The Effect of Bloodletting on Exercise Performance in a Subject With a High-Affinity Hemoglobin Variant

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We studied two young army recruits with erythrocytosis. One had a variant hemoglobin with high affinity for oxygen (hemoglobin Osler, also known as Fort Gordon and Nancy, \(\beta_{145}\) Tyr \(\rightarrow\) Asp). The other had normal oxygen affinity and erythrocytosis of undetermined etiology. Both were asymptomatic. We studied exercise capacity on a cycle ergometer before and after hemodilution. In the subject with high oxygen affinity, hemodilution resulted in reduced maximal work and increased heart rate at every work level. In addition, minute ventilation and arterial lactic acid increased, while anaerobic threshold decreased, indicating diminished oxygen supply to tissues. In contrast, the subject with normal oxygen affinity had no significant changes in exercise performance after hemodilution. These results suggest that when blood oxygen affinity is high, loss of efficiency in tissue oxygenation can be expected after phlebotomy or hemodilution. Therefore, it may be useful to measure blood oxygen affinity and exercise performance in polycythemic subjects in whom such procedures are intended to ameliorate symptoms of hyperviscosity.

More than 30 mutations of human hemoglobin cause polycythemia in carriers because of increased oxygen affinity. The presumed cause of polycythemia in such individuals is erythropoietin production, which results from reduced release of oxygen in tissues.

Carriers of these hemoglobins report symptoms that resemble those of polycythemia vera. These symptoms are attributed to increased blood viscosity, and physicians are often concerned about the possibility of vasoocclusion. Phlebotomy or hemodilution, procedures that are frequently beneficial to subjects with polycythemia vera, are sometimes recommended. Some authors have warned, however, that reduction of oxygen-carrying capacity might compromise tissue oxygenation because, in these cases, polycythemia is a normal physiologic response. Thus, no clear guidelines exist to determine the optimal hematocrit in such patients, and phlebotomy has been done largely by cautious trial and error.

Recently, an unusual opportunity arose that may help clarify this subject. Two young Army recruits were found, almost simultaneously, to have elevated and similar hematocrits. One was a carrier of hemoglobin Osler, \(\beta_{145}\) Tyr \(\rightarrow\) Asp, also named Fort Gordon or Nancy, a variant with very high oxygen affinity. The other had erythrocytosis of undetermined etiology and normal oxygen affinity. Their physical characteristics were remarkably well matched. To assess tissue oxygenation in these subjects, we carried out detailed studies of exercise capacity before and after hemodilution. The results provide objective information about the physiologic changes that result from bloodletting and allow formulation of a more rational approach.

Materials and Methods

Clinical Studies

Exercise and hemodilution studies were carried out according to a protocol approved by the Clinical Review Committee of the National Institutes of Health. After being informed of the potential risks and benefits of the procedures, the subjects gave their consent. All hematologic studies were performed according to standard clinical procedures. Red cell mass measurements were made using \(^{51}\)Cr-tagged red cells. Plasma volume was calculated from red cell mass and venous hematocrit.

Hemoglobin Structure

Methods for chemical modification of proteins and preparation of tryptic digests have been described. Peptide maps were constructed according to procedures reported by Bennett: the column chromatography methods of Jones and Schroeder were used to isolate peptides. The amino acid compositions of peptides were determined by the method of Spackman et al., after hydrolysis of peptides at 110°C for 24 hr in vacuo.

Whole Blood Oxygen Affinity

Oxygen equilibrium curves (OEC) of whole blood samples were measured as described previously. The blood samples were anticoagulated with heparin, kept at 4°C on wet ice, and air-shipped from Washington, D.C. to Atlanta, GA, where measurements were made within 24 hr of blood collection. Alternatively, whole blood OECs were measured using a Hemoscan (Aminco, Silver Spring, MD). Both procedures gave equivalent results under similar conditions.

Exercise Test

Exercise capacity was measured on a cycle ergometer (Pedalmate II, Collins, Braintree, MA), using a 1-min incremental protocol. After the subject was positioned on the ergometer, he pedaled at constant speed while the work increased each minute by 25 W. ECG and heart rate were monitored by a Lifepak-6 system (Physio-Control, Redmond, WA). During the last 15 sec of each minute, heart rate was recorded, expired gas was collected in meteorologic...
balloons, and blood was sampled through a radial artery catheter and placed into ice-cold tubes containing NaF.

After the test, volumes of the balloons were measured using a Singer flow meter (Collins). Concentrations of O₂ and CO₂ were measured using infrared (1B-2, Beckman, Fullerton, CA) and fuel cell (Applied Electrochemistry, Sunnyvale, CA) analyzers, respectively. Oxygen consumption and CO₂ production were calculated using the equations of Jones et al. Lactic acid concentrations were measured immediately, using reagent kits obtained from Calbiochem (La Jolla, CA).

**Hemodilution**

Isovolemic hemodilution was done using a Haemonetics model 30 discontinuous-flow cell separator (Haemonetics Corp., Natick, MA) with a 375-ml bowl. Blood was withdrawn from one arm, and fluid was replaced into the other. In one procedure, a standard anticoagulant, citrate phosphate dextrose (CPD) solution, was used, and in the second, heparin (20 U/ml) was used. Both were infused with a 1:8 ratio of anticoagulant:blood. Volume was maintained by the simultaneous infusion of 5% serum albumin in saline. Approximately 2 liters of whole blood were removed in each procedure. The flow rate ranged from 40 to 60 ml/min, as tolerated.

**RESULTS**

**Subjects**

L.S., the carrier of hemoglobin Osler, is a 24-yr-old black male soldier and racketball player who has smoked a pack of cigarettes a day for 4 yr. He has been normally active throughout his life. When examined before being stationed overseas, his hematocrit was 69%. His physical examination was unremarkable. Pulmonary function tests showed a mild restrictive defect compatible with cigarette use.

R.W. is a 25-yr-old black male soldier who has smoked a pack of cigarettes a day for 3 yr. He jogs 1 mile a day and is asymptomatic. On routine examination, he was found to have a hematocrit of 57%. Findings from his chest x-ray, electrocardiogram, bone marrow aspiration, liver-spleen scan, and intravenous pyelogram with nephrotomograms were normal. Leukocyte alkaline phosphatase and serum B₂ were normal. Computer-assisted tomography of the head showed no abnormalities.

The two subjects were remarkably well matched with regard to age, height, and weight (see Table 1). Predicted vital capacities are slightly different for the two subjects. The value for R.W., 4.81 liter, is 95% of predicted vital capacity, while for L.S. 3.93 liter is only 80% of his predicted vital capacity. No further evidence for a restrictive lung defect could be detected in L.S. by spirometry, history, or physical examination.

Both subjects had increased red cell mass. The values for hematocrit and hemoglobin, given in Table 1, were measured at the time of red cell mass measurements. Plasma volume for R.W. was normal, but both subjects had increased total blood volume. Thus, in the absence of a definitive diagnosis, R.W. was classified as having erythrocytosis of undetermined etiology and will be followed carefully for changes in his clinical status.

**Hemoglobin Structure**

Analysis of a tryptic digest of the aminoethylated β chain by peptide mapping showed the displacement of peptide Tp-14,15 to a position below peptide Tp-6. This change in mobility from a position below Tp-12b indicates a decrease in the hydrophobic nature of the peptide. The amino acid composition of the eluted peptide was Lys 0.9(1), His 1.6(2), Asp 2.2(1), Gly 1.0(1), Ala 3.9(4), Val 1.6(3), Leu 0.9(1), and Tyr 0(1); parenthetic numbers are the molar ratios of amino acids in normal peptide Tp-14,15. The value for valine is low because of the Val-Val sequence at positions 133–134. Similar results were obtained after isolation of Tp-14,15 by rechromatography on Dowex 50 × 2 of a peak that initially separated between Tp-4 and Tp-2 on Aminex A-5. Under normal conditions, Tp-14 coelutes with Tp-4. The fingerprint distribution of tryptic peptides and their elution from ion-exchange resins have been described previously.

Since there is only one residue of tyrosine in the combined peptides, and the variant resembles HbJ (β16 Gly-Asp) in both cellulose acetate and alkaline globin electrophoresis, it is apparent that the substitution is β145 Tyr-Asp. The neutral substitution, Tyr-Asn, would not lead to any alteration in the electrophoretic properties of the variant. The β145 Tyr-Asp substitution has been reported previously as Hb Osler, Fort Gordon, and Nancy.

**Blood Oxygen Affinity**

The whole blood oxygen equilibrium curves for the two subjects are shown in Fig. 1. The P50, the P0₂ at which hemoglobin is half-saturated with oxygen, was 10.9 torr for L.S. and 28.0 torr for R.W. The latter is in the normal range for the conditions under which the studies were performed, P0₂, 40 torr, and pH 7.4. The
shape of the OEC was distinctly biphasic for L.S., a finding that is characteristic of carriers of high-affinity hemoglobins, and is in agreement with previous studies with hemoglobins Osler\textsuperscript{22} and Fort Gordon.\textsuperscript{3}

**Hemodilution**

The hemodilution procedure required about 2 hr to complete and was well tolerated. The volumes of blood constituents removed and replaced are given in Table 2. The hematocrit and hemoglobin values listed were those measured at the time of the two exercise tests, and they differ slightly from those observed at the time of red cell mass measurement (Table 1) because therapeutic phlebotomy had been performed in the interim.

**Exercise Tests**

Figures 2–5 give the results of the exercise tests. Both subjects had increased heart rates after hemodilution, but the increase for R.W. was much less than that for L.S. (Fig. 2, top). Minute ventilation increased with exercise in both subjects (Fig. 2, bottom), but the pattern of change differed: L.S. responded briskly to increased work, and even more briskly after hemodilution. The nature of this response can be appreciated better by plotting minute ventilation against CO\textsubscript{2} production and O\textsubscript{2} consumption (Fig. 3). The initial slopes of VE/VO\textsubscript{2} were steeper for L.S. than for R.W. The response curves for R.W. were parallel, but higher after hemodilution; while for L.S., there was no effect of hemodilution.

Figures 4 illustrates some of the parameters of pulmonary gas exchange. The difference between inspired and expired gas concentrations (dF\textsubscript{CO\textsubscript{2}} and dF\textsubscript{O\textsubscript{2}}) were plotted against total ventilation. Thus, the higher the fractional difference, the greater the total lung-diffusing capacity. Initially, the exchange of both O\textsubscript{2} and CO\textsubscript{2} increased in R.W. with increasing ventilation. However, dF\textsubscript{O\textsubscript{2}} decreased past the anaerobic threshold. After hemodilution, the same pattern was followed, but the overall exchange was less. These changes probably reflect increases in both ventilation and lung perfusion with greater cardiac output. After hemodilution, the differences were less, because of decreased gas-carrying capacity of the blood.

**Table 2. Hemodilution**

<table>
<thead>
<tr>
<th></th>
<th>L.S.</th>
<th>R.W.</th>
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<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>65.8</td>
<td>65.8</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>18.8</td>
<td>15.3</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>53.2</td>
<td>42.3</td>
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<tr>
<td>WBC (10\textsuperscript{3}/cu mm)</td>
<td>3.4</td>
<td>4.1</td>
</tr>
<tr>
<td>Retic (%)</td>
<td>1.1</td>
<td>1.6</td>
</tr>
<tr>
<td>Blood removed (ml)</td>
<td>1,930</td>
<td></td>
</tr>
<tr>
<td>Albumin replaced (ml)</td>
<td>2,000</td>
<td></td>
</tr>
<tr>
<td>Net change (ml)</td>
<td>+ 70</td>
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Fig. 4. The efficiency of O₂ extraction (top) and CO₂ production (bottom) for L.S. (left) and R.W. (right). Values before (closed symbols) and after (open symbols) hemodilution are shown. Note that the fractional difference between inspired and expired gas concentrations is almost independent of minute ventilation and hemoglobin concentration for L.S.

The results for L.S. were quite different. As ventilation increased, the fractional extraction of O₂ and production of CO₂ decreased steadily, and there was no apparent effect of hemodilution on these curves.

The anaerobic threshold is the work rate or O₂ uptake, at which lactic acid begins to accumulate in the blood because the O₂ supply to exercising muscle is inadequate. This threshold has been detected either by direct measurement of arterial lactic acid, or by inference from the pattern of VCO₂/VO₂, the gas exchange ratio. Figure 5 shows both measurements.

In subject R.W., the lactic acid patterns before and after hemodilution were identical. Thus, hemoglobin concentration was apparently not a limitation to exercise after the dilution. In contrast, hemodilution in subject L.S. resulted in a substantial increase in arterial lactic acid, and the anaerobic threshold decreased by about 50 W, indicating that hemodilution did limit exercise.

The gas exchange ratios in both subjects were less accurate in demonstrating the anaerobic threshold. The ratio increased abruptly, however, in L.S. after hemodilution, suggesting a reduced anaerobic threshold. These results indicate that noninvasive detection of the anaerobic threshold in our subjects may be possible, but it is probably less quantitative than direct measurement of arterial lactic acid.

**DISCUSSION**

Some types of erythrocytosis, e.g., polycythemia vera, may be associated with vasooclusion and, by analogy, some authors have recommended phlebotomy in carriers of high-affinity hemoglobins to reduce blood viscosity. However, the latter subjects are unique because their oxygen equilibrium curves (OEC) are shifted markedly to the left (lower P50) and are biphasic. Elevated blood viscosity is solely derived from increased numbers of red cells. Moreover, arterial pO₂ is normal, providing a distinction from other types of secondary polycythemia. Thus, erythrocytosis seems to be a normal physiologic response, and it is not obvious that persons with high-affinity polycythemia are susceptible to any of the pathologic consequences of these different conditions.

The exercise protocol we selected is not designed to measure steady-state gas exchange or maximal oxygen uptake. Rather, it is useful to detect the anaerobic threshold, the point at which lactic acid increases in arterial blood. This point has been shown to be a highly reproducible index of the sufficiency of the total oxygen transport system to satisfy tissue oxygen demands.

Increased heart rate during exercise in L.S. suggests an increase in cardiac output to compensate for reduced oxygen-carrying capacity. However, this is not a unique interpretation of the data, because we have no direct assurance that the blood volume remained constant. For example, if the blood volume decreased, stroke volume would decrease and, with increased heart rate, cardiac output could be about the same. The hemodilution technique is probably the best method to assure that blood volume is held constant during the procedure. However, the rate at which the plasma volume is adjusted to match the new red cell mass is unknown, and we can only assume that since the procedures were carried out in the two subjects in an identical manner, the volume changes were parallel.

Although the physiologic basis for the ventilatory response to exercise is complex, it is mediated primarily by chemoreceptors that are sensitive to O₂ and CO₂. These receptors seem to have different “set points” in our two subjects. Unfortunately, no similar data are available for other subjects after hemodilution; we can only speculate that there is an effect of the
life-long polycythemia due to high oxygen affinity on the chemoreceptor control of ventilation. The failure to change the $VE/VCO_2$ or $VE/VO_2$ curves in L.S. (Fig. 3) seems to suggest that factors other than blood $O_2$ or $CO_2$ content may be responsible for controlling the sensitivities of his chemoreceptors. A group of persons with a similar problem, high altitude natives, has decreased chemoreceptor control. More data on more sensitivities of his chemoreceptors. A group of persons change the $VE/VCO_2$ and control of ventilation. With a similar problem, high altitude natives, has decreased chemoreceptor control.

In L.S. the fractional differences between inspired and expired $O_2$ and $CO_2$ were independent of exercise level or hemoglobin concentration. Interpretation of the total lung-diffusing capacity for $O_2$ depends on the hemoglobin saturation, but the dependence on the shape or position of the OEC has not been carefully studied. Our data suggest that there is some limitation to gas exchange in L.S. that we have not defined. Further detailed studies of gas exchange in the lung are needed to clarify this point.

A potential criticism of our study is that only two subjects were evaluated. Although studies of additional patients would be extremely useful, it is unlikely that other subjects with high-affinity hemoglobins could be matched in hematocrit, age, sex, and physical abilities with other subjects whose blood oxygen affinities are normal. Moreover, our experimental design was to compare subjects before and after hemodilution; each subject is, therefore, his own control. It is important to obtain as much information as possible from such unique opportunities because of the paucity of experimental data that are available in the literature.

Charache et al. studied individuals with hemoglobins Osler and McKees Rocks before and after phlebotomy and were unable to demonstrate any regular pattern of hemodynamic changes. Their study emphasizes the variability of physiologic compensations and stresses, once again, and emphasizes that all of these mechanisms are not understood.

Duvelleroy argued on theoretical grounds that small changes in $P_{50}$ can have large consequences in oxygen transport and that the ability of tissue capillary beds to expand or recruit is essential to compensate for the loss of oxygen release associated with reduced $P_{50}$. This concept is supported by the observation by Gau et al. that increasing $P_{50}$ by exchange transfusion in a subject with hemoglobin Malmo resulted in improved exercise performance, cardiac function, and symptoms.

Poyart et al. observed an individual with hemoglobin Creteil in whom exercise capacity was subjectively reduced after phlebotomy. They documented increased cardiac output and decreased mixed venous $pO_2$. After the frequency of phlebotomy was reduced in this subject, his exercise capacity returned to normal, even at a hematocrit of 60%. They pointed out that, since only about half of the hemoglobin is functional in oxygen delivery, phlebotomy results in a severe relative anemia, which can be compensated for only by increased cardiac output or decreased venous $pO_2$, or both.

A further case against bloodletting in subjects with high-affinity hemoglobins was made by Wade, who demonstrated paradoxically high cerebral blood flow in carriers of a high-affinity hemoglobin. Although the physiologic basis of this observation cannot be explained, it does suggest that bloodletting in these cases is not as urgent as it may be in subjects with other types of hyperviscosity.

We believe that a program of hemodilution in subjects with high-affinity polycythemia should not be based on symptomatic considerations alone. Increased risk of vasooclusion has not been proven for these subjects, and there are many reasons why their risk might be less than in other polycythemic states. It is not yet possible to predict the hematocrit that gives optimal viscosity and oxygen-carrying capacity in an individual. The only rational approach to therapy is an empirical test of oxygen delivery. While cerebral blood flow or other organ function might be more informative, exercise testing is convenient, noninvasive, and safe.

Finally, there is good evidence to suggest that in any individual for whom phlebotomy is considered, hemodilution is preferable to simple bloodletting. Some subjects with chronic lung disease and secondary polycythemia have died suddenly after phlebotomy. Cardiac output may suddenly drop in these cases because of contraction of the blood volume. It must be remembered that polycythemia reduces cardiac output, but the attendant expansion of blood volume can compensate, to some extent, and phlebotomy will remove this compensation. We believe that when reduction of the hematocrit is desired, blood volume should be maintained by replacement of the volume of blood removed with an appropriate volume expander.

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