# Sustained submaximal exercise does not alter the integrity of the lung blood-gas barrier in elite athletes

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**Hopkins, Susan R., Robert B. Schoene, William R. Henderson, Roger G. Spragg, and John B. West.** Sustained submaximal exercise does not alter the integrity of the lung blood-gas barrier in elite athletes. *J. Appl. Physiol.* 84(4): 1185–1189, 1998.—The extreme thinness of the pulmonary blood-gas barrier results in high mechanical stresses in the capillary wall when the capillary pressure rises during exercise. We have previously shown that, in elite cyclists, 6–8 min of maximal exercise increase blood-gas barrier permeability and result in higher concentrations of red blood cells, total protein, and leukotriene $B_4$ in bronchoalveolar lavage (BAL) fluid compared with results in sedentary controls. To test the hypothesis that stress failure of the barrier only occurs at the highest level of exercise, we performed BAL in six healthy athletes after 1 h of exercise at 77% of maximal $O_2$ consumption. Controls were eight normal nonathletes who did not exercise before BAL. In contrast with our previous study, we did not find higher concentrations of red blood cells, total protein, and leukotriene $B_4$ in the exercising athletes compared with control subjects. However, higher concentrations of surfactant apoprotein A and a higher surfactant apoprotein A-to-phospholipid ratio were observed in the athletes performing prolonged exercise, compared with both the controls and the athletes from our previous study. These results suggest that, in elite athletes, the integrity of the blood-gas barrier is altered only at extreme levels of exercise.

bronchoalveolar lavage; capillary stress failure; exercise-induced pulmonary hemorrhage; leukotriene $B_4$; surfactant

DURING MAXIMAL EXERCISE there are large increases in pulmonary arterial and pulmonary arterial wedge pressures (21, 31), and the calculated capillary pressure in the base of the human lung is >35 mmHg (38). These capillary pressures are sufficient to cause ultrastructural changes in the blood-gas barrier of the rabbit, including disruptions of the capillary endothelium, basement membrane, and alveolar epithelium (29). Bronchoalveolar lavage (BAL) fluid obtained from these animals contains increased concentrations of red blood cells, total protein and leukotriene $B_4$ ($LTB_4$) (30). Almost all thoroughbred horses in training have evidence of exercise-induced pulmonary hemorrhage (20, 39) and mechanical stress failure of pulmonary capillaries (37) caused by the extremely high capillary pressures that occur with exercise (8, 12). However, exercise-induced pulmonary hemorrhage is not limited to thoroughbred horses and has also been reported in Shetland ponies (3) and greyhound dogs (10).

Postexercise hemoptysis has been reported in humans (13, 33, 36), and we have previously shown that, in elite human athletes, short-term maximal exercise results in higher concentrations of red blood cells, total protein, and $LTB_4$ in BAL fluid compared with sedentary control subjects (7). Because these changes occurred in the absence of higher concentrations of inflammatory markers (other than $LTB_4$) in the BAL fluid, the findings are consistent with an effect of mechanical stress on the integrity of the blood-gas barrier. We hypothesized that stress-related impairment of the pulmonary blood-gas barrier occurs only after exposure to maximal physiological stresses. Therefore, we would expect that sustained heavy, but submaximal, exercise would not result in higher concentrations of red blood cells, total protein, and $LTB_4$ in BAL fluid. To test this hypothesis, we performed BAL on elite athletes after 1 h of submaximal exercise at 75–80% of maximal $O_2$ uptake ($\dot{V}O_{2max}$) and also on normal nonathletic controls who did not exercise before BAL.

## METHODS

This study was approved by the Human Subjects Committee of the University of California, San Diego. Fourteen subjects [6 male athletes and 8 controls (3 women, 5 men); athletes, 27.5 ± 1.0 (SE) yr; controls, 28.1 ± 1.9 yr] were recruited by advertisement. After giving informed consent, they agreed to further study. All were healthy nonsmokers and had a negative medical history. The athletes were highly trained cyclists and included one professional cyclist, two United States Cycling Federation Category 1 (National Level) cyclists, and three Category 2 (Regional Level) cyclists. These athletes were similar in caliber to those of our previous study (7).

A screening history and physical examination was performed. On a separate occasion, the subjects returned to the laboratory for further testing. To determine the appropriate workload for the 1-h ride, $\dot{V}O_{2max}$ was determined on an electronically braked cycle ergometer (Quinton Excaliber) equipped with a racing saddle and the subject's own pedals. After a 10- to 15-min warm-up at a self-selected workload and a 5-min warm-up at 150 W, the subjects rode in a progressive exercise test (30 W/min) until they were unable to continue. Heart rate was monitored by cardiac monitor (Lifepac 6). The subjects breathed through a nonrebreathing valve (Hans Rudolph 2700). Expired gas was sampled continuously from a heated 7.2-liter mixing chamber, and $O_2$ and $CO_2$ concentrations were measured (mass spectrometer 1100, Perkin-Elmer). Expired gas flow was measured by using a pneumotachometer (Fleisch no. 3), and the electrical signals from the mass spectrometer and the pneumotachometer were logged at 100 Hz by using a 12-bit analog-to-digital converter. Ventilation ($\dot{V}_E$), $O_2$ consumption ($\dot{V}O_2$), and $CO_2$ production ($\dot{V}CO_2$) were calculated by using a commercially available software package (Consentius Technologies, Salt Lake, UT). $\dot{V}O_{2max}$ was considered to be the average of the two highest consecutive 30-s measures of $\dot{V}O_2$. These results were used to calculate a workload that represented ~75–80% of $\dot{V}O_{2max}$.

On a subsequent occasion, the subjects returned to the laboratory and rode for 1 h at the previously determined workload. Using the previously described system, we measured $\dot{V}O_2$ and $\dot{V}E$ continuously for the first 5 min and at 10-min intervals thereafter. Small adjustments were made to the workload to maintain $\dot{V}O_2$ within 75–80% of $\dot{V}O_{2max}$.

Fiber-optic bronchoscopy was performed as soon as possible after exercise and in all cases was performed 60 min after the athletes completed the exercise test. The control subjects were nonathletic; they performed only normal activities in the 48-h period before the bronchoscopy. The subjects were premedicated with atropine (0.04–1.0 mg im), and an intravenous catheter was inserted in a peripheral forearm vein. Nebulized 4% lidocaine was used for topical anesthesia of the nasopharynx, and supplemental $O_2$ was administered via nasal prongs. The subject was monitored for cardiac rhythm and $O_2$ saturation. The 5-mm fiber-optic bronchoscope was introduced transorally and wedged in the right middle lobe. Four separate 30-ml aliquots of 0.9% saline were instilled and retrieved by gentle suctioning. The greatest possible care was taken not to abrade the airway. The lavage fluid obtained was poured through 4 × 4-in. gauze moistened with 0.9% saline to remove mucus and was placed in sterile 50-ml conical tubes maintained on ice.

The details of the BAL fluid assays have been previously described (7) and therefore are only briefly presented here. Cell counts were performed on the unspun BAL fluid, and differential cell counts were performed on cytospin preparations stained with Diff-Quik (Scientific Products, McGaw Park, IN). Additional slides were stained for hemosiderin in alveolar macrophages and were given a hemosiderin score, as has been previously described (4). The remainder of the fluid was spun at 200 *g* for 10 min at 4°C. The supernatant BAL fluid was pooled and frozen at −70°C for later biochemical analysis.

*BAL fluid proteins.* Total protein in unconcentrated BAL fluid was measured by the bicinchoninic acid method (25, 26). Immunoglobulin (Ig) M (mol wt 900,000), IgG (mol wt 150,000), and albumin (mol wt 67,000) were measured by radioimmunodiffusion with the use of commercially available kits (The Binding Site, San Diego, CA), as previously described (7, 24). The lower limits for detection of these assays are (in μg/ml) 2.5 IgM, 4.2 IgG, and 16.9 albumin. To enhance the sensitivity of the IgM assay, the immunodiffusion plates were double loaded with sample volume.

*BAL fluid eicosanoids.* $LTB_4$ and leukotriene $C_4$ ($LTC_4$) (42) were assayed by RIA to determine activation of the 5-lipoxygenase pathways of the arachidonic acid cascade, as previously described. Each RIA was performed in duplicate according to standard protocols. $LTB_4$ was assayed by using a commercial [$^3$H]$LTB_4$ RIA kit (NEN Research Products, Boston, MA). The $LTB_4$ antisera had a sensitivity of 12.5 pg/0.1 ml sample. Rabbit sera against $LTC_4$ (22) were kindly provided by Drs. Robert W. Egan and John L. Humes (Merck Research Laboratories, Rahaway, NJ); [$^3$H]$LTC_4$ was provided by NEN. The $LTC_4$ antisera had a sensitivity of 20 pg/0.1 ml sample.

*Surfactant proteins.* Surfactant apoprotein A (SP-A) was measured by using a capture enzyme-linked immunosorbent assay (7). Sample coefficients of variation averaged 8.6 ± 6.6%. Surfactant phospholipid (PL) concentration was calculated from phosphorus content (23) of extracted surfactant (1).

*Statistical analysis.* Student's *t*-test for independent means was used to compare the results between athletes and control subjects. An ANOVA was used to compare SP-A, PL, and the SP-A/PL ratio from the present study with those previously obtained from athletes who exercised for 6–8 min at maximal levels (7).

## RESULTS

All subjects tolerated the bronchoscopy procedure well. The details of the exercise test are presented in Table 1. A summary of results is given in Table 2. We have previously presented data from four of the control subjects (7). There was no significant difference between athletes and controls in the volume of BAL fluid recovered.

*BAL fluid cells.* There were no significant differences between athletes and control subjects for any of the cell constituents in BAL fluid. There were small amounts of red blood cells present in the BAL fluid from all of the athletes and from five of the eight control subjects.

*BAL fluid proteins.* There was no significant difference between athletes and control subjects for total protein, IgG, or albumin. IgM was not detected in the BAL fluid of any subjects.

*$LTB_4$ and $LTC_4$.* $LTB_4$ was detected in the BAL fluid of only one of the athletes and in none of the control subjects. $LTC_4$ was not detected in the BAL fluid of subjects from either group.

*Surfactant apoproteins (SPs).* There was no difference between athletes and control subjects for BAL fluid PL. However, athletes had a significantly greater concentration of SP-A ($P < 0.01$) and a higher SP/PL ratio ($P < 0.05$) than did control subjects. We also compared the concentrations of SP-A and PL and the SP-A/PL ratio with our previous data obtained from athletes who exercised for 6–8 min at maximal levels (7) (Fig. 1). The athletes in the present study had a significantly greater concentration of SP-A ($P < 0.05$) and a higher SP-A/PL ratio ($P < 0.05$) than did the athletes who performed short-term maximal exercise. The SP-A/PL ratio was correlated with the percentage of measured maximal heart rate sustained during the exercise test ($R = 0.89$, $P < 0.05$, Fig. 2). SP-A/PL ratio was not significantly related to other measures of cardiovascular fitness such as $\dot{V}O_{2max}$ or $\dot{V}O_2$ at the ventilatory threshold. SP-A/PL ratio was not significantly correlated with average $\dot{V}E$ or average tidal volume sustained during the exercise test.

## DISCUSSION

*Summary of results.* We have previously shown that 6–8 min of maximal exercise in elite cyclists impairs

Table 1. *Descriptive characteristics of subjects and exercise test*

| | Athletes | Controls |
|---|---|---|
| *n* | 6 | 8 |
| Age, yr | 27.5 ± 1 | 28.1 ± 1.9 |
| $\dot{V}O_{2m}$, l/min | 4.79 ± 0.14 | |
| $\dot{V}O_{2max}$, ml·kg$^{-1}$·min$^{-1}$ | 64 ± 3 | |
| Maximal heart rate, beats/min | 192 ± 5 | |
| Average $\dot{V}O_2$ during test, l/min | 3.70 ± 3.79 | |
| %$\dot{V}O_{2max}$ | 77 ± 1 | |
| Average heart rate during test, beats/min | 158 ± 6 | |
| Average $\dot{V}E$ during test, l/min, BTPS | 94 ± 4 | |

Values are means ± SE; *n*, no. of subjects. $\dot{V}O_2$, $O_2$ consumption; $\dot{V}O_{2max}$, maximal $\dot{V}O_2$; $\dot{V}E$, minute ventilation.

Table 2. *Summary of measurements in bronchoalveolar lavage fluid*

| | Athletes | Controls | *P* Value |
|---|---|---|---|
| *n* | 6 | 8 | |
| BAL recovery, % | 51.5 ± 2.6 | 69.3 ± 8.5 | 0.47 |
| Red blood cell concentration, $\times 10^5$ cells/ml | 4.2 ± 2.0 | 1.7 ± 1.1 | 0.13 |
| White blood cell concentration, $\times 10^5$ cells/ml | 1.8 ± 0.4 | 1.2 ± 0.2 | 0.10 |
| Alveolar macrophages, % | 95 ± 1 | 91 ± 2 | 0.20 |
| Neutrophils, % | 2 ± 1 | 7 ± 1 | 0.78 |
| Lymphocytes, % | 3 ± 1 | 1 ± 1 | 0.24 |
| Hemosiderin score | 12 ± 3 | 12 ± 7.1 | 1.00 |
| Total protein, μg/ml | 139.3 ± 30.9 | 110.4 ± 11.6 | 0.16 |
| Albumin, μg/ml | 63.2 ± 17.1 | 46.0 ± 4.5 | 0.30 |
| IgG, μg/ml | 14.9 ± 3.2 | 10.7 ± 1.3 | 0.10 |
| IgM, μg/ml | ND | ND | |
| $LTB_4$, pg/ml | † | ND | |
| $LTC_4$, pg/ml | ND | ND | |
| SP-A, μg/ml | 8.9 ± 2.3 | 3.9 ± 0.5 | 0.01* |
| PL, μg/ml | 78.0 ± 8.6 | 95.8 ± 11.0 | 0.25 |
| SP-A/PL ratio | 0.14 ± 0.05 | 0.05 ± 0.01 | 0.03* |

Values are means ± SE; *n*,no. of subjects. BAL, bronchoalveolar lavage; $LTB_4$, leukotriene $B_4$; $LTC_4$, leukotriene $C_4$; SP-A, surfactant apoprotein A; PL, surfactant phospholipid. IgG, immunoglobulin G; IgM, immunoglobulin M. ND, not detected; †detected in only one subject (150 pg/ml). *Significant, $P < 0.05$.

the integrity of the pulmonary blood-gas barrier so that higher concentrations of red blood cells, total protein, and $LTB_4$ are observed in the BAL fluid compared with sedentary controls (7). In the present study, we did not find significant differences in the concentrations of red blood cells, total protein, $LTB_4$, or $LTC_4$ in BAL fluid from the athletes after 1 h of heavy, but submaximal, exercise compared with controls. However, the athletes had higher concentrations of SP-A and a higher SP-A/PL ratio compared with the control subjects and compared with the subjects who performed 6–8 min of maximal exercise. The higher SP-A/PL ratio was correlated with cardiovascular fitness, as measured by the percentage of the measured maximal heart rate sustained during

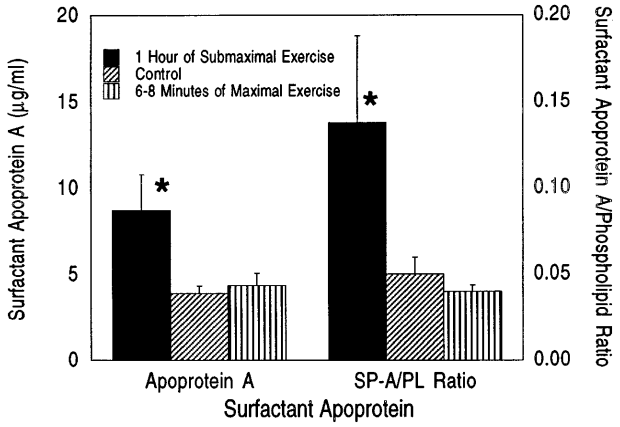


Fig. 1. Surfactant apoprotein-A (SP-A) and SP-A/phospholipid (PL) ratio in athletes from present study (1 h of submaximal exercise), control subjects, and subjects from previous study (6–8 min of maximal exercise; Ref. 7). Values are means ± SE. *$P < 0.05$ (2 tailed).

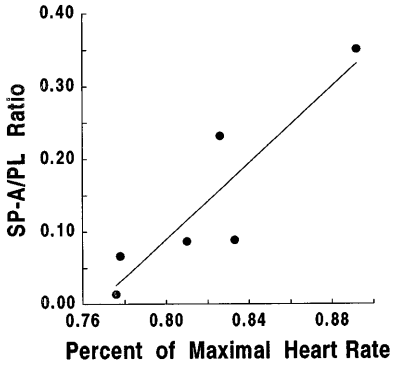


Fig. 2. Correlation between SP-A/PL ratio and %maximal heart rate sustained during exercise test. $R = 0.89$, adjusted $R^2 = 0.75$, $P = 0.02$.

the exercise test, but not with other measures of aerobic fitness.

*Rationale of the hypothesis.* There is now evidence that the blood-gas barrier is maintained to be as thin as possible to allow rapid exchange of respiratory gases but just strong enough to maintain structural integrity when subjected to maximal physiological stresses (see Ref. 35 for review). If that is correct, stress failure of the blood-gas barrier would be expected only under unusually high aerobic activity and not under less-extreme conditions.

Three primary mechanical forces act on the capillary wall: circumferential tension caused by capillary transmural pressure; longitudinal tension in the alveolar wall caused by high lung inflation; and surface tension, which is believed to support the capillaries and counteract the effects of circumferential and longitudinal tension (38).

Considerable data from several species demonstrate that pulmonary capillaries fail when exposed to high transmural pressures, whether induced in experimental preparations (29, 30) or as a result of extreme exercise (11, 37). Previously, we have shown higher concentrations of red blood cells, total protein, and $LTB_4$ in the BAL fluid of athletes who exercised at maximal levels for 6–8 min compared with sedentary controls (7). Those results suggested that short-term exercise alters the integrity of the blood-gas barrier so that the passage of red blood cells and protein is increased without altering the sieving function of the blood-gas barrier. The likely mechanism for this change in the blood-gas barrier is mechanical stress failure of the pulmonary capillaries.

There is good evidence that the strength of the blood-gas barrier is largely due to the extracellular matrix, which at the thinnest point is composed only of the basement membranes of the capillary endothelium and the capillary epithelium. The evidence includes the following. *1*) When the blood-gas barrier is exposed to high mechanical stress, breaks in the lung epithelium and endothelium are observed, whereas the basement membrane remains intact (38). *2*) When isolated rabbit renal tubules (consisting only of epithelium and basement membrane) are exposed to an increased transmural pressure, the mechanical properties are the same,

whether or not the epithelium is intact (34). *3*) The thickness of the basement membrane is greatest in the systemic capillaries of lower extremities, which are exposed to higher pressures than the rest of the body (40). *4*) The glomerular capillaries, which are exposed to systemic pressures, have a thicker basement membrane than the pulmonary capillaries (35). *5*) The compliance of mesenteric capillaries is consistent with the Young's modulus of basement membrane (27). *6*) The thickness of pulmonary capillary basement membrane is increased in mitral stenosis, in which pulmonary capillary pressure is chronically elevated (9). The last information suggests that the basement membrane is a dynamic tissue capable of remodeling in response to physiological stress, as is the case with pulmonary arteries (14, 15). In addition to the vulnerability of the blood-gas barrier to mechanical stress, there is evidence for diffusion limitation of pulmonary gas transport during exercise in both humans (5, 6, 28) and horses (32). The resistance to gas transport across the blood-gas barrier is proportional to its thickness; thus conflicting forces potentially affect regulation of strength of the blood-gas barrier.

*Effect of sustained submaximal exercise on red blood cells, protein, and $LTB_4$ in BAL fluid.* In the present study, we did not find any differences between athletes and control subjects in values for red blood cells, protein, or $LTB_4$ concentrations in BAL fluid. This suggests that 1 h of heavy but submaximal exercise is not sufficient to impair the blood-gas barrier, even though the exercise is of relatively long duration. This is not surprising, because it is expected that the blood-gas barrier would be exposed to lower transmural pressures in the present study than in our previous study, in which the subjects averaged 92% of their calculated maximal heart rate. During the 6–8 min of exercise at 90% of $\dot{V}O_{2max}$, a mean pulmonary arterial pressure of ~37 mmHg and a mean pulmonary arterial wedge pressure of ~21 mmHg are expected, and the calculated capillary pressure in the base of the lung is ~36 mmHg (31). This calculation is made by assuming that the pulmonary arterial wedge pressure is the same as left atrial pressure; that mean capillary pressure is half way between left atrial and pulmonary arterial pressure (likely to be a conservative estimate); and that the pressure is ~7 mmHg greater in the base of the lung, which is ~10 cm below the level of the heart. In the present study, our subjects were exercising at 82% of their maximal heart rate, and the expected capillary pressure at the base of the lung was between 25 and 30 mmHg. This calculation is made by using a value of 24–30 mmHg for pulmonary arterial pressure and a value of 12–16 mmHg for pulmonary arterial wedge pressure (31). In the rabbit lung, the capillaries consistently fail at a transmural pressure of ~39 mmHg (29), and the reduction in calculated capillary pressure during the prolonged submaximal exercise likely represents a considerable improvement in the safety factor before stress failure occurs.

We found small amounts of red blood cells in the BAL fluid of both athletes and control subjects in the present study. The presence of red blood cells in the BAL fluid likely resulted from abrasion of the airways during bronchoscopy. Great care was taken to avoid trauma to the airways, and identical bronchoscopy technique was followed for both groups of subjects. These findings underscore the need for control subjects studied under the same conditions when studies of this nature are interpreted.

*Effect of sustained submaximal exercise on SP in BAL fluid.* Pulmonary surfactant is synthesized in alveolar type II cells and is released by isolated type II cells in culture by a single mechanical stretch (41). Pulmonary surfactant is increased in the lung within minutes of increasing tidal volume in isolated perfused animal lungs (16, 18) and even within the first large breath (17). The release of pulmonary surfactant during hyperventilation is apparently a direct result of distortion of type II alveolar cells alone or in combination with acetylcholine and β-adrenergic mediators (18, 19). In human subjects, 30 min of exercise have been shown to increase the SP-A/PL ratio in aerobically fit subjects but to decrease it in less-fit subjects (2). We found a relationship between the SP-A/PL ratio and the percentage of the measured maximal heart rate sustained during the prolonged exercise test but no relationship to other measures of aerobic fitness. This result is probably because the athletic subjects are a very homogeneous group with respect to overall fitness, and the exercise task was chosen to maintain the subjects between 75 and 80% of $\dot{V}O_{2max}$. Also, only six of the subjects (the athletes) underwent the exercise test. Therefore, a very tight correlation is required before statistical significance is achieved.

*Conclusion.* Unlike short-term maximal exercise, sustained submaximal exercise does not result in higher concentrations of red blood cells, total protein, or $LTB_4$ in athletes compared with concentrations in nonexercising control subjects. This suggests that the integrity of the blood-gas barrier is only altered by maximal physiological stresses. Such a finding would be expected if the blood-gas barrier is continuously regulated to meet all but the most extreme physiological stresses.

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