Effect of Iron Injections on Aerobic-Exercise Performance of Iron-Depleted Female Athletes

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This investigation examined the effect of intramuscular iron injections on aerobic-exercise performance in iron-deficient women. Sixteen athletes performed a 10-min steady-state submaximal economy test, a \( \text{VO}_{2\text{max}} \) test, and a timed test to exhaustion at \( \text{VO}_{2\text{max}} \) workload. Subjects were randomly assigned to an iron-supplemented group (IG) receiving intramuscular iron injections or to a placebo group (PG). Twenty days after the first injection, exercise and blood testing were repeated. A final blood test occurred on Day 28. Postsupplementation, no differences were found between the groups’ submaximal or maximal \( \text{VO}_{2} \), heart rate, or blood lactate (\( P > 0.05 \)). Time to exhaustion was increased in the IG (\( P < 0.05 \)) but was not greater than that of the PG (\( P > 0.05 \)). The IG’s serum ferritin (SF) was significantly increased on Days 20 and 28 (mean ± standard error: 19 ± 3 to 65 ± 11 to 57 ± 12 µg/L; \( P < 0.01 \)), with a percentage change from baseline significantly greater than in the PG (\( P < 0.01 \)). It was concluded that intramuscular iron injections can effectively increase SF without enhancing submaximal or maximal aerobic-exercise performance in iron-depleted female athletes.

Key Words: economy, \( \text{VO}_{2} \), time to exhaustion, symptoms

Optimal physical performance depends on efficient oxygen delivery and utilization. Iron is a fundamental element to both of these factors—it serves as the functional component of hemoglobin and myoglobin (10), as well as being a critical constituent of mitochondrial enzymes and cytochromes that promote oxidative phosphorylation (22). Despite such functional significance, iron deficiency has become the world’s most prominent nutrient disorder (21) and can be categorized into 3 stages of severity:

1) Iron depletion: Iron stores in the bone marrow, liver, and spleen are depleted (serum ferritin [SF] < 35 µg/L; Hb > 115 g/L, transferrin saturation > 16%).
2) Iron-deficient erythropoiesis: Erythropoiesis diminishes as the iron supply to the erythroid marrow is reduced (SF < 20 µg/L, Hb > 115 g/L, transferrin saturation < 16%).

3) Iron-deficient anemia: Hemoglobin production falls, resulting in anemia (SF < 12 µg/L, Hb < 115 g/L, transferrin saturation < 16%).

During physical activity, iron losses can occur from several avenues, including red-blood-cell hemolysis, sweating, hematuria, and gastrointestinal bleeding (1, 3, 13, 24). In addition, female athletes suffer iron losses as a result of menstrual-blood loss (12). As such, athletes are highly susceptible to the development of iron deficiency (24). When an athlete’s serum ferritin levels fall below 35 µg/L, oral iron supplementation (100 mg/d) is generally recommended (14). Full iron repletion as a result of oral iron supplementation, however, will commonly require up to 3 months of treatment, and therefore the more direct method of intramuscular iron injections might be a more attractive means of iron supplementation for an elite athlete with a heavy competition schedule. It has been demonstrated that supplementation is effective in improving the physical performance of iron-deficient anemic individuals (21); however, there is still much debate on the efficacy of supplementation in enhancing physical performance in iron-deficient, nonanemic populations.

Recently, significant increases in running energy efficiency (23), 15-km running performance time (7), and VO$_{2\text{max}}$ (5) have been reported as a result of oral iron supplementation in iron-deficient, nonanemic athletic subgroups, with the proposition that the supplementation might be responsible for an increase in muscle oxidative capacity (5). Despite the positive effect of iron supplementation on serum ferritin levels, a number of studies have shown no effect on athletic performance (2, 4, 8). The exercise-testing regimens of those studies did not, however, incorporate any analysis of exercise economy. Furthermore, Powell and Tucker (17) found no effect of a high-dose, daily iron-supplementation regimen (130 mg elemental iron) on either iron status or athletic performance. This outcome, however, might be a result of the relatively short supplementation period (2 wk) employed during the investigation.

An alternative method of iron supplementation is intramuscular iron injection. Research from our laboratory has shown that this method can significantly increase (by 177%) serum ferritin concentration within 14 d (2), and it has been shown to positively influence the physical performance of iron-deficient, anemic, nonathletic populations (6, 15). When applied to a highly trained, iron-deficient, nonanemic population, however, improvements in physical performance were not seen (2), although the performance tests used primarily investigated anaerobic capacity and did not specifically examine any effects on submaximal economy, VO$_{2\text{max}}$, or time to exhaustion at supramaximal workloads. Hence, it was the purpose of this investigation to examine the effect of iron supplementation through intramuscular iron injection on the VO$_{2\text{max}}$, time to exhaustion, and submaximal exercise economy of iron-deficient, nonanemic female endurance athletes.

**Methods**

**Subjects**

Twenty well-trained female athletes were recruited from Western Australian running, cycling, and triathlon clubs and evaluated for iron status. Subject exclusion
criteria included a current pregnancy or pregnancy within the past year, an infectious illness in the preceding month or a fever in the preceding week, recent iron therapy, current smoking habits, recent blood donation, or severe asthma. After preliminary screening, 16 athletes were identified as iron depleted without anemia (Hb concentration > 115 g/L, SF < 35 µg/L) and recruited as subjects, then randomly assigned to an iron-treatment group (IG) or a placebo group (PG). Their physical characteristics are presented in Table 1. All participants were briefed on the purpose, requirements, and risks of involvement. Written informed consent was signed by all participants, and approval for the study was granted by the Human Ethics Committee of The University of Western Australia.

**Experimental Overview**

The study design incorporated a double-blind, placebo-controlled intervention. After completing initial blood screening, all subjects underwent 3 exercise-testing sessions before receiving a course of 5 × 2-mL intramuscular injections (into the upper quadrant of the gluteus maximus) over a 10-d period. The IG received 2 mL of Ferrum H (Sigma Pharmaceuticals, Australia) containing the equivalent of 100 mg of elemental iron, and the PG received 2 mL of saline solution with each injection. Twenty days after the first injection all subjects had a second blood test for analysis of iron status and repeated the 3 exercise tests. The second round of exercise testing was conducted at the same time of day (± 1 h) as the presupplementation testing; however, subjects were not necessarily in the same phase of the menstrual cycle. Finally, 28 d after the first injection, all subjects had a third and final blood test for iron status.

**Exercise-Testing Sessions**

Before exercise testing, all participants underwent a familiarization session in which they trialed the 3 exercise tests to be used: a steady-state submaximal economy test (~70% VO\(_{2\text{max}}\)), an incremental VO\(_{2\text{max}}\) test to exhaustion, and a time-to-exhaustion test at 100% VO\(_{2\text{max}}\) workload. Subjects were given the choice of completing the exercise tests by either cycling on a modified Monark cycle ergometer (model 814) or running on a motorized treadmill (IG \(n = 6\) running, \(n = 2\) cycling; PG \(n = 6\) running, \(n = 2\) cycling). Participants were encouraged to choose the exercise mode with which they were most familiar. At the conclusion of this session, the constant running speed or cycling workload at which participants could perform

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Iron group ((n = 8))</th>
<th>Placebo group ((n = 8))</th>
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<tbody>
<tr>
<td>Age (y)</td>
<td>25 ± 10</td>
<td>33 ± 7</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170.1 ± 7.0*</td>
<td>161.0 ± 5.3</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>61.41 ± 6.33</td>
<td>56.00 ± 5.73</td>
</tr>
<tr>
<td>Skinfolds(^a) (mm)</td>
<td>87.1 ± 39.2</td>
<td>73.9 ± 24.1</td>
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\(^a\)Sum of 7 sites.

\(^*P < 0.05\)—significantly different from the placebo group.
to elicit approximately 70% of their VO$_{2\text{max}}$ (for the submaximal economy test) was established.

When the subjects arrived at the laboratory for the first exercise test, a resting blood lactate (BLa) sample was taken before they performed a 5-min warm-up at 60% of maximal heart rate (HR) and a 5-min period of static stretching. After this, a continuous 10-min, constant-speed and -load, steady-state, submaximal economy test at approximately 70% VO$_{2\text{max}}$ was performed. During the test, HR and VO$_2$ were continuously recorded, and a BLa sample was taken 1 min postexercise. Subsequently, a 10-min rest period was allowed before a graded exercise test was performed to determine VO$_{2\text{max}}$. The graded exercise test was a continuous test with running-speed increases (1 km/h) or cycling-workload increases (20 W, constant cadence at 80 rpm) each minute until volitional exhaustion. Over the duration of the graded exercise test, HR and VO$_2$ were continuously recorded, and BLa samples were taken pre- and postexercise. The following day, participants reported back to the laboratory to complete a test requiring them to exercise until exhaustion at a work intensity equal to that achieved at VO$_{2\text{max}}$. During this test, VO$_2$ was again continuously recorded, as well as HR and BLa.

**Experimental Procedures**

**Iron Status.** Venous blood samples were drawn from an antecubital vein on each of the 3 occasions that blood screening for iron status was required and analyzed for serum levels of iron, ferritin, transferrin, transferrin receptor, transferrin saturation, and hemoglobin concentration. Serum iron concentration was measured using a BM/Hitachi 917 analyzer (Boehringer Mannheim). Transferrin concentration was determined through immunoturbidimetric assay (Boehringer Mannheim). Quantitative determination of serum ferritin in the sample was done using Chiron Diagnostics automated chemiluminescence system 180 using a 2-site sandwich immunoassay. Serum transferrin-receptor levels were measured with an enzyme immunoassay using the double-antibody sandwich method (Ramco sTfR test kit). The concentration of hemoglobin was determined in EDTA-anticoagulated Haemogard Vacutainers, using a Coulter Counter S880 automated hematology analyzer. Coefficients of variance recorded were 5–10% (serum transferrin receptor), 1.5% (serum iron), 3.3% (serum transferrin), 6.8% (serum ferritin), and 0.8% (hemoglobin concentration). To limit the chance of an acute phase response’s affecting these results, samples were taken at least 12 h after prior exercise.

**Blood Lactate.** Arterialized capillary blood samples (20 µL) were collected from the fingertip and analyzed for BLa concentration via an Accusport blood-lactate analyzer. The regression equation developed by Pinnington and Dawson (16) was applied to produce corrected values.

**Heart Rate.** Throughout all exercise testing sessions, HR was continuously recorded using a Polar heart-rate monitor (Accurex Plus, Finland).

**Oxygen Consumption.** During all trials, expired air was analyzed for concentrations of O$_2$ and CO$_2$ (Ametek gas analyzers, applied electrochemistry, SOV S-3A/1 and COV CD-3A, Pittsburgh, PA). Ventilation was recorded at 15-s intervals via a
turbine ventilometer (Morgan, 225 A, Kent, England). \( \text{VO}_{2\text{max}} \) was determined by summing the 4 highest consecutive 15-s \( \text{VO}_2 \) values.

**Written Inventories.** During the experimental period, all subjects were required to maintain their habitual exercise training and dietary regimens. Over the supplementation period, an exercise inventory was kept for 7 d for an analysis of physical activity patterns. Furthermore, a dietary record was also maintained over a 4-d period (inclusive of 1 weekend day) for analysis of iron and vitamin C intake. The dietary record allowed the athletes to maintain a consistent preexercise testing diet leading into the follow-up testing session. Finally, all participants were required to maintain a record of menstrual activity during the month of the study by tracking the number of sanitary napkins used on each day of menstruation, allowing us to determine a menstrual score as per the methods of Rowland and Kelleher (18).

**Statistical Analysis**

Results are expressed as mean and standard error unless otherwise stated. A MANOVA with repeated measures (treatment and time) was used to detect any significant changes in iron status and exercise-test performance. In the event of a significant main effect, a post hoc, independent \( t \)-test was used to analyze between-group differences. The alpha level was set at \( P \leq 0.05 \).

**Results**

**Hematology and Iron Status**

Hematological and iron-status data are presented in Table 2. At baseline, no significant between-group differences were evident for levels of serum iron, serum transferrin, serum transferrin receptor, or percentage of transferrin saturation (\( P > 0.05 \)). The serum ferritin concentration (SF) of the IG, however, was significantly lower (\( P < 0.05 \)) than the PG (19 ± 3 \( \mu \)g/L vs. 30 ± 2 \( \mu \)g/L). After the course of 5 intramuscular injections, a significant treatment-by-time effect on SF was found (\( P < 0.05 \)). Post hoc analysis showed that the SF of the IG was significantly (\( P < 0.01 \)) increased from baseline to Day 20 (19 ± 3 to 65 ± 11 \( \mu \)g/L) and was also significantly higher (\( P < 0.05 \)) than in the PG (33 ± 4 \( \mu \)g/L). No changes were seen in the PG. On Day 28, the SF remained significantly increased (\( P < 0.01 \)) from baseline in the IG (57 ± 12 \( \mu \)g/L). In addition, the SF of the PG also improved (\( P < 0.05 \)) from baseline (30 ± 2 \( \mu \)g/L to 37 ± 4 \( \mu \)g/L), and no significant difference was now evident between the 2 groups. The SF of the IG, however, was significantly greater than in the PG on Days 20 and 28 (\( P < 0.01 \)) when the percentage change from baseline was considered (276% ± 53% compared with 8% ± 10%, 243% ± 40% compared with 23% ± 8%).

**Steady-State Submaximal Economy Test**

The results of the steady-state submaximal economy test are presented in Table 3. During both the pre- and postsupplementation exercise testing sessions, there were no within- or between-group differences recorded for \( \text{VO}_2 \), respiratory-exchange ratio, \( V_e \), HR, or BLa in either of the 2 groups (\( P > 0.05 \)).
The results for the incremental VO\textsubscript{2max} test are presented in Table 4. At baseline, no significant differences were recorded between the 2 groups (\(P > 0.05\)). Postsupplementation, there were again no within- or between-group differences between the IG and the PG for any of the variables measured (\(P > 0.05\)).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Iron Group</th>
<th>Placebo Group</th>
</tr>
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<tbody>
<tr>
<td>VO\textsubscript{2} (L/min)</td>
<td>1.98 ± 0.09</td>
<td>2.04 ± 0.07</td>
</tr>
<tr>
<td>VO\textsubscript{2} (mL·kg\textsuperscript{-1}·min\textsuperscript{-1})</td>
<td>32.3 ± 1.9</td>
<td>36.2 ± 1.5</td>
</tr>
<tr>
<td>% VO\textsubscript{2max}</td>
<td>69 ± 1</td>
<td>70 ± 1</td>
</tr>
<tr>
<td>Respiratory-exchange rate</td>
<td>0.97 ± 0.01</td>
<td>0.94 ± 0.01</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>152 ± 3</td>
<td>144 ± 2</td>
</tr>
<tr>
<td>Lactate 1 min post (mM)</td>
<td>1.7 ± 0.3</td>
<td>1.4 ± 0.2</td>
</tr>
</tbody>
</table>

Incremental VO\textsubscript{2max} Test

The results for the incremental VO\textsubscript{2max} test are presented in Table 4. At baseline, no significant differences were recorded between the 2 groups (\(P > 0.05\)). Postsupplementation, there were again no within- or between-group differences between the IG and the PG for any of the variables measured (\(P > 0.05\)).

Time to Exhaustion at VO\textsubscript{2max} Workload

The results for time to exhaustion at VO\textsubscript{2max} workload are presented in Table 5. Before supplementation there were no differences between the test performances of the 2 groups (\(P > 0.05\)). After supplementation, no between-group differences
were evident for VO_{2peak}, total VO_{2}, or peak HR. In contrast, the postsupplementation total time to exhaustion was significantly increased in the IG (P < 0.05); however, this difference was not significant between the 2 groups (P > 0.05). In addition, the BLa levels recorded at 1 min and 5 min postexercise were significantly greater in the IG postsupplementation (P < 0.05), but this change in BLa was not different between groups (P > 0.05).

### Written Inventories

No differences were evident between the 2 groups’ estimated dietary energy intake (mean ± standard deviation; IG 8821 ± 2138 kJ/d, PG 8666 ± 2025 kJ/d) or total iron intake (IG 14.1 ± 5.8 mg/d, PG 14.4 ± 7.3 mg/d) from the dietary inventories they kept. From the exercise-training inventories, no differences were evident between the 2 groups in the number of exercise sessions completed each week (IG 7 ± 1, PG 6 ± 1) or in the average time spent exercising each day (IG 132 ± 44 min, PG 119 ± 41 min). No differences were recorded between the 2 groups for duration of menstrual flow (IG 5 ± 1 d, PG 4 ± 1 d) or the number of sanitary-napkin changes during the days of heaviest flow (IG 6.2 ± 1.8 napkins, PG 5.0 ± 2.2 napkins).
Discussion
The present study found that a course of intramuscular iron injections (5 × 2 mL; Ferrum H) over a 10-d period significantly increased SF from baseline levels at Day 20 and Day 28 postsupplementation in iron-deficient, nonanemic female athletes. During the testing and intervention periods, no between-group differences were found for estimated dietary iron consumption, weekly training volume, or menstrual activity, according to the written inventories.

Despite the improvements in SF, the intramuscular iron injections had a negligible effect on improving the economy, HR, and BLA recorded during a 10-min steady-state submaximal exercise test. Such findings contrast with those of Zhu and Haas (23), who showed a lower energy expenditure (2.0 kJ/min) during a 15-km cycling time trial as a result of 8 wk of oral iron supplementation when compared with a placebo-controlled group. Such an improvement might be explained by changes in Hb concentration. Although the Hb levels of the iron-treatment group in the Zhu and Haas study were not affected by iron supplementation, a closer analysis of the results shows that the Hb levels of the placebo group were significantly lower than baseline at the conclusion of the 8-wk intervention. Hinton et al. (7) found that total energy expended during a 15-km time trial was significantly and negatively associated with changes in Hb concentration. These authors suggested that for every 1-g/L increase in Hb, there was a 1.04-kcal decrease in energy expended. Using such a theory, the decrease in the placebo group’s Hb concentration from the Zhu and Haas (23) study was then equivalent to 5.1 g/L, or 5.304 kcal. It should also be recognized that the energy expenditure of the iron-supplemented group was not significantly different from baseline, but only different from that attained by the placebo group. Hence, the iron supplementation of the treatment group did not enhance exercise economy but actually prevented the decline in economy seen in the placebo group. In addition, the time-trial session of Zhu and Haas (23) was conducted at a greater intensity (~83% VO\textsubscript{peak}) than the submaximal test of the current investigation and for a longer duration of time (~30 min). Hence, it is possible that the beneficial effects of iron supplementation on exercise economy occur as the intensity and duration of exercise increases, and therefore a 10-min steady-state submaximal exercise test at ~70% VO\textsubscript{2max} might not be specific enough to detect performance-economy changes.

The present study also showed no significant improvements in VO\textsubscript{2max} after iron supplementation via intramuscular injection. Our results support those of previous investigations (8, 19, 23) that reported no significant differences in VO\textsubscript{2max} between treatment and placebo groups, despite finding treatment-group increases in SF over a supplementation period ranging from 8 to 12 wk. In contrast, Magazanik et al. (11) showed a 7.5% increase in VO\textsubscript{2max} after 21 d of iron supplementation (160 mg of ferrous sulphate per day), which was significantly greater than that seen in their placebo-controlled group. Magazanik et al. (11) also found, however, a concurrent significant improvement in Hb concentration, suggesting that the athletes might have been anemic at the beginning of the study. Because there were no changes in the Hb concentration of the current investigation’s IG, an improvement in VO\textsubscript{2max} would not be expected, and the results of Magazanik et al. (11) are not strictly relevant to athletes in the initial stages of iron deficiency.
The postsupplementation time-to-exhaustion test at VO\textsubscript{2max} workload used in our study showed a significant increase in the duration of exercise in the IG, but it was not significantly different between groups. These results agree with those of Klingshirn et al. (8), who showed no significant between- or within-group differences in the run time to exhaustion of a placebo and an iron-supplemented group. The significant exercise-duration increase seen in the IG was reflected by the increased BLa concentration at both 1 and 5 min postexercise. This increased BLa indicates that the subjects of the IG were able to work at a greater intensity than during presupplementation testing in order to achieve the longer exercise duration, whereas the PG maintained the same work intensity. Such an outcome contrasts with that of Lamanca and Haymes (9), who showed a significantly reduced BLa concentration at the conclusion of a run to exhaustion at 80% VO\textsubscript{2max} after 8 wk of iron supplementation. Their subjects also showed a significant Hb-concentration increase, however, potentially suggesting that the oxygen-delivery system of these subjects had improved, thereby reducing the anaerobic contribution to the work performed during the test to exhaustion.

The SF changes seen in the IG were significantly greater than those of the PG at Day 20. As a result of lower IG baseline SF levels, however, this difference was not significant at Day 28. To compensate for the baseline differences, we investigated the percentage change in SF from baseline. As a result, the IG percentage change in SF from baseline after supplementation was significantly greater than that of the PG on both Day 20 and Day 28. These results are in agreement with previous findings (2) that showed a 177% increase in SF as a result of a similar supplementation protocol. Furthermore, the present study supports the notion that intramuscular iron injections might increase SF at a faster rate than oral supplementation (2), because the percentage change in SF is comparable to that seen in a range of studies (5, 7, 23) using daily oral supplementation of 100–200 mg of elemental iron over a 6- to 12-wk period (SF increases averaging ~189%).

Previous studies dealing with athletes and iron depletion have used SF cutoff values of 12–40 µg/L to characterize iron deficiency (2, 4, 7, 8), yet no universally accepted guidelines exist. Therefore, the present study measured serum transferrin-receptor (sTfR) concentration because it is a reliable means of assisting in the diagnosis of iron deficiency (20). To our knowledge, this is the first investigation to have explored the effect of intramuscular iron injections on sTfR concentration. Previous studies have shown significant treatment-group sTfR-concentration decreases concurrent with significant increases in SF in response to oral iron supplementation ranging from 6 to 8 wk. Furthermore, the changes recorded in these markers were significantly different from a placebo group (7, 23). In contrast, the present study showed no significant within- or between-group changes in the concentration of sTfR in response to the course of intramuscular iron injections. These contrasting results might be a result of the total iron dosage over the supplementation period. The decrease in sTfR concentration shown by Hinton et al. (7) and Zhu and Hass (23) resulted from a total elemental-iron ingestion of 840 mg and 7650 mg, respectively, whereas the total elemental iron administered in the present study was 500 mg. The Week 3 data of Hinton et al. (7) show that at that point only 420 mg of elemental iron had been ingested, with no significant sTfR changes apparent in the treatment group. Furthermore, by Week 4 in the Zhu and
Haas (23) investigation, the treatment-group sTfR concentration was significantly lower than at baseline but was not significantly different from that of the placebo group. Therefore, it is possible that the effect of iron supplementation on sTfR concentration is a time- or dose-dependent relationship. Alternatively, the lack of change in sTfR concentration in response to the course of intramuscular iron injections might suggest that the sTfR levels of Stage 1 iron-deficient athletes are within the normal range and that the cellular requirements for iron in this specific population are not yet compromised. As such, a detriment to exercise performance might not be expected until an athlete reaches the subsequent stages of iron deficiency (Stages 2 and 3).

Conclusion

The findings of the present study show that a course of intramuscular iron injections (5 × 2 mL; Ferrum H) over a 10-d period provided a significant increase in SF from baseline levels to those seen at Day 20 and Day 28. Furthermore, it appears that intramuscular iron injections might increase SF at a faster rate than oral supplementation. Nonetheless, sTfr was not elevated, and despite the improvement in ferritin levels, exercise performance (submaximal and maximal) was not affected by marginal iron deficiency (Stage 1: iron depletion) when Hb is within the normal range. No significant positive effects in steady-state submaximal VO\textsubscript{2}, VO\textsubscript{2max}, or time to exhaustion at a VO\textsubscript{2max} workload were demonstrated after significant increases in body iron stores. Despite the lack of performance enhancement, measures to improve iron intake or iron supplementation in iron-depleted nonanemic female athletes should not be unduly discouraged, because these measures might act to prevent the deficiency from progressing to anemia.

References


