding to a decrease of 11% (0–20%) ($P < 0.05$).

In response to rowing, the concentration of endotoxins in plasma did not change significantly, and the concentrations of IL-1-α, IL-8 and TNF-α were below the level of detection (Table 1). The total protein concentration of plasma increased during the exercise bout. Correcting for this increase, the fluorescence due to tryptophan decreased, whereas that due to dityrosine did not alter significantly. Thus, the ratio of dityrosine to tryptophan fluorescence was elevated by 33% (12–82%) during rowing ($P < 0.05$). The ratio returned to pre-exercise levels 2 h later (Table 1). The different concentrations of bovine serum albumin resulted in fluorescences of 1825, 1595, 1500, 1325, and 2100, 2350, 2900, 3550, respectively.

During the first 3 h after rowing, the urinary excretion of 8-oxodG was increased 3–4 times in three of the subjects, but was unchanged in the others. Accordingly, the average excretion rate was 1.7 times the control value and not statistically significant when the whole group was evaluated (Table 1).

Discussion

We demonstrated a reduction in pulmonary diffusion capacity (DL_{CO}) after all-out exercise concomitant with an elevation of only one index of oxygen free radicals. The ratio of fluorescence due to dityrosine to that due to tryptophan in plasma proteins increased, while the urinary excretion of 8-oxo-7,8-dehydro-2-deoxyguanosine (8-oxodG), a repair product of oxidative damage to deoxyribonucleic acids (DNA) (Loft *et al.*, 1992), did not change significantly. Also, changes in plasma endotoxin failed to reach statistical significance and cytokines were below the level of detection.

Oxygen free radicals are substances with an unpaired electron in an orbital ring, which makes them highly reactive and short-lived. Even though the excretion of 8-oxodG was not significant, the 1.7-fold average increase might reflect a potential for the generation of oxygen free radicals in response to rowing. Amino acids may also be affected by oxygen free radicals provoking a change in the ratio of fluorescence due to dityrosine to that due to tryptophan. However, the fluorescence of bovine serum albumin mimicked the plasma fluorescences. Furthermore, tryptophan accounts for only 0.19% of the amino acids in albumin, whereas globulin-gamma and fibrinogen contain 5%. Tyrosine accounts for 3% in each of the three plasma proteins mentioned. Accordingly, the changes in fluorescences may also indicate an altered relative composition of plasma proteins after exercise.

Working skeletal muscles are the most likely site for the formation of oxygen free radicals during exercise as a result of greatly elevated oxygen uptake. A commonly suggested source of the oxygen free radicals is semiquinones located on the electron transport of the mitochondria, which reduce oxygen univalent to superoxide radicals (Sjödin *et al.*, 1990). Of the oxygen consumed by cells, 5% may result in oxygen free radicals.

The lung tissue may also be a site for the formation of oxygen free radicals. This is demonstrated when pulmonary hypoperfusion leads to non-hydrostatic pulmonary oedema, in part from xanthine oxidase (XO) generated superoxide radicals (O_2^-) directed at the capillary endothelium (Grosso *et al.*, 1989). Barnard and Matalon (1992) reported that XO causes a two-fold increase in the capillary filtration coefficient.

Table 1 Median levels (range in parentheses) of endotoxin, 8-oxodG, total protein, dityrosine and tryptophan in response to 6 min 'all-out' ergometer row

	Before	During	After
Endotoxin (pg ml^{-1})	4.8 (0.0–26.0)	4.8 (1.6–25.0)	1.6 (0.0–12.8)
8-oxodG/creatinine (nmol l^{-1})	2.5 (1.6–3.7)	2.2 (1.3–3.9)	1.6 (1.4–14.7)
Total protein (mg ml^{-1})	70 (42–86)	82[a] (66–101)	74 (39–96)
Dityrosine/total protein (D)	48 (27–145)	50 (15–100)	46 (13–155)
Tryptophan/total protein (T)	5 (3–18)	5[a] (2–11)	6[a] (2–19)
Ratio D/T	8.5 (4.8–11.2)	11.3[a] (5.3–20.4)	6.4 (5.6–12.0)

[a] Compared to rest (Before), $P < 0.05$. Values of 8-oxodG were obtained the day before rowing (Before), prior to rowing (During) and 3 h post-exercise (After).

Oxygen free radicals are also generated during reperfusion after a relative period of hypoperfusion. In fact, Schaffartzik *et al.* (1992) reported a mismatch of the ratio of alveolar ventilation to perfusion ($\dot{V}_A/\dot{Q}$) after exercise, which indicates local hypoperfusion in the lung tissue. This is reflected in the increased activity of XO demonstrated after exercise (Veera *et al.*, 1992). Hypoperfusion might also take place in response to rowing as the thoracic electrical impedance increases post-exercise (Rasmussen *et al.*, 1992; Hanel *et al.*, 1994). Oxygen free radicals generated in the lung tissue could react with pulmonary capillaries causing reduced diffusion capacity. However, since only one of the measured indices increased in this study, it would seem unlikely that oxygen free radicals play a major role in producing post-exercise reductions in DL_{CO}.

Other studies have demonstrated oxygen free radicals in response to exercise as indicated by the ratio of reduced to oxidized glutathione (GSH/GSSG) (Sastre *et al.*, 1992; Lew and Quintanilha, 1990; Viguie *et al.*, 1993) and the activity of glutathione peroxidase (Criswell *et al.*, 1993; Lew and Quintanilha, 1990). However, Viguie *et al.* (1993) reported an unchanged urinary excretion of the ribonucleic acid (RNA) adduct, 8-hydroxyguanosine, in response to consecutive days of submaximal exercise. Of particular relevance to the present results, Dernbach *et al.* (1993) concluded that there is no evidence of oxidative stress during high-intensity rowing training, as the thiobarbituric acid-reactive substances (TBARS) in plasma and muscle do not change significantly.

In relation to microvascular injury, pulmonary insufficiency is one of the main consequences of ARDS, with the oxygen free radicals, endotoxins and cytokines all being implicated (Runcie and Ramsay, 1990).

Oxygen free radicals related to ARDS have not been demonstrated by changes in 8-oxodG, dityrosine or tryptophan. However, Grum *et al.* (1987) reported that the activity of XO in plasma was higher than in non-ARDS patients, which indirectly reflects that oxygen free radicals are present. Changes in the concentration of endotoxins in plasma were demonstrated by Parsons *et al.* (1989), who reported that the mean levels of endotoxin were greater in patients with ARDS than in patients at risk (0.38 *vs* 0.06 ng ml^{-1}).

Furthermore, cytokines in the plasma of patients with ARDS have been noted at higher concentrations than in controls. The level of IL-8 was shown to be higher in ARDS patients (Chollet *et al.*, 1993), and the mean levels of TNF-α in the serum of patients were shown to be non-significantly higher than in normal subjects (Hyers *et al.*, 1991). In contrast, Suter *et al.* (1992) could not demonstrate increased levels of TNF-α and IL-1-β in their ARDS patients.

Even though other types of exercise have been demonstrated to induce increased levels of IL-1-α and endotoxins in plasma (Bosenberg *et al.*, 1988; Lewicki *et al.*, 1988), we were unable to detect any of the mediators involved in ARDS. Since only one index indicated oxygen free radicals in this study, this suggests that the analogy of the post-exercise reduction in pulmonary diffusion capacity to ARDS is doubtful. However, that undetectable damage around pulmonary capillaries takes place cannot be rejected, as we did not measure the substances in pulmonary venous blood. Taken together, a 6 min 'all-out' ergometer row reduces pulmonary diffusion capacity unrelated to the factors which provoke ARDS.

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