Haematological status of middle- and long-distance runners

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Summary

1. Haematological investigation and blood volume measurements were carried out on forty male middle- and long-distance runners and twelve non-athletes.

2. The distribution of haemoglobin concentration, packed cell volume, erythrocyte count, total iron-binding capacity, serum and erythrocyte folate and serum vitamin B₁₂ concentrations were essentially the same in athletes and non-athletes. The mean serum iron concentration was higher in non-athletes than in athletes. There was no difference in the above measurements between athletes taking iron and/or folate and athletes not taking these supplements.

3. Blood volume and total body haemoglobin were on average 20% higher in the athletes than in the non-athletes.

4. There was no correlation between haemoglobin concentration and blood volume in athletes. The evidence of this study suggests that haemoglobin concentration and blood volume are independently controlled.

5. 2,3-Diphosphoglycerate concentration in the erythrocytes was higher in the athletes than in the non-athletes; the mean values were 15.9 and 14.2 µmol/g of haemoglobin respectively.

Key words: haemoglobin, blood volume, athletes.

Introduction

There is widespread belief among athletes and sports physicians that a mild degree of anaemia is common among runners, and that correction of this by taking iron will raise the oxygen-carrying capacity of the blood and thus improve the runner's performance. There is no firm evidence for this belief, which may have arisen from the wide scatter of results obtained by haemoglobin determination on ear and finger blood with unstandardized methods. The subject attracted much interest at the time of the Olympic Games in Mexico City (altitude 2250 m) in 1968 because of the role of haemoglobin in acclimatization to altitude. Since then many if not most middle- and long-distance runners have taken iron supplements, although no information on the haematological status of runners has been available.

An investigation was conducted to establish the haematological status of middle- and long-distance runners. The questions posed were: (1) does the distribution of haemoglobin values in the athletes differ from normal? and (2) are the values at the lower end of the range optimum for the individual or do they imply some abnormality? To answer these questions it was necessary to carry out general haematological investigations, to measure blood volume and determine total body haemoglobin, as well as to exclude possible deficiency of nutritive factors and/or haemolysis.

Subjects and methods

Subjects

Forty male middle- and long-distance runners, and twelve control subjects, volunteered to take part in the study. All subjects were in good health and had not had any recent illness. The athletes were
running between 100 and 250 km a week in training. All of them had run at least once in a regional, national or international competition within 3 weeks of being examined. Sixteen runners had been taking iron within 6 months of the investigation, eight had taken iron and folic acid, and two folic acid alone. The iron and folate had been taken in varying doses in the form of multivitamin preparations and brewer’s yeast. None of the athletes had taken any supplements for a day before investigation. The non-athletes were laboratory personnel or students who did not participate in regular exercise.

Procedure

The investigation was carried out during the morning. Subjects came first to the Department of Haematology, St Bartholomew’s Hospital, where samples of venous blood and urine were collected, and then went to the Field Physiology Laboratory, N.I.M.R., Hampstead, where blood volume and other measurements were carried out.

Methods

**Haematological measurements.** Haemoglobin concentration (Hb), packed cell volume (PCV), erythrocyte count (EC), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) and leucocyte count (LC) were determined on sequestrenated blood by using a Coulter Counter model S. The accepted normal ranges for these measurements for male subjects are: Hb, 13.5-18.0 g/dl; PCV, 0.40-0.50; EC, 4.5 x 10^12/l to 6.5 x 10^12/l; MCV, 76-96 fl; MCH, 27-32 pg; MCHC, 32-36 g/dl; LC, 4 x 10^9 to 11 x 10^9/l. Morphological assessment of erythrocytes was carried out in peripheral blood films stained with May–Grünewald–Giemsa stain. Percentage of reticulocytes was estimated by counting 2000 cells in films stained with Brilliant Cresyl Blue. 2,3-Diphosphoglycerate concentration (2,3-DPG) in erythrocytes was measured by a modified automated method of Grisolia, Moore, Lugue & Grady (1969) with an AutoAnalyzer (Technicon). In the modified method the concentration of magnesium in the substrate was reduced tenfold and the addition of sodium EDTA was omitted. For the between-batch washing of AutoAnalyzer lines 0.5 mol/l NaOH was used. As a result of these changes better stability of the system and reproducibility of the results (coefficient of variation was 2.1%) was achieved. The normal range of 2,3-DPG is from 12.0 to 17.0 μmol/g of Hb (Y. Purcell, unpublished work).

Serum iron concentration was measured by an automated micromethod (Garry & Owen, 1967) with an AutoAnalyzer; normal values for males range from 12 to 30 μmol/l with the mean of 18.63 μmol/l (mn = 5.14 μmol/l; n = 73; B. Brozović, unpublished work). Unsaturated iron-binding capacity was measured by a semi-automated micromethod with radioactive iron (Brozović & Copestake, 1969). From these two measurements the total iron-binding capacity (TIBC) and transferrin saturation were calculated; the normal ranges are 52-73 μmol/l and 20-50% respectively. The measurement of serum folate and erythrocyte folate was carried out by a semi-automated microbiological assay using a chloramphenicol-resistant strain of *Lactobacillus casei* (Millbank, Davis, Rawlins & Waters, 1970). The normal ranges for serum folate and erythrocyte folate concentrations are from 9 to 40 nmol/l (4-18 μg/l) and from 360 to 1450 nmol/l (160-640 μg/l) respectively. Serum vitamin B₁₂ concentration was measured by a microbiological assay using *Euglena gracilis* strain Z (Anderson, 1964); the normal range is from 120 to 660 pmol/l (160-900 ng/l). The concentration of total bilirubin in serum was determined by standard automated methods initially with SMA 12/60 Technicon and, later, Vickers MC-3000 analysers; the upper limit of the normal range is 17 μmol/l (1 mg/100 ml). The concentration of haptoglobin in serum (Hp) was estimated by a photo- metric method, as described by Dacie & Lewis (1968a), with a Unicam SP.600 mark II spectrophotometer; the normal range is 0-3-2.0 g/l. The presence of haemosiderin in urine was detected in urinary sediment after staining with Prussian Blue (Dacie & Lewis, 1968b).

**Blood volume.** Blood volume was measured with carbon monoxide (CO) by a rebreathing method of Pugh (1964, 1969). The procedure was started after the subject had been sitting for about 40 min. Either 200 ml (for athletes) or 150 ml (for non-athletes) of dry CO was run into the rebreathing bag over a period of 1 min. Samples of venous blood were drawn from the heated forearm before rebreathing and exactly 10 min after starting to run in CO. The blood samples were analysed in duplicate by Roughton’s special method for small volumes of CO (Scholander & Roughton, 1943). The sp of a single analysis was 0.3 vol./l. The final concentration...
Haematological status of runners

Table 1. Particulars of the subjects

The mean values ± sd, with range and number of subjects, are presented. Values significantly different from the value for non-athletes (Student’s t-test): *P < 0.025; **P < 0.001.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>Skinfold thickness (mm)</th>
<th>Body fat (%)</th>
<th>Maximum oxygen intake (ml min⁻¹ kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Athletes</td>
<td>26.6 ± 4.1</td>
<td>175.0 ± 5.2</td>
<td>61.8 ± 4.3</td>
<td>4.9 ± 0.8 **</td>
<td>8.3 ± 1.8 **</td>
<td>73.0 ± 4.0 **</td>
</tr>
<tr>
<td>(n = 40)</td>
<td>21–36</td>
<td>165–186</td>
<td>52.6 ± 7.9</td>
<td>3.6 ± 6.7</td>
<td>5.2–12.2</td>
<td>63–80</td>
</tr>
<tr>
<td>Non-athletes</td>
<td>27.3 ± 3.4</td>
<td>176.4 ± 7.2</td>
<td>66.1 ± 8.5</td>
<td>6.6 ± 1.7</td>
<td>11.6 ± 3.0</td>
<td>50.4 ± 2.9</td>
</tr>
<tr>
<td>(n = 12)</td>
<td>21–32</td>
<td>165–186</td>
<td>51.8 ± 7.9</td>
<td>4.3–10.2</td>
<td>6.3–17.3</td>
<td>45–54</td>
</tr>
</tbody>
</table>

of carboxyhaemoglobin in the blood was between 30 and 35 vol./l. The haemoglobin concentration determined on both blood samples by the cyanmethaemoglobin method (Dacie & Lewis, 1968c), and PCV measured on a Hawksley microcentrifuge, were used for calculating blood volume (BV), erythrocyte mass, plasma volume (PV) and total body haemoglobin (TBH). PCV was not corrected for trapped plasma or for the ratio between whole-body and venous haematocrit. The reproducibility of the blood volume determination was within 100 ml in stable subjects examined two to four times within 10–30 days.

Other measurements. Skinfold thickness was measured with Harpenden calipers at four sites and the percentage of body fat calculated according to Durnin & Rahaman (1967). Maximum oxygen intake was measured on the treadmill and in a few athletes estimated from 5 km running times (Pugh, 1970).

Results

Subjects

The physical characteristics of the subjects are set out in Table 1. The athletes and non-athletes were, on average, of the same height, but the non-athletes were heavier and somewhat fatter. The maximum oxygen intake of the athletes was 46%
TABLE 2. Comparison of values for haemoglobin, packed cell volume, erythrocyte count, blood volume, erythrocyte mass and plasma volume in athletes and non-athletes

The mean values ± sd, with range and number of subjects, are presented. Value significantly higher than in non-athletes (Student’s t-test): *P < 0.001.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Haemoglobin conc. (g/dl)</th>
<th>Packed cell volume</th>
<th>10⁻¹² × Erythrocyte count (per l)</th>
<th>Blood volume (ml/kg body wt.)</th>
<th>Erythrocyte mass (ml/kg body wt.)</th>
<th>Plasma volume (ml/kg body wt.)</th>
<th>Total body haemoglobin (g/kg body wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Athletes</td>
<td>14.61 ± 0.72</td>
<td>0.43 ± 0.02</td>
<td>4.77 ± 0.27</td>
<td>93.1 ± 5.7*</td>
<td>39.8 ± 2.7*</td>
<td>53.4 ± 4.1*</td>
<td>13.6 ± 1.1*</td>
</tr>
<tr>
<td>(n = 39)</td>
<td></td>
<td>(n = 39)</td>
<td></td>
<td>(n = 34)</td>
<td>(n = 34)</td>
<td>(n = 34)</td>
<td>(n = 34)</td>
</tr>
<tr>
<td>Non-athletes</td>
<td>15.06 ± 0.98</td>
<td>0.44 ± 0.02</td>
<td>4.97 ± 0.45</td>
<td>74.5 ± 6.3</td>
<td>33.6 ± 3.6</td>
<td>40.5 ± 4.1</td>
<td>11.3 ± 1.2</td>
</tr>
<tr>
<td>(n = 12)</td>
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<td>(n = 12)</td>
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<td>(n = 12)</td>
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<td>(n = 12)</td>
<td>(n = 12)</td>
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</table>

higher than that of the non-athletes (Table 1; Fig. 1).

General haematological investigation

The mean values and distributions of Hb, PCV and EC for athletes and non-athletes were essentially the same (Table 2). The mean Hb both for runners taking iron and for runners not taking iron was 14.6 g/dl (ranges: 13.1–15.9 g/dl and 13.8–15.9 g/dl respectively). There was also no difference in the mean values and distributions of PCV and EC between these two groups of runners. The erythrocyte indices, MCV, MCH and MCHC, were normal in all the subjects investigated. The morphology of the erythrocytes was essentially normal in all the subjects, although in twenty-seven out of forty athletes a mild degree of anisocytosis was observed. The significance of this finding is not known.

The distribution of 2,3-DPG in the blood was higher for the athletes (mean ± sd: 15.9 ± 1.6 μmol/g of haemoglobin; range: 12.8–18.4 μmol/g; n = 28) than for the non-athletes (mean ± sd: 14.2 ± 1.0 μmol/g of haemoglobin; range: 13.2–15.7 μmol/g; n = 8); the difference was statistically significant (P < 0.01).

Blood volume

The mean blood volume was 5.752 l (range: 5.055–7.036 l; n = 34) for the athletes and 4.616 l (range: 3.975–6.430 l; n = 12) for the non-athletes. When expressed per kg body weight, the mean blood volume in the athletes (93.1 ml) was 25% higher than in non-athletes (74.5 ml) (Table 2; Fig. 1). In the athletes there was a proportional increase in both the erythrocyte mass and plasma volume. There was no correlation between blood volume and haemoglobin concentration.

Total body haemoglobin

The mean total body haemoglobin for the athletes was 840 g (range: 707–1130 g; n = 34) and for the non-athletes 747 g (range: 577–977 g; n = 12). The mean total body haemoglobin expressed per kg body weight was 20% higher in the athletes (13.6 g) than in non-athletes (11.3 g) (Table 2).

Serum iron and TIBC

The mean serum iron concentration for all athletes was 18.6 μmol/l, and essentially the same as for normal males (Table 3). The mean value for the non-athletes was 24.5 μmol/l. The higher value for the non-athletes was unexplained. There was no significant difference in serum iron between the athletes taking iron and those not taking iron; the respective mean values were 19.1 μmol/l (sd = 6.1 μmol/l) and 17.9 μmol/l (sd = 5.3 μmol/l). Two athletes had serum iron concentrations of 7.5 and 8.2 μmol/l, which were below normal range. One of these was taking iron, and both had normal TIBC.

The mean TIBC for all athletes (66.9 μmol/l) was slightly but not significantly different from that for non-athletes (59.4 μmol/l) (Table 3). There was also no significant difference in the mean TIBC between twenty-four athletes taking iron and nine athletes not taking iron. The TIBC values were above the accepted upper limit of normal in eight athletes not taking iron and in five taking iron. The increased TIBC values ranged from 73 to 82 μmol/l.
Haematological status of runners

TABLE 3. Comparison of values for serum iron, folate, vitamin B₁₂ and erythrocyte folate in athletes and non-athletes

The mean values±SD, with range and number of subjects, are presented. Value significantly different from the value for non-athletes (Student's t-test): *P<0·01 (see the text for explanation). Iron: 1 μmol/l = 5·6 μg/100 ml; folate: 1 nmol/l = 441 ng/l; vitamin B₁₂: 1 pmol/l = 1·36 ng/l.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Serum iron concn. (μmol/l)</th>
<th>Total iron-binding (μmol/l)</th>
<th>Serum folate concn. (nmol/l)</th>
<th>Erythrocyte folate concn. (nmol/l)</th>
<th>Serum vitamin B₁₂ concn. (pmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Athletes</td>
<td>18·65±5·73*</td>
<td>66·91±8·63</td>
<td>16·24±7·16</td>
<td>693·9±234·4*(1)</td>
<td>343·5±124·9</td>
</tr>
<tr>
<td>(n = 33)</td>
<td></td>
<td></td>
<td>(n = 35)</td>
<td>(n = 35)</td>
<td>(n = 40)</td>
</tr>
<tr>
<td>Non-athletes</td>
<td>24·54±3·72</td>
<td>59·39±6·85</td>
<td>13·52±2·15</td>
<td>559·7±167·4</td>
<td>318·8±95·9</td>
</tr>
<tr>
<td>(n = 6)</td>
<td></td>
<td></td>
<td>(n = 7)</td>
<td>(n = 7)</td>
<td>(n = 7)</td>
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</table>

(1) Excluding serum folate over 40 nmol/l found in three athletes taking folate and their respective erythrocyte folate values; see Results section for details.

Folic acid and serum vitamin B₁₂

There was no significant difference in mean serum folate concentration between athletes and non-athletes (Table 3). Similarly, there was no difference in mean serum folate between the athletes not taking and those taking folate supplements; the mean values were 13·4 and 16·9 nmol/l respectively. No significant difference in mean erythrocyte folate concentration was found either between athletes and non-athletes (mean values were 693·9 and 559·7 nmol/l respectively) or between athletes taking folate supplements and those not taking folate. However, the following values found in three athletes taking folate were excluded from the comparison: erythrocyte folate concentrations of 423, 1191 and 1332 nmol/l associated with serum folate over 40 nmol/l. Two athletes had serum folate of 6·1 and 6·8 nmol/l, which is below the normal range, but their erythrocyte folate concentrations were 467 and 376 nmol/l, thus excluding significant folic acid deficiency. All the subjects had essentially normal serum vitamin B₁₂ concentrations, the mean being 343·5 pmol/l for the athletes and 318·8 pmol/l for the non-athletes (Table 3).

Haemolysis

No measurable increase in blood destruction was found. The mean haptoglobin concentration in serum for athletes was 0·50 g/l (SD = 0·29 g/l; n = 24) and the range was 0·20–1·60 g/l. The mean haptoglobin for non-athletes was 0·49 g/l (SD = 0·16 g/l; n = 6) and the range was 0·37–0·80 g/l. Of all the subjects investigated only four athletes had serum bilirubin concentrations higher than 17·0 μmol/l (1 mg/100 ml). Three athletes had bilirubin concentrations of 18·8, 20·5 and 22·2 μmol/l but there was no other supporting evidence of haemolysis. The increased values were all observed within 1 week and a technical error in the determination of serum bilirubin could not be excluded. Another athlete whose serum bilirubin was persistently between 17·1 and 34·2 μmol/l was subsequently proved to have Gilbert's disease. Initially, reticulocyte counts and estimation of urinary haemosiderin were carried out. The results were invariably normal and later both tests were abandoned.

Discussion

The first question posed in the Introduction as to whether the distribution of haemoglobin values in athletes differs from normal may be answered in the negative. The haemoglobin concentration and other haematological indices of the runners and of the controls were closely comparable and within the accepted range of normal. The second question, as to whether the haemoglobin values in the lower half of the range were optimum for the individuals concerned, was answered by excluding possible deficiency of iron or folate. No difference in haematological status was found between those taking iron and/or folate and those not taking iron and/or folate. Nor was there any difference in distribution
of haemoglobin and other values within these groups. The absence of deficiency of iron and folate in these athletes argues against the belief that taking iron and folate can improve an athlete's haematological status. Such deficiencies may be encountered in young men (Höglund, Ehn & Lieden, 1970; Isager, 1969) and occasionally in young athletes (De Wijn, De Jongste, Mosterd & Willebrand, 1971). All the runners in the study presented were mature athletes who had been competing for at least 5 years.

Furthermore, there was no evidence of an abnormal rate of erythrocyte destruction. The haptoglobin concentration, which is the most sensitive of the tests applied, was normal in all but one subject. This subject, whose haptoglobin value was 0·20 g/l, was examined 3 days after a marathon race. Low haptoglobin values have been observed immediately after long-distance races of 42 km upwards (J. Brotherhood, unpublished work).

The most striking difference between the runners and the control subjects was in blood volume and total haemoglobin, the values of which were on average 20% higher in the runners. This finding confirmed earlier work on the relation of maximum oxygen intake with blood volume and TBH in athletes (Saltin & Astrand, 1967). The mean blood volume (74·5 ml/kg) for the control subjects was closely comparable with published data. Hobbs (1967) collected results on 677 male subjects from the world literature, showing a mean value of 77·6 ml/kg and range 59·8-101·7 ml/kg. The question of CO uptake by myoglobin and other sources of error in the determination of blood volume has been discussed elsewhere (Pugh, 1964, 1969; Glass, Edwards, De Garreta & Clark, 1969).

The TBH values of our control subjects, which averaged 11·39 g/kg, were somewhat higher than control values previously reported, namely 10·4 g/kg in twenty-six normal males (Ekelund, 1966) and the value of 10·6 g/kg in cyclists (Glass, Edwards, De Garreta & Clark, 1969); this is solely due to the calculation of TBH, in which the difference between whole body and venous packed cell volume has not been taken into account.

No correlation was found between haemoglobin concentration and blood volume, although there was a good correlation between TBH and blood volume \((r = 0·8630, P<0·001)\). The evidence of this study therefore suggests that haemoglobin concentration and blood volume are independently controlled. Under physiological conditions the haemoglobin concentration in the blood is related to, and is frequently equated with, the circulating erythrocyte mass. The circulating erythrocyte mass is maintained at an optimum size by appropriate adjustments in the rate of erythrocyte production (Gordon, Cooper & Zanjani, 1967; Gordon, 1971). The control mechanism is based on a feed-back system within which the renal tissue, inadequately supplied with oxygen, increases production of erythropoietic hormone, erythropoietin, which in turn stimulates the production of erythrocytes. The regulation of blood volume is incompletely understood. The increase in blood volume in response to metabolic demand is considered to be a two-step process in which the dimensions of the heart and vascular bed first increase, and the ensuing demand for a greater filling volume is satisfied as a second step (Gauer, Henry & Behn, 1970; Guyton, Coleman & Granger, 1972). This is mediated via stretch receptors in both atria of the heart, which are sensitive to change in central venous pressure. Stimulation of these receptors results in appropriate humoral and nervous responses, which effect water and electrolytes excretion by the kidney. However, it is not known whether the same mechanism controls blood volume over a long period of time. The finding of normal haemoglobin concentration and increased blood volume in the athletes would suggest that in patients with polycythaemia vera, in addition to erythroid proliferation, one of the regulatory mechanisms is impaired and may be responsible for raised haemoglobin concentration in the blood. It is highly probable that in both of these conditions a host of factors are at work, but no detailed information about their action is available.

The concentration of 2,3-DPG in the erythrocyte is usually increased in response to hypoxia and anaemia (Oski & Gottlieb, 1971; Bellingham & Grimes, 1973), apparently in association with other regulatory mechanisms which affect haemoglobin affinity for oxygen. A small increase of 2,3-DPG was found in non-athletes during short and strenuous exercise (Faulkner, Brewer & Eaton, 1970) and after 8 weeks of physical training (Shappell, Murray, Bellingham, Woodson, Detter & Lenfant, 1971). However, this increase apparently had no effect on the oxygen dissociation curve of haemoglobin in the subjects investigated. The increase of 2,3-DPG in athletes reported here was comparatively large, but information on oxygen dissociation curves was not obtained. At present it is not possible to evaluate
the practical significance of increased 2,3-DPG in terms of athletic performance since it is but one of many factors affecting oxygen transport in extreme exercise.

Acknowledgments

We are indebted to Mrs B. Malloy, who carried out some of the carboxyhaemoglobin determinations, Miss Y. Purcell for permission to quote results of 2,3-DPG measurements in normal subjects and athletes, Mrs L. Brown for estimating serum haemoglobin concentrations, staff of the Department of Haematology for carrying out general haematological investigations and microbiological assays, and Professor D. L. Mollin for advice and helpful suggestions.

References


