Greater erythrocyte deformability in world-class endurance athletes

JOHN A. SMITH,1 DAVID T. MARTIN,2 RICHARD D. TELFORD,3 AND SAMIR K. BALLAS4
1Department of Physical Therapy, Tennessee State University, Nashville, Tennessee 37209-1561; 2Department of Physiology and Applied Nutrition, Australian Institute of Sport, Belconnen, ACT 2616, Australia; 3School of Exercise Science, Griffith University, Gold Coast Campus 4017 QLD, Australia; and 4Cardeza Foundation for Hematologic Research, Department of Medicine, Thomas Jefferson University, Philadelphia, Pennsylvania 19107

Smith, John A., David T. Martin, Richard D. Telford, and Samir K. Balas. Greater erythrocyte deformability in world-class endurance athletes. Am. J. Physiol. 276 (Heart Cir. Physiol. 45): H2188–H2193, 1999.—Because athletes during endurance events require rapid uptake of oxygen, the ability of red blood cells (RBC) to move through capillaries may limit performance. Using ektacytometry, we determined whether RBC deformability (RCD) differed between elite road cyclists (n = 9) and sedentary controls (n = 5). Density profiles and standard hematological measurements were also performed. The deformability index (DI) was higher in the cyclists (0.723 ± 0.027) compared with that in controls (0.619 ± 0.040, P < 0.001). Cyclists also had a larger percentage of low-density RBCs (P < 0.001), and mean cell volume (MCV) was also higher (P = 0.013). These findings are indicative of a larger proportion of “young” RBCs in the blood of elite cyclists and provide further evidence that the turnover of RBCs in endurance athletes is higher than in the general population. With a younger more deformable RBC population and providing the destruction does not exceed replacement, performance potential should be enhanced. Furthermore, examination of factors that contribute to increased RBC turnover in athletes may help us understand the mechanisms that cause RBC aging.

THE ABILITY OF THE BLOOD to flow freely through the vascular bed is crucial for the maintenance of optimal cardiovascular health and, perhaps, athletic performance. Whereas the viscosity of whole blood governs flow in the macrocirculation, cellular properties control the passage through the microcirculation where blood cells must squeeze through capillaries that are smaller in diameter than the cells. Whole blood viscosity is determined by the combined influence of plasma constituents, hematocrit, and blood cell deformability and aggregation (23, 27). Red blood cell (RBC) deformability is influenced by internal fluidity, cell surface area-to-volume ratio, and the physical properties of the membrane and cytoskeleton (23, 27).

Although a large amount of research has focused on the physiological and biochemical contributors to athletic performance, little attention has been paid to variables that affect blood properties and flow. This is surprising considering that maximal aerobic power shows a strong positive association with hemoglobin mass in elite distance runners and rowers (8). As well as being affected by hereditary and acquired diseases, blood is a dynamic suspension that responds to changes in environmental conditions, such as altitude and heat, and stressors, such as physical activity and trauma (8, 17, 23). Endurance training causes chronic hemodilution by expanding plasma volume without significantly increasing RBC mass; this also reduces whole blood viscosity (23). In fact, we found that resting whole blood viscosity was inversely proportional to performance in a group of elite rowers (28). Plasma viscosity is also lower in active people (3), but RBC deformability (RCD) has not been examined by specific techniques in physically active people or elite athletes. Studies conducted by passing blood through micropore filters (filterability) have produced conflicting results (23).

Under normal conditions, RBCs have a limited life span of 120 days in the circulation of humans. Turnover is much higher in pathological conditions such as sickle cell anemia (4). The prevention of anemia requires the rate of replacement to keep pace with that of destruction. Although retention of its deformability appears to be the major determinant of RBC life span (4), the exact factors that regulate survival are not known. Intensive physical training does cause RBC destruction: much lower serum haptoglobin and ferritin concentrations and reticulocytosis are indicative of this (20). More direct evidence of accelerated RBC turnover during training is that autologous 51Cr-labeled RBCs survived for only 74 days in individuals running up to 130 km/wk compared with 114 days in untrained subjects (31). Given the strong evidence and theoretical rationale for a higher rate of RBC turnover in endurance athletes (21, 23, 31), we investigated whether RBC deformability was higher in a group of world-class endurance (road) cyclists compared with that in a group of untrained controls.

MATERIALS AND METHODS

Human subjects. Elite cyclists (n = 6 men, 3 women, and 5 untrained men) were recruited for the study. Their characteristics are shown in Table 1. The cyclists were members of the Australian national road cycling team, which was based at the Australian Institute of Sport. The study was carried out in the 7,000-ft altitude environment of Flagstaff, Arizona where the cyclists were preparing for the 1995 World Championship that were held at high altitude in Bogota, Colombia, 3 wk later. For the 2 wk before blood collection, the cyclists...
Table 1. Physical characteristics of subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cyclists</th>
<th>Controls</th>
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<tr>
<td>Age, yr</td>
<td>23 ± 5</td>
<td>31 ± 4</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>63.8 ± 7.13</td>
<td>75.6 ± 7.44</td>
</tr>
<tr>
<td>Height, cm</td>
<td>175 ± 7.70</td>
<td>180 ± 3.35</td>
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<tr>
<td>VO2max, ml·kg−1·min−1</td>
<td>70.1 ± 4.2</td>
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Data are means ± SD. VO2max, maximal O2 consumption.

trained about 25–29 h/wk covering 530–610 miles/wk. Support staff were working with the team served as controls. Some of the controls were former athletes of regional caliber; however, these subjects only exercised at low intensity for no more than 3 h/wk for the past year. The 2-wk acclimatization period would be more than enough time for any variable altered acutely by traveling and the increase in altitude to stabilize. The procedures used in this study were approved by the Ethics in Human Experimentation Committee of the Australian Institute of Sport.

Blood sampling. After 2 wk in Flagstaff, we collected blood by venipuncture from well-rested and 12-h fasted individuals. The blood was collected directly in vacutainers containing either acid-citrate dextrose (density, deformability) or EDTA (standard hematology). Acid-citrate dextrose-preserved samples were sent to Philadelphia, PA, in a refrigerated package by overnight express for RCD and density analyses, which were carried out the next morning. Previous studies showed that deformability index (DI) and RBC density did not vary over a 2-wk period (1). EDTA-anticoagulated blood was analyzed for standard hematological variables within 6 h of collection.

RBC deformability. The ektacytometer, a viscodiffractometer, designed and previously described by Mohandas et al. (2, 14), was used to determine RCD (in whole blood) under various experimental conditions. This device imposes a well-defined laminar shear stress field on erythrocytes while simultaneously monitoring the extent of cell deformation. The instrument consists of a concentric cylindrical viscometer in which one cylinder can be rotated to produce the desired level of shear stress. A laser beam is directed through the cell suspension that is contained within a 0.5-mm gap between the two cylinders. The cells, oriented by the shear stress field, then diffract the laser beam to produce a coherent diffraction pattern, from which cell deformation is determined, with the use of a photometric image-analysis system (2, 14). A “DI” is obtained that is equivalent to a measure of the ellipticity of a uniformly deforming cell population. The data output represents the mean DI of the whole RBC population; this is calculated from integrated measurements of individual RBC deformability (2, 9, 14). Irrespective of the technique used to measure RCD, it is generally assumed that the higher the DI value the more deformable and flexible the RBCs are, and the easier it is for them to pass through capillaries and deliver oxygen (9, 12, 17, 22). In the standard mode of operation, the DI is recorded continuously as a function of shear stress (9, 14). For measurement of intact cell deformability, 100 µl of 40% RBC suspension were thoroughly mixed with 3.0 ml of polyvinylpyrrolidone (PVP; mol wt 360,000, 4 g/dl wt/vol, 97.5°C), 290 mosmol/kg, pH 7.4. This suspension produced a maximum shear stress of 125 dyn/cm2 at the maximum applied shear rate of 100 rpm. Numerical values of the maximum deformability index reached, defined as DImax, were used to compare deformability of the different samples.

Separation of RBCs by density. Subpopulations of erythrocytes of uniformly defined densities were isolated on discontinuous Stractan II (Champion International, Tacoma, WA) density gradients by centrifugation of RBC obtained from sedentary controls and athletes (5). The gradients covered a density range from 1.065 to 1.120 g/ml in increments of 0.004 g/ml.

Measurement of the percentage of low-density cells. The proportion of low-density cells was measured by a simple two-step density gradient prepared from Stractan II by modification of the technique described by Clark et al. (6) for the determination of dense cells in sickle cell anemia. The gradients were prepared in 50-µl microhematocrit micropipettes by first introducing 20 µl of Stractan solution at a density of 1.120, then a small airspace, and then about 20 µl of Stractan at a density of 1.090 g/ml (the midpoint of the original 12 fractions). After another airspace was introduced, approximately 10–15 µl of well-mixed blood at room temperature were drawn into the tube. The entire gradient was then drawn to the dry end of the tube, which was then sealed with Critoseal. The tubes were then spun for 20 min in a standard microhematocrit centrifuge. Gradients were prepared in duplicate for each blood sample. At the end of the centrifugation, the tubes were removed and broken off to provide two fractions: cells that penetrated the 1.090 g/ml layer and cells that remained on top of this layer.

After the Stractan II was washed off, the collected fractions were dissolved in cytochrome c reagent (containing 0.1% Triton X-100 to ensure lysis) in a total volume of 2 ml. The percentage of cells in each fraction was calculated from the spectrophotometric measurement of hemoglobin concentration at 540 nm (6). As the sum of the fractions added up to 100%, the percentage of RBCs in each fraction could be determined.

Hematologic variables. RBC counts, hematocrit, mean cell volume (MCV), hemoglobin concentration, mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), and RBC distribution width (RCW) were determined using an automated hematolysis analyzer (Coulter Electronics, Hialeah, FL).

Statistical analysis. All data are presented as means ± SD. Training effects were analyzed by Student’s test for unpaired data. The significance level was set at P < 0.05. A correlation matrix using all dependent variables was also created to examine relationships between variables of interest.

RESULTS

RBC deformability Figure 1 shows that the deformability of isolated RBCs increased in a hyperbolic manner with rising shear stress. Once shear stress exceeded 25 dyn/cm², DI was clearly higher in the cyclist (Fig. 1). Analysis of the mean DImax showed that RBCs from all the cyclists were substantially more deformable than cells from the controls (Fig. 2; P < 0.0001). In fact, the DImax of all the cyclists (range: 0.700–0.785) was well above the values found in all the untrained controls (0.555–0.655). Elimination of the three female cyclists from the analysis did not alter this trend. There was a strong correlation between DImax and MCV (r = 0.55, P = 0.042) and between DI and hemoglobin concentration (r = −0.52, P = 0.042). No other significant correlations were found.

Standard hematology. Table 2 shows that significant differences were also found between the cyclists and controls for most hematological variables. Lower RBC
counts (P = 0.01) and hemoglobin concentrations (P = 0.01) were found in the cyclists. This is likely to be due to the lower hematocrit of the cyclists (P = 0.02). In contrast, MCV (P = 0.013) and MCH (P = 0.036) were higher in the cyclists. No significant differences were found between the groups with MCHC or RDW.

RBC density. Figure 3, A and B, shows a typical comparative profile of RBC density distribution between a cyclist and untrained control. The shift to the left (Fig 3B) illustrates the higher percentage of "younger" cells in the blood of the cyclist. Figure 4 shows that mean percentage of low-density (light) RBCs was much higher in all cyclists (P < 0.0001). Like DImax there was no overlap of the ranges between cyclists and controls (Fig. 4). Thus it is not surprising that a strong positive correlation between DImax and the percentage of low-density (young) cells (r = 0.81) was found.

DISCUSSION

Our results show that RBCs from elite cyclists are more deformable than cells from their untrained counterparts. Blood from the cyclists also contained a lower percentage of dense cells, and MCV was also higher; these are all indicative of a lower mean age of the RBC population (4) in these cyclists. Thus RBCs may move through the microcirculation more easily in athletes. This would increase the efficiency of oxygen delivery to working muscles during exercise and perhaps delay the onset of fatigue during vigorous exercise.

A variety of factors may contribute to the much higher DI in athletes. First, as shown by the much higher percentage of low-density RBCs, the mean age of the RBC population is lower in the cyclists. Numerous studies have shown that older (high density) RBCs are less deformable under shear stress than younger (low density) RBCs (4). Mairbaurl et al. (13) have also shown that mean RBC density is lower in moderately trained cyclists. Our group (24) and others (20) have shown that, compared with untrained controls, reticulocyte percentages are significantly higher in elite swimmers, runners, and basketball players. Cyclists were not studied, but we expect the values to be similar to those in swimmers because both sports have a high aerobic component, and they do not involve high mechanical impact. Deformability of RBCs is also influenced by the phospholipid composition of the membrane (11). RBC fluidity is significantly higher in RBCs isolated from endurance athletes, and RBC ghosts from active people have higher concentrations of polyunsaturated fatty acids (11). Dietary differences may influence this also. Most of the controls were former athletes of regional caliber. Because of the low RBC life span (120 days) and rapid loss of training adaptations in general once an athlete retires from competition (32), it is unlikely that former training history of the controls would affect these results. In fact, the differences would probably be greater if the control group had no athletic background and was totally sedentary.

In contrast to the benefits of regular endurance training (8, 23), single episodes of intensive exercise cause transient detrimental changes in blood rheology (16). Marathon running, for example, causes a transformation of a proportion of normal discocytes into stomatocytes that manifests as impaired filterability (18). In contrast, after a 100-km race, a significant increase in RBC filterability was found in RBCs isolated from runners; this suggests that a significant proportion of the oldest cells may have hemolyzed in response to

Table 2. Standard hematological variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cyclists (n = 9)</th>
<th>Controls (n = 5)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC count, ( \times 10^{12}/\text{ml} )</td>
<td>4.52 ± 0.45</td>
<td>5.24 ± 0.381</td>
<td>0.01</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>40.1 ± 2.62</td>
<td>43.8 ± 2.44</td>
<td>0.02</td>
</tr>
<tr>
<td>MCV, fl</td>
<td>86.0 ± 3.74</td>
<td>83.7 ± 1.95</td>
<td>0.01</td>
</tr>
<tr>
<td>Hemoglobin, g/dl</td>
<td>148.9 ± 9.6</td>
<td>162.6 ± 6.58</td>
<td>0.01</td>
</tr>
<tr>
<td>MCHC, g/dl</td>
<td>36.8 ± 0.62</td>
<td>37.1 ± 0.73</td>
<td>0.42</td>
</tr>
<tr>
<td>MCH, pg</td>
<td>32.8 ± 1.37</td>
<td>31.1 ± 0.95</td>
<td>0.04</td>
</tr>
<tr>
<td>RDW</td>
<td>12.6 ± 0.57</td>
<td>12.9 ± 0.44</td>
<td>0.45</td>
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</tbody>
</table>

Data are means ± SD. RBC, red blood cells; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; RDW, RBC distribution width.
repeated mechanical impact during the longer race (19). Lactate uptake by RBCs during exercise may also contribute to hemolysis because of the associated increase in MCV (26). Whereas the mechanical trauma associated with footstrike has been found to be the major cause of hemolysis during running (29), low-impact activities such as swimming and cycling also induce significant levels of hemolysis (20, 21). The high daily volumes of training (>5 h) undertaken by world-class athletes may increase RBC turnover dramatically. The associated high oxygen flux, which can increase 10- to 20-fold above basal during vigorous exercise (32), may cause concomitant rises in oxidative damage to susceptible RBCs. Under normal circumstances, autooxidation of hemoglobin to methemoglobin occurs at the rate of 3% per day, and this is accompanied by the release of superoxide and oxidative damage to the RBC (7, 10, 23). Although 1 h of cycling at moderate intensity increases RBC vulnerability to oxidative stress (25), probably because of glutathione depletion (7), we also found that RBCs from elite cyclists were more resistant to oxidative hemolysis in vitro compared with RBCs from untrained controls at rest (25). The small decrease in RCD induced by moderate exercise has recently been attributed to oxidative stress (16).

During exercise the cardiovascular system must be able to meet the demands of working muscles primarily. Cardiac output at rest is around 5 l/min with 15–20% of this directed to skeletal muscle (32). During vigorous exercise, however, cardiac output can increase 10-fold with 80% of this perfusing working muscles (32). The hypothesis that decreased RCD may contribute to exercise-induced fatigue is supported by studies done in vitro on hardened RBCs and cells from diseased patients that show impaired RCD impedes blood flow and oxygen utilization (12, 17, 22). Blood cell disorders such as sickle cell anemia and thalassemias cause substantial reductions in RCD that can, in turn, lead to vessel blockage and hypoxia inter alia (10). In sepsis, decreased RCD correlates closely with a lower arteriovenous oxygen difference and higher mixed venous oxygen saturation (17). In the microvasculature of rat striated muscle, large decreases in RCD increase RBC transit time by two- to threefold (12). Studies in vitro have shown that hardened RBCs enter capillaries of greater diameter and lower resistance (5). Thus, although capillaries of lower resistance may compensate for small reductions in RCD during exercise, fatigue may occur once these capillaries are overwhelmed.

Blood viscosity and RBC deformability may be optimal for the hematological demands of endurance competition in athletes with a higher percentage of young mature RBCs. Furthermore, these adaptations may provide a greater “window of protection” because RBCs will be able to cope better with detrimental changes induced by acute episodes of intense exercise. Even if
there are no physiological differences in oxygen transport and uptake at rest between athletes and controls, the differences in RCD detected may be physiologically significant (i.e., limit oxygen delivery and block capillaries) once exercise intensity reaches a critical threshold. We (26) have shown that lactate accumulation causes RBC swelling for example, and this may impede blood flow and oxygen uptake during intense exercise and thus lead to fatigue. These hypotheses could be investigated by undertaking longitudinal studies of athletes during different stages of training or untrained individuals who take up exercise programs.

As well as enhancing athletic performance, the combination of expanded plasma volume and higher RCD in physically active people may reduce the risk of acquired circulatory diseases by enhancing oxygen delivery and reducing the load on the cardiovascular system. There is a considerable body of evidence that suggests that regular exercise confers a “rheological advantage” on blood flow in active individuals (15, 23). Because whole blood and plasma viscosity are significantly higher in cardiac patients (30), these adaptations may explain, in part, why regular exercise lowers the risk of cardiovascular disease. Thus hematological techniques should be used to monitor responses to physical training and, perhaps, other lifestyle factors that may affect the blood and not just be confined to the pathological cases.

In conclusion, RCD is higher in world-class road cyclists. Because this is associated with a lower proportion of dense RBCs, these results support the view that, providing the individual does not become anemic, an increased rate of RBC turnover may be beneficial for the endurance athlete. Previous work suggests that old rigid RBCs may not be able to negotiate the microcirculation and enter capillaries. These cells may become trapped and cause local hypoxia. This is the case in conditions such as sickle cell disease (10), and it may occur during vigorous exercise. Because cyclists have more deformable RBCs and an apparent higher rate of RBC turnover than healthy controls, athletes may provide a unique physiological model (and provide a different or complementary perspective to pathological models) for understanding the mechanisms involved in regulating RBC properties and turnover.

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