An intensified training schedule in recreational male runners is associated with increases in erythropoiesis and inflammation and a net reduction in plasma hepcidin

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ABSTRACT

Background: Iron status is a determinant of physical performance, but training may induce both low-grade inflammation and erythropoiesis, exerting opposing influences on hepcidin and iron metabolism. To our knowledge, the combined effects on iron absorption and utilization during training have not been examined directly in humans.

Objective: We hypothesized that 3 wk of exercise training in recreational male runners would decrease oral iron bioavailability by increasing inflammation and hepcidin concentrations.

Design: In a prospective intervention, nonanemic, iron-sufficient men ($n = 10$) completed a 34-d study consisting of a 16-d control phase and a 22-d exercise-training phase of 8 km running every second day. We measured oral iron absorption and erythroid iron utilization using oral ^{57}Fe and intravenous ^{58}Fe tracers administered before and during training. We measured hemoglobin mass (mHb) and total red blood cell volume (RCV) by carbon monoxide rebreathing. Iron status, interleukin-6 (IL-6), plasma hepcidin (PHep), erythropoietin (EPO), and erythroferrone were measured before, during, and after training.

Results: Exercise training induced inflammation, as indicated by an increased mean ± SD IL-6 (0.87 ± 1.1 to 5.17 ± 2.2 pg/mL; $P < 0.01$), while also enhancing erythropoiesis, as indicated by an increase in mean EPO (0.66 ± 0.42 to 2.06 ± 1.6 IU/L), mHb (10.5 ± 1.6 to 10.8 ± 1.8 g/kg body weight), and mean RCV (30.7 ± 4.3 to 32.7 ± 4.6 mL/kg) (all $P < 0.05$). Training tended to increase geometric mean iron absorption by 24% ($P = 0.083$), consistent with a decreased mean ± SD PHep (7.25 ± 2.14 to 5.17 ± 2.24 nM; $P < 0.05$). The increase in mHb and erythroid iron utilization were associated with the decrease in PHep ($P < 0.05$). Compartmental modeling indicated that iron for the increase in mHb was obtained predominantly (>80%) from stores mobilization rather than from increased dietary absorption.

Conclusions: In iron-sufficient men, mild intensification of exercise intensity increases both inflammation and erythropoiesis. The net effect is to decrease hepcidin concentrations and to tend to increase oral iron absorption. This trial was registered at clinicaltrials.gov as NCT01730521. *Am J Clin Nutr* 2018;108:1324–1333.

Keywords: erythropoiesis, hepcidin, inflammation, iron absorption, physical exercise

INTRODUCTION

Iron is a critical nutrient for physical exercise and performance. Hemoglobin mass (mHb) is directly related to maximal aerobic oxygen uptake (VO_2max) (1). In women of reproductive age, iron supplementation results in increased maximal and submaximal exercise performance with the clearest effects in iron-deficient, trained subjects (2). Although effects are well established in anemic subjects, in nonanemic athletes a recent meta-analysis concluded that iron treatment to improve iron status and subjective perception of fatigue (3). Compared with sedentary counterparts, athletes have been reported to have lower iron status

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Supplemental Figures 1 and 2 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: AGP, α1-acid glycoprotein; CRP, C-reactive protein; EPO, erythropoietin; ERFE, erythroferrone; HR, heart rate; HR_{max}, maximal heart rate; LOD, limit of detection; MHb, hemoglobin mass; PF, plasma ferritin; PHep, plasma hepcidin; RCV, red blood cell volume; RPE, Borg Rating of Perceived Exertion; sTfR, soluble transferrin receptor; VO_2max, maximal oxygen uptake.

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(4, 5); however, at the same time, excessive iron supplement use has been reported in both recreational (6) and elite (7) athletes.

Exercise induces several physiologic changes that can affect iron status markers and their interpretation (8). In particular, at the beginning of a training intensification, plasma volume expands (9) and plasma ferritin (PF) and hemoglobin concentrations diminish. A decreased hemoglobin concentration has been suggested to be due to hemolysis through mechanical trauma (10, 11). However, although hemolysis can be detected during exercise, its impact on overall mHb may be negligible (12). Nonetheless, red blood cell life span has been reported to be shorter in athletes (13). Exercise may also increase iron losses, possibly through an increase in gastrointestinal bleeding and desquamation due to increased gut motility, trauma, and microischemia (14).

Low-grade inflammation with increases in IL-6 after exercise may also contribute to lower iron status in athletes, depending on training intensity (11), in particular in weight-bearing exercise modalities such as running (15). Plasma hepcidin (PHep), produced primarily by the liver, is the main regulator of iron metabolism (16). Exercise-induced inflammation has been shown to increase PHep concentrations postexercise (17). Such effects could decrease iron absorption and utilization in the hours postexercise and contribute to lower iron status in athletes. Acute and chronic inflammation can increase PHep and reduce iron export from enterocytes and macrophages, decreasing both oral iron absorption (18) and erythroid iron utilization (19).

The interaction of the inflammatory and erythropoietic stimuli on iron metabolism has been extensively studied in animals, but there are few studies in humans. Endurance exercise training may stimulate not only low-grade inflammation but also increase erythropoietic drive. Therefore, our study objective was to examine the interplay of inflammatory and erythropoietic influences on PHep and iron absorption during a 3-wk exercise schedule in iron-replete, recreationally trained men. Specifically, we hypothesized that intensified exercise training would increase inflammation and PHep concentrations and thereby decrease oral iron absorption.

METHODS

Subjects

Subjects were recruited from the student and staff population of ETH Zürich and the University of Zurich, Switzerland. Inclusion criteria were as follows: *1*) male sex, *2*) apparently healthy with no chronic diseases or medications (on the basis of self-report), *3*) no blood donation or significant blood loss in the 4 mo before the study, *4*) a recreational athlete who exercises between 1 and 4 times/wk for a total exercise load of not more than 5 h/wk, and *5*) nonsmoker.

The study was conducted in October–November 2012. The study was approved by the ethics committee of the ETH Zürich, Switzerland, and registered in the public clinical trial registry clinicaltrials.gov (NCT01730521). All of the subjects gave written, informed consent before participating in the study.

Exercise schedule

The study was divided in 2 phases (**Figure 1**): a control phase and an exercise phase. In the control phase subjects were instructed to maintain their usual frequency and intensity of physical exercise for 16 d. Before the start of the exercise phase, VO_2max and maximal heart rate (HR_{max}) were measured. The subjects then entered the exercise phase, which lasted 22 d, during which they ran 8 km every second day for a total of 11 training sessions. Subjects were instructed to conduct the training program in addition to their usual exercise schedule. The 4 initial training sessions were supervised by the study team and were performed on an outside circular track, and the subjects performed the exercise schedule at 80% of HR_{max}. Subjects were instructed about the Borg Rating of Perceived

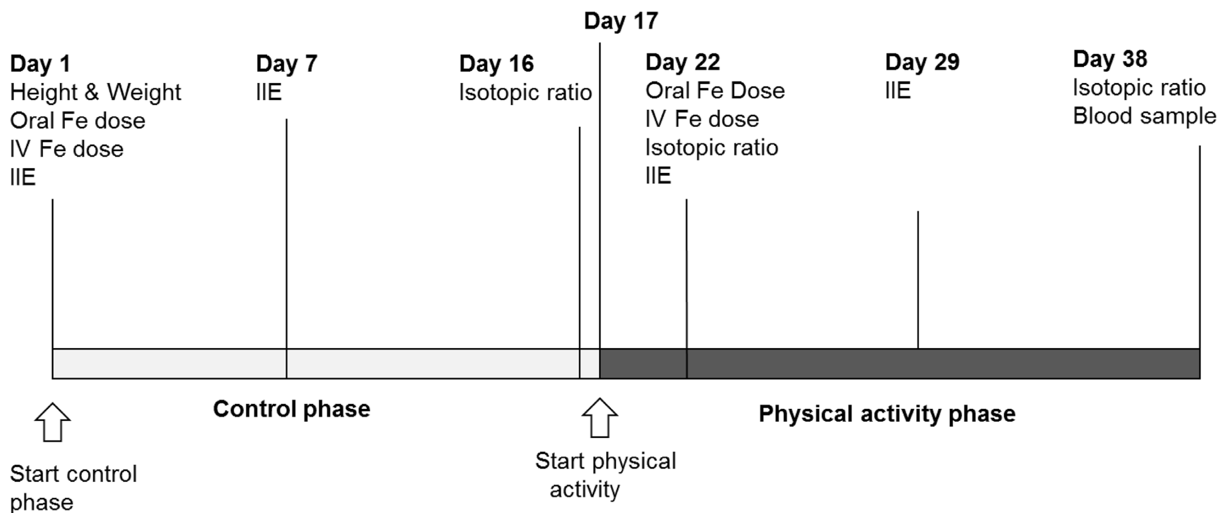

FIGURE 1 Study design. In the control phase, subjects followed their usual physical activity. In the intensified-activity phase, subjects performed 11 bouts of exercise (minimal distance, 8 km) every second day in addition to their usual training schedule. At the time points labeled IIE, iron status and inflammatory markers and erythropoietin were measured. IV, intravenous.

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Exertion (RPE) (20), and it was recommended that the subjects run at RPE levels of 4–6, a speed of running chosen to be physically demanding and on the edge of being challenging but being sustainable for the duration of the 8-km run, which was to be completed without walking. After completion of the 4 supervised exercise-training sessions, subjects were free to exercise independently provided they maintained the minimal duration, predefined heart rate (HR) zone (80% HR_{max}), and approximate intensity in RPE. During all exercise training, HR and exercise duration were monitored with an HR monitor watch (Polar RS 300 X; Polar Electro Oy) provided by the study team, which was used to transfer all exercise information for each subject to a spreadsheet (duration and average HR). In addition, the subjects separately kept a diary and noted the duration and timing of the exercise training sessions and their individual RPE.

Study procedures

At the start of the control phase (day 1), and after 3 training sessions during the exercise phase (day 22, 5 d after initiating the intensified training schedule), the subjects consumed a labeled test drink and received an intravenous infusion of a second stable iron isotope label. Our aim was to assess oral iron utilization and its determinants during training, and we chose to sample on day 5 to avoid measuring exclusively acute effects of individual training bouts. During the exercise phase, the subjects were running every second day as described above. A blood sample was taken 14 d after the administration of the iron labels to assess label utilization in red blood cells; iron absorption and erythroid utilization were quantified by assessing the shift in iron isotopic ratios at day 14 after label administration. At the beginning and at the end of the study (day 1 and day 36), total erythrocyte mass was quantified with the carbon monoxide–rebreathing method (21). At each isotopic administration, iron status markers, hemoglobin, inflammation, and erythropoietic markers as well as PHep were assessed. In addition, a sample was taken immediately after exercise and at a time-matched control time point at the midpoint of the control and exercise phases, respectively. The timing of the sampling was standardized [i.e., the control sample on day 7 was collected at the same time of day as the exercise sample of day 29 to minimize circadian effects on biochemical markers (PHep)] (22). The study overview is shown in Figure 1.

Administration of isotopic labels for oral iron absorption and intravenous erythroid iron utilization measurements

The standardized test drink consisted of 200 mL deionized water with 6 mg ^{57}Fe as aqueous $^{57}FeSO_4$ dissolved in solution and was administered at the Laboratory of Human Nutrition of the ETH Zürich. One hour after the ingestion of the test drink, the intravenous infusion to assess erythroid iron utilization was initiated. Briefly, 2 mL aqueous solution containing 100 μg ^{58}Fe as iron citrate was taken into a syringe containing 10 mL 0.9% saline solution and, via a 100-mL infusion bag leading into a 0.9% saline drip, was slowly infused through a venous catheter over 50 min. The rate of intravenous infusion of iron was based on an estimated 2-μg/min plasma appearance of iron normally absorbed from the gastrointestinal tract (23). The subjects were asked to not eat or drink for 3 h after ingestion of the oral test meal in order to avoid confounding on the absorption of the labeled iron by other dietary components.

Preparation of stable isotope labels

Isotopic label [^{57}Fe]-$FeSO_4$ was prepared from isotopically enriched elemental iron by dissolution in diluted sulfuric acid. The solutions were stored in Teflon containers (Savillex) and flushed with argon to keep the iron in the +II oxidation state. The intravenous test dose of [^{58}Fe]-citrate was produced according to the method described originally by Dainty et al. (23) and later applied in several other studies (24). The solution was divided into ampoules, sterilized, and evaluated for sterility and pyrogens (Kantonal Pharmacy, Zurich, Switzerland).

Analysis of isotopic composition of blood samples

Each isotopically enriched blood sample was analyzed for its iron isotopic composition under chemical blank monitoring. Whole-blood samples were mineralized using an HNO_3-H_2O_2 mixture and microwave digestion followed by separation of the sample iron matrix by anion-exchange chromatography and a solvent/solvent-extraction step into diethyl ether (25). All of the isotopic analysis were performed by negative thermal ionization mass spectrometry. Because of the high enrichment of the isotopically enriched labels and the low amounts of isotopic label utilization into red blood cells, data were normalized for the natural ^{56}Fe-to-^{54}Fe isotope ratio to correct for mass-dependent fractionation effects in the ion source.

Blood sample analyses

Hemoglobin concentration was measured by using a Hemoximeter (Radiometer abl800) on the day of blood collection. PF, soluble plasma transferrin receptor (sTfR), α1-acid glycoprotein (AGP), and C-reactive protein (CRP) were measured by using the multiplex immunoassay by Erhardt et al. (26). We calculated body iron stores for study participants at each time point by using the formula based on the sTfR-to-PF ratio proposed by Cook et al. (27). Erythropoietin (EPO) was measured by using an automated chemiluminescent immunoassay system (Immulite; Diagnostic Products Corporation). IL-6 was measured by using an ELISA kit (Quantikine; R&D Systems). PHep was measured by using a competitive ELISA method (28). Erythroferrone (ERFE) was measured by using a validated assay with a limit of detection (LOD) of 1.5 ng/mL and a limit of quantification of 14 ng/mL (29). To allow for continuous statistical analysis, values below the LOD (1.5 ng/mL) were assigned a random value between 0 and the LOD.

On the basis of the shift of the iron isotope ratios in the blood samples and the amount of iron circulating in the body, the amounts of ^{57}Fe isotopic label present in the blood 14 d after the first and the second test meal administration were calculated based on the principles of isotope dilution and considering that the iron isotopic labels are not monoisotopic (25).

Blood volume was calculated on the basis of hematocrit and measured mHb from which total circulating iron was derived from assuming iron content of hemoglobin (3.47 mg Fe/g hemoglobin). Because not all iron is incorporated in red blood

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cells, the measured utilization from the intravenous label was used to calculate the amount of absorbed iron finally present in red blood cells. Corrections for enriched baseline values were made when calculating iron absorption from the second test dose.

mHb and VO_2max determination

Total mHb was quantified by a modified version (30) of a carbon monoxide–rebreathing technique (21) at the beginning (day 1) and at the end (day 38) of the exercise phase. After the subjects had rested for 20 min in a semirecumbent position, 2 mL of blood was sampled from an antecubital vein without stasis and immediately analyzed for percentage of carboxyhemoglobin, hemoglobin concentration, and hematocrit (4 min at 13,500 rpm). The subjects then breathed 100% oxygen for 2 min to flush nitrogen from the airways. The breathing circuit (previously oxygen flushed) was then closed and a mixture of a 99.997% carbon monoxide bolus (1.25 mL/kg; CO 4.7; Linde Gas) and oxygen (200–250 mL/min) was then rebreathed for 8 min. At the end of the rebreathing period, another similarly obtained and analyzed 2-mL blood sample was collected. The change in percentage of carboxyhemoglobin between the first and second measurements was used to calculate mHb, taking into account the amount of carbon monoxide remaining in the rebreathing circuit at the end of the procedure (21). Red blood cell volume (RCV) and blood and plasma volumes were derived from mHb and hematocrit (21) and were assessed by the same operator. Previous studies using the same technique indicate that the mean CV for RCV is 2.5% (31, 32).

VO_2max was determined on a running treadmill with a starting speed of 5 km/h, with increments of 1 km/h every 90 s until exhaustion. Verbal encouragement was given toward the end of all trials. The treadmill had an ascending 15% gradient slope, which was kept constant during the test. Participants breathed through a mouthpiece (Hans Rudolph) with their noses occluded wearing a nose clip. Ventilatory variables were measured in breath by using an indirect calorimeter (Cosmed Quark CPET) consisting of a flow meter and fast-responding gas analyzers. The system was calibrated using a 3-L calibration syringe (Cosmed) and gas mixtures of known concentrations of oxygen and carbon dioxide. After the test, all data points were averaged over the last 30 s of each workload. The highest average value for oxygen uptake calculated over 30 s was taken as VO_2max.

Compartmental modeling

We investigated the relative contributions to circulating hemoglobin iron by *1*) dietary iron absorption and *2*) mobilization of body iron stores by estimating the amount of oral and intravenous stable isotopic iron incorporated in red blood cells during the study in both the control and exercise phases. We assumed the isotopic iron measurements to be generally representative of the entirety of the control and exercise periods.

Two methods were used to estimate the effect of exercise on the cumulative amounts of dietary iron absorbed using the ratio of oral iron absorption between the control and exercise phase ($FeAbs_{C/E}$) measured with water, as follows:

1) With the "dietary method," for each subject a dietary iron intake of 16 mg Fe/d (33) and an overall iron bioavailability from a mixed diet of 15% dietary iron were assumed in a hypothetical iron-depleted state, as proposed by Hallberg and Rossander-Hulten (34) for European diets and more recently by Armah et al. (35) for the US diet. Dietary iron absorption was then calculated by multiplying the estimated dietary bioavailability, adjusted for iron status (36), by $FeAbs_{C/E}$, the ratio of iron bioavailability between control and exercise phase, and summed for the days of duration of each phase.

2) With the "balance method," a daily absorbed iron requirement at steady state of 1.07 mg Fe in men was assumed (37). A correction factor between oral bioavailability and daily average requirement was derived using oral iron absorption from water during the control period. This factor was then used to calculate the additional iron absorbed during the exercise period by multiplying the correction factor by the oral iron absorption measured during exercise, yielding the additional amount of iron absorbed daily during exercise.

We subtracted daily losses and multiplied the difference for the duration of the exercise period. The amount of iron mobilized from body iron stores was estimated as the difference between the increase in circulating hemoglobin iron and the estimated amount of dietary iron absorption during the exercise phase. Body iron stores were estimated by using the formula by Cook et al. (27) on the basis of the ratio of serum ferritin and sTfR.

Statistical analysis

The study was conducted with a convenience sample size of 12 subjects and an estimated attrition of 20%. Ten subjects were estimated to be sufficient to test the 1-sided Ho hypothesis that iron absorption during training would be decreased compared with iron absorption during the control period assuming a 1-sided estimated reduction in iron absorption of 30%, an SD of 0.2 in log oral absorption, and a correlation for within-subject absorption of $r = 0.77$ (80% power and $\alpha = 0.05$ for paired *t* tests). Because we did not detect a decrease in iron absorption during training, we analyzed the study with a 2-sided statistical approach. With the same boundary conditions, using 2-sided paired *t* tests, a change in oral iron absorption of 40% can be detected with 10 subjects.

Data were analyzed with the SPSS software package (IBM SPSS Statistic, version 22). Data were examined visually for normal distribution and, if evident departures of normality were found, then the data were log-transformed. When biochemical markers were below the LOD, a random number between 0 and the detection limit for the biochemical marker in question was imputed. Normally distributed data were expressed as means ± SDs and nonnormally distributed data as medians and ranges. mHb, blood volume variables, iron absorption, and erythroid iron utilization pre- and postexercise were compared using paired-sample *t* tests. Biochemical markers were analyzed with a linear mixed model with time of blood sampling and training as fixed factors and with subject as a random factor. If an effect of training or timing was found, post hoc tests were conducted (Bonferroni-corrected paired comparisons).

Exploratory correlation analysis was conducted to investigate possible physiologic predictors of iron absorption and utilization, PHep concentrations, markers of inflammation, and erythropoiesis. Pearson and Spearman correlation coefficients were calculated between biochemical markers, oral iron absorption and utilization, mHb, and PHep. Three separate exploratory

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correlation analyses were conducted on samples obtained *1)* immediately after exercise and *2)* assessed at the time of isotopic administration and *3)* on the change in markers occurring before and after the exercise phase. No correction for multiple comparisons was undertaken. Significance was set at $P < 0.05$, and a trend was defined as $P < 0.10$. CIs for correlations were estimated using SPSS (version 23) using a bootstrapping procedure (bias corrected accelerated) with 1000 resamplings.

RESULTS

Twelve subjects were recruited for the study, but one subject declined participation before the study began and one became ill with a mild febrile upper respiratory tract infection on the day before the start of the study and withdrew. Therefore, 10 subjects started and all completed the study (**Supplemental Figure 1**). Two of the participants developed mild upper respiratory tract infections during the exercise phase and both missed 2 exercise training sessions but were able to complete the rest of the training program, completing 9 of the 11 planned exercise bouts.

The baseline characteristics of the men [as median (range) or mean ± SD] were as follows: age 35 y (21-50 y); height, 184 ± 9.7 cm; weight, 77 ± 8.6 kg; BMI (in kg/m^2), 22.9 ± 1.5; HR_{max}, 187 (170–199) beats/min; and $\dot{V}O_2$max, 56 ± 6.75 mL O_2 kg^{-1} min^{-1}. As assessed by $\dot{V}O_2$max, 5 subjects were characterized as having a "fair" level of fitness ($\dot{V}O_2$max: 48–53 mL O_2 kg^{-1} min^{-1}), 4 subjects as having "good" fitness ($\dot{V}O_2$max: 58–61 mL O_2 kg^{-1} min^{-1}), and 1 subject as having "excellent" fitness ($\dot{V}O_2$max: 72 mL O_2 kg^{-1} min^{-1}). Mean ± SD hemoglobin and body iron stores were 144 ± 7.2 g/L and 10.4 ± 1.96 mg Fe/kg body weight, respectively, and none of the subjects were anemic or iron deficient. During training, the mean ± SD duration for an exercise bout was 45.1 ± 6.3 min, the reported RPE was 4.8 ± 1.4, and the mean average HR and mean HR_{max} were 159 ± 14 and 174 ± 16 beats/min, respectively (**Supplemental Figure 2**).

Comparing baseline and final determinations, mHb increased by ∼3% ($P < 0.05$), increasing the mean ± SD amount of circulating hemoglobin iron by 94.6 ± 97.0 mg, whereas body iron stores decreased during the study ($P < 0.05$; **Table 1**). The estimated mean ± SD amounts of circulating hemoglobin iron derived from increased oral iron absorption were 11.1 ± 8.9 and 10 ± 16 mg Fe for the "dietary" and "balance" methods, ∼11% and 10% of the total, respectively (**Figure 2**).

TABLE 1
Blood volume, red blood cell mass, and total body iron at the beginning of the study (start of control phase, day 1) and at the completion of the exercise schedule (day 38)[1]

	Beginning of control phase (day 1) ($n = 8$)	End of training phase (day 38) ($n = 8$)
Blood volume, mL	6041 ± 1016	6143 ± 909
Plasma volume, mL	3473 ± 497	3419 ± 453
Red blood cell volume, mL/kg BW	30.7 ± 4.3	32.7 ± 4.6[2]
Hemoglobin mass, g/kg BW	10.5 ± 1.6	10.8 ± 1.8[2]
Body iron, mg/kg BW	10.42 ± 1.96	9.73 ± 2.09[2]

[1]Values are means ± SDs. BW, body weight.
[2]Different from the control phase, $P < 0.05$ (paired-samples *t* test).

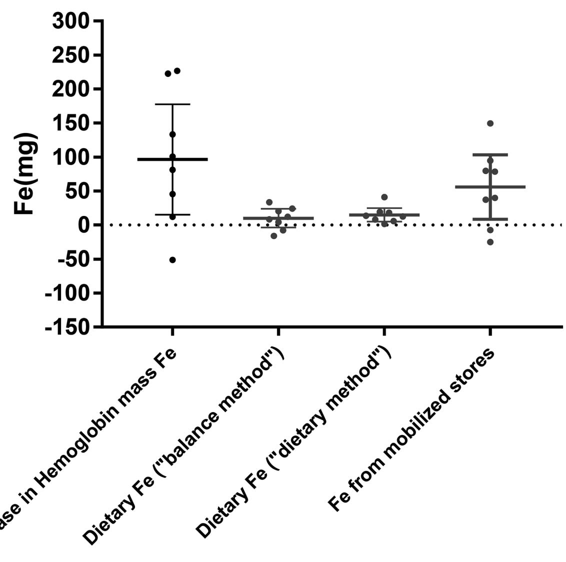

FIGURE 2 Relative contribution by dietary absorption and mobilization of stores (both depicted in gray) to the measured increase in hemoglobin mass iron of 94.6 mg ($P < 0.05$; $n = 8$, left, black symbols) during the study. Values are means (95% CIs). Mobilized iron stores provide a substantial contribution to the increase in hemoglobin mass and were estimated as proposed by Cook et al. (27). The contribution by dietary iron was estimated with 2 distinct methods (balance and dietary), yielding similar results.

RCV increased by ∼6% ($P < 0.05$). There was no significant change in overall blood volume and plasma volume between baseline and endpoint (Table 1). Erythroid iron utilization did not differ significantly between the control phase and the exercise phase. In contrast, we observed a 24% trend for an increase in oral iron absorption (19.3% compared with 15.6%; 2-sided $P = 0.083$) when comparing the exercise phase with the control phase (**Table 2**).

EPO increased during exercise ($P < 0.05$) and was significantly elevated in the blood sample collected immediately after the exercise bout on day 29 ($P < 0.01$; **Table 3**). In contrast, ERFE concentrations were below the limit of quantification (14

TABLE 2
Iron absorption and erythroid iron utilization during the control and exercise training phase in iron-replete young men[1]

	Iron bioavailability, %	
	Oral iron absorption	Erythroid iron utilization
Control phase (day 1)[2]	15.6 (8.5–28.6)	76.3 (67.4–86.3)
Training phase (day 22)[2]	19.3 (8.4–44.1)[3]	81.3 (66.5–99.4)
Estimated difference (day 22 − day 1)[4]	6.95 (−1.12, 15.03)	5.92 (−6.05, 17.89)

[1]$n = 10$. Iron absorption was measured at the beginning of the control phase after 3 training bouts of the exercise phase as the incorporated stable isotopic tracers in red blood cells from a 6-mg labelled $^{57}FeSO_4$ dose dissolved in water.
[2]Values are geometric means (geometric mean − SD, geometric mean + SD).
[3]$P = 0.083$ vs. control (day 1) (paired-samples *t* test).
[4]Values are mean estimated differences (95% CIs) with the use of a linear mixed model, with study phase [control (day 1) or training (day 22)] as a fixed factor and subject as a random factor. Differences are expressed as percentage of oral iron absorbed.

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TABLE 3
Iron status and inflammatory and erythropoietic markers during the control and the exercise phase[1]

Study phase and day	Days since exercise start	Sampling time	Hemoglobin, g/L	PF,[2] μg/L	sTfR,[3] mg/L	CRP mg/L	AGP, g/L	IL-6,[4] pg/mL	EPO,[5] iU/L	PHep,[6] nM	ERFE, ng/mL
Control											
Day 1	NA	Morning, fasting	144 ± 0.72[7]	107.0 (53.5–198)[8]	4.0 ± 0.78	0.34 (0.02–3.52)	0.64 (0.5–1.1)	1.09 ± 1.13	0.57 ± 0.42	4.39 ± 1.10	6.4 ± 3.1
Day 7	NA	Time-matched control for postexercise day 29 sample	ND	89.2 (53.1–184)[9]	4.1 ± 0.75	0.63 (0.2–4.8)	0.66 (0.4–1.1)	0.87 ± 1.13	0.66 ± 0.42	7.25 ± 2.14	7.7 ± 5.1
Training											
Day 22	3	Morning, fasting	ND	97.8 (56.7–224)	4.2 ± 0.77	0.55y (0.07–2.3)	0.70 (0.4–1.3)	1.22 ± 0.43	1.01 ± 1.53	3.78 ± 0.95	5.6 ± 4.3
Day 29	12	Postexercise	146 ± 0.91	107.7 (47.3–187)[10]	4.4 ± 0.78	0.41 (0.09–4.8)	0.72 (0.5–1.1)	5.17 ± 2.24[11]	2.06 ± 1.64	5.17 ± 2.24	1.8 ± 1.1

[1] $n = 10$. Assessed with linear mixed models with time of day and training as fixed factors and subjects as random factors. Individually time-matched samples were obtained at 2 time points during the day (morning and afternoon) in each of the phases. Several biochemical markers have a circadian pattern, and we differentiated the effect of time of sampling during the day and the effect of training. The postexercise samples were collected ≤30 min after the completion of a training session. AGP, α1-acid glycoprotein; CRP, C-reactive protein; EPO, erythropoietin; NA, not assessed; ND, not determined; PF, plasma ferritin; PHep, plasma hepcidin; sTfR, soluble transferrin receptor.

[2] Significant interaction training and sampling time ($P < 0.05$).

[3] Effect of training ($P = 0.060$).

[4] Significant interaction of training and sampling time ($P < 0.01$).

[5] Effect of training ($P < 0.01$).

[6] Effect of training ($P < 0.01$) and sampling time ($P < 0.05$). Interaction: NS.

[7] Mean ± SD (all such values).

[8] Geometric mean; range in parentheses (all such values).

[9] Different from day 1 (Bonferroni-corrected comparison, $P < 0.05$) in the same column.

[10] Different from day 7 (Bonferroni-corrected comparison, $P < 0.05$) in the same column.

[11] Different from all other values in the same column (Bonferroni-corrected comparison, all $P < 0.01$).

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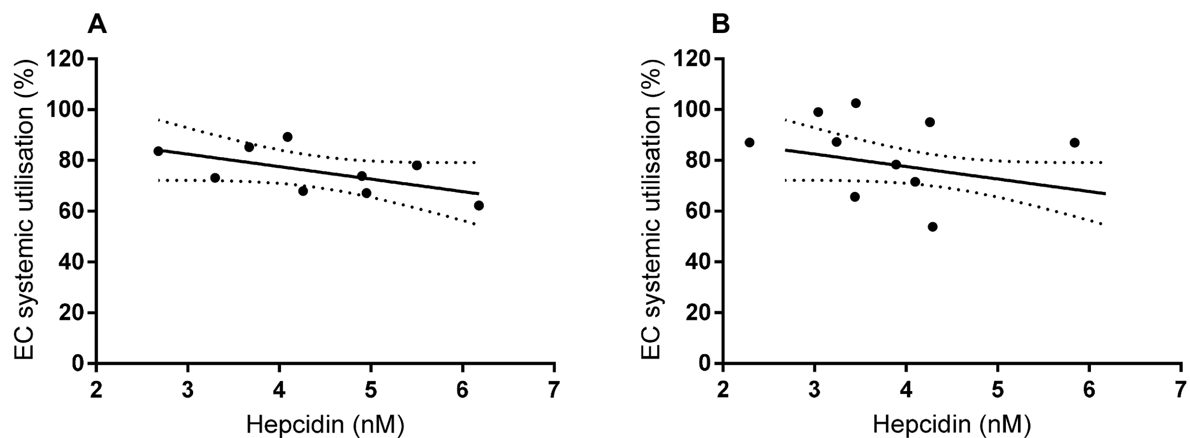

FIGURE 3 Negative correlation between erythroid (systemic) iron utilization assessed with intravenously administered stable isotopes and plasma hepcidin during the control (day 1; $n = 9$) (A) and the exercise (day 22; $n = 9$) (B) phase. Hepcidin was a significant predictor ($P < 0.05$) of systemic iron utilization in a linear regression controlled for subject factor. The correlation was also significant for day 1 alone ($r = -0.594$; 95% CI: −0.898, −0.17) (A) but not for day 22 alone (B). EC, erythrocyte.

ng/mL) in 36 of the 37 samples measured, and we detected no increase during the exercise phase compared with control.

Although serum CRP and AGP were higher during the exercise period than in the control period, the differences were not significant. In contrast, serum IL-6 was affected by the interaction of blood sampling time and exercise ($P < 0.01$). Serum IL-6 in the sample collected after exercise was significantly greater than in samples collected at all other time points ($P < 0.01$). Serum PHep decreased in samples collected during the exercise period ($P < 0.05$) compared with control. The time of sampling also had a significant effect, and serum PHep in the sample collected on day 29 postexercise was significantly lower than the time-matched sample collected during the control period ($P < 0.05$; Table 3). The interaction between training and sampling time was significant for PF ($P < 0.01$). Consistent with increased erythropoietic activity, we detected a trend for an increase in sTfR with training ($P = 0.060$).

In samples taken immediately post-training and in a time-matched control sample, PHep was negatively correlated with AGP ($r = -0.666$; 95% CI: −0.838, −0.424), with oral iron absorption ($r = -0.492$; 95% CI: −0.768, −0.169), CRP ($r = -0.486$; 95% CI: −0.749, −0.161), and EPO ($r = -0.478$; 95% CI: −0.796, −0.087). CRP was negatively correlated with mHb ($r = -0.586$; 95% CI: −0.835, −0.236; $P < 0.05$), RCV ($r = -0.613$; 95% CI: −0.850, −0.312), and blood volume ($r = -0.596$; 95%CI: −0.847, −0.313). When assessing nonparametric correlations (Spearman's ρ) in the post-training sample and time-matched control, IL-6 was positively correlated with EPO ($\rho_s = 0.558$; 95% CI: 0.243, 0.725) but negatively correlated with ERFE ($\rho_s = -0.623$; 95% CI: −0.949, −0.105), and tended to be negatively correlated with PHep ($\rho_s = -0.516$; 95% CI: −0.957, 0.001).

At the time of isotopic administration, PHep was negatively correlated with blood volume ($r = -0.554$; 95% CI: −0.800, −0.016) and plasma volume ($r = -0.548$; 95% CI: −0.799, −0.057). Similarly, CRP was negatively correlated with mHb ($r = -489$; 95% CI: −0.735, −0.207), blood volume ($r = -0.476$; 95% CI: −0.713, −0.156), and RCV ($r = -0.529$; 95% CI: −0.783, −0.157), but similar effects were not found for AGP. Erythroid iron utilization was correlated with PHep. In a linear regression including both time points of isotopic administration, hepcidin was a significant predictor of erythroid iron utilization ($P < 0.05$; **Figure 3**) when the model was controlled for subject.

We also explored correlations of the change in variables between the samples obtained during the training and control phase. Overall, throughout the study there was a trend for a significant correlation between the change in mHb and oral iron absorption ($r = 0.714$; 95% CI: −0.048, 0.980). When comparing biochemical samples obtained immediately after training and the time-matched control, the change in EPO correlated with the change in erythroid iron utilization ($r = 0.763$; 95% CI: 0.445, 0.978). The change in EPO immediately after training was also positively correlated with the change in PF ($r = 0.786$; 95% CI: 0.345, 0.990).

When analyzing the change in biochemical variables at the time of isotopic administration (control phase compared with training phase), the change in PHep was positively correlated with the change in RCV ($r = 0.773$; 95% CI: 0.136, 0.988). The change in oral absorption was inversely correlated with the change in sTfR ($r = -0.762$; 95% CI: −0.858, −0.751).

DISCUSSION

The overall aim of this study was to assess the effect of a 3-wk intensified exercise schedule on oral iron absorption, erythroid iron utilization, and their physiologic determinants. Exercise training increased inflammation, as evidenced by the increments in IL-6 concentrations, but contrary to our hypothesis, the erythropoietic effects of increased exercise were dominant, decreasing PHep and resulting in a borderline significant 24% enhancement in oral iron absorption. The mean mHb increased, with >80% of the hemoglobin iron obtained from mobilization of storage iron and the remainder from increased dietary iron absorption.

Iron absorption in athletes

Previous studies measuring iron absorption in athletes did not compare iron absorption directly with a well-characterized

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control group but reported a higher reference dose absorption in athletes, and the authors linked this finding to depleted iron stores (38). In a second study, in which long-term iron absorption via isotopic signal dilution was assessed, a higher iron elimination rate in athletes compared with control values from the literature was reported, suggesting higher iron absorption and more rapid radio tracer dilution (39), an effect possibly secondary to increased losses. In the current study, participants acted as their own controls, and our data support the notion that exercise upregulates oral iron absorption (8). However, in contrast to previous reports, our study suggests that the upregulation of iron absorption is not secondary to iron depletion (38, 39) but rather results from an increased erythropoietic drive due to exercise, which both decreases PHep and increases EPO and erythrocyte mass.

Mild erythropoietic drive supersedes the mild inflammatory stimulus on hepcidin

The erythroid compartment is a strong determinant of PHep and iron metabolism (40–42). ERFE has been characterized in an animal model of thalassemia intermedia, where it strongly and causally suppressed PHep (43, 44), and increases in ERFE have been confirmed in human blood donors upon EPO administration, and in both transfused and nontransfused β-thalassemia patients (29). However, in our study, we did not find correlations between ERFE, PHep, and other iron markers and ERFE concentrations were generally below the limit of quantification of 14 ng/mL. This lack of correlation could be analytical (insufficient sensitivity) or evidence of a minor biological role of ERFE in conditions of mild ("nonstress") erythropoiesis. Additional studies and refinement of analytical techniques are likely to provide clarification of the role of ERFE under these conditions, including the role of other signaling molecules (44, 45) and mechanisms (46). To our knowledge, this is the first study to examine these determinants and assess iron absorption in recreational runners before and during an intensified-training phase. Our data in exercise are consistent with previous reports that suggest that erythroid signaling is a potent PHep modulator (47) and high erythroid iron demand blunts potential PHep induction by a concomitant inflammatory response (48–50). In human infants with anemia, PHep did not differ between infected and noninfected individuals, whereas nonanemic infants with infection had higher PHep concentrations than did those without infection (51). Furthermore, EPO was the strongest predictor of PHep in severely anemic children with concomitant elevation of EPO and IL-6 or CRP (52). These studies are consistent with a strong regulatory role by erythropoiesis on PHep, suggesting that this stimulus can at least partially offset the opposing effect of inflammation. Our finding that the erythropoietic signals prevail in their effect on PHep and iron bioavailability in exercise is also suggested by the following: *1*) the counterintuitive negative correlation between PHep and AGP and CRP immediately post-training, coupled with the positive IL-6–EPO correlation, suggesting that exercise induces both erythropoiesis and inflammation and the sum of the effects decreases PHep but increases AGP and CRP; *2*) by the observed negative correlation between erythroid iron utilization and PHep (Figure 3); and *3*) by the trend for a correlation between mHb expansion and the change in oral iron absorption.

Our data confirm the observation made in animals that erythropoietic activity, but not anemia, is required for hepcidin suppression (42), a mechanism likely responsible for the effects we observed in this study because our study participants were iron replete and nonanemic. Tissue hypoxia induces hypoxia-inducible factor 2α (HIF-2α) (53) in the kidney, which can increase renal synthesis and secretion of EPO (54). In this study, erythropoietic drive is unlikely to have been due to exercise-induced hemodilution, because we detected no change in plasma volume. Thus, the trend for an increase in iron absorption in our study is likely due to the downregulation of hepatic hepcidin synthesis by increasing erythropoiesis. However, it has also been suggested that hypoxia can directly affect duodenal iron absorption, because HIF-2α is stabilized in hypoxic enterocytes (55) and activates the expression of iron acquisition genes (55).

Although we found a trend for exercise to affect oral iron absorption, the detected increase in mHb is likely to have been caused by the increased availability of iron from macrophage and hepatocyte stores, facilitated by the prevailing lower PHep concentration in exercise (56) (Figure 2). Our data suggest that mobilization of iron stores in this short-term study was the predominant (>80%) source for the increase in red blood cell mass, with the contribution of dietary iron absorption being relatively minor (57). Over the long term, if maintained, the observed upregulation in oral absorption and decrease in PHep may be a significant contributor to iron balance in regularly exercising subjects. Additional data with larger subject groups are necessary to determine the long-term interplay of erythropoietic and inflammatory signals on iron status.

Our study has several limitations. Because the study included only 10 subjects, the possibility of a β-error in our comparisons is increased, and we could not expand our correlation analysis into a multivariate regression analysis. Due to the nature of the intervention, we could not conduct the study with a crossover design, so we cannot fully exclude the possibility of time-related confounding. Thus, larger studies in parallel groups and adequate controls are required to confirm these findings. Our subjects had, on average, a fair level of fitness, and our results should not be extrapolated to untrained subjects or elite athletes. Finally, our subjects were iron-replete men, and our findings may not apply to iron-deficient athletes or female athletes.

Despite these limitations, our study has a number of strengths. We used stable iron isotope labels to measure oral iron absorption and intravenous iron utilization in combination with accurate measurement of mHb, before and after a well-controlled exercise period. Participants acted as their own controls, increasing the power of the comparisons and addressing one of the main methodologic concerns in previous iron absorption studies in exercising subjects. Compliance to the exercise schedule was excellent. We conducted several comparisons and explanatory correlations, theoretically increasing the chance for an α error. However, several distinct and independent measures are all consistent with the overall picture of higher iron availability despite subclinical inflammation and are collectively very unlikely to be due to chance. This study suggests that, in recreational runners, erythropoietic drive effectively offsets low-grade inflammation and downregulates hepcidin, with no indication of a decrease in oral iron absorption. Our findings, although they require confirmation in other athlete populations and longer-term studies, suggest that during a phase of intensified training, resulting in

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mild erythropoiesis, the inflammation-hepcidin axis is offset by erythropoietic stimuli, resulting in increased iron mobilization and possibly oral absorption (5). Combining erythropoiesis-stimulating training into schedules where increased inflammation is anticipated may have a beneficial impact on the marginal iron status in regularly exercising subjects.

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The authors' responsibilities were as follows—DM and MBZ: conceived the study and obtained funding; DM, SM, MBZ, CL, and GMB: contributed to the study design; DM, CZ, AM, AG-M, and DWS: conducted the study and performed the measurements; DM: conducted the statistical analysis and wrote the first draft of the manuscript; and all authors: edited and read and approved the final version of the manuscript. DWS and AG-M are employees of Radboud University and Medical Center, which offers high-quality hepcidin measurements to the scientific, medical, and pharmaceutical community on a fee-for-service basis. None of the other authors had a competing interest to declare.

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