Changes in haematological parameters and iron metabolism associated with a 1600 kilometre ultramarathon

K E Fallon, G Sivyer, K Sivyer, A Dare

Abstract

Objective—To investigate haematological variations and iron related changes in the serum of participants in a 1600 kilometre ultramarathon run.

Participants—Seven male and two female participants in a 1600 km foot race.

Methods—Blood samples were obtained from the participants before, after four and 11 days of running, and at the end of the event. Samples were analysed by standard methods for haemoglobin, packed cell volume, total red cell count, mean red cell volume, mean red cell haemoglobin, total white cell count and differential, platelets, reticulocytes, iron, ferritin, total iron binding capacity, percentage transferrin saturation, haptoglobin, and bilirubin and corrected for changes in plasma volume.

Results—The following variables decreased during the event (p<0.05): haemoglobin, packed cell volume, mean red cell volume, percentage lymphocytes, percentage monocytes, serum iron, total iron binding capacity, and percentage transferrin saturation. Increases (p<0.05) were found in plasma volume, total red cell count (day 4 only), total white cell count, percentage and absolute numbers of neutrophils and reticulocytes, absolute numbers of lymphocytes and monocytes (day 4 only), absolute numbers of eosinophils (day 11 and race end), platelets, ferritin, haptoglobin, and bilirubin (day 4 only).

Conclusion—Ultramarathon running is associated with a wide range of changes in haematological parameters, many of which are related to the normal acute phase response to injury. These should not be confused with indicators of disease.

Keywords: ultramarathon; haematology; iron; haemolysis; plasma volume

The marathon and ultramarathon events, generally those up to 24 hours’ duration, have been subjected to intense study. Little has been reported about the biochemical and haematological changes occurring during and after long ultramarathons. In the only study of haematological parameters in an event of 1000 km or longer the event was a staged race in which the participants covered only 50 km a day and had long periods of rest. In addition, previous studies have failed to correct findings for changes in plasma volume, which may be of the order of 10–20%. This paper describes haematological parameters and those related to iron metabolism and haemolysis in an event in which only short sleep breaks were taken, and these variables were individually corrected for changes in plasma volume.

Methods

Before the start of this study approval was obtained from the ethics committee of the South Queensland Regional Health Authority. All procedures conformed to the National Health and Medical Research Council guidelines for experimentation with human subjects, and all subjects gave their informed written consent before participation in this study.

Seven male and two female experienced ultramarathon runners participating in the 1600 km ultramarathon in Nanango, Australia volunteered for this study. The data of those who completed at least 1000 km were included in the analysis. The ultramarathon was a continuous event, the object being to cover 1600 km (1000 miles) in the shortest possible time. Runners competed on a 400 m oval grass track, the surface of which changed progressively to dirt after the first three days of the event. Temperature range during the 16 days of the event was 11.0–31.8°C and the relative humidity 17–100%. The runners were allowed free intake of their usual diets during the study period. A medical and training history was obtained before the race started.

Four runners completed 1600 km in less than 16 days, three completed 16 days of running but did not complete 1600 km, and two completed shorter distances (1364 km and 1044 km) in less than 16 days.

Twenty five millilitres of blood was drawn from an antecubital vein, using sterile technique, about 30 minutes before the start. Further samples were obtained between 9 and 10 am on days 4 and 11 and at the end of the run. The mean delay in sampling after the event was less than 30 minutes. Each sample was obtained with the subject in the seated position. All specimens were transported to the laboratory within one hour of venepuncture.

Full blood counts were performed on a Coulter Stack "G" autoanalyser. Serum iron concentration was measured by ascorbic acid reduction on a Boehringer Mannheim-Hitachi 917 analyser. Total iron binding capacity was calculated from serum transferrin concentration.
concentration, measured by immunoturbidimetry on a Boehringer Mannheim-Hitachi 917 analyser. Transferrin saturation was calculated from the formula: (serum iron/total iron binding capacity) × 100. Ferritin concentration was analysed by immunoassay performed by automated chemiluminescence on a Ciba-Corning ACS 180 analyser. Serum haptoglobin concentration was measured by immunoturbidimetry on a Boehringer Mannheim-Hitachi 917 analyser. Estimation of total bilirubin concentration was by performed on an Olympus AU 800 autoanalyzer using the sulphanilic acid with surfactant reagent method.

Plasma volume changes were derived from changes in haemoglobin and packed cell volume. All results were corrected for changes in plasma volume with the exception of red cell parameters, haemoglobin and packed cell volume, and variables expressed as percentages.

Statistical significance of paired differences in means and standard deviations of the meta-

Table 4 Changes in total and differential white cell counts

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-race</th>
<th>Day 4</th>
<th>Day 11</th>
<th>Post-race</th>
</tr>
</thead>
<tbody>
<tr>
<td>White cell count × 10^9/l</td>
<td>5.5 (0.7)</td>
<td>10.2* (2.8)</td>
<td>10.1* (3.6)</td>
<td>8.4* (1.9)</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>58 (12)</td>
<td>70* (8)</td>
<td>77* (10)</td>
<td>66* (7)</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>30 (10)</td>
<td>19* (7)</td>
<td>16* (7)</td>
<td>21* (6)</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>9 (2)</td>
<td>8 (2)</td>
<td>5* (2)</td>
<td>7* (1)</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>2 (1)</td>
<td>2 (1)</td>
<td>2 (1)</td>
<td>3 (1)</td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>1 (0)</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>2* (1)</td>
</tr>
<tr>
<td>Neutrophils × 10^9/l</td>
<td>3.2 (0.6)</td>
<td>7.2* (2.6)</td>
<td>7.9* (3.3)</td>
<td>8.0* (3.0)</td>
</tr>
<tr>
<td>Lymphocytes × 10^9/l</td>
<td>1.7 (0.7)</td>
<td>1.9* (0.6)</td>
<td>1.5 (0.7)</td>
<td>1.9 (0.4)</td>
</tr>
<tr>
<td>Monocytes × 10^9/l</td>
<td>0.5 (0.1)</td>
<td>0.8* (0.2)</td>
<td>0.4 (0.2)</td>
<td>0.6 (0.2)</td>
</tr>
<tr>
<td>Eosinophils × 10^9/l</td>
<td>0.13 (0.08)</td>
<td>0.16 (0.1)</td>
<td>0.20 (0.15)</td>
<td>0.31* (0.19)</td>
</tr>
<tr>
<td>Basophils × 10^9/l</td>
<td>0.05 (0.02)</td>
<td>0.20 (0.27)</td>
<td>0.06 (0.07)</td>
<td>0.017* (0.07)</td>
</tr>
</tbody>
</table>

* p<0.05 vs pre-race value.
† p<0.05 vs day 11 value.
Serum iron concentration was decreased below pre-race values on days 4, 11, and at the end of the race. Mean values remained within the normal range and on day 11 approached the lower limit of normal.

Serum ferritin concentration was increased compared with its pre-race value on days 4, 11, and at the end of the run. Mean values remained within the normal range.

Transferrin saturation decreased between pre-race and day 4, remained unchanged between days 4 and 11, and 11 and race end, at which time it remained significantly lower than its pre-race value. Mean values remained within the normal range but approached the lower limit of normal.

Total iron binding capacity was significantly different from pre-race values on day 11 only. Its mean value was above the normal range before the run but returned to the normal range during the race and then was again above the normal range at the end of the event.

Haptoglobin concentration increased between pre-race and day 4 and remained above pre-race values at both day 11 and at the end of the event. On days 4, 11, and at the end of the event mean values were above the normal range.

Bilirubin concentration increased between pre-race and day 4, when it was raised above the normal range, returning to a level not significantly different from pre-race levels on day 11 and at the end of the event.

**Discussion**

Very little has been reported on the biochemical and haematological changes occurring during and after very long ultramarathons, and in some cases the available data have been derived from staged rather than continuous events. This study provides baseline data for further research into events that test the outer limits of endurance. Caution needs to be exercised in the interpretation of the end of race data in this study as it represents a number of end points, which include completion of 1600 km, 16 days of running, and early retirement from the race. However, it should be noted that each of four runners completed 1600 km, three of the others finished on the 16th day, and the runner who finished on day 12 had completed 1364 km.

**Table 5 Changes in platelets and reticulocytes**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-race</th>
<th>Day 4</th>
<th>Day 11</th>
<th>Post-race</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets x 10^3/l</td>
<td>254 (51)</td>
<td>314* (54)</td>
<td>391* (73)</td>
<td>440* (125)</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>0.8 (0.2)</td>
<td>1.0 (0.5)</td>
<td>1.3* (0.6)</td>
<td>1.3* (0.6)</td>
</tr>
<tr>
<td>Reticulocytes x 10^9/l</td>
<td>38 (9)</td>
<td>50* (23)</td>
<td>63* (26)</td>
<td>64* (25)</td>
</tr>
</tbody>
</table>

* p<0.05 vs pre-race value.
† TIBC = total iron binding capacity.

**Table 6 Changes in parameters related to iron metabolism and haemolysis**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-race</th>
<th>Day 4</th>
<th>Day 11</th>
<th>Post-race</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron (µmol/l)</td>
<td>21 (3)</td>
<td>14* (5)</td>
<td>12* (2)</td>
<td>16* (5)</td>
</tr>
<tr>
<td>Ferritin (µg/l)</td>
<td>109 (76)</td>
<td>204* (102)</td>
<td>170* (86)</td>
<td>150* (77)</td>
</tr>
<tr>
<td>TIBC (µmol/l)†</td>
<td>72 (7)</td>
<td>69 (12)</td>
<td>66* (11)</td>
<td>76 (10)</td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
<td>30 (5)</td>
<td>20* (5)</td>
<td>19* (20)</td>
<td>21* (7)</td>
</tr>
<tr>
<td>Haptoglobin (g/l)</td>
<td>1.81 (0.73)</td>
<td>3.41* (0.89)</td>
<td>3.90* (0.64)</td>
<td>4.12* (1.19)</td>
</tr>
<tr>
<td>Bilirubin (µmol/l)</td>
<td>13 (7)</td>
<td>22* (17)</td>
<td>10 (5)</td>
<td>10 (3)</td>
</tr>
</tbody>
</table>

* p<0.05 vs pre-race value.
† TIBC = total iron binding capacity.

**Table 7 Changes in parameters related to iron metabolism and haemolysis**

**Table 8 Changes in platelets and reticulocytes**

**Discussion**

Serum iron concentration was decreased below pre-race values on days 4, 11, and at the end of the race. Mean values remained within the normal range and on day 11 approached the lower limit of normal.

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Bilirubin concentration increased between pre-race and day 4, when it was raised above the normal range, returning to a level not significantly different from pre-race levels on day 11 and at the end of the event.

Mean cell volume has been reported to be increased and mean cell haemoglobin concentration decreased after a marathon run, but no change was found in either variable after a 100 km run, 160 km run, and during or after a 20 day, 312 mile run. Mean cell haemoglobin concentration was found to be decreased, however, in a group who ran 1000 km in 20 days. The small but significant decrease in mean cell volume found in this study is unlikely to indicate iron deficiency as the haematological status of the runners was normal before the event, the mean cell volume remained in the normal range, and the development of microcytosis in iron deficient states can take several weeks to develop. It is unusual that a decrease in mean cell volume occurred at a time when reticulocytosis was also present and, possibly, this small change may not be truly significant if the normal variability of measurement of the analyser is taken into account.
PLATELETS
Increases in platelets have been reported after marathon running, but no change occurred after an ultratriathlon (7.5 km swim, 360 km cycle, and 85 km run). To our knowledge platelet count has not been previously measured in an event of this duration. The significance of the increase is unclear.

WHITE CELL COUNT
Increases in white cell count have been documented after the standard marathon, 160 km runs, and 24 hour runs. No change was found in white cell count after one 160 km run, but this may be explained by delayed sampling, which occurred 48 hours after the event. Neutrophilia occurring early in exercise is related to catecholamine induced margination of neutrophils, but a delayed increase is mediated by cortisol induced release of neutrophils from bone marrow or mobilisation due to chemotactic factors released from damaged tissues. Both these factors are operational during ultramarathon running.

Monocytes are known to increase two- to threefold during exercise, the rapidity of this increase indicating mobilisation rather than increased production. They produce interleukin-1, which is an important mediator of the acute phase response. This is consistent with the findings of this study.

RETICULOCYTES
Both percentage and absolute numbers of reticulocytes were increased on days 4, 11, and at the end of the event. All means remained within the normal range. No changes in reticulocytes in events of this duration have been reported, but no change in reticulocyte count was found after a 100 km run. The reticulocytosis has a number of possible causes, including gastrointestinal and urinary blood loss and haemolysis due to foot strike. The significant increase in bilirubin on day 4 may provide some evidence for a haemolytic state.

HAPTOGLOBIN
Haptoglobin is an acute phase protein and as prolonged exercise typically produces an acute phase response an increase during an event of this duration is not unexpected. Despite this, haptoglobin concentration has been found to be depressed after marathon running, 160 km ultramarathons, and a 24 hour run. This is thought to be related to intravascular haemolysis associated with mechanical trauma at foot strike. The rise shown during our study does not discount the possibility of intravascular haemolysis but probably indicates the intensity of the acute phase reaction consequent upon muscle damage and acute inflammatory processes occurring in other musculoskeletal tissues. Periods of intense training, which can be likened to strenuous daily running, also cause prolonged increase of both haptoglobin and serum ferritin concentrations owing to maintenance of a chronic acute phase response; concern has been expressed that this could mask early iron deficiency in athletes.

BILIRUBIN
Bilirubin concentration increased between pre-race and day 4, when it was raised above the normal range, returning to a level not significantly different from its pre-race value on day 11 and at the end of the event. After a 160 km run a significant increase in bilirubin concentration was found, which when broken down into direct and indirect types suggested intravascular haemolysis. No change was found after a 1000 km, 20 day run, but a significant increase was seen on day 3, a similar pattern to that of the current study. Although only total bilirubin was measured in that study, if taken in conjunction with low haptoglobin concentrations on day 3 of the 1000 km run, it is possible that this indicates maximum haemolysis occurring during the early stage of the event. This is consistent with the hypothesis that foot strike is the causative factor of haemolysis as runners in ultramarathons typically adopt a shuffling gait as the event progresses, which is likely to be accompanied by a lower force of foot strike and, therefore, potentially less damage to red blood cells. Another possible explanation for maximum haemolysis in the early stage of the event is that as older red cells are more fragile, those most vulnerable may be damaged early, and once these are removed from the circulation a steady state is reached.

SERUM IRON
Serum iron concentration was decreased below pre-race values on days 4, 11, and at the end of the race. Mean values remained within the normal range. Decreased serum iron concentration is consistent with an acute phase response, and this response has been shown after 160 km ultramarathons and 24 hour runs. Serum iron has been found to be raised on day 3 of a 1000 km run, but in that event runners only ran 50 km a day and then rested for periods of presumably greater than 16 hours. In that event a decrease from the pre-race value was seen at day 6. The mechanism of the fall in serum iron is controversial, but an increase in ferritin synthesis in the reticuloendothelial cells and diversion of iron to that store accompanied by inhibition of iron release, similar to that occurring in the anaemia associated with chronic disease, is a likely explanation.

TOTAL IRON BINDING CAPACITY
Total iron binding capacity was significantly different from pre-race values on day 11 only. Its mean value was above the normal range before the run, returned to the normal range during the race, and then was again above the normal range at the end of the event. Total iron binding capacity has rarely been measured during this type of event but it was found to be unchanged after a 160 km ultramarathon.

TRANSFERRIN SATURATION
Transferrin saturation decreased between pre-race and day 4, remained unchanged between days 4 and 11, and 11 and race end, at which time it remained significantly lower than...
Haematological parameters and iron metabolism changes in an ultramarathon

pre-race values. Mean values remained within the normal range but approached the lower limit of normal. No change but wide variation was seen after a 1000 km stage run, and a reduction has been documented after a 160 km triathlon. A reduction in transferrin saturation typically occurs in the anaemia associated with chronic disease and a short term acute phase response leading to low iron availability is the likely explanation for the reduction found in this study.

SERUM FERRITIN

Serum ferritin concentration was increased compared with pre-race values on days 4, 11, and at the end of the run. Mean values remained within the normal range. This is similar to the study of Seiler, who found an increase during the first three days, followed by a slight decrease without reaching initial values during a 1000 km run. This increase is consistent with an acute phase response. Serum ferritin concentration is often used as a single variable for the diagnosis of deficient iron stores in athletes. The acute phase response of this protein should be regarded in this situation, and therefore determination of serum ferritin should be performed at a time remote from strenuous exercise of long duration and ideally accompanied by other iron studies, including determination of percentage transferrin saturation, serum iron, and total iron binding capacity.

Conclusion

This study has documented haematological and iron related parameters during a continuous 1600 km foot race. Participation was shown to have no significant negative effect on the haematological system, but study of more subtle indicators of red cell production, including reticulocyte variables may indicate otherwise. There seem to be a number of differences in responses between staged and continuous races over similar long distances. These may be related to the group studied, the total distance covered, intensity of exercise, differences in the acute phase response to musculoskeletal tissue damage, or failure to consider the effects of plasma volume changes.

Analysis of pre-race samples indicates that in this group of relatively old, experienced runners, training an average of almost 200 km per week has no detrimental effect on haematological or iron related parameters as all of these were within the normal range of the general population. Haemodilution has been shown to occur in already well trained runners, and changes in haemoglobin, packed cell volume (whilst related to plasma volume change), serum iron concentration, percentage transferrin saturation, total iron binding capacity, and ferritin are similar to those found in the anaemia of chronic disease. They do not however, indicate the presence of such anaemia but reflect the acute phase response common to a variety of chronic illnesses and strenuous endurance exercise of long duration. Athletes can adapt to these changes and they are not deleterious to them. An awareness of the acute phase response and changes in plasma volume is important when assessing the significance of a wide range of haematological and biochemical parameters in athletes.

Commentary

Some research takes its subjects to such extremes that it provides unique opportunities to study the limits of human endurance and thereby to test hypotheses generated from less demanding work.

This paper represents a remarkable achievement by all the athletes taking part and provides an opportunity to determine whether the factors contributing to athletes' anaemia exert a greater effect after an exceptionally prolonged test of endurance. The results indicate that the well established prime cause—dilutional pseudo-anaemia—remains the main factor even at the limits of endurance. Although haemolysis from foot strike can cause iron loss through haemoglobinuria in susceptible subjects, haemolysis as measured by haptoglobin concentrations did not contribute to the “anaemia” in these experienced runners. The very slight increase in reticulocytes suggests that a mild degree of compensated haemolysis might be occurring but certainly insufficient to contribute to the anaemia. Experienced ultramarathon runners should have an efficient running style without excessive foot strike, which has been identified as a cause of haemolysis.

The iron studies and other parameters show that iron continues to be re-used as normal allowing for the acute phase response that can accompany severe physical stress and can also be the result of muscle damage.

E J Watts

Barbados to the BASM course, via the London marathon!

“You will never make it!” My father’s words rang in my ears as I sped up the motorway. The logistics of running in the London marathon on the morning of Sunday 13 April 1997 and then reaching Lilleshall Hall National Sports Centre in time for the start of the BASM introductory course (first lecture 7 30 pm) meant a rapid escape from the marathon finish!

I am an English GP, trained at the Middlesex Hospital, who has been working in Barbados since 1982, having fallen in love with the island on my medical student elective in 1980. The easy tropical climate, sports orientated friends, and body conscious society encouraged me to start running about eight years ago, although I had always enjoyed aerobics. I had completed the London marathon in 1994 and 1996, so thought that a third time would round it off nicely not realising the eventual clash of dates with the BASM course.

I flew back to England on Tuesday 8 April, hoping for (relatively) warm temperatures; memories of a freezing start in my bin liner at the 1994 marathon still haunt me! Fortunately, the weather was mild on Sunday 13th when I travelled to the Blackheath start on the sardine-like free train from Waterloo East. I soaked up the atmosphere as thousands of runners milled about, queued in the interminably long toilet lines, and rubbed copious amounts of liniment into vital anatomical places!

A long standing left plantar fasciitis, causing my heel to feel as if a red hot poker was being stuck into it (which gave me increased sympathy for similarly afflicted patients!) reduced the likelihood of a good time. A recently fitted orthotic unfortunately caused a huge blister on my inner left foot which made the last six miles a painful experience (although much kudos should go to those who provided me with excellent service at Headley Court). I ended up running the last mile hand in hand with a “clown” from Epsom, who was also suffering!

Maybe because I was attached to a tall sweaty man in makeup and wig, my two sisters who were to meet me failed to spot me crossing the finish line, causing a 45 minute delay at the “Repatriation Point”—a chaotic area, many painful yards from the finish, where runners on mobile phones try to locate their relatives!

Eventually we met up, made it through London’s underground system (so many steps to walk down!) to the car and then to Lilleshall.

I walked into the lecture room, to gruesome sports injury slides on the screen and a round of applause—I was still wearing my running kit, medal, and tracksuit!

Louise Berry
Barbados
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