The effect of iron and folate therapy on maximal exercise performance in female marathon runners with iron and folate deficiency

MICHELE MATTER, TESSA STITTFALL, JOHN GRAVES, KATHRYN MYBURGH, BRETT ADAMS, PETER JACOBS AND TIMOTHY D. NOAKES

Departments of Physiology and Haematology, Metropolitan Sport Science Centre and Leukaemia Centre, University of Cape Town Medical School and Groote Schuur Hospital, Cape Town, South Africa

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Summary

1. Of the 85 female marathon runners examined in this study, 14 (16%) had serum ferritin levels below 40 ng/ml but only two (2%) had iron deficiency anaemia (haemoglobin below 12 g/dl); 28 (33%) had serum folate levels below 4.8 ng/ml and of these two (2%) had haemoglobin levels below 12 g/dl and 13 (15%) had mean corpuscular volumes greater than 95 fl.

2. One week after treatment with oral folate (5 mg/day) or iron (50 mg of elemental iron/day), serum ferritin and folate levels were normal but maximum oxygen uptake, maximum treadmill running time, peak blood lactate levels and the running speed at the blood 'lactate turnpoint' were not changed from values measured during an identical test performed 1 week earlier. These parameters were also unchanged in a third exercise test performed after a further 10 weeks of treatment.

3. Serum folate or serum ferritin levels in a control (placebo-treated) group with initially high serum ferritin or folate levels fell with placebo treatment but maximum treadmill running time, maximum oxygen uptake values, peak blood lactate levels and the running speed at the blood 'lactate turnpoint' were unchanged.

4. We conclude that biochemical evidence of iron and folate deficiency is relatively common in female distance runners; that 1 week of treatment corrects the biochemical evidence of folate and iron deficiency but that such treatment does not influence maximal exercise performance nor does it alter blood lactate levels during exercise.

5. In the absence of iron deficiency anaemia, iron therapy for reduced serum ferritin levels, or folate therapy for low serum folate levels, may not improve maximal treadmill performance even in trained runners.

6. Serum ferritin or folate levels above the normal range are not associated with enhanced maximum oxygen uptake, maximum treadmill performance, lower blood lactate levels during exercise or higher running speeds at the blood 'lactate turnpoint'.

Key words: blood lactate, folate, folate therapy, iron, iron therapy, lactate turnpoint, marathon runners, maximum exercise performance.

Abbreviations: EC, erythrocyte count; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; PCV, packed cell volume.

Introduction

Iron deficiency anaemia reduces exercise performance [1–11] probably through combined effects on maximum oxygen uptake ($V_{O_2}$) [2, 3, 10, 11] and on muscle metabolism [4–7, 10–12]. Correction of the iron deficiency reverses these abnormalities [4, 8–10, 12]. However, whereas maximum $V_{O_2}$ returns rapidly to normal with correction of the anaemia, the correction of the metabolic defect in muscle lags behind the improvement in maximum $V_{O_2}$ [10].
Recent studies have shown that iron deficiency without anaemia is common amongst female runners so that as many as 80% of female runners have been considered to be iron deficient on the basis of low serum ferritin levels in the absence of iron deficiency anaemia in the majority [13–16]. The effects of tissue iron deficiency alone, shown by low serum ferritin levels in the absence of anaemia, and the effects of therapy on competitive performance in such athletes is not known, as previous studies have been performed in humans or animals in whom there was also iron deficiency anaemia [4, 9–12].

In this study we have compared the effects of 11 weeks of oral iron and folate therapy in a group of female marathon runners considered to be either iron- or folate-deficient or both but not anaemic, on the basis of conventional tests. We wished to determine whether correction of the biochemical evidence of iron and folate deficiency without anaemia enhances exercise performance capacity through effects on either maximum $V_O_2$, or on blood lactate levels during maximal treadmill exercise.

Methods

All subjects consented to participate in the experiment after the protocol had been described to them. The protocol for the investigation was approved by the Ethical Committee of the University of Cape Town Medical School.

Selection of subjects

In order to identify a group of iron-deficient women athletes we recruited a large group of local female marathon runners. This group of 85 runners comprised approximately 75% of all women marathon runners in the Cape Town area. After an overnight fast, these runners reported to the laboratory where venous blood samples were drawn for haematological analysis (see below).

On the basis of these haematological data, the subjects were placed into one of four groups according to the criteria given in Table 1.

From each group a sub-group of athletes were selected for further study (group B = 11 subjects; group C = 10 subjects; group D = 6 subjects). Subjects were selected on the basis of their willingness to participate in the extended study. The control group (group D) was made up of women with initially high serum ferritin levels in order to reduce the likelihood of an anaemia developing in the control group during the study. All groups contained runners of varying abilities. No attempt was made to match the groups for running ability.

Menstrual history

A detailed menstrual history was taken from all subjects.

Treatment protocol

Group B with low serum ferritin levels received 500 mg of amino acid chelate iron (Leppin; Albion Laboratories Inc., U.S.A.)/day containing 50 mg of elemental iron (278% of the recommended daily allowance). Group C with low serum folate levels received 5 mg of folic acid (G. W. Leppin Pty Ltd, Sandton, South Africa)/day. The control group D received 60 mg of lactose sugar placebo (G. W. Leppin Pty Ltd, Sandton, South Africa)/day. All the other runners were informed that their haematological data were normal and they were not required for further study.

Exercise testing protocol

The 27 subjects selected for the study underwent exercise testing in the laboratory on three different occasions at the onset of the study, 1 week later and again 10 weeks later. Participants were asked to fast

<table>
<thead>
<tr>
<th>Group</th>
<th>Haematological criteria</th>
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<tbody>
<tr>
<td>(A) Normal group (33 subjects)</td>
<td>Serum ferritin levels &gt; 40 ng/ml but &lt; 245 ng/ml and serum folate levels &gt; 5.6 ng/ml</td>
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<tr>
<td>(B) Low serum ferritin group (19 subjects)</td>
<td>Serum ferritin levels &lt; 40 ng/ml</td>
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<td>(C) Low serum folate group (23 subjects)</td>
<td>Serum folate levels &lt; 4.5 ng/ml</td>
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<tr>
<td>(D) Control (high serum ferritin) group (10 subjects)</td>
<td>Serum ferritin levels &gt; 245 ng/ml and serum folate levels &gt; 5.6 ng/ml</td>
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for 5 h before all laboratory tests, and not to undertake any strenuous or prolonged exercise for at least 5 days before each test. This precaution was necessary to allow full recovery from exercise and to prevent a false exercise-induced elevation of serum iron and ferritin levels [17].

On arrival in the laboratory the subjects were weighed on a Seca 770 Alpha Personal Scale (Vogel and Halke, Hamburg, West Germany). They then undertook a 5 min warm-up run on the treadmill at 6 km/h after which disposable electrodes were placed on the chest in the CM5 configuration for measurement of heart rate with a Life Trace 12 monitor (Albury Instruments Ltd, London). A Jelco intravenous catheter placement unit (Critikon; Tampa, FL, U.S.A.) was inserted into a subcutaneous forearm vein and connected via preheparinized tubing to an Eyela Microtube Pump MP-3 (Rikakikai Co. Ltd, Tokyo, Japan). The pump drew blood continuously at a rate of 2 ml/min. During the test the forearm was kept warm with a hairdryer.

The test protocol was that previously described [18]. Subjects were exercised progressively to exhaustion on a Quinton Treadmill (Tiernay Electrical Co., Seattle, WA, U.S.A.). All subjects ran with a model no. 2766 counterbalance head support holding a model no. 2770 Rudolf Valve (both by Hans Rudolph Inc., Kansas City, KS, U.S.A.). A noseclip prevented nasal breathing. Air was exhaled through clear-bore 35 mm tubing into a 15 litre Perspex mixing chamber with baffles. Expired air from the mixing chamber was continuously sampled through Drierite anhydrous CaSO₄ (Vacumed Inc., Ventura, CA, U.S.A.) to the pick-up heads of a Beckman OM-11 O₂ analyser model 242B and a Beckman LB-2 medical gas analyser model 240M (Beckman Instruments Inc., IL, U.S.A.). The outputs from the analysers were recorded on a Beckman respiratory recorder RR-2. Both analysers were calibrated before and after each test using gases of known composition that had been calibrated previously using the Haldane technique.

Inspiratory volume and respiratory rate were recorded from a Morgan ventilation monitor (P. K. Morgan Ltd, Kent, U.K.) that had been calibrated against a Collins chain-compensated gasometer (Collins Inc., Braintree, MA, U.S.A.).

Subjects began exercising at 6 km/h with incremental increases of 0.5 km/h every 30 s until exhaustion. During the test, heart rate, ventilation (l/min) and respiration rate (breaths/min) were recorded at the end of each minute. \( F_{EO₂} \) and \( F_{CO₂} \) were recorded continuously on the paper recorder and individual blood samples were collected each minute of the test in tubes containing ice-cold 70% perchloric acid for later analysis of blood lactate levels.

Rates of \( V_{O₂} \), carbon dioxide production and respiratory exchange ratio were calculated for each minute using conventional equations [19]. The blood 'lactate turnpoint' was determined visually from individual graphs of blood lactate levels at the different treadmill running speeds. The treadmill speed at which the first blood lactate level was clearly elevated above the preceding values, was taken as the 'lactate turnpoint'.

**Blood parameters**

Blood samples were assayed for serum ferritin levels by immunoassay (Amersham International, U.K.) [20], serum \( B_{12} \) and folate levels by radiodilution (Amersham International, U.K.) [21], serum iron, total iron binding capacity and percentage saturation were determined by the International Committee for Standardization in Haematology recommendations [22, 23]. Erythrocyte count (EC), haemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin content (MCHC) were measured by Coulter counter [24]. Blood lactate levels were measured by enzymatic assay based on the reduction of NAD to NADH [25].

**Statistical methods**

Paired data for pre- and post-treatment trials were compared in each group with the Student's t-test. Comparison between the two groups was performed with the Scheffe test. The level of significance was taken at \( P < 0.05 \) for both tests.

**Results**

**Initial haematological values**

Table 2 lists the results of haematological parameters measured in four groups comprising 85 marathon runners. There were no intergroup differences in mean Hb, EC, PCV, MCH, MCHC, total iron binding capacity and serum \( B_{12} \) levels. MCV was elevated in the folate-deficient (group C) compared with both the normal group (group A) and the low serum ferritin group (group B). Serum ferritin and serum iron levels and percentage saturation were low in the low serum ferritin group (group B) and high in the high serum ferritin group (group D), whereas serum folate levels were low in the folate-deficient group (group C).

The abnormal findings included 14 subjects (16%) with serum ferritin levels lower than 40 ng/ml. Iron deficiency anaemia (Hb less than 12 g/dl)
was present in only two of these subjects (2%). Twenty-eight subjects (33%) had serum folate levels below 4.8 ng/ml; of these 13 (15%) presented with macrocytic anaemia with an MCV greater than 95 fl.

**Menstrual patterns**

Of the 85 women in the study, 18 (21%) were taking the contraceptive pill. The distribution of 'pill' takers in the different groups was not different: group A = 6 (18%), group B = 5 (26%), group C = 5 (22%) and group D = 2 (20%).

Of the remaining 67 women, five were perimenopausal and two had had hysterectomies. Oligomenorrhoea (menstrual cycles between 35 and 90 days) was present in only five runners, and none was amenorrhoeic. Menstrual cycles were normal in the remaining 55 women (65%).

All of the oligomenorrhoeic runners had either low serum folate (three runners) or ferritin levels (two runners). Another runner whose oligomenorrhoea was controlled by contraceptive medication was also folate-deficient.

**Effects of iron or folate supplementation on haematological parameters and on maximum $V_O^2$, and blood lactate levels during exercise**

**Haematological values.** Table 3 shows the effects of 1 and 11 weeks of iron therapy on Hb, PCV, serum ferritin and iron levels, total iron binding capacity and percentage saturation.

Within 7 days of iron therapy, serum ferritin levels and percentage saturation rose significantly in the low serum ferritin group, and remained normal for the remainder of the study. Hb and PCV were not influenced by iron therapy, nor were serum iron levels or total iron binding capacity. Serum ferritin and iron levels and percentage saturation fell progressively in the control, placebo-treated group so that after 11 weeks there were no significant differences in any of these parameters between the groups and both had normal values for all parameters.

Table 4 shows that the mean serum folate levels rose significantly at 1 and 11 weeks in the group with low serum folate levels treated with oral folate, whereas levels in the control group dropped significantly after 1 week of placebo therapy. Hb, PCV and MCV were not influenced by folate therapy.

**Maximal exercise data.** Table 5 shows that neither 1 nor 11 weeks of iron or folate therapy for the subjects whose serum ferritin or folate levels were initially low, influenced either maximum $V_O^2$, maximum heart rate, peak blood lactate levels, blood lactate levels or treadmill running speed at
### Table 3. Effect of 1 and 11 weeks of iron therapy on haemoglobin concentration, packed cell volume, serum ferritin, serum iron, total iron binding capacity and percentage saturation in iron-deficient runners (group B) compared with placebo-treated controls (group D)

Values are expressed as means ± SD. Groups B and D are as described in the legend to Table 2. Test 1, initial value; test 2, re-test after 7 days of therapy; test 3, re-test after a total of 11 weeks of therapy. Statistical significance: *P < 0.05 for test 2 or test 3 vs test 1.

<table>
<thead>
<tr>
<th></th>
<th>Hb (g/dl)</th>
<th>PCV</th>
<th>Serum ferritin (ng/ml)</th>
<th>Serum iron (g/dl)</th>
<th>Total iron binding capacity (g/dl)</th>
<th>Saturation (%)</th>
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<tr>
<td><strong>Test 1</strong></td>
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<td><strong>Test 2</strong></td>
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<td><strong>Test 3</strong></td>
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<td><strong>Group B</strong></td>
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<tr>
<td>(n = 11)</td>
<td>13.1 ± 0.7</td>
<td>13.4 ± 0.8</td>
<td>14.0 ± 0.9</td>
<td>0.38 ± 0.03</td>
<td>0.39 ± 0.03</td>
<td>0.41 ± 0.03</td>
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<td><strong>Group D</strong></td>
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<tr>
<td>(n = 6)</td>
<td>13.7 ± 0.7</td>
<td>13.4 ± 0.8</td>
<td>14.3 ± 0.8</td>
<td>0.40 ± 0.02</td>
<td>0.37 ± 0.05</td>
<td>0.39 ± 0.03</td>
</tr>
</tbody>
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### Table 4. Effect of 1 and 11 weeks of folate therapy on haemoglobin concentration, packed cell volume, serum folate levels and mean corpuscular volume in folate-deficient runners (group C) compared with placebo-treated controls (group D)

Values are expressed as means ± SD. Groups C and D are as described in the legend to Table 2. Test 1, initial value; test 2, re-test after 7 days of therapy; test 3, re-test after a total of 11 weeks of therapy. Statistical significance: *P < 0.05 for test 2 or test 3 vs test 1.

<table>
<thead>
<tr>
<th></th>
<th>Hb (g/dl)</th>
<th>PCV</th>
<th>Serum folate levels (ng/ml)</th>
<th>MCV (g/dl)</th>
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<tr>
<td><strong>Test 1</strong></td>
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<td><strong>Test 2</strong></td>
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<td><strong>Test 3</strong></td>
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<tr>
<td><strong>Group C</strong></td>
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<td>(n = 10)</td>
<td>13.7 ± 0.7</td>
<td>13.3 ± 0.8</td>
<td>14.2 ± 1.0</td>
<td>0.39 ± 0.04</td>
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<tr>
<td><strong>Group D</strong></td>
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<tr>
<td>(n = 6)</td>
<td>13.7 ± 0.7</td>
<td>13.4 ± 0.6</td>
<td>13.3 ± 0.8</td>
<td>0.40 ± 0.02</td>
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</tbody>
</table>

### Table 5. Effects of 1 and 11 weeks of iron or folate therapy in iron- or folate-deficient subjects on maximum oxygen uptake, maximum heart rate, peak blood lactate levels, blood lactate levels and treadmill speed at the 'lactate turnpoint; and maximum treadmill running speed compared with placebo-treated controls

Values are expressed as means ± SD. Groups B, C and D are as described in the legend to Table 2. Test 1, initial value; test 2, re-test after 7 days of therapy; test 3, re-test after a total of 11 weeks of therapy. Statistical significance: *P < 0.05 for test 2 or test 3 vs test 1.

<table>
<thead>
<tr>
<th></th>
<th>Maximum $\dot{V}$O$_2$ (ml min$^{-1}$ kg$^{-1}$)</th>
<th>Maximum heart rate (beats/min)</th>
<th>Peak blood lactate levels (mmol/l)</th>
<th>Blood lactate level at lactate turnpoint (mmol/l)</th>
<th>Treadmill speed at lactate turnpoint (km/h)</th>
<th>Peak treadmill running speed (km/h)</th>
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<tbody>
<tr>
<td><strong>Test 1</strong></td>
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<td></td>
<td>47.4 ± 3.6</td>
<td>47.9 ± 4.9</td>
<td>47.7 ± 4.6</td>
<td>179.5 ± 8.9</td>
<td>181.5 ± 6.5</td>
<td>179.8 ± 8.0</td>
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<td><strong>Group C</strong></td>
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<td></td>
<td>47.9 ± 4.3</td>
<td>47.8 ± 4.0</td>
<td>48.7 ± 3.8</td>
<td>180.9 ± 6.9</td>
<td>182.6 ± 10.2</td>
<td>180.7 ± 6.7</td>
</tr>
<tr>
<td><strong>Group D</strong></td>
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<tr>
<td></td>
<td>45.6 ± 3.5</td>
<td>45.6 ± 4.0</td>
<td>46.7 ± 4.3</td>
<td>184.5 ± 9.4</td>
<td>182.5 ± 11.7</td>
<td>180.2 ± 9.2</td>
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</table>
the 'lactate turnpoint', or peak treadmill running speed. Values for all these parameters were also similar in all the tested groups.

In order to detect any shifts in the 'lactate turnpoint' during progressive exercise, composite graphs have been constructed (Fig. 1). The blood lactate level at the 'lactate turnpoint' was identified in each subject and the blood lactate levels at the four speeds below, and four speeds above, the turnpoint speeds were summed for the three treadmill tests for the three different study groups. There was no difference in the blood lactate levels at any of these running speeds, indicating that therapy had not influenced blood lactate levels.

Discussion
There were two essential findings in this study. First was the high incidence of biochemical evidence considered to indicate folate or iron deficiency. Thus 16% of the sample had low serum ferritin levels and 33% had low serum folate levels. The incidence of iron deficiency anaemia, shown by low Hb and low serum ferritin levels, was 2%; the incidence of macrocytic anaemia, shown by an MCV greater than 95 fl with low serum folate levels, was 15%.

Second, neither low nor high serum ferritin or folate levels appeared to influence maximum treadmill exercise performance or the biochemical and physiological parameters measured during maximum exercise. Thus these parameters did not change during the 11 week study in the groups whose serum ferritin or folate levels were initially low (groups B and C respectively), but whose biochemical evidence of folate or iron deficiency was reversed with therapy. Nor did these parameters change in the group whose serum ferritin levels were initially high but which fell to the normal range with placebo treatment. Thus it would seem that serum ferritin levels elevated beyond the normal range are not associated with enhanced maximal exercise performance, a view which is in accord with the general belief that vitamin and mineral hyper-supplementation does not enhance exercise performance (L. M. Weight, K. H. Myburgh & T. D. Noakes, unpublished work).

Previous studies have shown changes in the maximum $\text{VO}_2 [10]$, in peak heart rates [4, 8] and in peak blood lactate levels [4, 11], which parallel the rate of reversal of anaemia in iron-deficient subjects. No such changes occurred in our subjects. The most obvious explanation for this discrepancy would be that our subjects were not sufficiently iron deficient to be anaemic, in which case iron therapy would likely have shown an effect. Alternatively, their low serum ferritin levels might not indicate a true iron deficiency. Evidence for this possibility comes from the work of Magnusson and his colleagues [26-28], who compared whole body iron status in two groups of long distance runners, the
one group considered to be iron deficient because of low serum ferritin levels and low bone marrow haemosiderin content, the other with normal values for these parameters. There was no other evidence for iron deficiency in the group with low serum ferritin levels. In particular, the bone marrow sideroblast counts, Desferal tests, MCV, erythrocyte protoporphyrin values and serum iron levels were all normal in this group. In addition, the dietary iron intake was high (>18 mg/day) and the urinary iron excretion was not increased.

The authors concluded that the low serum ferritin levels in that group did not indicate iron deficiency. Rather they suggest that the reduced bone marrow haemosiderin and low serum ferritin levels reflect a shift in the catabolism of senescent erythrocytes undergoing intravascular haemolysis during exercise, from the reticulo-endothelial system to the hepatocytes. It remains to be shown why some but not all runners show this adaptation.

The findings of our study are therefore compatible with the interpretation that the low serum ferritin levels measured in our runners did not indicate iron deficiency sufficiently severe to impair performance as (i) the physiological variables measured in the first test were not different between the groups with either high or low serum ferritin levels, and (ii) the increase in serum ferritin levels that resulted from iron therapy did not cause an increase in exercise performance, in contrast to findings when true iron deficiency is present [4, 8, 10]. Similarly, it would seem that the correction of low serum folate levels in the absence of anaemia will not enhance athletic performance.

In contrast to the findings of Magnnusson et al. [27], our runners with low serum ferritin levels (group B) also had low serum iron levels. These did not increase with iron therapy whereas serum ferritin levels did. Interestingly, even after 11 weeks of iron therapy, serum ferritin levels in the group with initially low serum ferritin levels (group B) were lower than levels in the normal group (A) (48.3 ± 9.3 vs 128.2 ± 30.1 µg/dl), further suggesting that there are differences in iron kinetics between runners with normal and with low serum ferritin levels.

Conclusion

This study shows that the correction of low serum ferritin levels in female runners who are not anaemic is not associated with any measurable change in either maximal treadmill performance or any physiological and biochemical parameters measured during progressive exercise to exhaustion. This finding is in keeping with the hypothesis that low serum ferritin levels are not, by themselves, an absolute indication of iron deficiency in runners, but reflect an altered catabolic pathway for the handling of senescent erythrocytes by the hepatocytes rather than by the reticulo-endothelial system. Similarly, the correction of low serum folate levels in the absence of anaemia is not associated with enhanced performance.

Acknowledgments

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References


