Effect of Exercise on Erythrocyte Count and Blood Activity Concentration After Technetium-99m In Vivo Red Blood Cell Labeling

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SUMMARY We studied the effect of exercise on blood radiotracer concentration after technetium-99m in vivo red blood cell labeling. After red blood cell labeling, 13 subjects underwent maximal supine bicycle exercise. Radioactivity, analyzed with a well counter, was measured in heparinized venous blood samples drawn at rest and during peak exercise. Changes in activity were compared with changes in erythrocyte count. Activity and erythrocyte counts increased during exercise in all 13 subjects. Percent increase in activity correlated with percent increase in erythrocyte count ($r = 0.78$), but did not correlate with either duration of exercise or maximal heart rate. Twenty minutes after termination of exercise, activity and erythrocyte count had decreased from peak exercise values but remained higher than preexercise values. In nine nonexercised control subjects, samples drawn 20 minutes apart showed no change in activity or in erythrocyte count. We conclude that exercise increases blood activity, primarily because of an increase in erythrocyte count. During radionuclide ventriculography, blood activity must be measured before and after any intervention, particularly exercise, before a change in left ventricular activity can be attributed to a change in left ventricular volume.

EQUILIBRIUM radionuclide ventriculography is a useful method of estimating left ventricular volume and output. After blood pool labeling, which may be achieved by erythrocyte tagging with technetium-99m ($^{99m}$Tc), the ratio of left ventricular activity to peripheral blood activity concentration is proportional to left ventricular volume. The effect of exercise on left ventricular volumes has been examined by observing the changes in left ventricular counts. As long as the radiotracer remains in constant concentration within the circulation during exercise, no measurement of blood activity is needed to assess the percent change in left ventricular volume. Furthermore, using a single determination of blood activity, absolute ventricular volumes can be determined at rest and during exercise. However, if blood activity changes during exercise, the magnitude of the change must be known before alterations in ventricular volumes can be determined based on changes in ventricular activity.

Studies in animals and in man have demonstrated an increase in hemoglobin concentration during exercise. We studied the effect of exercise on blood activity concentration after $^{99m}$Tc in vivo red blood cell labeling, and compared changes in blood activity with changes in erythrocyte count.

Methods

The study population consisted of 22 subjects who had been referred for radionuclide ventriculography. Red blood cells were labeled in vivo by intravenously injecting unlabeled stannous pyrophosphate, followed by 15–25 mCi of $^{99m}$Tc as pertechnetate 15–20 minutes later. In each of the 13 subjects who underwent maximal (symptom-limited) supine bicycle exercise, two venous blood samples were drawn. The first was drawn at rest, 10 minutes after pertechnetate injection, and the second during peak exercise, approximately 15–20 minutes later. The initial work load was 25 W, and work load was increased by 25 W every 2 minutes. In seven of these patients, blood samples were also drawn 15–25 minutes after termination of exercise. In nine nonexercised controls, blood samples were drawn 10 and 30 minutes after pertechnetate injection. Each 10-ml venous blood sample was drawn through a separate venipuncture in the arm not used for pertechnetate injection and was injected into an evacuated heparinized glass tube. Both radioactivity and red blood cell counts were measured in duplicate. Erythrocyte counts were measured using a Coulter counter, and radioactivity was measured in 50-µl aliquots using a well counter. The linearity of the well counter was adequate over the range of activity measured ($r = 0.997$, coefficient of variation = 3.3%).

The significance of changes in erythrocyte count and in blood activity was determined using the $t$ test. Percent change in blood activity was correlated with percent change in erythrocyte count, duration of exercise and maximum heart rate, using standard linear regression analysis.

Results

Exercise duration, reason for exercise termination, maximal heart rate, and percent change in blood radioactivity concentration and in erythrocyte count are listed in table 1. Both activity concentration and erythrocyte count increased during stress in all exercised patients (fig. 1). Mean activity concentration, expressed as counts per second per µl blood (cps/µl), increased from 196 ± 7 cps/µl at rest to 209 ± 7 cps/µl (± SEM) ($p < 0.0001$), and the erythrocyte count increased from 4.70 ± 0.13 $10^6$ cells/mm$^3$ to 4.99 ±
TABLE 1. Subject Data

<table>
<thead>
<tr>
<th>Pt</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Medications</th>
<th>Exercise duration (min:sec)</th>
<th>Maximal work load (W)</th>
<th>Reason for stopping</th>
<th>Maximal heart rate (beats/min)</th>
<th>Percent increase blood activity</th>
<th>Percent increase erythrocyte count</th>
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<tr>
<td>1</td>
<td>58</td>
<td>F</td>
<td>MR</td>
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<td>50</td>
<td>Dyspnea</td>
<td>95</td>
<td>3.9</td>
<td>2.9</td>
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<tr>
<td>2</td>
<td>71</td>
<td>M</td>
<td>CAD</td>
<td>Nitr, asp</td>
<td>4:20</td>
<td>75</td>
<td>Fatigue, dyspnea</td>
<td>126</td>
<td>5.4</td>
<td>6.5</td>
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<tr>
<td>3</td>
<td>46</td>
<td>M</td>
<td>CAD</td>
<td>Lidof, prop</td>
<td>6:00</td>
<td>75</td>
<td>Fatigue, dyspnea</td>
<td>91</td>
<td>19.5</td>
<td>12.6</td>
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<td>M</td>
<td>CAD</td>
<td>Prop, thia</td>
<td>4:21</td>
<td>75</td>
<td>Fatigue</td>
<td>166</td>
<td>9.4</td>
<td>3.4</td>
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<tr>
<td>5</td>
<td>65</td>
<td>M</td>
<td>CAD</td>
<td>Thia</td>
<td>8:18</td>
<td>125</td>
<td>Fatigue</td>
<td>144</td>
<td>6.1</td>
<td>5.5</td>
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<tr>
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<td>64</td>
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<tr>
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<td>50</td>
<td>M</td>
<td>CAD</td>
<td>Lidof</td>
<td>10:30</td>
<td>150</td>
<td>Fatigue</td>
<td>120</td>
<td>4.1</td>
<td>5.2</td>
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<tr>
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<td>26</td>
<td>M</td>
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<td>125</td>
<td>Fatigue</td>
<td>158</td>
<td>6.7</td>
<td>5.9</td>
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<tr>
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<td>57</td>
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<td>CAD</td>
<td>Lidof</td>
<td>10:00</td>
<td>125</td>
<td>Fatigue</td>
<td>100</td>
<td>9.7</td>
<td>5.8</td>
</tr>
<tr>
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<td>60</td>
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<td>CAD, CHF</td>
<td>Fur, asp, dig</td>
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<td>75</td>
<td>Dyspnea, hypertension</td>
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<td>10.1</td>
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<tr>
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<td>100</td>
<td>Fatigue</td>
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<td>2.2</td>
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<td>CAD</td>
<td>Lidof</td>
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<td>125</td>
<td>Fatigue</td>
<td>110</td>
<td>6.2</td>
<td>5.9</td>
</tr>
</tbody>
</table>

Abbreviations: MR = mitral regurgitation; CAD = coronary artery disease; AVR = status after aortic valve replacement; CP = atypical chest pain syndrome; AR = aortic regurgitation; nitr = nitrates; asp = aspirin; lidof = lidoflazine; prop = propranolol; thia = thiazide diuretic; fur = furosemide; CHF = congestive heart failure.

0.17 $10^6$ cells/mm³ ($p < 0.0001$). Mean percent increases in activity concentration (6.6%) and in erythrocyte count (6.2%) were similar. The percent increase in activity concentration with exercise correlated with the percent increase in red blood cell count ($r = 0.78$) (fig. 2). There was no significant correlation between percent increase in activity concentration or erythrocyte count during exercise and either maximal heart rate or duration of exercise, whether the patients receiving propranolol were included or excluded (fig. 3).

In the seven patients in whom additional blood samples were drawn after exercise, the mean activity concentration was 192 ± 12 cps/µl before exercise, 204 ± 12 cps/µl at peak exercise, and 196 ± 12 cps/µl 20 minutes after termination of exercise. The mean erythrocyte count was 4.66 ± 0.16 $10^6$ cells/mm³ before exercise, 4.93 ± 0.17 $10^6$ cells/mm³ at peak exercise, and 4.80 ± 0.18 $10^6$ cells/mm³ after exercise (fig. 1). Activity concentration and erythrocyte count 20 minutes after exercise remained significantly higher than respective values preexercise ($p < 0.005$ and $p < 0.0005$) but declined significantly compared to peak exercise values ($p < 0.005$ and $p < 0.05$).

In nine nonexercised controls, blood samples drawn 20 minutes apart showed no significant change in activity (sample 1, 172 ± 24 cps/µl; sample 2, 172 ± 24 cps/µl) or in erythrocyte count (sample 1, 4.52 ± 0.16 $10^6$ cells/mm³; sample 2, 4.56 ± 0.14 $10^6$ cells/mm³) (fig. 4).

Discussion

Our study demonstrates that exercise increases blood activity concentration after $^{99m}$Tc in vivo red blood cell labeling. This increase appears to result largely or totally from an increase in erythrocyte count.

Animal and human studies have shown increases in hematocrit and in hemoglobin concentration during exercise. One factor that contributes to the increase in hematocrit during exercise, at least in dogs, is an increase in the number of circulating erythrocytes due to splenic contraction. Vatner et al. found that during running, mean hematocrit in dogs increased from 40% to 49% and that this effect of exercise was abolished by splenectomy. By comparing large-vessel hematocrit and plasma volume at rest and during exercise in dogs, Kraan et al. estimated that one-third of all erythrocytes are stored in the spleen at rest, and that splenic erythrocyte stores may be nearly emptied into the vascular circulation during vigorous swimming exercise. The effect of exercise on splenic erythrocyte storage may in part be mediated by circulating catecholamines. Guntheroth et al. found that epinephrine injection caused a rapid reduction in splenic dimension in dogs, followed by a rise in splenic vein hematocrit and blood flow and a subsequent increase in systemic hematocrit. A second factor in the increase in hematocrit during exercise is a reduction in plasma volume. Dia et al. found that plasma volume decreased 11% in humans during supine bicycle exercise. The magnitude of the change in hematocrit depends, in part, on the type and degree of exertion. Senay et al. found that the increase in hematocrit was greater during upright cycle exercise than during treadmill exercise and that during cycle exercise the rise in hematocrit was proportional to the rise in oxygen consumption.

After labeling of red blood cells with $^{99m}$Tc, an increase in erythrocyte count due to splenic contraction and/or hemoconcentration would result in an increase in blood radioactivity concentration. The contribution of splenic contraction to this increase depends on the extent of equilibration of labeled red blood cells within...
FIGURE 1. Effect of exercise on blood activity concentration (A) and on erythrocyte count (B). In all subjects, activity concentration and erythrocyte count were greater during peak exercise than during rest (p < 0.0001, p < 0.0001). In the patients in whom additional blood samples were drawn 20 minutes after termination of exercise, activity concentration and erythrocyte count remained higher than preexercise values (p < 0.005 and p < 0.0005) but were less than peak exercise values (p < 0.005 and p < 0.05).

the splenic storage pool. Kraan et al.\textsuperscript{11} found that 10 minutes after injection of chromium-51 ($^{51}$Cr)-labeled red blood cells in dogs, these cells had equilibrated with approximately 50\% of the splenic storage volume. Other workers have found that complete equilibration occurs within 20–30 minutes after injection.\textsuperscript{16, 17} Baker and Remington\textsuperscript{16} compared the rise in hematocrit and blood activity resulting from epinephrine-induced splenic contraction 60 minutes after injection of $^{51}$Cr-labeled red blood cells in dogs. They found a similar percent increase in hematocrit and blood activity, suggesting complete equilibration of the labeled cells within the splenic storage volume.

In our study, the absence of a significant difference between activity in blood samples drawn 10 and 30 minutes after pertechnetate injection in nonexercised controls suggests that there is rapid equilibration of $^{99m}$Tc in vivo labeled erythrocytes within the splenic storage volume in humans as well. Therefore, any rise in erythrocyte count that results from splenic contraction should induce a proportional increase in blood activity. We found a similar mean percent increase in red blood cell count and in blood activity during supine bicycle exercise, suggesting that equilibration had been achieved. The extent of exercise-induced splenic contraction in humans has not been directly assessed, however, and its contribution to the rise in erythrocyte count is not known.

Variations in the response of the splenic erythrocyte pool or plasma volume to stress is suggested by the lack of correlation between change in blood activity or erythrocyte count and duration of exercise or maximal heart rate. This variation may in part be due to differences in the hormonal response to stress. Since catecholamine levels are likely to play a role in mediating splenic contraction,\textsuperscript{15, 16, 18} differences in response may reflect variability in catecholamine release, influenced by the patient’s emotional response to testing. Fright produces vigorous splenic contraction in dogs.\textsuperscript{15, 18} In addition, difference in hydration state may have contributed to the variation in our observed responses to exercise. Gaeblein and Senay\textsuperscript{14} found that the preexercise level of hydration plays a major role in determining the direction and magnitude of plasma volume shifts during stress, probably through an effect on the threshold for vasopressin release.

The changes induced by exercise do not immediately reverse fully upon termination of stress. Twenty minutes after exercise, the blood activity and erythrocyte count both fell toward, but did not reach, preexercise values. These findings suggest that either circulating erythrocytes had not reached equilibrium with the splenic pool, or plasma volume had not returned to preexercise levels, or both.

Radionuclide ventriculography, using red blood cells labeled with $^{99m}$Tc by either the in vivo or in vitro method, has been used to estimate left ventricular volumes and outputs, presuming that left ventricular activity is proportional to volume.\textsuperscript{2-4, 9, 19} Several investigators have used this method to study the effect of

\[ y = 1.23x - 0.12 \]
\[ r = 0.78 \]

FIGURE 2. Comparison of percent increase in blood activity concentration with that in erythrocyte count with exercise.
various interventions, particularly exercise, on left ventricular volumes.\(^8\) We have shown that exercise increases blood activity after \(^{99m}\)Tc red blood cell labeling; it will be essential in future studies to measure blood activity during each radionuclide ventriculogram before attributing a change in left ventricular activity to an exercise-induced change in left ventricular volume. The calculated ventricular volume is inversely proportional to the assumed or measured blood activity concentration. Therefore, the calculation of left ventricular volumes in our subjects during rest and exercise, based on a single blood activity measurement at rest, would result in an overestimation of exercise ventricular volume of 0.8–19.5\% (or 1–14 ml/m\(^2\)) for a normal end-diastolic volume of 70 ml/m\(^2\), with a mean 6.6\% (or 5 ml/m\(^2\)) for an end-diastolic volume of 70 ml/m\(^2\). The magnitude of the overestimation of ventricular volumes cannot be predicted by either exercise duration or maximal heart rate. When the effect of any intervention, such as drug administration, on left ventricular volume or output is measured by radionuclide ventriculography, blood activity should be monitored to avoid errors due to unexpected alteration in blood activity during the intervention. For example, administration of catecholamines and of drugs that influence catecholamine levels or effects may alter the erythrocyte counts, thereby changing the blood activity concentration.\(^1\) Changes in blood activity would not affect left ventricular ejection fraction measurement, which is based on relative end-diastolic and end-systolic count rates, since such changes would alter the end-diastolic and the end-systolic count rates proportionally.

References
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