Footstrike is the major cause of hemolysis during running

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Footstrike is the major cause of hemolysis during running, according to Telford et al. (2003). This study aimed to test the hypothesis that footstrike is the major cause of hemolysis during exercise. The researchers found that footstrike was significantly associated with increased plasma free hemoglobin and reduced haptoglobin concentrations, indicating exercise-induced hemolysis. The authors concluded that footstrike is the major contributor to hemolysis during running.

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EXERCISE-INDUCED HEMOLYSIS has been reported for more than 50 years (11). In particular, distance running has been associated with significant destruction of red blood cells (RBC) with RBC turnover being substantially higher in runners compared with untrained controls (29). Several groups have suggested that mechanical damage to RBC occurs as they pass through the capillaries of the foot during the footstrike phase (5, 8, 10, 20, 29). However, studies on athletes involved in sports in which foot impact does not occur have also found evidence of exercise-induced hemolysis. These activities include swimming (25), weight lifting (2, 24), and rowing (9). Given that factors other than footstrike cause hemolysis during exercise, these variables as causes of hemolysis during running cannot be eliminated. Thus the contribution of footstrike per se to running-induced hemolysis is unclear.

Apart from footstrike, several other mechanisms may contribute to hemolysis during exercise. Because of their continuous exposure to high-oxygen flux, RBC are extremely vulnerable to oxidative damage (3, 26). Under normal conditions, the superoxide radical is generated from the autooxidation of oxyhemoglobin to methemoglobin in RBC at a rate of 3%/day (17). Because superoxide generation appears to be proportional to oxygen flux (26), oxidative stress may in turn increase in proportion to the oxygen uptake (VO2) associated with exercise. Oxidative stress has been implicated in the normal “aging” of RBC (3), and there are numerous reports of increases in “footprints” of oxidative damage and antioxidant depletion in RBC after exercise (26). Perturbation of osmotic homeostasis is another potential contributor to exercise-induced hemolysis. Because exercise-induced changes in RBC density (16) and mean cell volume (13) have been reported, these osmotic changes may render the RBC more susceptible to membrane damage and hemolysis, especially during their passage through the microcirculation where cells undergo a constant swelling and shrinking cycle. Finally, it is also possible that compression of large muscle groups on capillaries may accelerate hemolysis of older RBC (18).

The aim of this study was to test the hypothesis that footstrike is the major cause of hemolysis during run-
ning. To measure the effect of footstrike, it was necessary to control other potential hemolytic factors, namely oxidative and other stresses imposed in transit through the circulation. To do this, we have compared the hemolytic responses in athletes as they ran and cycled at equivalent metabolic loads, with the main difference and aspect of interest being in the uniqueness of footstrike to the running medium.

METHODS

Subjects. Ten male triathletes volunteered for the study. The physical, hematological, and training characteristics of the subjects are presented in Table 1. RBC concentration, hematocrit, and hemoglobin concentration were measured before the exercise tests, and all individuals’ values fell within the normal reference ranges. The study was approved by the Ethics Committee of the Australian Institute of Sport, and subjects gave written, informed consent.

Exercise trials. Each subject attended the laboratory on three separate occasions. First, a graded running test to exhaustion on the treadmill (AusTreadEx, Preston, Victoria, Australia) was used to determine peak VO₂ (VO₂peak). An initial speed of 9 km/h at 0% gradient was increased 2 km/h every minute to 15 km/h followed by a 1% increase in gradient every minute until the individual reached volitional exhaustion. A Hans Rudolph 2700 valve (Hans Rudolph, Kansas City, MO) was used, and volume of inspired air was measured by a Morgan Ventilometer (PK Morgan, Gillingham, Kent, UK). Samples of inspired air were drawn continuously through a desiccant (calcium chloride) and into Applied Electrochemistry oxygen and carbon dioxide analyzers (AEI Technologies, Pittsburgh, PA). Pre- and posttest calibration of the ventilometer and gas analyzers occurred by using a Tissot spirometer (Warren E. Collins, Braintree, MA) and gravimetric standard gases (BOC Gases, Wetherill Park, NSW, Australia). Heart rates were recorded via ECG transmitters to a Quinton Instruments (Bothell, WA) ECG data computer. VO₂peak was designated as the highest rate of oxygen consumption recorded in a 30-s period, and every subject exceeded a respiratory exchange ratio of 1.10. The oxygen consumption recorded in a 30-s period, and every minute to 15 km/h followed by a 1% increase in gradient every minute until the individual reached volitional exhaustion. A Hans Rudolph 2700 valve (Hans Rudolph, Kansas City, MO) was used, and volume of inspired air was measured by a Morgan Ventilometer (PK Morgan, Gillingham, Kent, UK). Samples of inspired air were drawn continuously through a desiccant (calcium chloride) and into Applied Electrochemistry oxygen and carbon dioxide analyzers (AEI Technologies, Pittsburgh, PA). Pre- and posttest calibration of the ventilometer and gas analyzers occurred by using a Tissot spirometer (Warren E. Collins, Braintree, MA) and gravimetric standard gases (BOC Gases, Wetherill Park, NSW, Australia). Heart rates were recorded via ECG transmitters to a Quinton Instruments (Bothell, WA) ECG data computer. VO₂peak was designated as the highest rate of oxygen consumption recorded in a 30-s period, and every subject exceeded a respiratory exchange ratio of 1.10. The cycling ergometry was performed by using a Repco air-braked ergometer (Repco Cycle, Mulgrave, Victoria, Australia) modified for racing cycling body positioning and pedals. The standardized running and cycling trials began ~1 wk later. Each subject completed two 1-h exercise bouts (running and cycling) at 75% of their VO₂peak; one-half of the group was randomly selected to perform the cycle test first, thus preventing any bias due to order. The alternative exercise bout was performed 1 wk later. Whereas individual athletes varied in their weekly training programs, each was careful not to vary their own training in the weeks leading up to their cycling and running trials. A further condition was that no training was carried out 24 h before testing. Athletes were also instructed to consume the same preexercise diet on the day of both of their trials. All subjects wore medium-weight training shoes of a similar type, with all shoes being in good condition. The warm-up was consistent and consisted of 10 min of jogging at ~14 km/h or cycling at ~150 W followed by four efforts of 30 s at the speed or power of their 1-h following effort. The speed of the treadmill required to elicit 75% VO₂peak was estimated from the graded treadmill test, and the cycle ergometer power required was estimated from tables of cycling oxygen cost (1). Our aim was to elicit very similar oxygen consumption in the 1-h period for each mode of exercise on an individual basis to closely approximate potential oxidative and circulatory stress to the RBC. VO₂peak was measured after 5 min of exercise for a duration of 5 min, after which adjustments were made if necessary to the speed or power. The VO₂peak was then checked again in the following 5 min and adjusted again if necessary to produce the designated 75% VO₂peak. Measurement of oxygen consumption was repeated at the 20- and 40-min marks of exercise. Trials with several subjects had indicated that this method successfully elicited the required VO₂peak values using each mode of exercise. In addition, heart rates were measured via telemetry (Sports Tester PE 4000, Polarelectro). To verify that the hemolysis found after running and cycling was caused by exercise and not due to temporal variation or damage during blood collection, methemoglobin, free hemoglobin, and hemoglobin were also analyzed in blood samples collected from a nonexercising group over the same time course as the exercising group. As we were interested in controlling any potential temporal effect and the handling of blood samples, we did not need to involve the triathletes in further blood sampling, thus eliminating any potential bias due to order. The alternative exercise protocol was used to control other potential hemolytic factors, namely oxidative and other stresses imposed in transit through the circulation. To do this, we have compared the hemolytic responses in athletes as they ran and cycled at equivalent metabolic loads, with the main difference and aspect of interest being in the uniqueness of footstrike to the running medium.

Table 1. Characteristics of the 10 male triathletes

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>27.8</td>
<td>6.6</td>
</tr>
<tr>
<td>Height, cm</td>
<td>181.5</td>
<td>4.2</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>74.7</td>
<td>7.4</td>
</tr>
<tr>
<td>VO₂peak, ml·kg⁻¹·min⁻¹</td>
<td>64.5</td>
<td>5.2</td>
</tr>
<tr>
<td>RBC, 10⁹/l</td>
<td>5.11</td>
<td>0.25</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>45.3</td>
<td>1.9</td>
</tr>
<tr>
<td>Hemoglobin concentration, g/dl</td>
<td>15.3</td>
<td>0.9</td>
</tr>
<tr>
<td>Plasma ferritin, µg/l</td>
<td>71.8</td>
<td>13.9</td>
</tr>
<tr>
<td>Training volume, km/wk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Run</td>
<td>64.8</td>
<td>12.6</td>
</tr>
<tr>
<td>Cycle</td>
<td>180</td>
<td>106</td>
</tr>
<tr>
<td>Swim</td>
<td>7.2</td>
<td>2.9</td>
</tr>
</tbody>
</table>

The blood measurements shown were taken at rest, just before the treadmill test to measure the peak oxygen uptake (VO₂peak) on the treadmill. RBC, red blood cell count.
Plasma free hemoglobin was assessed spectrophotometrically by the cyanomethemoglobin method (21). Serum haptoglobin concentrations were determined in the clinical pathology laboratory of the Canberra Hospital by using rate nephelometry (Array 360, Beckman Coulter, Miami, FL). RBC count, hematocrit, and hemoglobin concentration were measured by using a semiautomated 5550 Coulter Counter (Beckman Coulter). Blood lactate concentration was determined by using the YSI model 1 lactate analyzer (Yellow Springs Instrument). Methemoglobin was measured by using a 270 Corning CO-oximeter (Ciba Corning Diagnostics, Medfield, MA) into which 125 μl of blood from a heparinized syringe were injected within 2 min of sampling. In this machine, dilution, calibration, and calculation of results, using five measurements per sample, are performed automatically under microprocessor control. This instrument detects the optical absorbance of each of the hemoglobin fractions (oxyhemoglobin, deoxyhemoglobin, carboxyhemoglobin, and methemoglobin) from which it calculates the values of interest to our study (total hemoglobin and methemoglobin as a fraction of total hemoglobin). Plasma ferritin was measured as previously described (27).

Statistical analysis. The statistical analysis involves a comparison between profiles of repeated measurements (15) obtained in the running and cycling trials. The overall observed significance level is determined by undertaking an appropriate repeated-measures analysis of variance on the pooled data. Mean profiles are shown graphically, and variability between means is represented by least significant difference (LSD). (LSD is 2 × standard error, and any pair of means differing by more than the LSD are significantly different at P = 0.05). The statistical package Genstat (2000: 5th edition; VSN International, Oxford, UK) was employed for statistical computation.

RESULTS

No significant differences were found in \( \dot{V}O_2 \) at any of the equivalent sampling points, which, over all of the measurements, averaged 48.5 ± 3.1 (SE) ml·kg⁻¹·min⁻¹ for running and 47.6 ± 2.5 ml·kg⁻¹·min⁻¹ for cycling. Similarly, no significant differences were found in post-exercise blood lactate concentration, which averaged 3.4 ± 0.3 mM for the running and 3.4 ± 0.2 mM for cycling exercise trials, indicating that potential oxidative and circulatory stress on the RBC was similar for each mode of exercise. In other words, these potential hemolytic effects on the RBC were well controlled. Heart rates averaged 164 ± 3.2 and 161 ± 2.6 beats/min for the running and cycling trials, respectively, with negligible drift and no significant difference between the profiles (P = 0.81). There was no significant difference between the running and cycling trials in methemoglobin formation, although there was a twofold increase (P < 0.05) in methemoglobin accumulation in both modes of exercise, which was maximal immediately after the 1-h effort (Fig. 1). These values fell progressively back to preexercise levels 24 h after both exercise tests. No significant differences (P > 0.05) occurred in any of the hematological variables (hematocrit, RBC concentration, and hemoglobin concentration) before and after each of the exercise trials. However, we did note small, but nonsignificant, increases in the means of hematocrit, RBC concentration, and hemoglobin concentration immediately after both forms of exercise followed by small, nonsignificant reductions in these variables at the 1-h sampling point (data not shown). Most relevant to this investigation was that the statistical comparison of profiles of the means of each hematological variable for cycling and running was not significantly different (P values of 0.29, 0.48, and 0.37, respectively). Consequently, we were able to assume that, even if a small change in plasma volume had occurred, the similarity of such occurrence for each mode of exercise would indicate that any such change would have no impact on the interpretation of our comparative results.

Although plasma free hemoglobin concentration (an indication of hemolysis) increased significantly (P < 0.05) after both running and cycling, the mean increase was fourfold greater (P < 0.05) after running (Fig. 2). At 1 h postexercise, mean free hemoglobin was not significantly different from the preexercise mean for the cycling and running trials.

The greater increase in plasma free hemoglobin concentration immediately after running was subsequently reflected by a greater decrease in haptoglobin concentration. Haptoglobin concentration reached its lowest point 1 h after running (Fig. 3), and analysis of the integrated profiles indicated a significant difference between running and cycling (P = 0.014). The average change in haptoglobin concentration between preexercise and 1-h postexercise samples was 0.016 g/l for cycling and 0.085 g/l for running.
rum haptoglobin concentrations were found after the free hemoglobin concentrations and decreases in samples taken from the nonexercising group (nonsignificant differences (P > 0.05) revealed no significant differences at P = 0.05).

The investigation of temporal variation in the blood samples taken from the nonexercising group (n = 5) revealed no significant differences (P > 0.05) in any hemolytic variable in the 2-h measurement period (Table 2), indicating that the previously demonstrated significant changes in this time period were exercise related.

DISCUSSION

Compared with cycling, greater increases in plasma free hemoglobin concentrations and decreases in serum haptoglobin concentrations were found after the running trial. Because foot impact is the major difference of relevance between running and cycling, these results provide solid evidence that mechanical trauma to RBC during footstrike is the factor responsible for the greater hemolysis found after running. Because there were no significant differences between the running and cycling trials in the means of measures of \( \dot{V}O_2 \), heart rate, and lactate accumulation, it is highly unlikely that variance in general circulatory stresses to the RBC can explain the differences. Of interest is a previous finding that shoe type may be an influencing factor in RBC damage because reticulocyte levels were 29% higher in runners training with hard-soled shoes after 18 days of running that covered 429 km, compared with a matched control group that trained with soft-soled shoes (7). The present study, in confirming that footstrike is in fact the major cause of the hemolysis, is consistent with finding of increased erythropoiesis after the endurance running with less cushioning in the shoes.

Many reports have appeared describing sports anemia in athletes (13, 19, 28), with most cases apparently due to an expanded plasma volume and hemodilution (pseudoanemia) rather than true RBC deficiency. However, it has been reported that rein fused autologous \(^{51}\)Cr-labeled RBC survive for an average of only 74 days in runners compared with 114 days in sedentary controls (29), indicating that running reduces RBC life span, which is consistent with a higher rate of RBC destruction and increased reticulocytosis (23). This study confirms that the stress of footstrike has a significant influence on the life span of runners’ RBC and might be suspected to increase the susceptibility of those involved in running sports to anemia should responsive erythropoietic mechanisms be inadequate. However, Green et al. (12) have reported that total body RBC volumes do not differ between trained runners and cyclists. This suggests, in general, that, despite a greater rate of RBC destruction in runners, replacement keeps pace and thus prevents anemia.

Although most iron is salvaged from senescent or damaged RBC and reutilized under normal conditions (6), these conservation mechanisms may fail when subjected to the stress of intensive training. For example, should blood haptoglobin stores be overwhelmed or incompletely replenished during the recovery period, excess hemoglobin will be excreted or broken down. Although a single hemolytic episode is unlikely to cause iron loss of clinical significance (20), daily or

### Table 2. The variation of blood measurements in samples taken 1 h apart in nonexercised subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate, mM</td>
<td>0.88 ± 0.03</td>
<td>0.90 ± 0.02</td>
<td>0.86 ± 0.02</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>Methemoglobin, %</td>
<td>0.34 ± 0.01</td>
<td>0.35 ± 0.05</td>
<td>0.37 ± 0.03</td>
<td>0.038 ± 0.02</td>
</tr>
<tr>
<td>Plasma hemoglobin, mg/l</td>
<td>13.6 ± 3.7</td>
<td>14.4 ± 2.4</td>
<td>15.9 ± 4.5</td>
<td>3.2 ± 1.9</td>
</tr>
<tr>
<td>Haptoglobin, g/l</td>
<td>0.68 ± 0.2</td>
<td>0.70 ± 0.2</td>
<td>0.70 ± 0.2</td>
<td>0.1 ± 0.01</td>
</tr>
</tbody>
</table>

Values are means ± SD. There were no significant differences (P > 0.05) in the means of any one variable at any of the sample points.
twice-daily hemolytic episodes during hard training may have a cumulative effect that might be significant; athletes such as marathoners with high training volumes would be particularly at high risk. Several studies have indicated that distance runners have compromised iron stores (4, 8, 14, 22). In some cases, iron supplementation is required to prevent or correct anemia. The suggestion that the mechanical trauma of footstrike may be associated with iron deficiency in runners is consistent with evidence that athletes who participate in other foot-impact sports such as basketball and tennis have lower iron stores than cyclists and rowers (27), even after correction for body size and gender.

This present study indicates that hemolysis occurred for reasons other than footstrike, with a small but significant increase in plasma free hemoglobin concentration evident after the cycling trial. This increase was considerably less than that related to running. In fact, there was not a sufficient release of hemoglobin during the cycling to significantly reduce serum haptoglobin concentration at the time points measured. Another possible cause of hemolysis that we have suggested previously to be common to running and cycling is oxidative stress. The small increase in methemoglobin that we observed after running was also found to an equivalent degree after cycling, indicating that the hour of exercise did to some extent increase oxidation of hemoglobin. Taking this into account, together with the finding of only a minor amount of hemolysis resulting from cycling compared with running, we can deduce that oxidative influences alone have little effect, if any, on the hemolysis associated with running.

In conclusion, in controlling for the potential influences of oxidative and circulatory challenges to the RBC, we have demonstrated that the mechanical trauma associated with footstrike is the major cause of hemolysis during running.

REFERENCES