Intrapulmonary arteriovenous shunts of >15 μm in diameter probably do not contribute to arterial hypoxemia in maximally exercising Thoroughbred horses

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Manohar, Murli, and Thomas E. Goetz. Intrapulmonary arteriovenous shunts of >15 μm in diameter probably do not contribute to arterial hypoxemia in maximally exercising Thoroughbred horses. J Appl Physiol 99: 224–229, 2005. First published March 17, 2005; doi:10.1152/japplphysiol.01230.2004.—The present study examined whether Thoroughbred horses performing strenuous exercise exhibit intrapulmonary arteriovenous shunting that may contribute to the observed arterial hypoxemia. Experiments were carried out on seven healthy, exercise-trained Thoroughbreds at rest, maximal exercise (galloping at 14 m/s on a 3.5% uphill grade for 120 s), and submaximal exertion (8 m/s on a 3.5% uphill grade for 150 s). Along with blood gas/hemodynamic parameters, intrapulmonary arteriovenous shunting was studied by injecting 15-μm-diameter microspheres, labeled with different stable isotopes, into the right atrium while simultaneous blood samples were being withdrawn at a constant rate from the pulmonary artery and the aorta. Arterial hypoxemia was observed only during maximal exercise. Also, despite significant pulmonary arterial hypertension during submaximal and maximal exertion, 15-μm microspheres did not traverse the pulmonary microcirculation to appear in the aortic blood. Thus our findings did not support a role for intrapulmonary arteriovenous shunts of >15 μm in diameter in the exercise-induced arterial hypoxemia in racehorses. Interestingly, our observation that, in going from 30 to 120 s of maximal exertion, arterial O2 tension had remained unchanged despite significant reductions in mixed venous blood O2 tension, hemoglobin-O2 saturation, and O2 content also discounts the importance of intrapulmonary arteriovenous shunts in causing arterial hypoxemia. This is because, assuming that a constant fraction of total pulmonary blood flow bypasses the gas-exchange areas of the equine lungs via intrapulmonary arteriovenous shunts during 30–120 s of maximal exertion, the observed significant reductions in mixed venous blood oxygenation should cause a significant reduction in arterial O2 tension, which was not the case in our horses. Thus it is suggested that intrapulmonary arteriovenous shunting probably does not contribute to the exercise-induced arterial hypoxemia in racehorses.

blood gas tensions during exertion; arterial desaturation during exercise; pulmonary microcirculation; microspheres

IT IS WELL KNOWN THAT STRENUOUSLY EXERCISING RACEHORSES experience significant arterial hypoxemia and desaturation of hemoglobin, and it is reported that these changes in arterial oxygenation limit athletic performance (1, 3, 8, 13–16, 24). A significant arterial hypercapnia is also observed in strenuously exercising Thoroughbred horses despite significantly increased alveolar ventilation. Although the inadequate alveolar hyperventilation during maximal exertion contributes to the observed reduction in arterial O2 tension, this mechanism does not account for the entire decrease in arterial O2 tension.

Multiple inert-gas elimination studies indicated that most of the exercise-induced arterial hypoxemia in racehorses is the result of diffusion limitation and that there is essentially no contribution from intrapulmonary arteriovenous shunting (24).

Exercise-induced arterial hypoxemia is also observed in healthy human athletes, wherein it limits maximal whole body O2 consumption (3, 4, 17). Diffusion limitation, inadequate alveolar hyperventilation, rightward shift of the hemoglobin-O2 dissociation curve, and ventilation-to-perfusion inhomogeneity are principal factors contributing to the arterial hypoxemia in exercising human athletes (3, 4, 17). Although multiple inert-gas elimination studies have discounted intrapulmonary arteriovenous shunts as contributing to the exercise-induced hypoxemia in human athletes (3), recently Eldridge and coworkers (5) demonstrated, using agitated saline contrast echocardiography, that increasing exercise intensity caused contrast bubbles to traverse the pulmonary circulation, thereby suggesting recruitment of dormant intrapulmonary arteriovenous shunts in healthy human subjects. Because the shunted mixed venous blood is deoxygenated, and becomes more so during strenuous exertion as O2 extraction by the working muscles increases with increasing work intensity, it was suggested that the contribution of intrapulmonary arteriovenous shunting in bringing about the exercise-induced arterial hypoxemia in human subjects cannot be discounted and may indeed be much more significant than previously thought (4, 5, 22a).

Furthermore, because increased pulmonary arterial blood pressure during exertion probably facilitates opening of the otherwise dormant intrapulmonary arteriovenous shunts, it was suggested that these shunts may also act as a protective parallel vascular network limiting the rise in regional pulmonary vascular pressure while preserving cardiac output during exercise (5). In light of these observations (5, 22a) and considering the facts that 1) pulmonary arterial blood pressure of exercising Thoroughbred horses has been demonstrated to increase to a much greater extent [mean pulmonary artery blood pressure of Thoroughbred horses increases from near 30 mmHg at rest to approach, and often even exceed, 100 mmHg during maximal exercise (Refs. 10–12)] and 2) the exercise-induced arterial hypoxemia tends to be more pronounced in racehorses [arterial O2 tension and hemoglobin O2 saturation in maximally exercising racehorses usually decreasing (from near 100 mmHg and ~98–99%, respectively, at rest) to ~75 mmHg and ~85%, respectively; Refs. 13–16], it was hypothesized that dormant intrapulmonary arteriovenous shunts similar to those described in exercising human subjects (5, 22a) may become...
functional in strenuously exercising racehorses, thereby contributing to the observed arterial hypoxemia and desaturation of hemoglobin. Thus our primary objective in the present study was to determine whether intrapulmonary arteriovenous shunts become functional in the lungs of Thoroughbred horses performing short-term, high-intensity exercise capable of eliciting maximal heart rate, stress failure of pulmonary capillaries leading to exercise-induced pulmonary hemorrhage (EIPH), and significant arterial hypoxemia and desaturation of hemoglobin. Toward this goal, we injected 15-μm-diameter microspheres labeled with various stable isotopes into the right atrium of healthy, exercise-trained Thoroughbred horses standing quietly at rest as well as during submaximal and maximal exertion, while simultaneously sampling blood from the aorta and the pulmonary artery at a constant rate. It was reasoned that the appearance of labeled microspheres into the aortic blood would be indicative of the presence of functional intrapulmonary arteriovenous shunts of >15 μm in diameter. We have extensive experience with the microsphere technique for studying tissue/organ blood flow in large domestic animals at rest and during exertion (9).

MATERIALS AND METHODS

Horses

Experiments were carried out on seven healthy, sound Thoroughbred horses (2 fillies, 5 geldings), 3–6 yr old, and weighing 443 ± 24 kg. They were housed in an air-conditioned building and were accustomed to being handled by people. The horses were maintained on alfalfa hay and oats, and free access to water was provided. The horses were dewormed periodically and were inoculated with tetanus toxoid and strangles vaccine. Our protocols and procedures were approved by the Institutional Animal Care and Use Committees.

Exercise Training and Work Intensity Eliciting Maximal Heart Rate

After initial familiarization with walking, trotting, cantering, and galloping on the high-speed treadmill for 1 wk, all horses were exercise trained 3 days/wk for 7 wk as described previously (10–16). Thereafter, separate trials were undertaken to determine the work intensity eliciting maximal heart rate and stress failure of pulmonary capillaries (25) leading to EIPH. In agreement with previous work, it was observed that galloping at 14 m/s on a 3.5% uphill grade not only elicited maximal heart rate but also induced EIPH in all horses, as demonstrated by the presence of fresh blood in the trachea on postexercise airway endoscopic examination (7, 10, 23), and could not be sustained for >120 s despite vigorous human encouragement. Thus this workload was selected for further experimentation as representing maximal exertion for our horses.

Experimental Procedures

The procedures have been described in detail previously (9–16). Briefly, on the day of the study, after local anesthesia in the 16th or 17th intercostal space, a 9-Fr catheter was advanced into the thoracic aorta. Thereafter, using local infiltration of 2% lidocaine HCl, cardiac catheters (8 Fr) with tip-manometer (Millar Instruments, Houston, TX), fluid-filled lumen, and a thermistor (Edward Laboratories, Santa Clara, CA) were advanced into the pulmonary artery via introducers inserted into the left jugular vein. The catheter locations were confirmed by monitoring the characteristic phasic blood pressure waveforms on a physiograph (Gilson Medical Electronics, Middleton, WI). These catheters permitted simultaneous sampling of the aortic and pulmonary arterial (mixed venous) blood as well as continuous monitoring of the pulmonary arterial blood pressure and temperature during the experiments. Another 10-Fr cardiac catheter was positioned into the right atrium via an introducer in the left jugular vein; this catheter was used for injecting 15-μm-diameter labeled microspheres to determine the existence of functional intrapulmonary arteriovenous shunts (see below). After catheter placement, horses stood quietly on the treadmill for ~45 min before initiating preexercise measurements.

Blood gas variables were determined using a calibrated blood gas analyzer/Co-oximeter (ABL520, Radiometer, Copenhagen, Denmark), and the blood gas tensions/pH data were corrected to the simultaneously measured pulmonary artery blood (core) temperature. O2 extraction was calculated as (arterial-to-mixed venous blood O2 content gradient/arterial O2 content) × 100.

For detecting intrapulmonary arteriovenous shunting in the present study, a vigorously agitated, ultrasonicated suspension of ~8 × 10⁸ (for preexercise measurements), 15 × 10⁸ (during submaximal exercise), or 20 × 10⁸ (during maximal exertion) microspheres, 15 μm in diameter and labeled with the five stable isotopes of gold, samarium, lanthanum, ytterbium, or lutitium (BioPal, Worcester, MA), was rapidly injected into the right atrium followed by 30–35 ml of physiological saline. This was done while simultaneous blood samples were being withdrawn from the aorta and the pulmonary artery at a constant rate (25.00 ml/min each) using a dual, parallel withdrawal pump (Harvard Apparatus, Holliston, MA). The sequence of various microsphere labels was randomized for every horse. During exercise steps of the protocol, simultaneous injections of two different microsphere labels (maximal exertion: 20 × 10⁸ microspheres of each label; submaximal exercise: 15 × 10⁸ microspheres of each label) were made. Aortic and pulmonary arterial blood withdrawal began 15 s before microsphere injections and continued for at least 90 s postinjection. All blood samples were transferred into sodium-free polypropylene vials (BioPal), dried overnight at a temperature of 70°C in an oven, and submitted for neutron activation analysis (BioPal). Neutron activation is a well-known analytical technique wherein neutrons activate the stable isotopes embedded within the microspheres contained in the sample, rendering them radioactive (19). The resulting radioactivity is quantified using high-resolution radiation counting equipment. It has been reported that neutron activation methodology is capable of detecting the presence of even a single microsphere in a myocardial tissue sample (19). We have extensive experience with the microsphere technique for studying tissue/organ blood flow in large domestic animals at rest and during exercise (9).

Experimental Design and Protocol

In these experiments, all horses were studied at rest as well as during submaximal and maximal exercise, as described below, on two separate occasions, 1 wk apart. At first, blood gas/hemodynamic studies were carried out, and the experience thus gained was used to refine the settings for microsphere studies, which were carried out 1 wk later. All experimentation was carried out at an ambient temperature of 19–21°C. Maximal exercise in these studies was followed by a rest period of at least 120 min.

Preexercise (resting) measurements. In quietly standing horses, ~45 min after instrumentation, along with baseline blood gas/hemodynamic measurements, a vigorously agitated, ultrasonicated suspension of 15-μm-diameter stable isotope-labeled microspheres was injected into the right atrium during steady hemodynamic conditions while simultaneous aortic and pulmonary arterial blood samples were being obtained at a constant rate to detect intrapulmonary arteriovenous shunting. Aortic and pulmonary arterial blood withdrawal began 15 s before microsphere injections and continued for 90 s postinjection.

Maximal exertion. On completion of preexercise measurements, incremental exercise began at 2 m/s on the high-speed treadmill, which had been preset at a 3.5% uphill grade. After 120 s of walking at 2 m/s, the belt speed was increased 1 m/s every 60 s until the horses trotted at 6 m/s for 60 s. Thereafter, belt speed was increased to 8 m/s for 60 s and then to 14 m/s. Horses galloped at 14 m/s on a 3.5%
uphill grade for 120 s. Then, the belt speed was decreased to 5 m/s, and the horses trotted for 60 s. This was followed by a walk at 2 m/s for 300 s before the treadmill was stopped. Thereafter, the horses were allowed to stand quietly on the treadmill for at least 120 min.

In this exercise protocol, simultaneous blood withdrawal from the aorta and the pulmonary artery began at 15 s of galloping at 14 m/s on a 3.5% uphill grade. Vigorously agitated, ultrasonicated suspension of 15-μm-diameter stable isotope-labeled microspheres was injected into the right atrium at 30 s of galloping at 14 m/s on a 3.5% uphill grade, when exercise-induced arterial hypoxemia is known to be well developed in Thoroughbred horses (13–16). Blood withdrawal from the aorta and the pulmonary artery continued until 90 s postinjection of microspheres. In all experiments, using a flexible fiber-optic endoscope (Pentax Fiberscopes, Orangeburg, NY), the nasopharynx, larynx, and trachea (up to the carina) were examined 45–50 min postexercise to detect the occurrence of EIPH (7, 10, 23).

Submaximal exertion. With 120 min of rest elapsing after maximal exertion, baseline measurements were made on standing horses. Thereafter, incremental exercise began on the treadmill preset at a 3.5% uphill grade. Exercise started in the same manner as described above for maximal exercise. On reaching the belt speed of 8 m/s, the horses were cantered for 150 s. Then, the belt speed was decreased to 5 m/s and the horses were trotted for 60 s. This was followed by a walk at 2 m/s for 300 s before the treadmill was stopped. In this incremental exercise protocol, 15-μm-diameter stable isotope-labeled microspheres were injected into the right atrium at 50 s of exertion at 8 m/s on a 3.5% uphill grade. Simultaneous blood withdrawal from the aorta and the pulmonary artery began 15 s before injection of microspheres and was continued until 90 s postinjection.

Data Analysis

In the present study, the blood gas/hemodynamic data were subjected to repeated-measures analysis of variance followed by Newman-Keuls multiple range test (22) to determine the significant effects of work intensity/duration (SAS statistical software version 8.2, SAS Institute, Cary, NC). For all statistical analyses, the level of significance was set at $P < 0.05$. The data are presented as means ± SE.

RESULTS

Preexercise values of arterial as well as mixed venous blood gas/pH variables are given in Table 1. In standing horses, arterial $O_2$ tension, hemoglobin $O_2$ saturation, $CO_2$ tension, and pH were 95.7 ± 1.5 Torr, 98.6 ± 0.1%, 45.0 ± 0.7 Torr, and 7.421 ± 0.002, respectively. Neutron activation analysis revealed the presence of labeled microspheres in the pulmonary arterial blood sampled from quietly standing horses; however, none of the aortic blood samples were found to contain the 15-μm-diameter, stable isotope-labeled microspheres.

During maximal exertion, all horses exhibited a significant drop in arterial partial pressure of $O_2$ and hemoglobin $O_2$ saturation; at 30 s of galloping at 14 m/s on a 3.5% uphill grade, corresponding values were 77.1 ± 2.8 Torr and 94.1 ± 0.8%, respectively (both $P < 0.0001$ vs. preexercise values). As exercise duration progressed to 120 s, further significant changes in arterial $O_2$ tension of galloping horses were not observed (Table 1); however, arterial hemoglobin $O_2$ saturation decreased significantly as hyperthermia, acidosis, and hypercapnia intensified (Table 1). At 120 s of galloping at 14 m/s on a 3.5% uphill grade, arterial hemoglobin $O_2$ saturation had dropped to 84.1 ± 1.6%, whereas $O_2$ tension was 74.7 ± 2.4 Torr. Despite these changes, because of a dramatic increase in hemoglobin concentration, arterial blood $O_2$ content of galloping horses significantly exceeded the preexercise value (Table 1). Mixed venous blood $O_2$ tension, hemoglobin-$O_2$ saturation, and $O_2$ content were also found to have decreased significantly at 30 s of maximal exertion in all horses, and as exercise duration progressed to 120 s further significant reductions in these variables were observed (Table 1). Although neutron activation analysis detected the presence of stable isotope-labeled microspheres in the pulmonary arterial blood samples obtained during maximal exertion, none of the aortic blood samples were found to contain the 15-μm-diameter labeled microspheres. Airway endoscopic examination revealed that galloping at 14 m/s on a 3.5% uphill grade was attended by the occurrence of EIPH in all horses.

During cantering at 8 m/s on a 3.5% uphill grade (submaximal exertion), although arterial $O_2$ tension and hemoglobin-$O_2$ saturation were well maintained near preexercise values, arterial blood $O_2$ content had increased significantly due to the increase in hemoglobin concentration (Table 1). During submaximal exertion in the present study, a significant reduction

### Table 1. Blood gas/pH data from 7 Thoroughbreds before and during exertion

<table>
<thead>
<tr>
<th></th>
<th>Preexercise</th>
<th>30 s</th>
<th>60 s</th>
<th>90 s</th>
<th>120 s</th>
<th>Submaximal Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Arterial blood</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Core temperature, °C</td>
<td>37.0 ± 0.1a</td>
<td>38.9 ± 0.1</td>
<td>39.5 ± 0.1b</td>
<td>40.2 ± 0.1b</td>
<td>40.7 ± 0.1b</td>
<td>38.7 ± 0.1d</td>
</tr>
<tr>
<td>$O_2$ tension, Torr</td>
<td>95.7 ± 1.5a</td>
<td>77.1 ± 2.8</td>
<td>74.6 ± 3.2</td>
<td>74.4 ± 2.6</td>
<td>74.7 ± 2.4</td>
<td>99.7 ± 2.4a</td>
</tr>
<tr>
<td>$CO_2$ tension, Torr</td>
<td>45.0 ± 0.7a</td>
<td>47.8 ± 2.3</td>
<td>53.4 ± 1.6b</td>
<td>57.2 ± 1.9c</td>
<td>59.1 ± 2.0c</td>
<td>41.9 ± 1.5d</td>
</tr>
<tr>
<td>pH</td>
<td>7.421 ± 0.002</td>
<td>7.313 ± 0.009</td>
<td>7.231 ± 0.011b</td>
<td>7.128 ± 0.013b</td>
<td>7.049 ± 0.013b</td>
<td>7.417 ± 0.012a</td>
</tr>
<tr>
<td>Hemoglobin concentration, g/dl</td>
<td>12.5 ± 0.4a</td>
<td>20.7 ± 0.4</td>
<td>21.1 ± 0.3b</td>
<td>21.6 ± 0.2b</td>
<td>22.0 ± 0.3b</td>
<td>19.5 ± 0.3d</td>
</tr>
<tr>
<td>Hemoglobin $O_2$ saturation, %</td>
<td>98.6 ± 0.1a</td>
<td>94.1 ± 0.8</td>
<td>90.9 ± 1.3b</td>
<td>87.4 ± 1.5b</td>
<td>84.1 ± 1.6b</td>
<td>96.9 ± 0.2a</td>
</tr>
<tr>
<td>$O_2$ content, ml/dl</td>
<td>17.0 ± 0.5a</td>
<td>26.6 ± 0.5</td>
<td>26.2 ± 0.5</td>
<td>25.8 ± 0.4</td>
<td>25.4 ± 0.4c</td>
<td>26.2 ± 0.7c</td>
</tr>
<tr>
<td>$O_2$ extraction, %</td>
<td>25.6 ± 2.3a</td>
<td>82.0 ± 1.5</td>
<td>88.8 ± 1.0b</td>
<td>92.3 ± 0.8b</td>
<td>94.0 ± 0.6b</td>
<td>54.0 ± 2.5d</td>
</tr>
<tr>
<td><strong>Mixed venous blood</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$O_2$ tension, Torr</td>
<td>36.7 ± 1.4a</td>
<td>18.8 ± 0.6</td>
<td>16.2 ± 0.8</td>
<td>13.5 ± 1.1c</td>
<td>11.4 ± 1.2c</td>
<td>26.5 ± 0.9d</td>
</tr>
<tr>
<td>Hemoglobin $O_2$ saturation, %</td>
<td>75.0 ± 2.4a</td>
<td>16.4 ± 1.4</td>
<td>9.9 ± 1.0b</td>
<td>6.5 ± 0.8c</td>
<td>4.9 ± 0.6c</td>
<td>44.4 ± 2.5d</td>
</tr>
<tr>
<td>$O_2$ content, ml/dl</td>
<td>12.7 ± 0.7a</td>
<td>4.8 ± 0.4</td>
<td>2.9 ± 0.3b</td>
<td>2.0 ± 0.2c</td>
<td>1.5 ± 0.2c</td>
<td>12.1 ± 0.8a</td>
</tr>
</tbody>
</table>

Values are means ± SE. $^a$Statistically significant difference from all data for maximal exertion, $P < 0.05$. $^b$Statistically significant difference from immediately preceding data for maximal exertion, $P < 0.05$. $^c$Statistically significant difference from values at 30 and 60 s of maximal exercise, $P < 0.05$. $^d$Statistically significant difference from preexercise as well as all data for maximal exertion, $P < 0.05$. $^e$Significantly different from preexercise value, $P < 0.05$.  

J Appl Physiol • VOL 99 • JULY 2005 • www.jap.org
in mixed venous blood O2 tension and hemoglobin O2 saturation was also observed, but the mixed-venous blood O2 content remained similar to its preexercise value (Table 1). Neutron activation analysis detected the presence of labeled microspheres in the pulmonary arterial blood samples obtained during submaximal exercise, but none of the aortic blood samples were found to contain the 15-μm-diameter microspheres.

DISCUSSION

Our findings regarding development of significant hyperthermia, arterial hypoxemia, desaturation of hemoglobin, hypercapnia, and acidosis during galloping at 14 m/s on a 3.5% uphill grade in the present study (Table 1) are consistent with previously reported data in maximally exercising Thoroughbred horses (13–16). It has also been reported that submaximal exercise performed at 8 m/s on a 3.5% uphill grade did not result in arterial hypoxemia (13–16); this was also true in the present study (Table 1). Mixed venous blood gas data in standing and exercising horses in the present study (Table 1) also mirrored those reported previously (13–16). Because significant pulmonary arterial, capillary, and venous hypertension is known to occur in exercising Thoroughbreds (10–12), our primary objective in the present study was to determine whether, similar to observations in exercising human subjects (5, 22a), Thoroughbred horses performing strenuous exercise also develop intrapulmonary arteriovenous shunting, which may contribute to the observed arterial hypoxemia and desaturation of hemoglobin. In this regard, our principal finding was that the 15-μm-diameter stable isotope-labeled microspheres injected into the right atrium did not traverse the pulmonary microcirculation of racehorses at rest, submaximal exercise, or during maximal exertion as demonstrated by their absence in the aortic blood sampled for up to 90 s postinjection. This was the case despite the development of significant pulmonary arterial hypertension; similar to previous reports (10–12), pulmonary arterial blood pressure approached 75.9 ± 3.8 and 96.2 ± 2.4 mmHg, respectively (vs. 29 ± 1.5 mmHg at rest) during exercise performed at 8 and 14 m/s on a 3.5% uphill grade. Whereas this finding regarding the absence of intrapulmonary arteriovenous shunting of 15-μm-diameter microspheres in quietly standing horses is also similar to findings in human subjects at rest (5, 22a), our observations regarding the absence of intrapulmonary arteriovenous shunting of 15-μm-diameter microspheres in Thoroughbreds performing submaximal and maximal exercise are in sharp contrast with findings in exercising human subjects (5, 22a). Based on our observations, it is concluded that intrapulmonary arteriovenous shunts of >15 μm in diameter are unlikely to be functional in Thoroughbred horses at rest or while performing submaximal and maximal exertion. However, we cannot rule out the presence of intrapulmonary arteriovenous shunts of <15 μm in diameter. Based on direct measurements, West et al. (25) reported the mean radii of curvature of the pulmonary capillaries in Thoroughbred horses to range from 2.75 to 4.35 μm. Thus pulmonary gas-exchange vessels in the equine lungs are probably <10 μm in diameter. In our studies, since 15-μm-diameter microspheres did not traverse the pulmonary microvasculature to appear in the aortic blood even during maximal exertion when pulmonary artery pressure reaches its highest value (8–10), it is suggested that intrapulmonary arteriovenous shunts, if present in the lungs of maximally exercising racehorses, would be in the range of ~10 to <15 μm in diameter.

Our selection of 15-μm-diameter microspheres for the present study was influenced by previous reports examining the distribution of pulmonary blood flow in resting and exercising horses with this technique (2, 6, 21). However, in these reports, intrapulmonary arteriovenous shunting of 15-μm-diameter microspheres was not examined. Although, in these experiments, cardiac (2, 6, 21) and other tissues may have been removed at necropsy, investigators did not report on the absence/presence of intravenously injected microspheres in the heart and/or other tissues.

Although Eldridge and coworkers (5) suggested that increasing exercise intensity caused recruitment of dormant intrapulmonary arteriovenous shunts in healthy human subjects, the exact dimensions of the intrapulmonary arteriovenous shunts remained unknown because the size/diameter of the injected contrast bubbles was not precisely known. Eldridge et al. (5) estimated the diameter of the injected contrast bubbles entering the pulmonary microvasculature to be in the range of 60 to 90 μm, and it was reported that these bubbles quickly traversed the lungs of exercising human subjects (presumably via intrapulmonary arteriovenous shunts) so as to appear in the left heart with a delay of only three cardiac cycles. In view of the rapid transit of the contrast bubbles through intrapulmonary arteriovenous shunts in exercising human subjects (5, 22a) as well as the fact that, in exercising racehorses (26), transit time for blood to traverse from the pulmonary artery to the carotid artery is extremely brief (3.03 ± 0.54 and 2.67 ± 0.4 s during exercise performed at 75 and 100% of the maximal heart rate, respectively; Ref. 26), in the present study aortic blood sampling began 15 s before injection of microspheres into the right atrium and was continued for 90 s postinjection. This was done to ensure that we would not miss the potential shunted microspheres. Our finding that 15-μm-diameter microspheres injected into the right atrium did not traverse the pulmonary microvasculature to appear in the aortic blood, even during maximal exertion when pulmonary artery blood pressure had reached 96.2 ± 2.4 mmHg, indicates that large intrapulmonary arteriovenous shunts probably do not exist in the lungs of exercising horses. This is in agreement with previous work using multiple inert gases that intrapulmonary arteriovenous shunting does not contribute to the exercise-induced arterial hypoxemia in racehorses (24). An important issue pertaining to the divergent findings in maximally exercising horses is that, whereas the microspheres used in the present study would not have deformed and/or otherwise changed their size/diameter, this is unlikely to be the case with intravenously injected agitated saline contrast bubbles in the experiments of Eldridge et al. (5). The latter could not only deform while traversing through the pulmonary microcirculation but also were likely constantly changing (likely decreasing) in size as O2 diffused out into the deoxygenated blood, thereby causing increased partial pressure of N2 inside the bubbles, which, in turn, would promote N2 diffusion into blood. Such changes in the form/size of the agitated saline contrast bubbles may have contributed to their rapid appearance in the left heart, which was interpreted as suggesting the presence of intrapulmonary arteriovenous shunting in exercising human subjects (5, 22a).

It has been demonstrated that pulmonary vascular resistance decreases significantly in exercising racehorses (12) and hu-
man subjects (18) because of the capillary recruitment and distension occurring in response to the high pulmonary vascular pressures and blood flow. Pulmonary capillary recruitment is generally thought to invoke not only the opening of previously unperfused/perfused capillaries but also the opening of supernumerary arteries in the lungs (5, 20) that arise at a right angle from the conventional pulmonary arteries and have a baffle valve at their origin (20). However, the extent/magnitude of pulmonary capillary distension during maximal exertion is largely unknown. Because 15-μm-diameter microspheres did not traverse the pulmonary vasculature to appear in the aortic blood even during maximal exertion when pulmonary arterial blood pressure had increased dramatically, it is suggested that pulmonary capillaries of even the maximally exercising Thoroughbred horses are unlikely to distend to diameters exceeding 15 μm. In fact, with high distending pulmonary capillary pressures observed in exercising Thoroughbreds, disruption of the capillary walls (stress failure of pulmonary capillaries) resulting in EIPH (25) is commonly observed, as was the case in the present study.

Another significant finding in human subjects (5, 22a) was that intrapulmonary arteriovenous shunting of the injected contrast bubbles could be easily demonstrated during submaximal exercise in conjunction with the appearance of arterial hypoxemia, and it was suggested that the increase in pulmonary arterial blood pressure and flow during exercise were important in the opening of dormant intrapulmonary arteriovenous shunts. In contrast with these findings (5, 22a), we observed in Thoroughbreds performing submaximal exertion, neither intrapulmonary arteriovenous shunting of 15-μm-diameter microspheres nor arterial hypoxemia/desaturation of hemoglobin had developed (Table 1). This, along with our finding in maximally exercising Thoroughbreds that despite an even greater magnitude of pulmonary arterial hypertension (mean pulmonary artery pressure: 96.2 ± 2.4 mmHg) 15-μm microspheres injected into the right atrium did not cross the pulmonary microcirculation to appear in the aortic blood, suggests that species differences may exist in the exercise-induced intrapulmonary arteriovenous shunting, not only in terms of the size/dimensions of the shunts but also regarding its magnitude. In this context, Eldridge et al. (5) have suggested that there may also be significant variability among individual human subjects regarding the pulmonary arteriovenous shunts as well as the effect of exercise on blood flow through them, which may explain the reported wide intersubject variability in the exercise-induced arterial hypoxemia (3, 4, 17).

Opening of the dormant intrapulmonary arteriovenous shunts as pulmonary vascular pressures and blood flow increase during exercise is an appealing hypothesis (4, 5, 22a) to explain the exercise-induced arterial hypoxemia and desaturation of hemoglobin. This is because the admixture of even a small fraction of the mixed venous blood, which is severely deoxygenated during high work intensities (Table 1), bypassing the gas-exchange area of the lungs with the oxygenated blood exiting pulmonary capillaries would readily depress the arterial O2 tension and hemoglobin O2 saturation. In this context, whereas Eldridge et al. (5) presented evidence that intrapulmonary arteriovenous shunts (perhaps, 60- to 90-μm diameter) may become functional during exercise in healthy human subjects and could possibly contribute to the arterial hypoxemia observed during submaximal and maximal exertion, our data indicate that intrapulmonary arteriovenous shunts of >15 μm probably do not exist in exercising Thoroughbred horses. However, we cannot rule out the existence of functional intrapulmonary arteriovenous shunts of <15 μm in diameter in maximally exercising racehorses. Thus, although further work is necessary to define the size and role of intrapulmonary arteriovenous shunts in bringing about the exercise-induced arterial hypoxemia in racehorses, an associated interesting observation argues against a major role for intrapulmonary arteriovenous shunting in the arterial hypoxemia observed in maximally exercising racehorses. In previous studies (13–16) as well as in the present study, we have observed that the arterial O2 tension of racehorses galloping at 14 m/s on a 3.5% uphill grade did not change significantly between 30 and 120 s (Table 1) but that the mixed venous blood O2 tension, hemoglobin O2 saturation, and O2 content decreased significantly as maximal exercise progressed from 30 to 120 s (Table 1). For example, in our horses, although the mixed-venous blood O2 tension, hemoglobin-O2 saturation, and O2 content had decreased (all P < 0.0001) from 18.8 ± 0.6 Torr, 16.4 ± 1.4%, and 4.8 ± 0.4% volume, respectively, at 30 s of maximal exertion to reach 11.4 ± 1.2 Torr, 4.9 ± 0.6%, and 1.5 ± 0.2% volume, respectively, at 120 s, the arterial O2 tension had not changed significantly (Table 1). Assuming that between 30 and 120 s of maximal exertion a constant fraction of the total pulmonary blood flow (i.e., cardiac output) bypasses the gas-exchange areas of the lungs via the intrapulmonary arteriovenous shunts, the progressive significant drop in mixed venous blood oxygenation observed with increasing exercise duration (Table 1) should cause the arterial O2 tension to decrease significantly. However, this was not the case in the present (Table 1) or previous studies (13–16).

In summary, the results of the present study did not support a role for functional intrapulmonary arteriovenous shunts of >15 μm in diameter in bringing about the exercise-induced arterial hypoxemia in racehorses. However, the presence of intrapulmonary arteriovenous shunts of <15 μm in diameter was not ruled out. Our observation that the arterial O2 tension of horses galloping at 14 m/s on a 3.5% uphill grade remained unchanged between 30 and 120 s, despite significant reductions in mixed-venous blood O2 tension, hemoglobin O2 saturation, and O2 content, also discounts the importance of intrapulmonary arteriovenous shunting in the development of exercise-induced arterial hypoxemia in racehorses. This is because, assuming that a constant fraction of the total pulmonary blood flow bypasses the gas-exchange areas of the lungs via intrapulmonary arteriovenous shunts between 30 and 120 s of maximal exertion, the significant drop in mixed venous blood oxygenation observed with increasing exercise duration should cause the arterial O2 tension to decrease significantly. However, this was not the case in the present study. Thus it is concluded that intrapulmonary arteriovenous shunting probably does not contribute to the exercise-induced arterial hypoxemia in racehorses.

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