Hemodynamic Response to Normovolemic Polycythemia at Rest and during Exercise in Dogs

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SUMMARY. Very little is known about the influence of polycythemia on oxygen transport during exercise. We studied chronically instrumented dogs trained to run on a treadmill before and after their hematocrit had been increased by isolvolemic exchange transfusion with packed red blood cells. With normovolemic polycythemia, cardiac output fell in a linear fashion as hematocrit was increased to 65%, but these changes were balanced by an increasing oxygen content resulting in constant systemic oxygen transport. Oxygen consumption was unchanged both at rest and during exercise after induction of polycythemia. To investigate the effect of polycythemia on oxygen transport further, we measured both mixed venous P\textsubscript{O\textsubscript{2}} and lactate. Mixed venous P\textsubscript{O\textsubscript{2}} increased and lactate remained unchanged both at rest and in exercising polycythemic dogs. Thus, we conclude that, in conscious animals, systemic oxygen transport is well preserved with increasing hematocrit to at least 65%. (Circ Res 56: 793-800, 1985)

ALTHOUGH polycythemia is a fundamental mammalian response to hypoxemia, it has often been assumed to be detrimental. Whereas it would seem that the increased capacity of blood to carry oxygen might be useful, this may be negated by the fall in cardiac output which occurs with polycythemia. Indeed, previous studies in resting, anesthetized dogs concluded that systemic oxygen transport (cardiac output \times oxygen content) is maximal at a normal hematocrit and falls with either anemia or polycythemia (Richardson and Guyton, 1959; Murray et al., 1962, 1963). The decreased systemic oxygen transport with polycythemia was due to a fall in cardiac output that exceeded the rise in oxygen content. The fall in cardiac output has been attributed to increased viscosity of the polycythemic blood which, in turn, could have increased vascular resistance and limited venous return (Guyton and Richardson, 1961). However, these studies were performed in barbiturate-anesthetized animals in which resting vascular resistances are abnormally high and reflexes blunted; both conditions might exaggerate the effects of viscosity. In addition, there are several reasons why polycythemia might not prove disadvantageous during exercise. Muscle pumps may overcome the effects of viscosity on venous return (Stegall, 1966), arterial vasodilation in exercising muscle might overcome the increased vascular resistance imposed by increased viscosity; and, finally, increased flow rates alone should decrease the effects of viscosity in both the venous and arterial beds (Whittaker and Winton, 1933). If so, polycythemia might not have a detrimental effect on systemic oxygen transport during exercise. Thus, we sought to determine the effects of polycythemia on systemic oxygen transport during exercise, using conscious, chronically instrumented dogs trained to run on a treadmill.

Methods

Surgical Techniques

In an initial series, 11 mongrel dogs weighing 22-37 kg were trained to run easily for several minutes on a level treadmill. Subsequently, a splenectomy was performed under sterile conditions, using halothane anesthesia to minimize large variations in hematocrit and blood volume in the dog (Barcroft and Florey, 1931; Vatner et al., 1974). The dogs were allowed to recover for a minimum of 7 days, after which each underwent a left thoracotomy with placement of 18-gauge polyvinyl catheters in the descending aorta, the main pulmonary artery, and the left atrium. A solid state pressure transducer (Konigsberg P 18) was placed through a stab incision in the apex of the left ventricle, and a Zepeda electromagnetic flow probe was placed around the ascending aorta. This surgery was followed by a recovery period of at least 2 weeks. Two days before each study, under light pentobarbital anesthesia, one external jugular vein was used to place a 14-gauge polyvinyl catheter used for infusion of packed red blood cells. All catheters were tunneled subcutaneously and exteriorized at the back of the neck.

Because high hematocrits could influence the accuracy of electromagnetic flow probe readings, in an additional eight dogs (24-36 kg), radioactive microspheres were used for study of cardiac output measurements. These dogs underwent the same surgical procedures, except the solid-state pressure transducer and flow probe were not placed during the thoracotomy.

Treadmill Training

Each dog was trained to run on a level treadmill at a speed between 5 and 9 miles/hr. No dog was run for more than 10 minutes, three times a week, to avoid conditioning effects.
Donor Blood

Blood was removed from anesthetized donor dogs via a 13-gauge steel needle placed in the apex of the left ventricle and drained into vacuum bottles containing either 5000 U of heparin or 60 ml of ACD for each 500 ml of whole blood and transferred to transfer packs and spun at 1200 g for 20 minutes to pack the red blood cells to a hematocrit of 75-80%. Donor blood was acquired within 24 hours of the study day and stored at 4°C in ACD. All packed blood cells were filtered through a blood filter prior to transfusion. Blood which demonstrated visible hemolysis was not used. Blood was warmed to body temperature just before transfusion. There was no evidence of major or minor transfusion reactions during these studies.

Hematocrit, Lactate, Blood Gases, and Oxygen Content

Hematocrits were drawn from the pulmonary artery catheter and were measured by the microhematocrit centrifuge method. No correction was made for trapped plasma. Blood lactate was measured by a standard technique (Henry, 1968). Blood gas tensions for oxygen, carbon dioxide, and measurement of pH were obtained using polarographic electrodes (Radiometer) calibrated against standard buffers. All blood gas values were corrected to normal saline at room temperature. Arterial oxygen content was measured with the Lex-O2-Con device (Lexington Instruments) which, in our hands, agrees with Van Slyke measurements within 0.1 volume %.

Blood Volume

On the day before each study, 6 ml of whole blood were removed from each dog. Two milliliters of red blood cells were incubated with Na153CrO4 in N.I.H. A-ACD (ICSH panel) for 15 minutes at room temperature. The cells were washed three times in normal saline and diluted in plasma to the original volume. After mixing, three small aliquots were taken to determine the total number of counts injected. The remaining blood was added to a preweighed syringe and reweighed to determine the total weight of the blood. The day of the study, we removed 2 ml of blood from the dog and determined its exact volume by using its weight and the specific gravity of blood at that hematocrit. Each sample was counted in a well-type γ-counter. Initial blood volume was then determined by the formula

\[
\frac{\text{Total } ^{51}\text{Cr counts injected}}{\text{Blood volume}} = \frac{\text{counts per sample}}{\text{ml per sample}}
\]

Subsequent blood volumes were estimated by measuring all blood removed from the dog and counting aliquots of this blood to determine the total amount of 51Cr removed. By knowing the total amount of 51Cr injected initially and the amount removed, blood volume was determined at each level of the experiment without relabeling red blood cells by the formula

\[
\frac{\text{Total } ^{51}\text{Cr counts remaining}}{\text{Blood volume}} = \frac{\text{counts/sample}}{\text{ml/sample}}
\]

To ensure further that normovolemia was maintained, all transfusions were isovolemic exchange transfusions, a method previously shown to maintain a constant blood volume (McGrath and Weil, 1978).

Cardiac Output and Pressure

In the dogs with electromagnetic flow probes stroke volume was calculated using the integral of the aortic flow signal (Horwitz, 1972). Cardiac output was determined by averaging stroke volume for at least six consecutive beats to reduce the effects of respiratory variation. Each electromagnetic flow probe was calibrated in vitro before insertion in each dog. Calibrations were made with normal saline at room temperature. Flow was assumed to be zero at end-diastole using an electromagnetic flowmeter (Ze-peda, EPD2). We compared cardiac output obtained by electromagnetic flowmeter to those determined by microspheres over a hematocrit range of 35-66 and found the cardiac outputs by electromagnetic flow probe were not consistently different from those obtained by microspheres; thus, no correction was made for hematocrit.

All signals were recorded on an eight-channel Beckman dynagraph recorder. Mean arterial and left atrial pressures were measured through the implanted catheters with Statham P23Db manometers using the mid chest level as reference. Zero references were recorded both at rest and during exercise. Mean left atrial and arterial pressures were recorded continuously during exercise. A dose of 1 ml of 0.02% Tween-80 was injected before each study. If a reaction occurred, the studies were not begun for 30 minutes after heart rate and blood pressure had returned to normal.

In the microsphere studies, cardiac output was measured by microspheres, 15 ± 3 μm in diameter, (New England Nuclear) labeled with 46Sc, 95Nb, 103Ru, 113Sn, 51Cr, and 153Gd. The microspheres were suspended in 10% dextran and 0.02% Tween 80. At rest, after a steady withdrawal of blood was established from the descending aortic catheter with a Cole-Parmer Masterflex pump set at a single constant flow rate between 7 and 10 ml/min, microspheres were injected through the left atrial catheter, followed by a 7-ml flush of normal saline. Each injection contained 2-5 × 104 microspheres, depending on the activity of the nuclide. Arterial blood was collected directly into preweighed counting tubes. This collection of blood began 10 seconds before injection of microspheres and lasted for a total of 200 seconds. Before all injections, microspheres were continually agitated by vortex mixer for 1 minute. During exercise, two different microspheres were injected through the left atrial catheter, the first at approximately 3 minutes of exercise and the second microsphere 80 seconds later. Each injection was followed by a 7-ml flush of normal saline. Arterial blood was collected into counting tubes at a constant flow rate (7-10 ml/min) beginning 10 seconds before injection of the first microsphere and lasting for a total of 300 seconds. The interval for injection of the two microspheres was chosen to avoid overlap between the two microspheres. All cardiac output tubes were counted consecutively in a well-type γ-counter with appropriate energy levels for each nuclide. Each tube was counted for 4 minutes. Cardiac output was calculated by the formula:

\[
\text{CO} = I(Qar/\text{larr}),
\]

where CO = cardiac output, I = total counts injected, Qar = the arterial withdrawal flow rate, and larr = the total activity in the arterial sample collected (Archie et al., 1973, Fixler et al., 1976). "1 was calculated as the total counts in the preweighed microsphere syringe, minus the counts remaining in the syringe after injection. During exercise, the dogs continued to run at the same speed until the microspheres collections were completed. The two values
for cardiac output during exercise showed no consistent or significant differences. Because they were obtained during steady state exercise, an average of the two values for cardiac output during exercise was used.

Study Design

In the group of 11 dogs in which cardiac output was measured by electromagnetic flow probe, hemodynamic measurements were made at rest and at 3 minutes of steady state exercise. Each dog was first studied at its baseline hematocrit, both at rest and during exercise. Isovolemic exchange transfusion then was performed, removing 200–400 ml of whole blood from the dog and replacing it with an equal volume of donor-packed red blood cells. Blood volume and hematocrit determinations were made at least one-half hour after each transfusion to assure normovolemia. At least 45 minutes after each transfusion, all measurements were repeated at rest, and exercise was again performed at precisely the same treadmill speed as during the control run. This procedure was repeated between four and six times on any one day at several levels of hematocrit. Exercise blood samples for arterial oxygen content, and for arterial and mixed venous Po2, PCO2, pH, and lactate were drawn within 15 seconds of the end of exercise. This protocol was so lengthy that we could not achieve high levels of hematocrit in a single day. Thus, in 4 of the 11 dogs, we studied only normal and very high hematocrits (64–70%). The dogs were studied at the initial hematocrit and then again after multiple exchange transfusions when the hematocrit reached at least 65%.

In the second group of eight dogs in which microspheres were used for cardiac output, the dogs were studied only at the normal hematocrit and then at a hematocrit of 64–70%. The study design differed in only two ways from the study done in animals with electromagnetic flow probes. Microspheres were injected for cardiac output measurements and blood samples for arterial oxygen content, arterial Po2, PCO2, pH, and for mixed venous oxygen content, and for mixed venous Po2, PCO2, and were drawn simultaneously after 3 minutes of exercise. The lactate samples (aorta, mixed venous) were drawn within 15 seconds of the end of exercise.

Control Studies

To control the effects of transfusion and time, and because of the nonrandom sequence of increasing hematocrits, we prepared three dogs as described above, including electromagnetic flow probes. All measurements were performed in the same way, and the study design was the same as described above. However, transfusions were different, in that 200–400 ml of whole blood were removed and an equal amount of donor whole blood was reinflused to keep both hematocrit and blood volume constant. Donor whole blood was first packed by centrifugation but then was resuspended in plasma to a normal hematocrit in these control studies to mimic exactly the blood preparation in the polycythemia studies.

Statistics

The group of 11 dogs studied with electromagnetic flow probes was divided into two groups for statistical analysis. Because each dog had different levels of hematocrit in the group of seven animals studied at multiple hematocrits, each dog was tested for hematocrit effects, and the results were combined by a cumulative p-test (Fisher test) (Steel and Torrie, 1960). For purposes of illustration, these
dogs are grouped in hematocrit ranges of five points: that is, 35–40%, 40–45%, 45–50%, 50–55%, 55–60%. All dogs studied at only two hematocrit levels were analyzed by paired t-test (Steel and Torrie, 1960).

In the eight dogs studied at two hematocrits, using cardiac output by radioactive microspheres, statistical analysis was performed by paired t-testing. The cumulative p-test (Fisher test) was used for evaluation of the control dogs.

Results

Heart Rate

No significant changes in heart rate were detected in dogs with polycythemia, at rest or during exercise, whether studied with electromagnetic flow probes (Fig. 1A) or radioactive microspheres (Fig. 1B).

Cardiac Output and Systemic Oxygen Transport

Cardiac output fell significantly in dogs with polycythemia both at rest and during exercise (Fig. 2). The fall in cardiac output occurred in a linear fashion as a function of hematocrit (Fig. 2A). Despite this fall in cardiac output, there were no significant changes in systemic oxygen transport (Fig. 3). Since heart rate did not change either at rest or during

FIGURE 1. Heart rate vs. hematocrit. Heart rate ± 1 SE (bar) as a function of hematocrit. Panel A is the group of 11 dogs with cardiac output measured by electromagnetic flow probe. ○ represents the seven dogs with sequential measurements. △ represents four dogs studied only at normal and high hematocrits. Panel B is the group of eight dogs with cardiac output measured by radioactive microspheres.
exercise, the fall in cardiac output in both groups of dogs was due entirely to a fall in stroke volume.

**Arterial-Venous Oxygen Difference and Oxygen Consumption (Table 1)**

Arterial-venous oxygen difference was measured in the group of eight dogs studied at two levels of hematocrit with radioactive microspheres. The arterial-venous oxygen difference increased after polycythemia, both at rest and during exercise, balancing the fall in cardiac output. There were no significant changes in oxygen consumption (AVO2 difference × CO) with polycythemia.

**Mean Arterial Pressure and Systemic Vascular Resistance**

In the 11 dogs studied with electromagnetic flow probes, no significant changes were seen in mean aortic pressure with polycythemia (Fig. 4A). In the second group of dogs, there was a significant rise in aortic pressure only with exercise (Fig. 4B). Polycythemia led to a rise in systemic vascular resistance both at rest and during exercise (Fig. 5).

**Mixed Venous PO2 and Arterial and Mixed Venous Lactate (Table 2)**

Mixed venous PO2 levels were unchanged in the animals studied at several levels of hematocrit. However, in the two groups of dogs studied at just two levels of hematocrit, PO2 levels in mixed venous blood were higher with polycythemia, both at rest and during exercise. Mixed venous PO2 was lower during exercise than at rest (P < 0.01 for both control and polycythemia in all groups). Both arterial and mixed venous lactate levels were unaffected by polycythemia at rest or during exercise. Mixed venous and arterial lactate levels were not different at rest or with exercise. Resting lactates were significantly lower than exercise lactates before and after polycythemia (P < 0.01 in all groups).

**Blood Volume**

Blood volume as measured by 51Cr labeling showed no significant changes in either of the groups of dogs studied with flow probes (1840 ± 135 vs. 1955 ± 173) or in the second group of dogs studied with microspheres (2153 ± 90 vs. 2240 ± 132) (mean ± SEM).

**Table 1**

<table>
<thead>
<tr>
<th>Arterial-Venous Oxygen Difference and Oxygen Consumption</th>
<th>Control</th>
<th>Polycythemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVO2 difference (vol %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>8.2 ± 0.4</td>
<td>10.5 ± 0.6*</td>
</tr>
<tr>
<td>Exercise</td>
<td>12.9 ± 0.4</td>
<td>17.0 ± 0.8†</td>
</tr>
<tr>
<td>O2 consumption (ml/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>351 ± 23</td>
<td>295 ± 26</td>
</tr>
<tr>
<td>Exercise</td>
<td>1065 ± 89</td>
<td>997 ± 70</td>
</tr>
</tbody>
</table>

*P < 0.05.
†P < 0.01.
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**Control Dogs (Table 3)**

Three dogs underwent a control run followed by four exchange transfusions with whole blood leaving hematocrit unchanged. Cardiac output, measured by electromagnetic flow probe, did not increase in any animal.

**Discussion**

We found that acutely induced normovolemic polycythemia to hematocrits up to 68% caused no decrement in systemic oxygen transport. Although cardiac output fell progressively with rising hematocrit, this was offset by comparable increases in oxygen content, and thus there was no change in systemic oxygen transport.

These results differ from most previous data, the bulk of which was collected in anesthetized dogs, and led to the conclusion that the optimal hematocrit for oxygen transport is the normal hematocrit, and that at higher hematocrits the fall in cardiac output leads to a fall in systemic oxygen transport (Richardson et al., 1959; Murray et al., 1963). Cardiac output fell much less with polycythemia in our study than in previous studies in anesthetized dogs. There are several possible reasons for this difference. In anesthetized, artificially ventilated animals, circulatory reflexes may be blunted or totally abolished (Kontos et al., 1965), and vascular resistance is often quite high (Murray et al., 1963). Thus, in previous studies, decreases in stroke volume with polycythemia may have been accentuated by the extremely high baseline systemic resistance. In many of these studies, only very high and very low hematocrit ranges were studied. Our particular interest was in hematocrits between 30% and 65%, a range that has not been carefully investigated. Others have speculated that the fall in cardiac output with polycythemia is due primarily to increased viscosity, leading to decreased venous return and, possibly, to an increased afterload (Guyton and Richardson, 1961). Viscosity does increase as hematocrit rises; however, several studies have shown that viscosity in vivo is lower than viscosity measured in vitro (Whittaker and Winton, 1933; Levy and Share, 1953; Djososugio et al., 1970), and that the effects of hematocrit on viscosity are much less in vivo than in vitro. In particular, an elevation in hematocrit appears to have a negligible effect on in vivo viscosity in a vasodilated vascular bed in exercising muscle (Gustafsson et al., 1980). In the hematocrit range of our study (up to 68), in vivo viscosity changes are quite small (Whittaker and Winton, 1953; Gustafsson et al., 1980). In anesthetized animals with very high resting vascular resistances, viscosity may play a much more important role.

Only one previous study deals with polycythemia in the awake, exercising animal (Weisse et al., 1964). In that study, three dogs were studied during exercise—two had a fall in systemic oxygen transport with polycythemia and one had an increase. As in our study, these investigators found that the fall in cardiac output with polycythemia is much less in conscious animals than has been reported in anesthetized animals.

Normovolemia was maintained during this study because hypervolemia itself may increase cardiac output (Murray et al., 1963; Keroes et al., 1969).
Normovolemia was ensured by the process of isovolemic exchange transfusions (McGrath and Weil, 1978) and by blood volume measurements.

Systemic oxygen transport measures the amount of oxygen delivered to the body but may not indicate the adequacy of delivery relative to demand. To assess O₂ utilization, we measured P₀₂ and lactate in the mixed venous blood. Mixed venous P₀₂ was well preserved with rising hematocrit, and mixed venous lactate levels did not rise with induced polycythemia. Thus, it would appear that oxygen delivery to exercising muscle was intact during normovolemic polycythemia at this modest level of exercise.

Whether polycythemia improves exercise performance in humans has been the subject of several studies. The earliest studies concluded that both VO₂max and exercise capacity were improved one
day after reinfusion of autologous frozen red blood cells (Ekblom et al., 1972, 1976). These studies lacked control groups and blinded design. Subsequent studies (Videman and Rytomaa, 1977; Williams et al., 1978) reported that induced polycythemia ("blood doping") did not lead to significant improvements in exercise performance. However, these studies led to only very small changes in hemoglobin after blood reinfusion. Two recent studies with adequate controls suggest that polycythemia may indeed improve exercise performance. Horstman et al. (1980) used altitude to induce polycythemia in nine male volunteers and demonstrated an improved \( VO_2_{max} \), systemic oxygen transport, and endurance time at high altitude, despite a fall in cardiac output. They concluded that polycythemia contributes to increased work capacity at high altitude. In another study of 11 highly trained runners, \( VO_2_{max} \) and running time to exhaustion were significantly improved after infusion of 900 ml of autologous freeze-preserved red blood cells (Buick et al., 1980). No such improvements were seen in these same subjects before phlebotomy or with a sham infusion of 50 ml of saline. The results of both of these studies may have been influenced by an increase in total blood volume, so that it is difficult to separate the effects of an increase in hematocrit alone.

According to our results, for hematocrits as high as 68\%, systemic oxygen transport is well preserved at rest and during exercise in spite of a lower cardiac output. The increase in mixed venous \( PO_2 \) following polycythemia supports the notion that systemic oxygen transport may actually improve. Our data are insufficient to determine whether or not there is an eventual fall-off in systemic oxygen transport when hematocrits are above 68\%.

Our study fails to indicate the mechanism of the decrease in cardiac output with polycythemia. Previous investigators have suggested that increasing viscosity may be the cause (Guyton and Richardson, 1961). However, several factors suggest that this is not the case. Myocardial performance does not appear to be altered with polycythemia, and tachycardia is typically absent in polycythemia, suggesting that the cardiac output is not working with either a preload deficiency or an afterload burden (Horstman et al., 1980). Mean arterial pressures remain relatively constant at both rest and exercise, a finding described in previous studies (Weisse et al., 1966). Systemic oxygen transport also appears to be remarkably constant with increasing hematocrit. A major question remains as to whether it is homeostasis of mean arterial pressure, systemic oxygen transport, a combination of these, or a factor previously undefined, which modulates cardiac output with polycythemia.

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