Exercise-induced Arteriovenous Intrapulmonary Shunting in Dogs

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***Rationale*: We have previously shown, using contrast echocardiography, that intrapulmonary arteriovenous pathways are inducible in healthy humans during exercise; however, this technique does not allow for determination of arteriovenous vessel size or shunt magnitude.**

***Objectives*: The purpose of this study was to determine whether large-diameter (more than 25 μm) intrapulmonary arteriovenous pathways are present in the dog, and whether exercise recruits these conduits.**

***Methods*: Through the right forelimb, 10.8 million 25-μm stable isotope-labeled microspheres (BioPAL, Inc., Worcester, MA) were injected either at rest (n = 8) or during high-intensity exercise (6–8 mph, 10–15% grade, n = 6). Systemic arterial blood was continuously sampled during and for 3 minutes after injection. After euthanasia, tissue samples were obtained from the heart, liver, kidney, and skeletal muscle. In addition, 25- and 50-μm microspheres were infused into four isolated dog lungs that were ventilated and perfused at constant pressures similar to exercise.**

***Measurements and Main Results*: Blood and tissue samples were commercially analyzed for the presence of microspheres. No microspheres were detected in the arterial blood or tissue samples from resting dogs. In contrast, five of six exercising dogs showed evidence of exercise-induced intrapulmonary arteriovenous shunting, as microspheres were detected in arterial blood and/or tissue. Furthermore, shunt magnitude was calculated to be 1.4 ± 0.8% of cardiac output (n = 3). Evidence of intrapulmonary arteriovenous anastomoses was also found in three of four isolated lungs.**

***Conclusions*: Consistent with previous human findings, these data demonstrate that intrapulmonary arteriovenous pathways are functional in the dog and are recruited with exercise.**

Keywords: intrapulmonary arteriovenous anastomoses; shunt; pulmonary gas exchange; pulmonary circulation

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Previous studies, using contrast echocardiography, suggest that intrapulmonary shunts are recruited during exercise in health. However, the size of the contrast is unknown, and thus arteriovenous diameter is undefined.

What This Study Adds to the Field

Large-diameter intrapulmonary arteriovenous shunts are functional in healthy animals, and exercise recruits these vessels.

The classic understanding of the normal pulmonary circulation is that all the blood leaving the right ventricle passes through the pulmonary microcirculation via capillaries before returning to the left side of the heart through the pulmonary venous circulation. Studies using saline contrast echocardiography in humans have demonstrated that exercise results in the transpulmonary passage of contrast bubbles, suggesting that large-diameter intrapulmonary (I-P) arteriovenous pathways are recruited with exercise (1–3). These findings are controversial in that they are in contrast with prior research, using inert gas and/or inspired 100% oxygen (4–8), that has failed to detect substantial right-to-left physiological I-P shunt during exercise.

Despite an absence of right-to-left I-P shunt during exercise as determined by functional, gas exchange–dependent techniques (4–8), there is substantial research documenting anatomic arteriovenous anastomoses in the isolated lung. Studies have demonstrated direct vascular anastomoses between pulmonary arteries and veins in many mammals including cats (9), dogs (9–11), and healthy humans (9, 12, 13). More recently, we have demonstrated that I-P arteriovenous pathways greater than 50 μm are functional in fresh healthy ventilated and perfused isolated human lungs (14).

A limitation of the previous work in exercising humans, using the contrast echocardiography technique, is that the exact size of the contrast bubbles that traverse the pulmonary circulation is unknown, and therefore arteriovenous vessel diameter is undefined. Furthermore, this technique does not allow determination of the magnitude of cardiac output that is bypassing the pulmonary microcirculation, the primary site of pulmonary gas exchange. Accordingly, this project was designed to determine whether exercise recruits arteriovenous vessels in canines by using a large-diameter polymer microsphere of known diameter such that vessel diameter could be characterized. This approach would also allow us to measure the amount of blood (i.e., percentage of cardiac output) that is bypassing the pulmonary capillaries via these large-diameter arteriovenous vessels. In addition, to confirm that the conduits allowing microspheres to bypass the pulmonary microcirculation were not cardiac in origin, separate experiments were performed whereby excised dog lungs were perfused with a solution containing 25- and 50-μm microspheres.

On the basis of the previous human work, it was hypothesized that (*1*) exercise recruits large-diameter pulmonary arteriovenous pathways and, therefore, 25-μm microspheres would bypass the pulmonary microcirculation and be detected within the systemic circulation; (*2*) similarly sized microspheres would bypass the pulmonary microcirculation and be found in the pulmonary

(*Received in original form February 7, 2007; accepted in final form May 1, 2007*)

Supported by a Grant-in-Aid from the American Heart Association (0550176Z), by the Natural Sciences and Engineering Council of Canada (M.K.S.), and by NHLBI Training Grant T32 HL07654 (A.T.L.).

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Am J Respir Crit Care Med Vol 176. pp 300–305, 2007
Originally Published in Press as DOI: 10.1164/rccm.200702-206OC on May 3, 2007
Internet address: www.atsjournals.org

venous outflow of the isolated dog lung, providing confirmation that these conduits were pulmonary arteriovenous pathways and not an intracardiac shunt. Preliminary results of this study were previously published in abstract form (15).

METHODS

All procedures were approved by the Institutional Animal Care and Use Committee at the University of Wisconsin–Madison (Madison, WI) and conducted in accordance with the *Guiding Principles in the Care and Use of Animals* (American Physiological Society, Bethesda, MD). Dogs were studied because of their ability to perform high-intensity exercise with positive reinforcement, and the low species prevalence of intracardiac shunt (16). Data were collected from a total of 18 mixed-breed dogs (17.2 ± 1.2 kg); 8 dogs were studied exclusively at rest, 6 during exercise, and isolated lungs from 4 experimentally naive dogs were also examined.

Intact Dogs

Dogs were trained to stand quietly and/or run vigorously on a motorized treadmill, and after training, most dogs were instrumented through two surgical procedures. Similar to previous studies (17) from our laboratory, dogs were instrumented with an ascending aortic (Transonic Systems, Inc., Ithaca, NY; n = 6) and renal artery flow probe (Transonic Systems, Inc.; n = 1) for beat-by-beat measurement of blood flow, and an abdominal aortic catheter (n = 10) for arterial blood sampling. Two of the dogs studied at rest and four dogs studied during exercise were instrumented with flow probes, whereas four of the dogs studied at rest and six of the dogs studied during exercise were instrumented with an abdominal aortic catheter. For both surgeries, animals were induced with sodium pentothal (20 mg/kg), and a surgical plane of anesthesia was maintained with isoflurane (1–1.5%). Strict sterile techniques were used during all surgical procedures, and appropriate antibiotics and analgesics were used postoperatively. Animals were allowed to recover for at least 2 weeks before data collection.

Arterial Blood Gases

Arterial blood samples (3 ml/sample) were taken in four resting dogs, and all six exercising dogs. Blood gas measurements were made in duplicate and analyzed on an automated gas analyzer (model ABL-505; Radiometer, Brønshøj, Denmark). Samples were corrected for body temperature and systematic error was determined by tonometry data. In the resting dogs (n = 4), blood gases were obtained before microsphere injection, and 15 minutes after injection. In the exercising dogs (n = 6), three blood gases were obtained: (*1*) at rest before exercise; (*2*) during exercise, immediately before microsphere injection; and (*3*) at rest, 15 minutes after microsphere injection.

Microsphere Methodology

All dogs received a bolus injection of 10.8 million 25-μm stable isotope-labeled (lutetium or samarium) neutron-activated microspheres suspended in 10 ml of saline containing 0.01% Tween 80 and 0.01% Thimerosal (BioPAL, Inc., Worcester, MA) either at rest or during exercise. Microspheres were injected via an acutely placed 20-gauge catheter into a left forelimb vein. Immediately after injection, the 10-ml syringe was refilled with saline and the catheter was rapidly flushed three times.

In the dogs that had a chronic systemic arterial catheter (rest, n = 4; exercise, n = 6), three arterial blood samples were obtained for microsphere analysis: (*1*) a 10-ml sample obtained immediately before microsphere injection (negative control 1); (*2*) a continuous blood draw for 3 minutes beginning at the time of injection (withdrawal rate, about 10 ml/min); and (*3*) a 10-ml sample obtained at rest, 15 minutes after microsphere injection (negative control 2, used to verify that microspheres were not damaged or broken into pieces small enough to travel through pulmonary capillaries).

All tissue and blood samples were analyzed for the presence of microspheres (commercial analysis by BioPAL, Inc.). Per recommendations of BioPAL, Inc., arterial blood samples were centrifuged for 1 minute at 2,000 rpm. The supernatant was aspirated and the blood was resuspended in sanSaLine (BioPAL, Inc.). Centrifugation and aspiration steps were then repeated, and subsequently blood samples were dried in a warming oven at 70°C for at least 48 hours.

After euthanasia, 5- to 15-g tissue samples were obtained from most dogs (rest, n = 5; exercise, n = 5). Tissues sampled included the following: right kidney, liver, right gluteal muscle, right quadriceps muscle, and epicardium of the left ventricle. Sections of the arterial and peripheral vein catheter and tubing were also obtained for microsphere determination. Samples were dried in a warming oven at 70°C for at least 48 hours, and later analyzed (BioPAL, Inc.).

Shunt Quantification

When microspheres were detected in arterial blood samples, shunt as a fraction of cardiac output could be calculated on the basis of the following data: total counts injected, counts remaining in the venous catheters and injection syringe, cardiac output, arterial blood sample volume, and counts detected in arterial sample. For the animal with a renal flow probe, shunt magnitude was similarly calculated by integrating total kidney counts per fraction of cardiac output received by the kidney.

Protocols

Resting dogs (n = 8). Each animal was guided onto a motorized treadmill and stood quietly while instrumentation was connected. All dogs remained standing throughout the experiment. After 5 minutes of quiet standing, baseline data were obtained. Microspheres were then injected as described above. Fifteen minutes after injection, additional data were acquired. Dogs were promptly killed, and tissue samples were taken from six of the dogs as described above. Once all tissue samples were obtained, the heart was removed and thoroughly inspected for evidence of atrial and/or ventricular septal defects, including a patent foreman ovale. Specifically, both atria and ventricles were located, and the septum was carefully examined by probing with a combination of fingers and small surgical instruments. No cardiac defects that would contribute to right-to-left shunting were found.

Exercising dogs (n = 6). As with the resting dogs, each animal was guided onto the treadmill and stood quietly while instrumentation was connected. After 5 minutes of quiet standing, resting data were acquired. The treadmill was then started, and the speed was increased to the highest speed that the dog could maintain with positive reinforcement (5–8 mph, 10–15% grade). After 2–3 minutes of steady state exercise, exercise data were obtained, and microspheres were then injected. Once arterial sampling was complete, the treadmill speed was lowered to 4 mph, promptly stopped, and the dog remained standing on the treadmill. Fifteen minutes after microsphere injection, additional data were acquired. As in the resting studies, dogs were promptly killed, tissue samples were taken (n = 5), and careful *post mortem* examination of the heart revealed no cardiac defects.

Isolated Lungs

Whole lungs were harvested from four additional dogs who were not previously involved in any experiments or surgeries. Using an experimental setup and methodology previously described (14), lungs were ventilated via an endotracheal tube to a peak inflation pressure of 11 mm Hg, with 3.7–mm Hg positive end-expiratory pressure at 20–25 breaths/minute and an inspiratory duration/total breath duration of 0.5. The main pulmonary artery and left atrium were cannulated and the lungs were perfused at 14.7 mm Hg (n = 2) or 29.4 mm Hg (n = 2) with phosphate-buffered saline (5% albumin), using a nonpulsatile reservoir system. Microspheres with diameters of 25 ± 2.5 and 50 ± 4 μm (mean ± SD) and fluorescently labeled (red and green, respectively) (250,000 of each size; Duke Scientific, Fremont, CA) were combined in solution and continuously injected together over 5 minutes (1 ml/min), while the entire venous outflow was collected. The vials containing the venous outflow were vacuum filtered, the filters were imaged by fluorescence microscopy, and microspheres were counted.

Data Analysis

Group data are expressed as means ± SE. For all inferential analyses, the probability of type I error was set at 0.05. Within each protocol, cardiovascular and blood gas data were evaluated with either a paired *t* test (resting dogs) or by one-way repeated measures analysis of variance (exercising dogs). On detection of an effect by analysis of variance,

TABLE 1. CARDIOVASCULAR DATA FROM DOGS INJECTED WITH MICROSPHERES AT REST AND DURING EXERCISE

	Resting Dogs ($n = 2$)		Exercising Dogs ($n = 4$)		
	Preinjection	15 min Postinjection	Resting, Preinjection	Exercise	Resting, 15 min Postinjection
Cardiac output, L	5.4 ± 0.6	5.5 ± 0.1	5.7 ± 0.4	11.9 ± 1.8*	6.0 ± 0.4
Stroke volume, ml	44 ± 3	43 ± 2	47 ± 4	53 ± 4	46 ± 3
Heart rate, beats/min	123 ± 6	128 ± 4	126 ± 10	224 ± 14*	134 ± 7

Data are presented as means ± SE.
* $p < 0.05$ versus preinjection. No differences in resting cardiovascular function were observed within each group before (preinjection) or 15 minutes after injection of microspheres.

comparisons were made, and a Bonferroni correction factor was applied to maintain the family-wise error rate at 0.05.

RESULTS

Intact Dogs

Grouped cardiovascular, blood gas, and microsphere data are presented in Tables 1, 2, and 3, respectively.

Resting Dogs

In the two dogs with chronic flow probes, cardiac output, heart rate, and stroke volume were stable before and after microsphere injection (*see* Table 1). No significant difference was found in arterial blood gases before or after microsphere injection (*see* Table 2). No microspheres were detected in any of the arterial blood samples, either before, during, or after microsphere injection, and no microspheres were detected in any of the tissue samples from the resting dogs (*see* Table 3).

Exercising Dogs

Exercise caused significant ($p < 0.05$) increases in heart rate and cardiac output from preinjection resting baseline. Exercise resulted in significant ($p < 0.05$) increases in arterial pH and P_{O_2}, and a decrease in arterial P_{CO_2}. Fifteen minutes after microsphere injection, resting cardiovascular parameters had returned to preexercise baseline values. Resting blood gases obtained 15 minutes after microsphere injection were not different from baseline preexercise values.

Microspheres were not detected in arterial blood at rest before or 15 minutes after microsphere injection. However, microspheres were found in the arterial blood of two of six exercising dogs obtained immediately after microsphere injection. Of the five exercising dogs from which tissues samples were also acquired for microsphere analysis, microspheres were detected in four. In total, microspheres were detected in arterial blood and/or tissue in five of six exercising dogs.

In two dogs in which microspheres were detected in the arterial blood, the percentage of cardiac output shunted through vessels greater than 25 μm could be determined. In addition, the percentage of cardiac output shunted could be calculated in the dog with a renal flow probe, as microspheres were detected in the kidney of this animal. The percentage of cardiac output shunted in these three dogs averaged 1.42 ± 0.75% (range, 0.2–3.1%).

Isolated Lungs

At perfusion pressures of 14.7 and 29.4 mm Hg, blood flow was 116 ± 33 and 240 ± 12 ml/minute, respectively, before injection of microspheres, and blood flow did not decrease after microsphere injection (167 ± 89 and 245 ± 20 ml, respectively), suggesting that embolization was minimal. Three of the four dogs showed evidence of I-P arteriovenous shunts, as microspheres were found in the pulmonary venous outflow. The average shunt fraction in the three dogs was 0.007% (range, 0–0.02%) for 25-μm microspheres, and 0.023% (range, 0.001–0.05%) for 50-μm microspheres.

DISCUSSION

We found that dogs developed exercise-induced I-P arteriovenous shunting, as microspheres injected into a left forelimb vein during exercise were detected in systemic arterial blood and/or tissue (liver, kidney, right quadriceps, right gluteal muscle, and heart). In contrast, arterial blood and tissue of dogs injected at rest did not contain microspheres, indicating that I-P arteriovenous shunts greater than 25 μm were unlikely to be functional at rest. Moreover, I-P right-to-left shunt magnitude during exercise was estimated at about 1.4% (range, 0.2–3.1%) of cardiac output. No dog was found to have an intracardiac shunt during postmortem examination, which, combined with the isolated lung data demonstrating 25- to 50-μm microspheres in the pulmonary venous outflow, indicates that the conduits allowing microspheres

TABLE 2. ARTERIAL BLOOD GAS DATA FROM DOGS INJECTED WITH MICROSPHERES AT REST AND DURING EXERCISE

	Resting Dogs ($n = 4$)		Exercising Dogs ($n = 6$)		
	Preinjection	15 min Postinjection	Resting, Preinjection	Exercise	Resting, 15 min Postinjection
pH	7.392 ± 0.011	7.387 ± 0.017	7.397 ± 0.013	7.442 ± 0.018*	7.399 ± 0.015
Pa_{CO_2}, mm Hg	32.1 ± 1.4	32.2 ± 1.1	35.6 ± 1.9	30.1 ± 1.9*	36.8 ± 2.8
Pa_{O_2}, mm Hg	98.9 ± 2.3	100.8 ± 3.0	92.5 ± 2.5	106.4 ± 2.9*	95.7 ± 5.1
HCO_3	18.3 ± 0.8	18.7 ± 0.8	20.4 ± 1.1	20.0 ± 1.4	21.1 ± 1.0

Data are presented as means ± SE.
* $p < 0.05$ versus preinjection.

TABLE 3. FREQUENCY OF MICROSPHERE DETECTION IN ARTERIAL BLOOD AND TISSUE SAMPLES FROM DOGS INJECTED WITH MICROSPHERES AT REST AND DURING EXERCISE

	Resting Dogs ($n = 8$)	Exercising Dogs ($n = 6$)
Arterial blood samples		
Before injection	0/4	0/6
Immediately after injection	0/4	2/6
15 min postinjection	0/4	0/6
Tissue samples		
Liver	0/5	3/4
Kidney	0/5	3/4
Quadriceps muscle	0/5	1/4
Gluteal muscle	0/5	1/4
Left ventricle	0/5	2/5
Dogs with microspheres detected in samples	0/8	5/6

to bypass the pulmonary microcirculation in the intact animal were I-P arteriovenous pathways, and not of cardiac origin. Consistent with previous human exercise studies (1–3), these data confirm that large-diameter (> 25 μm) I-P arteriovenous pathways are recruited with exercise.

Methodologic Considerations

In addition to the recruitment of I-P arteriovenous pathways, other possibilities could explain our findings in the exercising dog. As mentioned above, no dog was found to have an intracardiac shunt on postmortem examination, which is consistent with previous data showing an extremely low prevalence of intracardiac shunt in the dog (16). Moreover, if an intracardiac shunt were functional in our animals, it is likely that microspheres would have been observed in the systemic circulation of the resting dogs. It is also possible that microspheres could break, travel across normal pulmonary capillaries, and be detected in the systemic circulation. This is doubtful, however, because broken microspheres would recirculate and thus be detected in the arterial blood samples collected 15 minutes after microsphere infusion. Postinjection resting pulmonary gas exchange data and cardiac output were not different from preinjection rest in the dogs injected during exercise, demonstrating no evidence of physiologically significant overembolization. Finally, abnormal microvascular distention could allow microspheres to traverse the pulmonary microcirculation. However, previous studies in isolated dog lungs have shown that pulmonary vessels distend less than 3% for each millimeter of mercury increase in transmural pressure (18, 19), and distensibility is about 2% · mm Hg^{-1} in exercising humans (20). Pulmonary arterial or venous pressures were not measured in our dogs; however, previous studies have shown that mean pulmonary arterial and mean left atrial/pulmonary venous pressures are about 20 mm Hg (21) and 11 mm Hg (22), respectively, in resting healthy dogs, and do not exceed 40 (21) and 11 mm Hg (22), respectively, during maximal exercise. If we assume a capillary diameter of 10 μm at rest, and that capillary pressure is midway between pulmonary artery and pulmonary venous pressure (23), the 10–mm Hg increase in transmural pressure would distend the capillary to no more than 14 μm at peak exercise, well below the 25-μm microspheres used in the present study. Thus, it is highly unlikely that our exercise results can be explained by anything other than exercise-induced recruitment of I-P arteriovenous pathways greater than 25 μm in diameter.

Previous Exercise Studies Examining I-P Shunt

Manohar and Goetz (24) investigated whether I-P arteriovenous shunts are recruited in the exercising thoroughbred horse. Manohar and Goetz used a similar technique whereby 15-μm microspheres were injected into the right atrium, while blood was simultaneously withdrawn at a constant rate from the aorta. In contrast to our findings, no microspheres were detected in the aortic blood samples, suggesting no exercise-induced arteriovenous shunts in these animals. It is important to note that thoroughbred horses have a somewhat unique pulmonary vasculature in that they are the only mammal that consistently demonstrates exercise-induced pulmonary hemorrhage (25) and, contrary to humans (3, 7) and dogs (26), thoroughbred horses do not demonstrate a reduction in pulmonary vascular resistance above moderate intensity exercise (27). Alternatively, methodologic limitations of Manohar and Goetz (24) may have also reduced the sensitivity needed to detect arteriovenous shunts in thoroughbred horses. It is estimated that the peak cardiac output in the maximally exercising horses examined by Manohar and Goetz (24) would have been about 300 L · min^{-1} (27), and therefore their aortic sampling rate of 25 ml · min^{-1} represents 0.008% of cardiac output. Manohar and Goetz (24) injected 20 million microspheres into the right ventricle during maximal exercise, which, assuming a 2% anatomic shunt, would result in 400,000 microspheres getting across the pulmonary circulation. Under ideal conditions, sampling 0.008% of cardiac output would retrieve about 30 microspheres in the aortic arterial sample. In contrast, the current study was designed such that about 180 microspheres would be detected in our 10-ml arterial blood samples and, importantly, tissue samples were also obtained. Despite substantially greater sensitivity with our arterial sampling technique, we detected microspheres in the arterial blood of only two of six dogs. Moreover, in the one dog that failed to demonstrate exercise-induced arteriovenous shunting, only arterial blood samples were obtained, and therefore we cannot exclude that microspheres may have been detected in the tissue of this animal. Our results indicate that tissue samples are vital to detect exercise-induced arteriovenous shunts, and that although species difference cannot be excluded, the arterial blood sampling used by Manohar and Goetz (24) may have lacked the sensitivity necessary to accurately and reliably detect a small shunt fraction in thoroughbred horses.

Isolated Preparation

The purpose of our isolated lung studies was to confirm that the vessels allowing microspheres to bypass the pulmonary microcirculation were not cardiac in origin. Studies have previously documented large-diameter arteriovenous shunts in the dog lung (9–11). Niden and Aviado (11) purposely overembolized the lung, whereas Parker and coworkers (10) observed an increase in mean pulmonary artery pressure and reduction in systemic arterial pressure, indicative of substantial embolization. Whereas previous isolated lung studies do not report the amount of microspheres injected, 500,000 microspheres were injected in the present study, which resulted in a much less severe embolization as demonstrated by little change in pulmonary flow after microsphere injection. These findings extend our work in fresh human lungs (14), demonstrating that 25- to 50-μm I-P arteriovenous pathways are functional, and that overembolization is not a requirement for I-P arteriovenous recruitment.

The percentage of flow passing through arteriovenous vessels greater than 25 μm in diameter was found to be 0.001–0.05% in the isolated dog lung, which is substantially lower than the mean shunt fraction calculated in the exercising dog (1.4 ± 0.8%). Perfusion pressure in the isolated dog lung was limited to 29.4 mm Hg, as this was similar to, but did not exceed, the highest estimated driving pressure in the exercising dog of 36 mm Hg (mean pulmonary artery pressure, 40 mm Hg [21]; left atrial pressure, 4 mm Hg [28]). Despite a similar driving pressure,

flow through the isolated lungs was no greater than 0.25 L/minute; substantially lower than pulmonary blood flow (i.e., cardiac output) either at rest or during exercise in the intact dog, indicating greater vascular resistance and an altered pulmonary vasculature in the isolated lung. Pulmonary arterial wedge/left atrial pressure has been reported to be 4 mm Hg (28) to 10 mm Hg (22) during exercise in healthy dogs. Reeves and Taylor (29) have shown that increased pulmonary venous pressure plays an important role in pulmonary vascular recruitment and vascular resistance during exercise, and perhaps arteriovenous recruitment as well. A limitation of the current work is that pulmonary venous pressure was not increased above atmospheric pressure in the isolated lung, as preliminary work demonstrated that increasing outflow pressure produced pulmonary edema during experimentation. Moreover, in the isolated lung experiments end-expiratory pressure was set at 5 cm H_2O, and thus the lung was under zone II conditions (alveolar pressure greater than venous pressure), reducing vessel caliber and flow. Finally, isolated lungs were perfused with phosphate-buffered saline containing 5% albumin, which may have altered microcirculatory dynamics as compared with the intact lung. Our data confirm previous studies (9–11, 14), and further demonstrate that pressures and flows obtained in the isolated lung, which mimic zone II conditions, are sufficient to recruit I-P shunt pathways. However, because of the substantially lower flow rates and pulmonary venous pressures in the isolated lung, this preparation likely substantially underestimates shunt magnitude in the intact exercising dog.

Shunt Consequence

On the basis of previous morphometric data, 25-μm microspheres would be passing through at least first-order (i.e., terminal) or second-order arterioles (30, 31). Hsia and coworkers (32) have shown that normal dogs exercising at a similar intensity to the present study (i.e., mean cardiac output of about 12 L/min) increased alveolar–arterial O_2 pressure difference [$(A–a)D_{O_2}$] from about 4 (SD, ~2) mm Hg at rest to about 14 (SD, ~9) mm Hg during exercise. Of note, Hsia and coworkers (32) found that the widened $(A–a)D_{O_2}$ was secondary to greater ventilation–perfusion mismatch, and that the calculated I-P/intracardiac shunt fraction was less than 1% in all dogs during exercise. Oxygen consumption and expired carbon dioxide were not measured in the present study, and therefore it is not possible to calculate the respiratory exchange ratio. If we assume a respiratory exchange ratio of 0.8 at rest, and 1.1 at peak exercise, mean $(A–a)D_{O_2}$ is estimated to be about 10 mm Hg both at rest and during exercise. A 1.9% shunt of pure mixed venous blood (*see* Lovering and coworkers [33] for calculations) would explain the entire $(A–a)D_{O_2}$ during exercise; however, the lack of change in estimated $(A–a)D_{O_2}$ with exercise in our dogs indicates that any negative consequence of arteriovenous shunt recruitment was counteracted by other exercise-induced improvements in gas exchange.

Our findings in the exercising dog, that is, that exercise recruits anatomic arteriovenous shunts, despite a previous inert gas study that has not found physiological pulmonary shunt in the dog (32), is consistent with previous human work demonstrating I-P shunt as assessed by contrast echocardiography (1–3), even though prior gas exchange–dependent techniques have not documented substantial right-to-left shunt (4–8). Indeed, we would suggest that the magnitude of the exercise-induced anatomic arteriovenous shunting in the human may be greater than in the dog (32), because of the larger relative increases in cardiac output, pulmonary vascular pressures, and $(A–a)D_{O_2}$ typically observed in humans with exercise. The lack of right-to-left shunt documented during exercise by gas exchange–dependent techniques, despite solid evidence of an anatomic I-P shunt, is controversial and may be explained by either gas exchange occurring proximal to (34) or within (35) arteriovenous anastomotic vessels (36). Notably, a substantial range in shunt fraction (range, 0.2–3.1% of cardiac output) was found in the three exercising dogs for which shunt fraction could be calculated. Technical error cannot be excluded; however, this heterogeneous shunt response would help explain the $(A–a)D_{O_2}$ variability in the exercising dog (32) and the substantial variability in the human $(A–a)D_{O_2}$ response to exercise (37). Additional investigations are required to better understand the relationship between the recruitment of anatomic I-P arteriovenous shunts determined with anatomically based techniques and how these vessels may contribute to the components of gas exchange (right-to-left physiological shunt, diffusion limitation, ventilation–perfusion matching) as evaluated by gas exchange–dependent techniques (36).

As mentioned previously (3, 14, 36), recruitment of anatomic I-P arteriovenous shunts would negatively impact the role of the lungs as a biological filter. Microspheres were detected in the epicardium of the left ventricle in two of our exercising dogs, suggesting that recruitment of I-P shunts could play a role in embolic heart disease. In addition, previous work has shown that stroke patients have a higher prevalence of patent foramen ovale (38–40), and hereditary hemorrhagic telangiectasia patients are more susceptible to embolic stroke and neurologic symptoms (41), indicating that blood flow bypassing the pulmonary microcirculation may have important physiological relevance. Isolated lung work has shown that I-P arteriovenous shunts are up to 420 μm in diameter in the dog (11), 390 μm in the cat (9), and 200 μm in the adult human (13). Further characterization of I-P shunts will determine whether exercise recruits similar large-diameter vessels that could have important neural and/or cardiovascular consequences.

Conclusions

We found that exercise recruits large-diameter (> 25 μm) I-P arteriovenous pathways in the healthy dog. These findings in an animal model are consistent with previous human studies, using contrast echocardiography, that indicate that I-P arteriovenous shunts are recruited with exercise. Furthermore, it was found that about 1.4% (range, 0.2–3.1%) of cardiac output is being directed through these arteriovenous vessels during exercise, which would impair gas exchange and impact the filtration ability of the pulmonary microcirculation.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

Acknowledgment: The authors thank Dr. Curtis A. Smith, Kathy Henderson, Ben Soriano, Jose Andres Vasconcellos, and Dr. Juan Carlos Robles for assistance.

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