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Conflict of interest disclosure

The authors declare no competing financial interests.

Abstract

Purpose: Iron deficient athletes are often treated with long term, low dose iron therapy. Such treatments may be efficacious in correcting iron deficiency, however the effect on acute and chronic iron metabolism and subsequent endurance capacity is less clear. **Methods:** Fifteen national and international standard runners were identified as iron deficient non-anaemic (IDNA) and assigned to either an intravenous iron treatment or placebo group. Participants completed 3 exercise tests to volitional exhaustion; before treatment, within 24 h and 4 weeks after treatment. **Results:** Serum ferritin, serum iron, transferrin saturation were significantly improved in the iron group following intervention and compared to placebo ($p < 0.05$). Hepcidin levels were significantly greater before and after exercise following the iron injection ($p < 0.05$) and this was independent of changes in interleukin-6. There were no differences between groups in red cell indices, total haemoglobin mass, $\dot{V}O_{2max}$, sub-maximal blood lactate, running economy, ratings of perceived exertion or time to exhaustion ($p > 0.05$). **Conclusion:** A single 500 mg intravenous iron injection is effective for improving iron status for at least 4 weeks but this does not lead to an improved aerobic capacity. This investigation suggests that iron availability supersedes inflammation in the regulation of hepcidin in IDNA endurance athletes following acute intravascular iron injection treatment.

Key Words: Endurance, VO_{2max}, haemoglobin, hepcidin, ferritin, interleukin-6

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Introduction

Iron deficiency is commonly identified in endurance runners due to prolonged training and repeated ground impacts causing iron losses through a number of potential mechanisms (1, 37). Iron deficiency is associated with oxidative stress (16), impaired mitochondrial electron transport and protein synthesis (1) and can lead to compromised erythropoiesis (iron deficient anaemia, IDA; 35). It is well established that when iron deficiency causes IDA it leads to impaired oxygen carrying capacity and endurance performance (8). Although, the effect of iron deficiency non-anaemia (IDNA) is less clear, iron treatment in the form of either oral iron supplementation or parenteral iron are appealing interventions for endurance athletes with IDNA.

Iron therapy is widely reported to be effective at replenishing body iron stores (3, 8, 9, 11, 24, 40), and in some reports has led to improvements in $\dot{V}O_{2max}$ (9), sub-maximal cycling efficiency and time trial performance (11), fractional utilisation of peak $\dot{V}O_2$ (40) and total haemoglobin mass (tHb-mass; 9) in IDNA endurance athletes. However, the restoration of iron stores does not guarantee an improvement in aerobic capacity or indices of endurance performance (3, 11, 24). Moreover, improvements in iron status following treatment are often transient, with observations of serum ferritin (sFer) returning to pre-treatment levels within weeks (23), the cause of which is currently unknown. Previous investigations into iron supplementation have largely described the outcome of the intervention but have not addressed the regulation of iron metabolism or the impact that the iron treatment may have upon it.

The peptide hormone hepcidin is the master regulator of iron metabolism. Hepcidin controls iron absorption from the diet and the export of iron from hepatocytes and macrophages (18). Regulation of hepcidin is strongly mediated by iron availability, inflammation and hypoxia (20), stimuli that endurance athletes may be regularly exposed to. The link between hepcidin and

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inflammation (7) has been established in both mice (20) and human models (17, 18). Indeed, exercise induced increases in the inflammatory marker interleukin-6 (IL-6) have preceded increases in hepcidin in active (19), trained (25) and well trained runners (26, 33). However, elite athletes may experience less of an inflammatory response following exercise than those who are less well trained due to adaptation (12), which may hypothetically alter the hepcidin response in this population. In addition, post-exercise hepcidin is correlated with baseline sFer but not IL-6, suggesting that iron availability supersedes inflammation in the regulation of hepcidin in individuals who are iron deficient (26). Therefore, the influence of iron availability on hepcidin is a key factor in the regulation of iron metabolism in iron deficient endurance athletes, yet it is currently not known how treating iron deficiency with intravenous iron will influence hepcidin and iron metabolism in elite endurance athletes.

The aim of this study was firstly to characterise the response of hepcidin and iron metabolism to iron treatment in the form of a single, high dose intravascular injection. Furthermore, given the lack of consensus on the value of iron repletion at improving endurance performance in previous studies, a second aim was to test the hypothesis that the iron injection would enhance aerobic capacity in IDNA elite distance runners.

Materials and methods

Participants

National and international standard endurance runners from the London Marathon endurance performance group at St Mary's University, London received a full blood test for assessment of red cell indices and iron status. Of 45 athletes screened, 15 (9 female, age 21 ± 2 years; height 169.1 ± 4.0 cm; body mass 59.3 ± 4.3 kg; $\dot{V}O_{2max}$ 64.5 ± 5.7 ml·kg^{-1}·min^{-1}; 6 male,

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age 20 ± 1 years; height 178.8 ± 5.9 cm; body mass 65.2 ± 3.5 kg; $\dot{V}O_{2max}$ 76.7 ± 2.7 ml·kg^{-1}·min^{-1}) were classified as iron deficient but not anaemic (females: sFer < 30.0 μg·L^{-1}, Hb > 12.0 g·dL^{-1}; males: sFer < 40 μg·L^{-1}, Hb > 12.0 g·dL^{-1}). It is acknowledged that there is no universal consensus for the classification of IDNA in athlete populations, with a variety of cut-offs for sFer appearing in the literature (3, 9, 29). An upper limit of 40 μg/L is used in the present investigation for males as this is considered iron deficient by the national governing body for Track and Field to which the athletes are affiliated, and whereby medical practitioners would treat and monitor the iron status of each athlete. All participants gave written informed consent prior to participating in the study, and met the exclusion criteria of current or recent pregnancy (12 months), recent illness or blood donation. The study protocol was approved by both the National Health Service (NHS; 12/LO/0513) and the St Mary's University ethics committees. Participants were instructed not to deviate from their normal dietary practices, to refrain from iron supplementation throughout the study and to attend all laboratory trials in a well-hydrated state, at the same time of day, and to refrain from strenuous exercise during the preceding 24 hours (h).

Experimental Overview

The study used a double-blind, randomised controlled design. Participants were assigned to either an iron treatment group (*n* = 7) or placebo group (*n* = 8), ensuring males and females were split evenly between groups and completed three discontinuous incremental treadmill tests to volitional exhaustion. Treadmill test one was performed prior to receiving either an intravenous iron injection (500 mg Ferinject, Vifor Pharma Ltd, Opfikon, Switzerland) or placebo injection (0.9% sterile saline solution), carried out in hospital conditions and under the

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supervision of a doctor. Treadmill test 2 was completed 7 days later and within 24 h of the participants receiving the treatment; and treadmill test 3 took place 4 wks post treatment. Venous blood samples were taken immediately before, immediately after, 3 hrs after and 24 hrs after each treadmill test for the assessment of sFer, serum iron (sFe), transferrin (sTf), transferrin saturation (Tsat), soluble transferrin receptor (sTfR) and hepcidin. Additionally, measurements of tHb-mass were taken after each of the treadmill tests.

Treadmill Tests

The treadmill test consisted of two parts: 1) a discontinuous incremental test of 3 minute stages, beginning at an initial predetermined intensity of 12-15 $km \cdot h^{-1}$ and increasing by 1 $km \cdot h^{-1}$ at the start of each stage until blood lactate concentration exceeded 4 $mMol \cdot L^{-1}$. Participants completed an average of 6 stages (range 5-7). Running was interrupted for 30 s at the end of each stage to allow for earlobe capillary blood sampling, whereby 20 μl of blood was obtained and immediately dropped into haemolysing solution for subsequent analysis of blood lactate using an automated analyser (Biosen C-Line, EFK Diagnostic, Barleben, Germany). Heart rate was recorded continuously throughout the test at 5 s intervals (Polar S610, Polar electro, Kempele, Finland); 2) Following a 10 min active recovery period of treadmill walking at a self-selected speed, subjects completed a further incremental test for the determination of $\dot{V}O_{2max}$, which consisted of 1% gradient increases each minute at a constant running speed (1-2 $km \cdot hr^{-1}$ below the running speed during the final stage of part one) until volitional exhaustion. Oxygen uptake ($\dot{V}O_2$), expired carbon dioxide ($\dot{V}CO_2$) and ventilation ($\dot{V}E$) were sampled and measured continuously throughout both parts of the treadmill test using an on-line breath-by-breath analyser (Oxycon Pro, Jaeger, Hoechberg, Germany). The gas analyser was calibrated before

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each test using oxygen and carbon dioxide gases of known concentrations (Cryoservice, Worcester, UK) and the flow sensor was calibrated using a 3-litre syringe (Viasys Healthcare GmbH, Hoechberg, Germany) according to manufacturers instructions. Sub-maximal $\dot{V}O_2$ was determined via a final minute average for each stage. $\dot{V}O_2$max established via a rolling 30 s average, after eliminating values that are outside four standard deviations of the midpoint of a rolling 20 breath mean (attributed to 'noise'; 30). Rate of perceived exertion (RPE; 4) was assessed during the 30s interruption for blood sampling, by asking participants to point to a number on a scale from 6 (no exertion) to 20 (maximal exertion) in order to rate their overall effort over the previous 3 minute running bout.

Iron Treatment

Athletes were randomised to iron treatment or placebo injection by a researcher blinded to the treadmill test data. Athletes were blinded to the intervention received. The iron or placebo injection was given by slow intravenous injection over 10 minutes through covered syringe and tubing and the athlete's arm was shielded from vision, perfomed at a hospital location (Hospital of St John and St Elizabeth, London). The researcher (NP) who performed the iron injection did not disclose which intervention the athlete had received to the physiology testers (RB and CP) until the end of the trial period.

Haematology and Biochemistry

Venous blood was collected via venepuncture of an anticubital vein in the forearm. A 10 ml SST gel Vacutainer (BD, Oxford, England) was filled and allowed to clot for 30 min at room temperature. The sample was then centrifuged at 4 °C and 3000 rpm for 10 min. Serum was then

drawn off by syringe and divided into 1 ml aliquots and immediately frozen at -80 °C until analysis. Serum iron (sFe), sFer and transferrin (sTf) were assayed on the Architect ci6200 platform (Abbott Diagnostics Ltd, Maidenhead, UK), with inter and intra-assay coefficients of variation (CV) of 5% and 6% respectively. Serum iron was measured using a colourmetric method, sFer was measured using a solid-phase; two-site chemiluminescent immunometric assay (ICMA), and sTf was measured using a turbidimetric method. Transferrin saturation (Tsat) was calculated using the following formula

$$Iron \times 100/transferrin \times 12.57 \times 2$$

Hepcidin was measured using an in-house immunoassay with antibodies specific for human hepcidin-25 (5). Soluble transferrin receptor (sTfR) and IL-6 were measured using commercial enzyme immunosorbent immunoassays (ELISA) using two specific monoclonal antibodies (R&D Systems, Abingdon, UK).

Blood Volume and Total Haemoglobin Mass

Blood volume and tHb-mass were assessed and calculated using the optimised carbon monoxide (CO) rebreathing method described by Schmidt and Prommer (31). Briefly, a bolus of a known dose of CO (0.8-1.0 ml·kg^{-1}) was rebreathed for 2 min using a specifically designed spirometer (Bloodtec, Bayreuth, Germany). Capillary blood samples were taken from an earlobe prior to and at 6 and 8 min after rebreathing began, and analysed for carboxyhaemoglobin (%HbCO) using spectrophotometry (OSM-80, Radiometer, Copenhagen, Denmark). CO not taken up by the subject was calculated using the volume of the rebreathing bag and the CO concentration left in it using a hand held CO gas analyser (Pac700, Drager, Lubeck, Germany). Seven minutes after commencing rebreathing end tidal CO concentration was measured using the

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same CO gas analyser. tHb-mass and blood volume were then calculated using SpiCO measurement software (Bloodtec, Bayreuth, Germany). All measurements were taken 1 h following completion of the treadmill tests, with participants in a seated position 15 min prior to and throughout the procedure.

Statistical Analysis

Data were analysed using the Statistical Package for the Social Sciences (SPSS, Inc., Chicago, IL, USA). Measures of central tendency and spread are presented as mean ± *SD*. Sub-maximal $\dot{V}O_2$, and blood lactate ([La]) during the three treadmill tests and changes in sFer, sFe, sTf, Tsat, sTfR and hepcidin were analysed using a mixed analysis of variance (MANOVA) with repeated measures (Treatment condition x measurement time point). A one-way analysis of variance (ANOVA) was used to analyse the effect of the iron treatment on tHb-mass, blood volume and $\dot{V}O_{2max}$. When the assumption of sphericity was violated, the Greenhouse-Geisser correction was used. In the event of a significant outcome a *post-hoc* test with Bonferroni adjustments was applied to identify where differences lay. The level of significance was set at $p < 0.05$ for all analyses. In order to provide a more insightful interpretation of the data, the actual *p* value is given for significant outcomes.

Results

Iron Indices

There were no significant between-group differences prior to treatment (baseline) in sFer, sFe, sTf, Tsat, sTfR, Il-6 or hepcidin (figures 1, 2, 3 and table 1). After treatment a significant main effect of iron treatment group was revealed for sFer (*p*=0.001). Significant effects of time

point (p=0.001) and time point x treatment group interaction (p=0.001) revealed that sFer increased within 24 h of the iron injection in the iron group and this was maintained during all remaining time points up to 4 weeks after treatment (figure 1). Serum ferritin did not change in the placebo group.

Prior to treatment no significant main effect of treatment group (p=0.995) or treatment group x time point interaction (p=0.943) was observed for hepcidin. However, there was a significant main effect of time point (p=0.009) but *Post hoc* tests were unable to identify where the effects of time point were. Twenty-four hours after the iron injection a significant main effect of treatment group was observed for hepcidin (p=0.004), with the iron group having significantly higher hepcidin values at all time points compared to the placebo group (figure 2). There was also a significant effect of time point (p=0.016) but no significant time point x treatment group interaction (p=0.398). The Bonferroni adjustments were unable to identify where the significant time point effects lay. Four weeks after the treatment there were significant main effects of treatment group (p=0.009), time point (p=0.004) and time point x treatment group interaction (p=0.033), with the iron group having a significantly higher hepcidin response 3 h post-exercise.

Interleukin-6 increased immediately after each exercise test in both the iron and placebo groups (p=0.001; figure 3) but there were no treatment effects (p=0.659) or significant time point x treatment group interactions (p=0.569). Serum iron (p=0.001) and Tsat (p=0.001) were significantly affected by treatment and both measures observed significant time point (p=0.001) effects and treatment group x time point interactions (p=0.001). Following the iron injection both sFe and Tsat (table 1) increased significantly within 24 h compared to the placebo group but by 4 weeks after both had returned to pre-treatment levels.

Serum transferrin was not affected by treatment (p=0.150) and there was no treatment group x time point interaction (p=0.174). A significant effect of time point (p=0.001) was observed revealing an increase in sTf in both groups immediately after the pre-treatment treadmill test when compared to pre-test levels (table 1). There was a significant effect of treatment group on sTfR (p=0.014) but no significant effect of time point (p=0.121) or a significant treatment group x time point interaction (p=0.163; table 1).

Red Cell Indices & Treadmill Data

Red cell indices and treadmill data are presented in tables 2 and 3 respectively. There were no significant differences between groups in red cell indices, $\dot{V}O_{2max}$, $v\dot{V}O_{2max}$, economy, speed at 2 mmol·L [La], speed at 4 mmol·L [La], time to exhaustion, RPE or tHb-mass at baseline or following iron treatment (p>0.05).

Discussion

Iron treatments are clearly effective at improving the iron status of IDNA endurance athletes in the short-term, however the improvements in sFer levels are often transient and return to iron deficient levels within weeks (23). Previous investigations into iron supplementation have largely described the outcome of the intervention but have not addressed the regulation of iron metabolism or the impact that the iron treatment may have upon it. The peptide hepcidin is proposed to be the major regulator of iron absorption (18) and is influenced by inflammation, iron and hypoxia (20); these are stimuli with clear relevance to endurance athletes. Following an inflammatory response, possibly caused by exercise, hepcidin increases, blocking iron absorption and iron release from macrophages (18). Conversely, during periods of iron deficiency or

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hypoxia, hepcidin is down regulated to allow for increased iron release and absorption. In the present investigation, prior to treatment, there was a significant effect of time and although the statistical analysis was unable to confirm which pre-treatment time point this related to, the effect is likely to have occurred 3 h after exercise, which is in line with reports from previous investigations in iron replete recreationally active (19) and trained athlete populations (26, 33); a likely result of an exercise induced inflammatory response and significant increases in IL-6 (19, 25).

The major finding, however, was the observation that when IDNA athletes are treated with intravenous iron the significant increase in sFer results in a post-exercise increase in hepcidin that is independent of changes in IL-6 (figure 2 & 3). A recent study suggested the post-exercise hepcidin response is mediated by resting sFer levels, with low iron stores overriding inflammation resulting in attenuated hepcidin (26). Our data support the assertion that the level of iron stores supersedes inflammation in the regulation of hepcidin, with greater responses evident as the sFer levels were increased. However, the absence of a hepcidin response following exercise in individuals with sFer values commonly associated with IDNA (i.e. sFer <30 μg/L), was not seen here. Significant increases in hepcidin occurred after exercise at 24 h and 4 weeks after intravenous iron treatment (figure 2), yet there were no significant differences in IL-6 between groups at any time point pre- or post-treatment (figure 3). This coincided with significant elevations in sFer (figure 1), sFe and Tsat (table 1) within 24 h of treatment and the maintenance of an elevated sFer at 4 wks post-treatment. The up-regulation of hepcidin, independent of inflammation has been shown in other settings (22, 26, 27) but this is the first study to show this interaction and the role hepcidin plays in iron metabolism in response to intravenous iron treatment in elite IDNA endurance athletes. Furthermore, the changes in

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hepcidin regulation and iron metabolism following intravenous iron treatment had no effect on red cell indices or surrogate measures of endurance capacity, rejecting the hypothesis that an iron injection would improve the aerobic capacity of IDNA endurance athletes.

The mechanism by which iron mediates hepcidin is currently not clear but hepcidin expression has shown good correlation with increased sFe and Tsat in iron-loaded mice (15). Talbot et al. (36) reported a suppression of hepcidin in iron replete individuals during a sojourn to altitude, which was overcome by prior iron loading. The decrease in hepcidin occurred until Tsat, but not sFer, had normalised. Indeed, in the current investigation Tsat and sFe were significantly elevated 24 h post iron treatment. However, this does not explain the hepcidin response to exercise 4 wk after iron treatment because sFe and Tsat had returned to pre-treatment levels. The up-regulation of hepcidin has previously been reported to occur in times of iron overload in healthy, non-endurance trained, volunteers following oral iron supplementation of 65 mg per day for 3 days (18). The increased hepcidin response in the IG following exercise at 24 h and 4 wks post-iron treatment in the present study coincided with significantly increased sFer. Ferritin has been shown to regulate the signalling molecule bone morphogenetic protein 6 (BMP6) in mice, thereby serving as an iron store regulator, mediating hepcidin expression in response to iron overload (6). It is plausible that despite the athletes having initial sFer levels well below the clinical criteria for IDNA (24), this actually represents a state of normal iron metabolism for this population. The substantial increase in iron after the injection may therefore have caused an unnecessary increase in iron availability, reminiscent of iron overload, and the stimulation of hepcidin in order to prevent further iron absorption. Moreover, sFer is reported to correlate with markers of hydroxyl radical formation, an indicator of oxidative damage (38). It is argued that sFer originates from cell damage (13) and does not contain its full compliment of

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iron (21), which suggests that the lost iron initiates oxidative stress. It is possible that the IL-6 independent increases in hepcidin following exercise observed in the present study are also in response to oxidative stress, although we do not have any data to support this contention.

Soluble transferrin receptor is considered to be an accurate marker of iron deficiency (2) and benefits from being unaffected by exercise-induced inflammation (35). Previously, oral iron supplementation in IDNA subjects has resulted in a significant decrease in sTfR accompanied by increases in sFer (40). Conversely, sTfR was reportedly unchanged in female IDNA endurance athletes following an iron injection (24). The present investigation also found no significant changes in sTfR in either the iron or placebo group following treatment, despite increases in sFer in the iron group. The lack of change in sTfR in response to the iron injection might suggest that sTfR levels in IDNA individuals are within the normal range and despite low sFer, iron requirements and availability are not yet compromised. Furthermore, the lack of an impact upon $\dot{V}O_{2max}$, $v\dot{V}O_{2max}$, running economy, [La] at sub-maximal running speeds, TTE, RPE, tHb-mass (table 3) or red cell indices (table 2) following the iron injection provides additional evidence that low sFer levels prior to iron treatment do not lead to any negative outcomes, notwithstanding the limitations of the present study. Moreover, the current evidence raises the possibility that clinical ranges for sFer are redundant in endurance athletes, particularly when used as a single marker of iron storage. The lack of any improvement in aerobic capacity and surrogate measures of endurance performance in the present study is in contrast to a recent investigation which reported likely improvements in tHb-mass and $\dot{V}O_{2max}$ following iron injection but not oral supplementation (9). However, the increases in tHb-mass observed by Garvican et al. (9) following iron injection (2.7% after 6 weeks & 1.9% after 8 weeks) fall close to and within the typical measurement error associated with CO rebreathing (2.2%; 10). Additionally, the average

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oscillation for tHb-mass in athletes over a year is 4.6% (28), which suggests it may be difficult to associate very small changes in tHb-mass to intravenous iron treatment.

The present study provides further evidence that the up-regulation of the post-exercise hepcidin response following iron treatment represents a major consideration when prescribing iron treatments for IDNA athletes. It is accepted that the up-regulation of hepcidin inhibits iron absorption from the diet and iron release to the circulation (14, 18) and the rise in hepcidin observed following an acute exercise bout appears to be greater following IV iron treatment, as demonstrated in the current investigation. This effect may inadvertently result in a reduction in iron absorption, which if continued over a prolonged period, may be contrary to the intended goal of enhancing the iron status of the athlete. Further in depth pharmacokinetic studies may be necessary to evaluate the mechanistic relationship between hepcidin, sFer and IV iron absorption. One consideration is the dose protocol utilised. It is possible that the single dose of 500 mg used in the current investigation was too high and the increased hepcidin response was the consequence, whereas a smaller dose may not have elicited a response of such magnitude, potentially causing less disruption to iron homeostasis. It would be beneficial for future research to focus on the long-term hepcidin response to iron treatment and the dose – response relationship in markers of iron status. In addition, a previous investigation has proposed an sFer cut-off value of <30 μg/L to classify IDNA based on the lack of a post-exercise response in this population (26). Although these are useful data to begin to determine a precise classification, which is currently elusive, confounding factors in the study are apparent, such as a variety of exercise intensities and durations used; a small number of non-elite athletes and the individual variation in the sFer/hepcidin interaction. Indeed, substantial hepcidin responses were observed in the present investigation in some individuals despite sFer values <30 μg/L. Therefore, caution

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is warranted and the individual variability in the hepcidin response needs to be considered, particularly if using a sFer cut-off of <30 μg/L as a basis for the diagnosis and treatment of IDNA in elite athletes.

The present investigation is limited by a lack of dietary control. The athletes were instructed to continue with their regular dietary practice and therefore there was no control of iron intake from food sources. However, in practice it is unlikely that athletes at this level of performance would deviate dramatically from their normal diet at any one time and vast changes would be required to significantly alter total iron intake during the time course of this study. Another limitation is the potential impact that menstrual cycle phase can have on indices of iron metabolism (34) and again, this was not controlled. However, although iron indices may be lower during menses, $\dot{V}O_{2max}$, tHb-mass and the other measures of endurance capacity are unlikely to be affected by menstrual cycle phase (39). The training that the athletes undertook on a daily basis was also not under the control of this investigation, other than the standard pre-test requirement to reduce training intensity in the 24 hours prior to treadmill testing, which could have potentially influenced aerobic capacity. However, tHb-mass remains relatively stable in athletes of a highly trained status (32) and the measures of iron metabolism are also unlikely to be affected.

Conclusion

Iron treatment in the form of a single 500 mg dose of parenteral ferric carboxymaltose significantly raised sFer within 24 h and this was maintained for 4 weeks after treatment. However, the hypothesis that improved iron status would result in the enhancement of aerobic capacity and indices of endurance capacity is rejected, as there were no significant effects on

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$\dot{V}O_{2max}$, sub-maximal [La], running economy, RPE or TTE. Additionally, this investigation revealed for the first time that iron availability supersedes inflammation in the regulation of hepcidin in IDNA endurance athletes following acute intravascular iron injection treatment.

Conflict of interest disclosure

Acknowledgements

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Figure Legends

Figure 1. Serum ferritin values for the Iron group (A) and Placebo group (B) measured pre-treadmill test, immediately post-treadmill test and three hours post-treadmill test. Measurements were replicated pre-treatment, 24 hours post-treatment and 4 weeks post-treatment. * Significant difference ($p < 0.05$) between iron and placebo groups; † significant difference ($p < 0.05$) between baseline and post-treatment time points.

Figure 2. Hepcidin values for the Iron group (A) and Placebo group (B) measured pre-treadmill test, immediately post-treadmill test and three hours post-treadmill test. Measurements were replicated pre-treatment, 24 hours post-treatment and 4 weeks post-treatment. * Significant difference ($p < 0.05$) between iron and placebo groups; † significant difference ($p < 0.05$) between pre-exercise and post-exercise time points.

Figure 3. Interleukin-6 values for the Iron group (A) and Placebo group (B) measured pre-treadmill test, immediately post-treadmill test and three hours post-treadmill test. Measurements were replicated pre-treatment, 24 hours post-treatment and 4 weeks post-treatment. * Significant difference ($p < 0.05$) between iron and placebo groups; † significant difference ($p < 0.05$) between baseline and post-treatment time points.
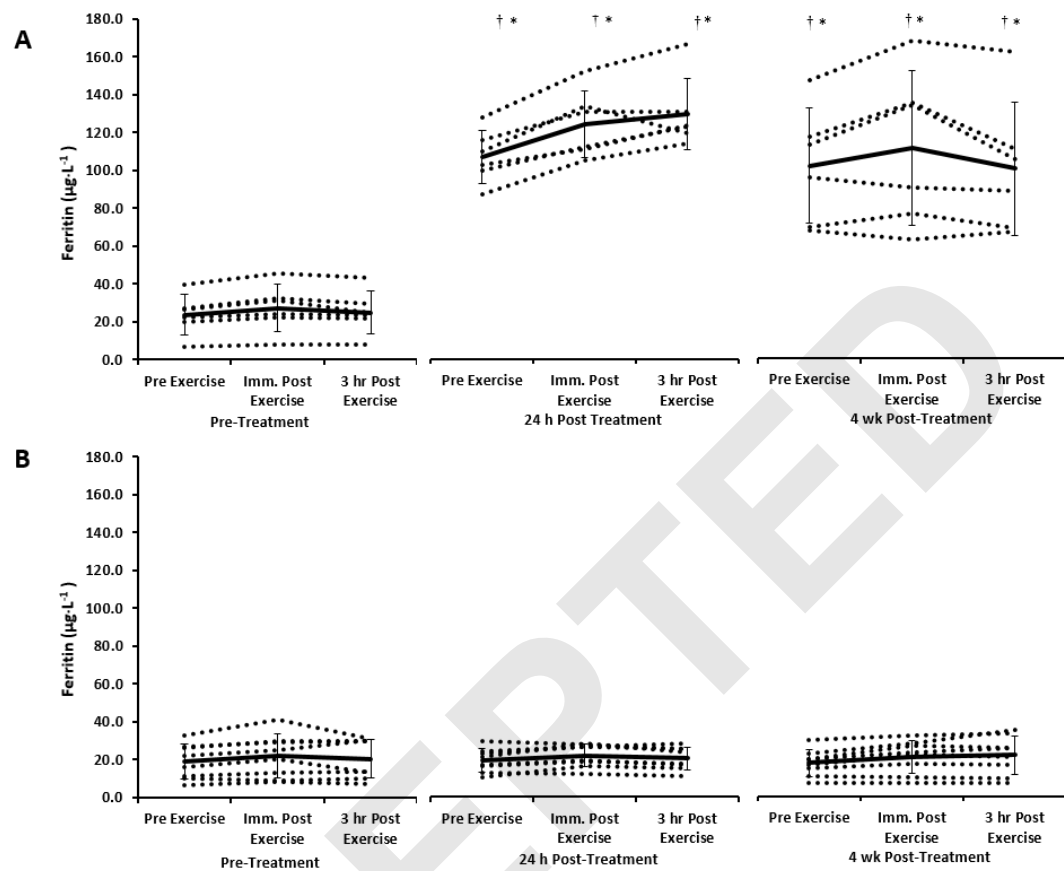
Table Legends

Table 1. Iron metabolism data measured pre-exercise, immediately after exercise and 3 hours after exercise pre-treatment, 24 hours post-treatment and 4 weeks post-treatment.
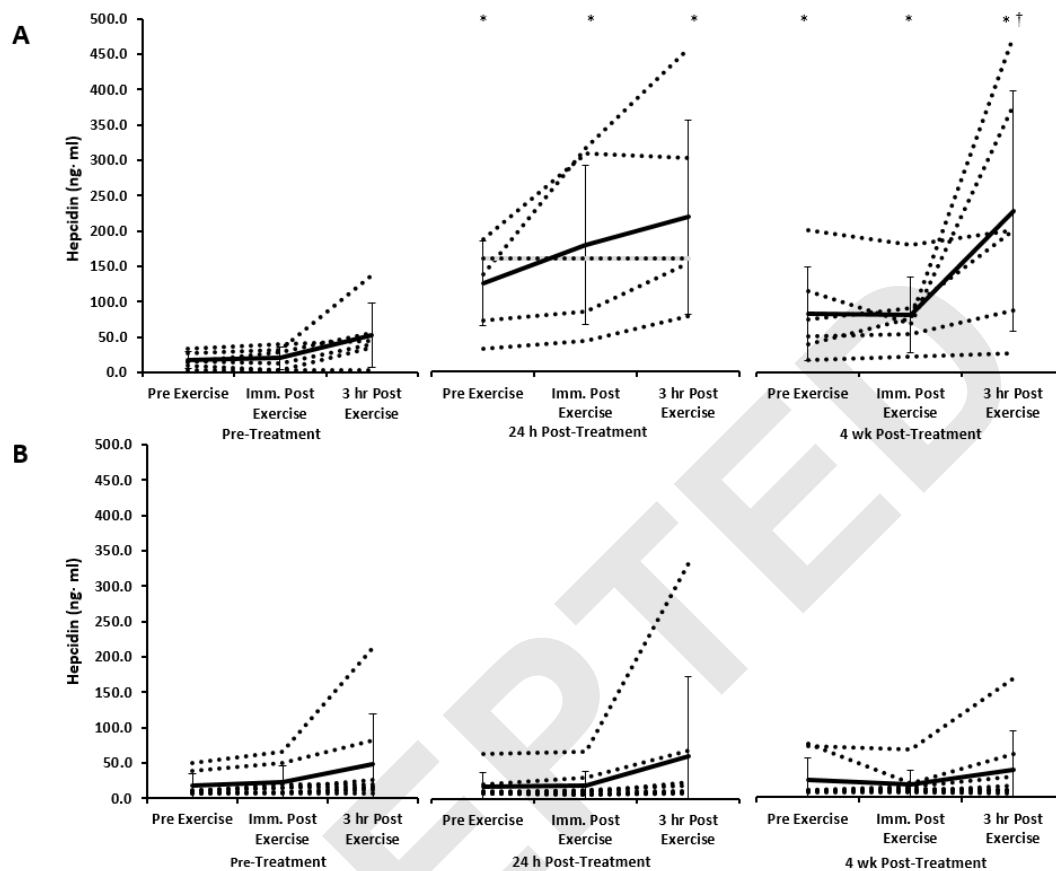
Table 2. Red cell indices measured pre-treatment and 4 weeks post-treatment.

Table 3. Total Haemoglobin mass and exercise test data taken pre-treatment, 24 hours post-treatment and 4 weeks post-treatment
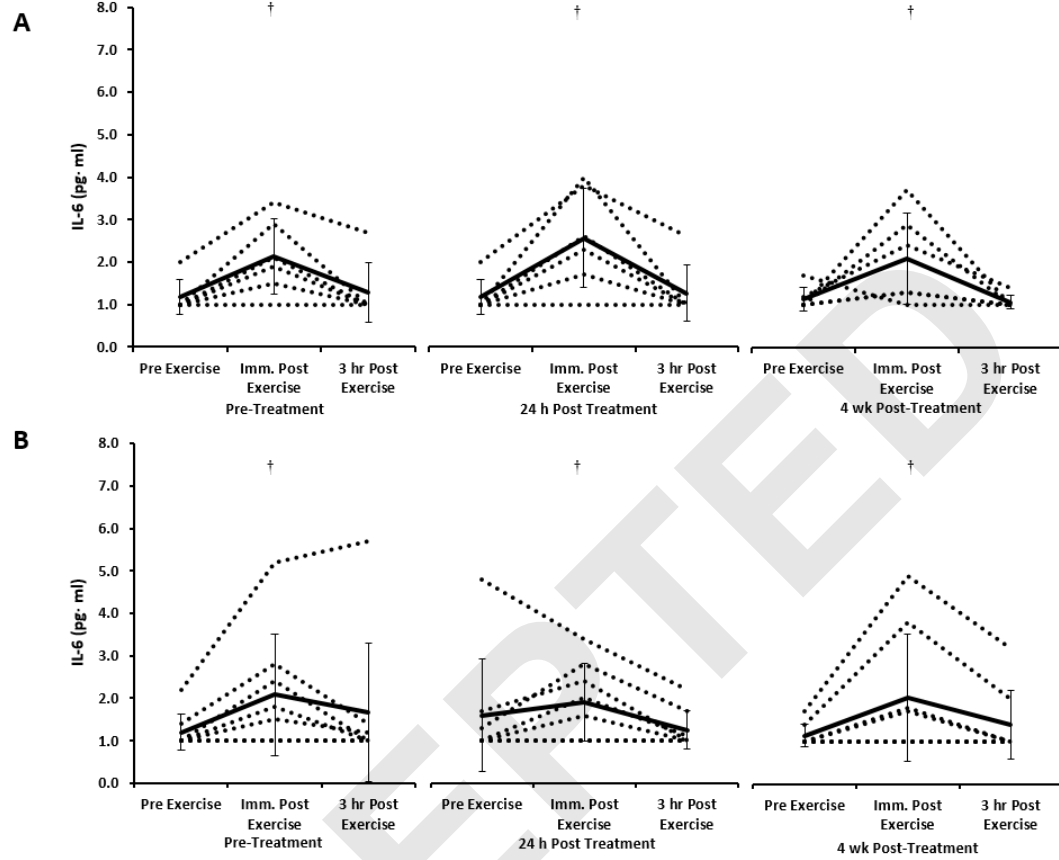
Fig. 1

A

B

Fig. 2

Fig. 3

Table 1. Iron metabolism data measured pre-exercise, immediately after exercise and 3 hours after exercise pre-treatment, 24 hours post-treatment and 4 weeks post-treatment.

Measure	Group	Pre-Treatment			24 Hours Post-Treatment			4 Weeks Post-Treatment		
		Pre	24 h Post	4 wks Post	Pre	24 h Post	4 wks Post	Pre	24 h Post	4 wks Post
Serum Iron, umol·L	IG	20.3	23.2	20.6	70.7*†	72.8*†	64.0*†	23.4	25.7	21.2
		(7.2)	(8.2)	(9.5)	(10.0)	(8.5)	(11.9)	(4.0)	(3.3)	(4.9)
	PG	19.3	22.5	21.8	20.6	23.4	21.3	15.1	17.9	17.3
		(6.9)	(7.3)	(8.0)	(14.3)	(15.5)	(11.9)	(6.2)	(9.0)	(6.9)
Tsat, %	IG	33.1	34.3	32.4	113.2*†	110.6*†	104.7*†	43.2	45.1	39.2
		(12.0)	(13.9)	(15.0)	(8.4)	(6.9)	(5.9)	(6.3)	(6.0)	(8.4)
	PG	29.1	31.2	31.4	30.3	31.5	30.9	20.9	24.8	24.8
		(11.3)	(11.8)	(12.6)	(19.7)	(19.6)	(17.4)	(11.2)	(12.0)	(10.5)
sTf, g·L	IG	2.5	2.7	2.6	2.5	2.6	2.4	2.2	2.3	2.2
		(0.3)	(0.2)	(0.2)	(0.3)	(0.3)	(0.4)	(0.4)	(0.3)	(0.3)
	PG	2.7	2.9	2.8	2.8	3.0	2.8	2.7	2.9	2.8
		(0.4)	(0.4)	(0.6)	(0.5)	(0.4)	(0.4)	(0.4)	(0.4)	(0.4)
sTfR, nm·L	IG	19.4	21.2	19.6	20.4	21.3	20.3	19.4	19.9	19.0
		(2.9)	(2.1)	(2.8)	(3.1)	(2.2)	(3.1)	(2.6)	(2.4)	(2.4)
	PG	23.2	23.4	21.8	21.4	23.1	21.3	22.1	23.4	22.1
		(3.0)	(2.1)	(2.5)	(2.7)	(3.1)	(3.1)	(2.1)	(2.8)	(2.0)

* Significant difference ($p < 0.05$) between iron and placebo groups; † significant difference ($p < 0.05$) between pre-treadmill test, pre-treatment time point.

Table 2. Red cell indices measured pre-treatment and 4 weeks post-treatment.

	Placebo Group		Iron group	
	Pre	4 wks Post	Pre	4 wks Post
Haemoglobin, g·L^{-1}	143.6	138.7	140.2	137.2
	(9.0)	(11.7)	(15.4)	(13.8)
Haematocrit, L·L	0.426	0.406	0.415	0.414
	(0.021)	(0.024)	(0.032)	(0.025)
Mean Cell Volume, fL^{-1}	89.13	88.29	90.27	92.98
	(3.72)	(2.75)	(4.46)	(5.95)
Mean Cell Hb, pg	30.01	30.06	30.38	30.70
	(1.37)	(1.28)	(1.59)	(1.51)
Mean Cell Hb Conc, g·L^{-1}	337.25	341.29	337.17	330.83
	(8.38)	(11.59)	(13.93)	(15.14)
RDW, %	13.08	13.04	12.70	12.92
	(0.78)	(0.68)	(0.68)	(0.58)
Red Cell Count, 10*12·L^{-1}	4.7938	4.6171	4.6083	4.4750
	(0.3046)	(0.3541)	(0.4271)	(0.4428)
Red Cell Folate, μg·L^{-1}	269.63	245.19	265.70	238.42
	(133.51)	(118.18)	(110.41)	(67.22)
B12, ng·L^{-1}	482.75	476.29	567.00	573.00
	(117.66)	(172.44)	(175.03)	(175.87)

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Table 3. Total Haemoglobin mass and exercise test data taken pre-treatment, 24 hours post-treatment and 4 weeks post-treatment.

	Placebo Group			Iron Group		
	Pre Treatment	24 h Post Treatment	4 wks Post Treatment	Pre Treatment	24 h Post Treatment	4 wks Post Treatment
tHb, g·kg^{-1}	12.15	12.16	12.43	14.13	13.88	13.12
	(1.53)	(1.84)	(2.10)	(1.93)	(1.77)	(1.72)
VO$_{2max,}$ ml·kg^{-1}·min^{-1}	64.40	64.83	64.27	73.02	71.60	70.30
	(10.22)	(9.56)	(7.35)	(4.80)	(6.78)	(5.29)
Economy, ml·kg^{-1}·km^{-1}	220.13	216.63	215.29	221.33	217.00	218.00
	(3.14)	(10.02)	(10.11)	(6.12)	(9.83)	(22.27)
vVO$_{2max,}$ km·h^{-1}	17.51	17.93	17.90	19.83	20.33	19.80
	(2.60)	(2.50)	(1.95)	(1.53)	(1.55)	(1.61)
Speed @ 2mmol·L, km·h^{-1}	14.42	14.55	15.10	16.43	16.67	16.54
	(2.58)	(2.55)	(1.96)	(1.63)	(1.24)	(1.52)
Speed @ 4mmol·L, km·h^{-1}	16.20	16.15	16.82	18.23	18.24	18.01
	(2.43)	(2.47)	(1.91)	(1.80)	(1.26)	(1.50)
TTE, s	370.25	365.50	352.86	350.86	369.43	373.33
	(29.79)	(35.66)	(64.14)	(26.46)	(42.16)	(48.10)
RPE, Borg 6-20	12.88	12.68	12.29	13.89	12.96	12.95
	(0.95)	(1.12)	(1.47)	(2.25)	(1.86)	(1.99)

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