Haemolytic effects of exercise

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SUMMARY

1. Exercise-induced haemolysis has been implicated in the sub-optimal iron status of endurance-trained athletes. Accordingly, erythrocyte survival studies using 51Cr were performed on male and female distance runners (n = 20) and sedentary control subjects (n = 10) in order to determine whether the rate of erythrocyte destruction was altered as a consequence of repetitive exercise training.

2. The chromium half-disappearance time of the male (25.4 ± 3.6 days, mean ± sd) but not the female (28.3 ± 4.6 days) athletes was significantly lower than that of the male (33.1 ± 4.5 days) and female (32.3 ± 2.6 days) control subjects (P < 0.01). The mean erythrocyte lifespan of the male and female distance runners (67.2 ± 22.2 and 72.4 ± 26.0 days, respectively) was significantly shorter than that of the non-exercising male and female subjects (113.4 ± 31.0 and 114.1 ± 29.0 days, respectively) (P < 0.01).

3. There was no correlation between the mean erythrocyte lifespan and the haemoglobin concentration, serum ferritin levels, body mass, weekly training distance, number of years running or daily protein intake. The mean cell volume and reticulocyte count measured in the same athletes before and after completing a standard 42 km marathon race were within the normal range, whereas the plasma haemoglobin levels were elevated (77.0 ± 50.5 mg/l) and the serum haptoglobin levels were decreased (0.89 ± 0.4 g/l) at rest, with a further significant decrease after running (0.69 ± 0.4 g/l) in the latter measurement (P < 0.05).

4. It is concluded that the demonstrated increase in erythrocyte turnover may be sufficient to precipitate an iron deficiency in endurance athletes when dietary intake or absorption does not meet the accelerated erythropoietic demands.

Key words: distance runners, erythrocyte destruction, exercise, haemolysis, iron status, plasma haemoglobin, serum haptoglobin.

Abbreviations: MRCL, mean erythrocyte lifespan; T50Cr, chromium half-disappearance time.

INTRODUCTION

Microscopic haematuria and haemoglobinuria after walking and running ('march haemoglobinuria') was first described over 100 years ago (see refs in [1, 2]) and ascribed to increased erythrocyte destruction due primarily to mechanical trauma [3] compounded by an erythrocyte membrane-protein abnormality [4].

Significantly decreased serum haptoglobin levels have been described in distance runners [5], triathletes [6] and even swimmers [7] after endurance events. Persistently decreased serum haptoglobin levels, suggesting a chronic haemolytic state, has also been documented in recreational [8] and competitive middle- and long-distance [1, 9–11] athletes.

Several studies provide evidence to suggest that exercise results in a negative iron balance [3, 12, 13], and possibly the accelerated erythrocyte breakdown and haemoglobinuria in runners who train regularly could account for this [1, 8–11]. Indeed, Godal & Refsum [14] and Banga et al. [4] identified athletes with hereditary spherocytosis, who became anaemic as a consequence of persistent haemoglobinuria whenever they commenced rigorous training. However, as the iron liberated during erythrocyte turnover is generally efficiently re-utilized, a shortened lifespan of the erythrocyte should not directly contribute to iron deficiency. Nevertheless, there is frequently an increased amount of haemosiderin lost in urine in haemolytic states [15]. It is thus plausible that even a mildly increased rate of erythrocyte catabolism could create and sustain a negative iron balance [10], especially in subjects with low ferrous stores and impaired dietary absorption.

Nevertheless, some studies have shown the increased rate of erythrocyte destruction to be only slight, if at all
[16, 17], possibly due to the advent of modern running shoes designed specifically for shock absorption [18]. There is also evidence of iron deficiency, anaemia and intravascular haemolysis in athletes in non-impact sports [7], which suggests that mechanisms other than footstrike are responsible for this phenomenon [19-21].

Reliable ferrokinetic studies have never been carried out on athletes, so that the evidence for a haemolytic effect is largely circumstantial. In this study, erythrocyte survival rates were determined in male and female distance runners by using radiolabelling techniques. We also attempted to document evidence of a haemolytic episode consequent upon sustained strenuous exercise by measuring those haematological parameters associated with erythrocyte destruction before and after marathon running.

METHODS AND MATERIALS

Erythrocyte survival studies

Subjects. Erythrocyte survival data were obtained on 10 males and 10 female athletes, who had all been training at 50–120 km/week for more than 2 years. The control group comprised five males and five females, who did not perform any regular exercise. No subject smoked, and none was on prescription medication.

The calculated effective dose equivalent for 125I-labelled human albumin and 51Cr-labelled erythrocytes was 5.5 mrem (0.055 mSv) and 23.4 mrem (0.234 mSv), respectively [22], which in total amounted to 10% of the annual maximal permissible radiation dose for the public [23, 24]. The study was approved by the University of Cape Town Medical School Ethics and Research Committee, and the radiation dose administered to the subjects conformed to the recommendations of the South African Atomic Energy Board.

Radiolabelling of erythrocytes and sample collection. The procedure followed was a modification of that described by the International Committee for Standardization in Haematology [25]. The subjects were instructed not to perform any vigorous exercise in the 24 h preceding the study. On arrival at the laboratory they were seated in the upright position for 15 min before venipuncture. Thereafter 16 ml of blood was withdrawn without stasis into a syringe containing 4 ml of anti-coagulant (acid citrate dextrose; NIH Formuiae A), and was transferred into a 20 ml sterile glass vial with a serum cap. After centrifugation at 1000 g and separation from the plasma fraction, the erythrocytes were labelled with 30 μCi (1.1 MBq) of sodium 51Crchromate solution (B.P.; Code CJS.IP; Amersham International PLC, Amersham, Bucks, U.K.). In addition to the 10 and 20 min post-reinfusion samples (day 0), an additional 5 ml blood sample was taken without stasis into a heparinized vacu-tainer the next day (day 1) and thereafter once a week for 3 weeks (days 7, 14 and 21). Portions of whole blood were placed in counting tubes containing saponin and stored frozen (−20°C) until the last sample had been collected (day 21), whereafter all samples (days 0, 1, 7, 14 and 21) were counted in a Minaxi Auto-Gamma 5000 series γ-counter (Packard Instrument Co., Downers Grove, Ill., U.S.A.).

Erythrocyte survival calculations. These were performed according to the equations described by Dornhorst [26] and the International Committee for Standardization in Haematology [25].

The percentage erythrocyte survival for all samples taken after day 0 was calculated as:

\[
\text{MRCL}_{\text{day} t} = \frac{(\text{c.p.m./ml of blood for day } t - \text{BG})}{(\text{c.p.m./ml of blood for day } 0 - \text{BG})} \times 100
\]

where BG is background count.

(i) Chromium half-disappearance time \((T_{50Cr})\). The goodness of fit of the exponential survival curve as measured by the residual standard error was better than the goodness of fit for the linear survival plot in all cases. Therefore the reported \(T_{50Cr}\) values are all derived from the exponential plot fitted by the method of least squares.

The \(T_{50Cr}\) was thus calculated as:

\[
T_{50Cr} = \frac{\ln 2}{k}
\]

where \(k\) is the slope of the line.

(ii) Mean erythrocyte lifespan (MRCL). After correcting for elution [25], the percentage survival was re-calculated. Once again the goodness of fit of the exponential survival curve, as measured by the residual standard error, was better than that of the linear survival plot, so that the MRCL values were all derived from the exponential plot fitted by the method of least squares. The MRCL was calculated by multiplying the half-life of the fitted line by 1.4427 [25].

Effects of prolonged running on erythrocyte destruction

Subjects. Twenty male distance runners competing in a standard 42 km running race (marathon) were selected to participate in this investigation. All had been training between 50 and 120 km/week and competing in races of distances from 10 to 100 km for at least 2 years.

Haematological measurements. Blood and mid-stream urine samples were collected 24 h before the marathon race, within 10 min of completing the event, and thereafter at 24 and 48 h and 6 days after the race. Portions of serum and urine samples were stored frozen for later determination of serum haptoglobin levels using a commercial kit on a Behring nephelometer (Behring, Marburg, F.R.G.), and urinary haemosiderin was determined by using Perl's Prussian Blue stain [27]. The mean cell volume was measured on the Coulter Counter Model S Plus (Coulter Electronics, Hialeah, FL, U.S.A.) [28]. The reticulocyte count was performed on EDTA-treated blood using New Methylene Blue stain [27], while the
plasma haemoglobin concentration was determined within 2 h of blood sampling by the method of Crosby & Furth [29].

Statistical analysis

A robust test for the equality of variance was performed on each parameter before applying a one-way analysis of variance and the Bonferroni test to determine the degree and position of any statistical differences between the groups [30]. Linear regression was used to establish the correlation coefficients for various parameters. The level of significance was taken as \( P < 0.05 \).

RESULTS

Erythrocyte survival studies

There was no difference between \( T_{50}\text{Cr} \) or the MRCL values of the males and females in either group. The \( T_{50}\text{Cr} \) of the male athletes \( (P < 0.01) \), but not of the female athletes, was significantly lower than that of their sedentary counterparts, but both sexes had a shorter MRCL \( (P < 0.01) \) than their respective control subjects (Table 1, Fig. 1). There was a significant correlation between the \( T_{50}\text{Cr} \) and MRCL \( (r = 0.8623, P < 0.05) \), but not between the MRCL and the plasma haemoglobin level, body mass, weekly mileage or protein intake in the distance runners. The MRCL, but not the \( T_{50}\text{Cr} \), was markedly lower than the normal range (Table 1).

Effect of prolonged running on erythrocyte survival

The demographic and haematological data are presented in Table 2. The baseline serum haptoglobin levels were below the normal range for this laboratory \( (1-3 \text{ g/l}) \), and were further significantly decreased immediately after the race (Fig. 2). After 24 h the mean serum haptoglobin levels were elevated over pre-race levels, but were still at the lower end of the normal range. The plasma haemoglobin levels were persistently and markedly above normal, but did not increase after the race (Fig. 3). The mean cell volume and reticulocyte count were entirely normal at all times and were not changed after exercise. Negligible traces of haemosiderin were demonstrated in the urine specimens of two athletes after the race.

DISCUSSION

Although the results of \( ^{51}\text{Cr} \)-labelling studies have in the past usually been expressed as the \( T_{50}\text{Cr} \), it is now generally agreed that the MRCL value is of greater clinical value in assessing erythrocyte survival [31]. Therefore erythrocyte survival rates will be discussed here in terms of the MRCL.

The primary and novel observation of this study is that the \( T_{50}\text{Cr} \) and the mean MRCL of well-trained distance runners is significantly shortened compared with that of sedentary persons of similar sex, age and physique (Table 1, Fig. 1). Erythrocyte survival \( per se \) has not previously been measured in athletes using appropriate radioisotopes. Although Steenkamp et al. [17] report that marathon running does not alter the rate of erythrocyte destruction in trained women, they do not report the data in terms of standard \( T_{50}\text{Cr} \) or MRCL values. In this study, the MRCL of the male and female athletes was on average 42% shorter than that of the male and female control subjects, which is comparable with the findings of Ashida [32], who reported the erythrocyte lifespan to be 40% shorter in exercising rats.

That variations in the rate of elution in different individuals and for different labelling techniques [31, 33] may seriously affect the estimation of mean erythrocyte survival, and the possibility that this process may be altered in athletes due to plasma volume shifts and haemoconcentration effects is recognized. However, as no athlete significantly altered their training regimen during the study period and serial determinations of plasma haemoglobin levels and packed cell volume over a 3 week period before and after the erythrocyte survival studies indicated that the athletes and control subjects were indeed in a steady state, we consider the established

### Table 1. Demographic characteristics, \( T_{50}\text{Cr} \) and MRCL of trained athletes and control subjects

<table>
<thead>
<tr>
<th></th>
<th>Male athletes ((n = 10))</th>
<th>Female athletes ((n = 10))</th>
<th>Male control subjects ((n = 5))</th>
<th>Female control subjects ((n = 5))</th>
</tr>
</thead>
<tbody>
<tr>
<td>( T_{50}\text{Cr} ),(days)</td>
<td>25.4 ± 3.6†</td>
<td>28.3 ± 4.6</td>
<td>33.1 ± 4.5†</td>
<td>32.3 ± 2.6</td>
</tr>
<tr>
<td>MRCL ,(days)</td>
<td>67.2 ± 22.2‡</td>
<td>72.4 ± 26.0*</td>
<td>113.4 ± 31.0†</td>
<td>114.1 ± 29.0*</td>
</tr>
<tr>
<td>Haemoglobin concn. ,(g/l)</td>
<td>143.1 ± 19.5</td>
<td>130.3 ± 17.8</td>
<td>147.0 ± 4.7</td>
<td>130.3 ± 5.6</td>
</tr>
<tr>
<td>Serum ferritin concn. ,(µg/l)</td>
<td>52.25 ± 37.9</td>
<td>29.6 ± 29.6</td>
<td>113.0 ± 105.3</td>
<td>24.0 ± 10.0</td>
</tr>
<tr>
<td>Age ,(years)</td>
<td>34.3 ± 8.2</td>
<td>32.8 ± 8.4</td>
<td>37.2 ± 8.4</td>
<td>27.3 ± 2.2</td>
</tr>
<tr>
<td>Body mass ,(kg)</td>
<td>68.0 ± 6.2</td>
<td>55.7 ± 6.6</td>
<td>78.4 ± 8.7</td>
<td>64.6 ± 9.8</td>
</tr>
<tr>
<td>Weekly training distance ,(km)</td>
<td>81.8 ± 21.8</td>
<td>73.2 ± 27.6</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Number of years running ,(years)</td>
<td>8.2 ± 6.2</td>
<td>4.9 ± 3.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Daily protein intake ,(g)</td>
<td>82.2 ± 12.9</td>
<td>61.6 ± 13.6</td>
<td>105.8 ± 21.3</td>
<td>54.3 ± 20.0</td>
</tr>
</tbody>
</table>
Table 2. Effects of sustained strenuous exercise on haemolytic parameters in trained athletes

Data are expressed as means ± SD. Statistical significance: *P < 0.05, †P < 0.01 compared with before race.

<table>
<thead>
<tr>
<th></th>
<th>Before race</th>
<th>After race</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 min</td>
<td>24 h</td>
</tr>
<tr>
<td>Serum haptoglobin concn. (g/l)</td>
<td>0.89 ± 0.4</td>
<td>0.69 ± 0.4*</td>
</tr>
<tr>
<td>Plasma haemoglobin concn. (mg/l)</td>
<td>77.0 ± 50.5</td>
<td>112.5 ± 74.0</td>
</tr>
<tr>
<td>10^{-9} x Reticulocytes count (I^{-1})</td>
<td>0.031 ± 0.02</td>
<td>0.036 ± 0.02</td>
</tr>
<tr>
<td>Mean cell volume (fl)</td>
<td>92.8 ± 3.3</td>
<td>92.1 ± 3.4</td>
</tr>
</tbody>
</table>

Fig. 1. $T_{51}$Cr (●) and MRCL (□) in male and female athletes and sedentary control subjects. Values are means with bars indicating SD. Statistical significance: †P < 0.01, male athletes versus control subjects; *P < 0.01, female athletes versus control subjects.

elution factors for acid citrate dextrose (NIH Formulae A) [25] to be applicable in this study and the data to be valid in the sense that the subjects were in steady state.

The mechanisms of this shortened mean erythrocyte survival are not clear, and in this study no correlation was demonstrated between the mean erythrocyte lifespan, body mass or weekly training distance. The classic explanation, based on multiple observations of haemolysis associated with sustained contact-type exercise, particularly walking and running ('march haemoglobinuria') [1-3, 10] is that of increased erythrocyte destruction due to the mechanical trauma of repeated footstrokes.

The increased erythrocyte destruction as a result of exercise is probably due to an intravascular haemolysis, which may be associated with a weakening of the structural integrity of the cell membrane [4], possibly due to the traumas of increased circulatory rate, increased body temperature, compression of the erythrocytes by muscular activity [7], acute exercise acidosis, or the pressure from body weight in weight-bearing activities such as running [1, 3, 10]. Elevated levels of catecholamines observed with exercise may also increase both the osmotic and mechanical fragility of erythrocytes and the potential for accelerated destruction [13, 21].

That a degree of intravascular haemolysis is also observed in non-contact sports [7, 21] lends credence to the proposals by Japanese authors [12, 21] that a 'haemolysing factor', possibly lysolecithin [20], is released from the spleen during exercise, causing erythrocytes to become osmotically fragile and susceptible to haemolysis. Moreover, other authors [10, 34, 35] have also described a compensated haemolysis of older erythrocytes ('runner's macrocytosis'), which are more osmotically fragile than younger cells [21]. The observation of increased erythrocyte deformability in trained persons may reflect the age-related shift in the erythrocyte population, so that there is a predominance of younger, less structurally rigid erythrocytes [10, 38]. Another consideration is that younger cells preferentially take up the $^{51}$Cr label, which could influence estimates of erythrocytes survival in an athlete.

Shiraki et al. [12, 21] have proposed that this compensated haemolysis is in fact an ingenious adaptive mechanism to the demands of exercise training. That is, the exercising muscle protein requirements are met at the expense of the erythrocytes, which in effect form a large and mobile 'protein pool'. Thus athletes on high-protein diets apparently do not show the same degree of erythrocyte destruction as do those on a low-protein diet [21].

The protein nutrition of most, but not all, of the subjects in whom erythrocyte survival rates were assessed, was determined from a 7-day dietary record kept by the subjects. The data was analysed by using the Floro Diet program, which is based on the food composition tables compiled by the National Research Institute for Nutritional Disease. In all cases, the daily protein intake exceeded 1 g day^{-1} kg body weight (Table 1), which is also in excess of the Recommended Daily Allowance [39]. There was also no correlation between the protein intake and haematological status of the athletes in this study, and it is unlikely that erythrocytes, with their significant role in oxygen transport, would be catabolized to meet the protein requirements of exercising muscle, as suggested by Shiraki et al. [21]. It is even more unlikely to occur in
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well-trained athletes such as those in our study, who were in a steady state as regards muscle breakdown and regeneration with no further muscle hypertrophy and whose protein intake met the Recommended Daily Allowance [39].

The second part of this study examined the acute effects of strenuous exercise on erythrocyte survival. In these experienced athletes, who trained and competed in modern cushioned running shoes, there was no evidence of a haemolytic episode consequent upon sustained running exercise (Table 2, Figs. 2 and 3). Possibly, the decrease in serum haptoglobin levels due to haemolysis was mediated by a concomitant increase in hepatic production of this protein, owing to its role as an acute-phase reactant. Indeed, serum haptoglobin levels have been shown not to decrease to the same extent when there is inflammation associated with haemolysis [40].

Our findings support earlier observations of significantly elevated plasma haemoglobin levels in runners at rest and after strenuous exercise [8], although there was no further increase after marathon running (Table 2, Fig. 3). The method of venepuncture is critical when plasma haemoglobin levels are to be measured, and although all the blood samples were taken without stasis, we cannot exclude the possibility that much of the observed increase at rest was due in some part to lysis during venepuncture. Given these methodological limitations, it is not possible to quantitatively assess the extent of erythrocyte destruction due to marathon running per se.

It would appear therefore that a number of factors other than impact stress maintain a chronic and almost imperceptible degree of haemolysis in trained athletes. The resultant decrease in mean erythrocyte lifespan can be implicated in the comprised haematological status of some athletes, particularly when iron intake and absorption are inadequate to meet the consequentially accelerated erythropoietic demand.

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REFERENCES


36. Reference deleted.

37. Reference deleted.

