

Iron supplementation limits the deleterious effects of repeated blood donation on endurance sport performance but not on iron status

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Background - Every day, blood banks worldwide face the challenge of ensuring an adequate blood supply. Iron deficiency is by far the most common cause of deferral of blood donors. The aim of the present study was to determine the effect of iron supplementation after repeated blood donation on iron status and physiological performance.

Materials and methods - Forty-four moderately trained and iron-replete subjects were randomly divided into a whole blood donation (n=36) and a placebo donation (n=8) group. One third of the donation group received no iron supplementation, whereas one third received 20 mg iron and one third received 80 mg iron daily for 28 days. The subjects were intended to make three donations 3 months apart, and recovery of endurance capacity, assessed by an incremental maximal cycling test, and haematological parameters was monitored up to 28 days after each donation.

Results - Negative effects of repeated blood donation were found for markers of iron storage, markers of functional iron and/or iron metabolism regulation, and physiological markers. Iron supplementation did not affect iron storage but did limit, at the highest dose of 80 mg, the effect of blood donations on functional iron and/or iron metabolism regulation, and at both 20 and 80 mg the negative effects on maximal power output and peak oxygen consumption.

Discussion - Iron supplementation limited the deleterious effects of repeated blood donation on endurance sport performance but not on decline in iron status in iron-replete young men. These results underline the importance of iron supplementation to minimise the deleterious effects of blood donation on physiological functions, and the necessity to optimise the supplementation strategy to preserve iron status.

Keywords: erythropoietin, ferritin, maximal oxygen consumption, maximal power output, donor selection.

INTRODUCTION

Every day, red cell transfusions are needed for people undergoing surgery or giving birth, but also for patients who suffer from leukaemia or anaemia. As red cells cannot be made artificially, blood banks depend on whole blood donations to ensure an adequate blood supply. The availability of blood donors is influenced not only by the recruitment of new

Arrived: 8 April 2020

Revision accepted: 11 May 2020

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blood donors but also by changes in deferral policies. One of those policies concerns the iron status before donation. Iron deficiency is by far the most common cause of deferral of blood donors^{1,2}. The donation itself increases the chance of becoming iron-deficient^{2,3} and replenishment of iron stores takes many months, leading to a high rate of iron depletion, especially in frequent blood donors⁴. There are also concerns about the functional impact of donation on, for instance, brain function and development^{5,6}, although there is a lack of scientific data on this functional impact. It is therefore not surprising that different iron supplementation strategies have been tested⁷⁻¹¹, even if none seems really effective with regards to full recovery of ferritin levels within 3 months, the usual inter-donation recovery period, in non-iron deficient populations^{7,9}. Blood banks have developed their own iron supplementation strategy in the absence of evidence-based recommendations, but the impact of supplementation remains unclear. It is therefore important to test new strategies rigorously and to verify that the strategies are effective also for repeat donors, as only one study has tested the effects of iron supplementation in subjects giving repeated donations over a period of 2 years¹². In the search for new donors, moderately trained athletes are of particular interest as they are likely to be healthy and fit. The impact of blood donation on sports performance has been evaluated before by submaximal constant testing^{13,14}, submaximal¹⁵⁻¹⁷ or maximal^{13,18-32} progressive testing, a time-to-exhaustion test^{24,25,33} or time trial tests^{22,34}. These studies indicate that maximal but not submaximal performance is clearly decreased from immediately up to 2 weeks after blood donation. Only two of the studies had a proper placebo simulating blood donation without actual blood withdrawal^{16,21}, and only one study looked at the effect of repeated blood donation on exercise performance²¹. In these studies, no iron supplementation, which could help to limit the impact of repeated donation on blood parameters reflecting iron status, was given. The aim of the present study was therefore to determine the effect of iron supplementation after repeated blood donation on both iron status and endurance performance in a sample of non-iron-deficient volunteers at the start of the study, compared to an appropriate placebo group. This is the first study looking at the functional and physiological effects

of repeated blood donation coupled or not with iron supplementation.

MATERIALS AND METHODS

Subjects

Sample size was estimated using the software PASS 15 and the two-sample t-test using ratios. Eight subjects in the placebo group and 12 subjects in the blood donation group were sufficient to detect a 10% reduction of the mean (one-sided) in the blood donation vs the placebo groups with a power of 80%. The estimated standard deviation was 8.5%, the alpha value was 0.05 and the ratio between blood donation and placebo groups was 1.5. A 10% reduction corresponds to the expected changes in VO₂ peak values between 1 to 3 days post-donation and pre-donation^{20,25,26}. Forty-four young men (n=8 in the placebo group, n=12 in the donation + no iron group, n=12 in the donation + 20 mg iron group and n=12 in the donation + 80 mg iron group, randomly assigned) volunteered to participate in a single-blind longitudinal study (*Online Supplementary Content, Table SI*). Most of the volunteers were staff members or students from the UCLouvain. None of them was a previous blood donor. To provide 20 mg and 80 mg of elemental iron, the subjects were given 174 mg and 695 mg iron gluconate (Losferron®), respectively. The placebo and the donation + no iron groups received a maltodextrin tablet as a placebo for iron. The subjects had to take one tablet (iron or placebo) a day for 28 days post-donation or simulation of donation. The subjects were asked to take the tablet in the fasted state and at least 15 minutes before breakfast together with a glass of orange juice. A defined number of tablets was given to each subject, with a few tablets given in excess on purpose in order to test compliance at the end of each of the three experimental trials. The study drugs were labelled as required by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice (ICH-GCP) Guideline E6 (European Commission 2/3/2010). Inclusion criteria were as follows: recreational sportsmen (1 to 8 hours of sport a week), age 18 to 40 years, body mass index 20 to 28 kg·m⁻², no contraindication to performing exercise at maximal intensity. All participants provided written informed consent after all the potential risks of the study had been explained to them and they had been informed of their right to withdraw at any time.

This study was conducted at the UCLouvain in Belgium from November 2017 to June 2018. The Ethics Committee of the UCLouvain approved the study. The investigation was performed according to the principles outlined in the Declaration of Helsinki. Four subjects (1 in each group) gave up before the end of the study (**Figure 1**). Three subjects got injured independently of the study and one subject from the donation + 80 mg iron group did not end the second series of tests because of ferritin levels below 12 µg/L. The study (RK2017) was registered at clinicaltrialsregister.eu and received the identifier 2017-003406-40.

Exercise testing

One week before the first test series, subjects reported to the laboratory to perform a maximal incremental test (*Online Supplementary Content, Figure S1*). Fifteen minutes after

blood sampling, a peak oxygen consumption (VO₂ peak) test was performed on a bicycle ergometer (Avontronic, Cyclus 2, Leipzig, Germany). The protocol started at 70 Watts, followed by incremental loads of 30 Watts every 2 minutes until exhaustion. Blood lactate was measured before, during (at 190 Watt) and at the end of the test by taking a capillary blood sample (5 µL) from an earlobe (Lactate Pro 2, Arkray, Japan). The VO₂ peak test and blood sampling were repeated 2 days, 1 week, 2 weeks and 4 weeks after each of the three blood donations (see below) following exactly the same conditions (*Online Supplementary Content, Figure S1*). Due to ethical considerations, the VO₂ peak test was not performed on the day of donation.

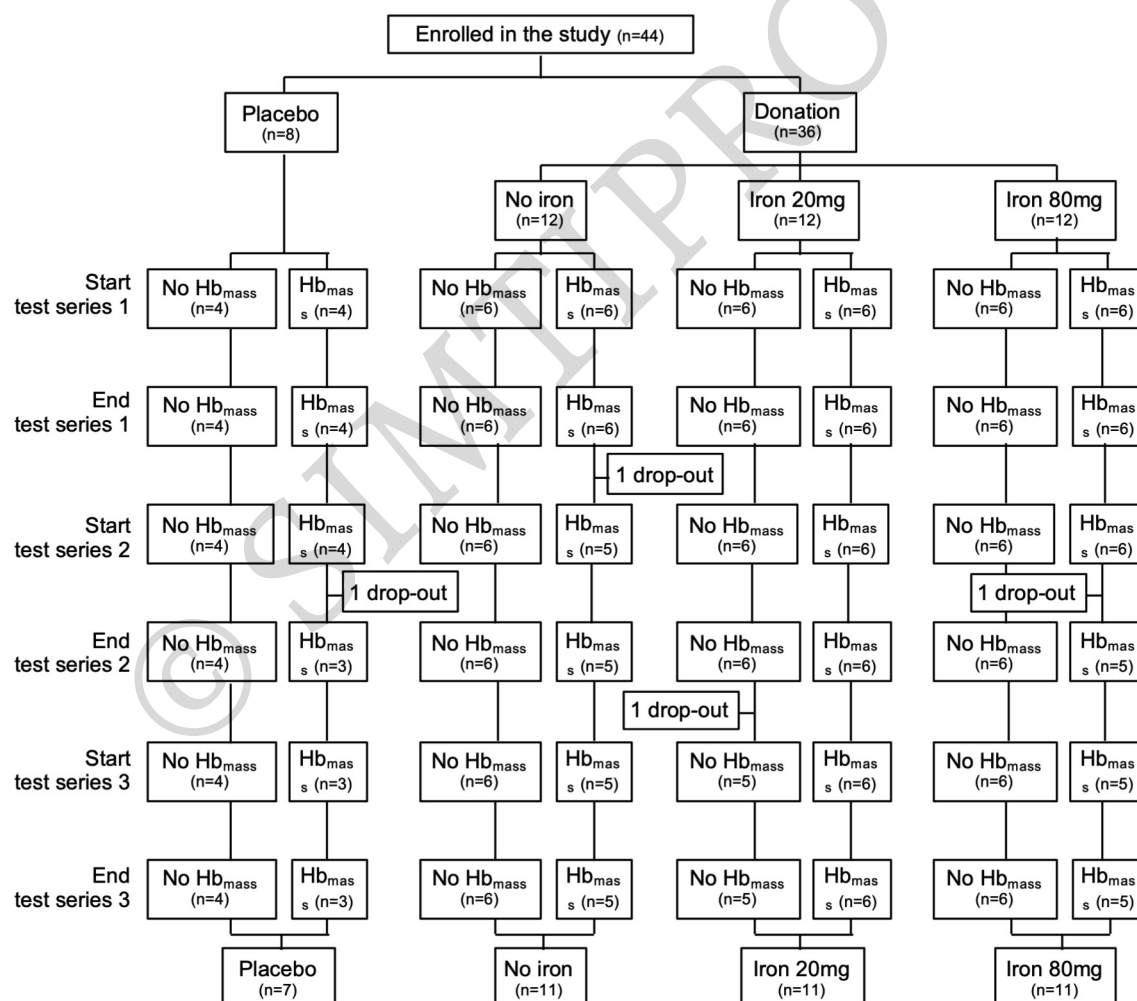


Figure 1 - Flow chart of the subjects in the study

Haemoglobin mass measurement

Due to practical issues, haemoglobin mass was determined in half of the subjects ($n=4$ in placebo and $n=6$ in each of the 3 donation groups). Haemoglobin mass was determined 30 min after the end of the incremental test to ensure that ventilation had returned to basal levels. It was measured using a modified version³⁵ of the optimised carbon monoxide (CO) re-breathing method developed by Schmidt and Prommer³⁶.

Blood donation

One week after exercise pre-testing, participants reported to the Red Cross donor center in Leuven (Belgium). They underwent a blood donation of 470 mL according to the Belgian Law of 01/02/2005 (Donation) or had an experience similar to undergoing a blood donation (Placebo) as described in Howell *et al.*¹⁶. During each donation or simulated donation, subjects were blinded and listened to music through a headset. To study the effect of repeated donations, the blood collection procedure or simulation was repeated after 3 months (second test series) and after 6 months (third test series).

Blood analyses

The following parameters were analysed using automated devices: platelet count, haematocrit, haemoglobin concentration, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, mean corpuscular volume, mean platelet volume, red blood cell count, red blood cell distribution width, white blood cell count (ABS Micros 60, Horiba, Diegem, Belgium), and ferritin, iron, and transferrin concentrations (Pentra C200, Horiba, Diegem, Belgium). The clot activator tubes were centrifuged at the end of each experimental day for 6 min at 3,000 g at 4 °C. The supernatant was collected and stored at -80 °C. Serum erythropoietin, hepcidin and soluble transferrin receptor (sTfR) levels were determined by enzyme-linked immunosorbent assay using Human Erythropoietin (DEP00), Hepcidin (DHP250) and soluble Transferrin Receptor (DTFR1) Quantikine Kits (Bio-Techne, Abingdon, UK), respectively. Log (sTfR/ferritin) was calculated as the base 10 logarithm of the ratio of sTfR (in $\mu\text{g/L}$) to ferritin (in $\mu\text{g/L}$)³⁷. sTfR/log(ferritin) was calculated as the ratio of sTfR (in $\mu\text{g/L}$) to the base 10 logarithm of ferritin (in $\mu\text{g/L}$)³⁸.

Statistical analysis

All values are expressed as the mean $\pm$ standard error of mean. Differences in subjects' characteristics at baseline were tested with one-way analysis of variance (GraphPad Prism Software, San Diego, CA, USA). The p-values can be found in **Table I**. A mixed model for repeated measures (computed with SAS Statistical Software 9.4, SAS Institute, Cary, NC, USA) was used with the subjects as a random variable and groups (placebo, donation alone, donation + 20 mg iron and donation + 80 mg iron) and condition (time) as fixed independent variables. The p-values of the main effects can be found in **Table I**. The model used Kenward-Roger approximation of degree of freedom with compound symmetry variance-covariance structure. When appropriate, contrast analyses were performed to compare means, applying a Sidak correction. Linear mixed models for repeated measures give unbiased results in the presence of missing data. Statistical significance was set at $p < 0.05$.

RESULTS

Lack of effect of iron supplementation on haemoglobin mass and plasma ferritin levels after repeated blood donation

Haemoglobin mass (Hb_{mass}) ($p=0.0065$) and plasma ferritin levels ($p < 0.0001$) evolved differently between the four groups after repeated blood donation (main group effect, **Table I** and **Figure 2A and B**). Hb_{mass} values were globally lower in the no iron group ($p=0.0064$), the 20 mg iron group ($p=0.0235$) and the 80 mg iron group ($p=0.0284$) compared to the placebo group. Similarly, plasma ferritin levels were globally lower in the no iron group ($p < 0.0001$), the 20 mg iron group ($p < 0.0001$) and the 80 mg iron group ($p=0.0005$) compared to the placebo group. Contrary to Hb_{mass} , which returned to basal values between donations, ferritin levels dropped from donation 1 to donation 2 and further from donation 2 to donation 3 in all donation groups ($p < 0.05$ – 0.001) (**Table II**). No protective effect of iron supplementation, whatever the dose, was measured for Hb_{mass} and ferritin levels.

Serum erythropoietin levels tended to evolve differently between the four groups after repeated blood donation (main group effect, $p=0.0707$) (**Table I** and **Figure 2C**). Erythropoietin levels tended to be higher in the no iron group ($p=0.0598$), but not in the 20 mg iron group ($p=0.2379$) or the 80 mg iron group ($p=0.4498$), compared to the placebo group.

Table I - p-values from the global analyses of variance

				No iron	20 mg	80 mg	20 mg	vs 80 mg	vs 20 mg
	Group	Time	G*T						
				vs P	vs P	vs P	No iron	No iron	80 mg
Hb mass	0.0065	<0.0001	0.1314	0.0064	0.0235	0.0284	0.9637	0.9528	1.0000
EPO	0.0707	<0.0001	<0.0001	0.0598	0.2379	0.4498	0.9813	0.8744	0.9995
Haematocrit	0.0192	<0.0001	<0.0001	0.1628	0.0119	0.2926	0.8654	0.9994	0.6129
Haemoglobin	0.3549	<0.0001	<0.0001	1.0000	0.5170	0.9810	0.6248	0.9720	0.9553
Iron	0.1376	0.1503	0.1082	0.6934	0.9990	0.9932	0.2794	0.1972	1.0000
Ferritin	<0.0001	<0.0001	0.0399	<0.0001	<0.0001	0.0005	1.0000	0.9790	0.9722
Hepcidin	0.0153	<0.0001	0.7980	0.0348	0.0164	0.1208	0.9998	0.9931	0.9426
sTfR	0.0280	<0.0001	<0.0001	0.0180	0.2190	0.2559	0.8465	0.8558	1.0000
log (sTfR/Fer)	<0.0001	<0.0001	<0.0001	<0.0001	0.0004	0.0128	0.9973	0.4487	0.7951
sTfR/(log Fer)	0.0006	<0.0001	<0.0001	0.0003	0.0122	0.0432	0.6942	0.3616	0.9968
Transferrin	0.6380	<0.0001	0.0003	0.8095	0.9954	0.9999	0.9784	0.8964	0.9999
MCH	0.1535	<0.0001	0.1072	0.9593	0.6855	0.9873	0.9905	0.5155	0.1819
MCHC	0.7320	<0.0001	0.5245	1.0000	0.9782	0.9819	0.9722	0.9607	1.0000
MCV	0.1471	<0.0001	0.0005	0.2368	0.9992	0.9954	0.3435	0.4508	1.0000
MPV	0.4385	<0.0001	0.0383	0.9973	0.9999	0.8409	0.9522	0.9816	0.5541
RBC	0.0295	<0.0001	<0.0001	0.2424	0.0187	0.3300	0.8270	1.0000	0.6941
RDW	0.6125	<0.0001	0.1564	0.9985	0.7491	0.9921	0.9266	1.0000	0.9728
WBC	0.5964	0.3657	0.8460	0.9656	1.0000	0.9988	0.9603	0.7004	0.9953
Platelets	0.2254	<0.0001	0.4117	0.8485	0.2590	0.9812	0.8936	0.9981	0.6171
Pmax	0.0198	<0.0001	0.6651	0.0193	0.7841	0.8811	0.2399	0.1551	1.0000
VO ₂ peak	0.0907	<0.0001	0.4482	0.0819	0.6340	0.3163	0.7617	0.9787	0.9954
Lactate 190W	0.0806	<0.0001	0.0774	0.9995	0.2323	0.9989	0.1521	1.0000	0.2751
Lactate post	0.3731	<0.0001	0.3522	0.9648	0.9592	1.0000	0.3982	0.9349	0.9388
HRmax	0.8017	<0.0001	0.5305	0.9692	1.0000	1.0000	0.9773	0.9496	1.0000
VEmax	0.9508	0.0085	0.1975	1.0000	1.0000	0.9983	0.9999	0.9999	0.9958
Total time	0.0753	<0.0001	0.5344	0.7970	0.4751	0.9964	0.9962	0.3163	0.1112
Oxygen pulse	0.1949	<0.0001	0.3094	0.2284	0.5420	0.9317	0.9931	0.7327	0.9777

Hb_{mass}: haemoglobin mass; EPO: erythropoietin; sTfR: soluble transferrin receptor; Fer: ferritin; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; MCV: mean corpuscular volume; MPV: mean platelet volume; RBC: red blood cell count; RDW: red blood cell distribution width; WBC: white blood cell count; Pmax: maximal power output; VO₂ peak: peak oxygen consumption; HR: heart rate; VE: ventilation.

Serum hepcidin ($p=0.0153$) and sTfR levels ($p=0.0280$) evolved differently between the four groups after repeated blood donation (main group effect) (Table I and Figure 2D, E). Hepcidin levels were globally lower in the no iron group

($p=0.0348$) and the 20 mg iron group ($p=0.0164$), but not in the 80 mg iron group ($p=0.1208$), compared to the placebo group. Compared to all other haematological parameters studied here, which were either unchanged, increased or

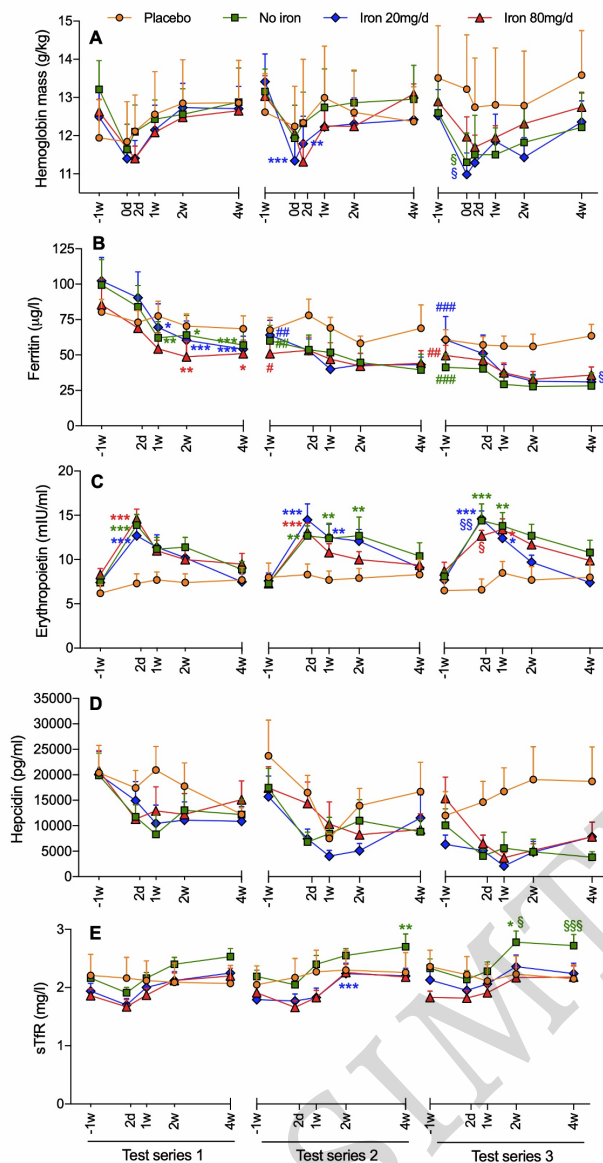


Figure 2 - Haemoglobin mass, plasma ferritin, serum erythropoietin, hepcidin and soluble transferrin receptor
Evolution of the haemoglobin mass (A), plasma ferritin (B), serum erythropoietin (EPO, C), hepcidin (D) and soluble transferrin receptor (sTfR) levels before and after each of the three test series in the placebo and donation groups.

Data are expressed as means $\pm$ standard error of mean. $n=8$ in the placebo group, $n=12$ in each of the three donation groups for test series 1; $n=5$ in the placebo group, $n=12$ in donation 20 mg group and $n=11$ in the donation no iron and donation 80 mg iron groups for test series 2; $n=5$ in the placebo group, $n=11$ in each of the three donation groups for test series 3. $^{\circ}p<0.05$, $^{\circ\circ}p<0.01$, $^{\circ\circ\circ}p<0.001$ vs placebo, same time. $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ vs -1w of test series 1, same group. $^{\ast}p<0.05$, $^{\ast\ast}p<0.01$, $^{\ast\ast\ast}p<0.001$ vs 1 week before the same test series, same group. -1w: one week before the test series; 0d: day of donation; 2d: two days post-donation; 1w: one week post-donation; 2w: two weeks post-donation; 4w: four weeks after blood donation.

decreased after blood donation, sTfR levels were bi-phasically regulated by blood donation. Up to 1 week after donation, sTfR levels were decreased before increasing beyond the baseline level at weeks 2 and 4 after donation (Figure 2E).

sTfR levels were globally different in the no iron group ($p=0.0180$), but not in the 20 mg iron group ($p=0.2190$) or the 80 mg iron group ($p=0.2559$), compared to the placebo group. These results showed that iron supplementation, whatever the dose, limited the effects of repeated blood donation on sTfR levels and that only the highest dose, i.e. 80 mg iron, reduced the decrease in hepcidin levels after blood donation. No cumulative effect of repeated donation was observed for erythropoietin, hepcidin and sTfR, as they all returned to basal values between donations.

Similarly to Hb_{mass} and ferritin, log (sTfR/ferritin) ($p<0.0001$) and sTfR/log (ferritin) ($p=0.0006$) evolved differently between the four groups after repeated blood donation (main group effect) (Table I). Log(sTfR/ferritin) and sTfR/log (ferritin) values were globally lower in the no iron group ($p<0.0001$ and $p=0.0003$), the 20 mg iron group ($p=0.0004$ and $p=0.0122$) and the 80 mg iron group ($p=0.0128$ and $p=0.0432$) compared to the placebo group. In the donation groups, log (sTfR/ferritin) and sTfR/log (ferritin) values at baseline increased from donation 1 to donation 3 ($p<0.05$ - 0.001) (Table II). No protective effect of iron supplementation, whatever the dose, was measured for log (sTfR/ferritin) and sTfR/log (ferritin) values.

Haematocrit ($p=0.0192$) and red blood cell count ($p=0.0295$) evolved differently between the four groups after repeated blood donation (main group effect, Tables I and II). Haematocrit was globally lower in the 20 mg iron group ($p=0.0119$) but not in the no iron group ($p=0.1628$) and the 80 mg iron group ($p=0.2926$) compared to the placebo group. Similarly, red blood cell counts were globally lower in the 20 mg iron group ($p=0.0187$) but not in the no iron group ($p=0.2424$) and the 80 mg iron group ($p=0.3300$) compared to the placebo group. No group effect was measured for haemoglobin ($p=0.3549$), iron ($p=0.1376$), transferrin ($p=0.6380$), mean corpuscular haemoglobin ($p=0.1535$), mean corpuscular haemoglobin ($p=0.7320$), mean corpuscular volume ($p=0.1471$), mean platelet volume ($p=0.4385$), red cell distribution width ($p=0.6125$), white blood cell count ($p=0.5964$) and platelet count ($p=0.2254$) (Tables I and II).

Table II - Effect of repeated blood donation on haematological parameters

	Test series 1					Test series 2				
	-1w	2d	1w	2w	4w	-1w	2d	1w	2w	4w
Hct (%)	P	41.8±1.1	40.4±1.0	41.0±0.8	40.2±0.8	38.9±0.9	39.8±1.3	39.6±0.8	39.9±0.9	39.8±1.3
	D0	43.9±0.7	39.0±0.6***	39.4±0.9***	40.1±0.8***	41.2±0.8***	39.0±0.5***	39.5±0.7***	39.4±0.8***	40.5±0.8***
	D20	42.4±1.0	37.2±0.7***	37.9±0.9***	38.9±0.8***	40.2±0.7***	37.0±0.5***	37.5±0.7***	38.5±0.6***	39.1±0.7***
	D80	43.0±0.7	39.0±0.6***	39.3±0.4***	40.7±0.6***	41.4±0.8***	38.3±0.5***	38.5±0.7***	39.9±1.1***	40.0±0.8***
Hb (g/dL)	P	15.3±0.4	15.0±0.4	15.0±0.3	14.8±0.3	14.4±0.3	14.7±0.3	14.6±0.3	14.7±0.3	14.8±0.3
	D0	16.0±0.3	14.4±0.2***	14.6±0.3***	14.6±0.3***	15.0±0.3***	14.4±0.2***	14.5±0.3***	14.6±0.3***	14.9±0.3***
	D20	15.6±0.3	13.8±0.2***	14.0±0.3***	14.3±0.3***	14.8±0.2***	13.8±0.2***	13.9±0.3***	14.2±0.2***	14.4±0.2***
	D80	15.5±0.3	14.3±0.2***	14.4±0.2***	14.7±0.2***	15.0±0.3***	14.2±0.2***	14.1±0.2***	14.6±0.4***	14.7±0.2***
Iron (µg/dL)	P	129±10	115±19	120±23	109±13	109±14	112±18	89±7	109±12	106±16
	D0	130±18	139±19	103±14	83±12	102±20	108±20	64±8	87±16	100±16
	D20	121±13	122±15	103±8	122±16	112±9	105±13	104±12	113±14	135±17
	D80	105±10	124±13	100±11	128±11	105±11	111±16	126±26	132±21	99±11
Tf (g/L)	P	2.89±0.08	2.83±0.05	2.59±0.07	2.59±0.08	2.43±0.05***	2.41±0.05***	2.57±0.06	2.51±0.05	2.52±0.06
	D0	2.92±0.09	2.66±0.08**	2.64±0.08**	2.69±0.07	2.52±0.06***	2.53±0.10	2.64±0.10	2.70±0.09	2.77±0.10
	D20	2.84±0.08	2.63±0.09	2.53±0.08**	2.65±0.08	2.58±0.10*	2.38±0.09	2.58±0.10	2.61±0.11	2.67±0.10
	D80	2.87±0.09	2.69±0.06	2.56±0.07**	2.67±0.07	2.54±0.07**	2.44±0.09	2.54±0.08	2.63±0.11	2.61±0.11
log (sTfR/Fer)	P	1.42±0.09	1.45±0.08	1.43±0.09	1.46±0.09	1.48±0.08	1.48±0.07	1.50±0.08	1.58±0.07	1.56±0.11
	D0	1.39±0.07	1.41±0.07	1.59±0.07	1.66±0.08***	1.72±0.09***	1.62±0.08	1.79±0.11	1.84±0.09	1.92±0.10***
	D20	1.31±0.06	1.33±0.07	1.54±0.09	1.63±0.09***	1.67±0.07***	1.50±0.09	1.71±0.08	1.79±0.08***	1.74±0.07**
	D80	1.47±0.08	1.52±0.09	1.67±0.09	1.78±0.08***	1.75±0.08***	1.68±0.09	1.73±0.08	1.85±0.07	1.81±0.08
sTfR/ (log Fer)	P	1.19±0.21	1.18±0.22	1.16±0.22	1.16±0.20	1.15±0.19	1.14±0.18	1.25±0.20	1.31±0.19	1.31±0.23
	D0	1.12±0.06	1.03±0.06	1.26±0.08	1.43±0.10	1.56±0.13**	1.31±0.10	1.26±0.11	1.70±0.15	1.87±0.19***
	D20	0.99±0.07	0.91±0.06	1.17±0.10	1.29±0.11	1.36±0.09	1.05±0.09	1.21±0.12	1.47±0.14***	1.41±0.10
	D80	1.06±0.08	1.01±0.09	1.22±0.12	1.42±0.13	1.43±0.12	1.24±0.11	1.23±0.10	1.54±0.12	1.47±0.13*
MCH (pg)	P	31.6±0.5	31.9±0.5	31.5±0.6	31.7±0.5	31.9±0.5	32.2±0.5	32.2±0.6	32.1±0.5	31.9±0.6
	D0	31.7±0.4	32.1±0.4	32.3±0.4	31.8±0.4	31.6±0.4	32.0±0.5	31.8±0.5	32.2±0.4	31.8±0.5
	D20	31.7±0.3	31.8±0.4	31.8±0.4	31.6±0.4	31.8±0.3	32.0±0.3	31.9±0.3	31.9±0.4	31.7±0.4
	D80	30.9±0.2	31.5±0.3	31.4±0.2	31.1±0.3	31.4±0.3	31.5±0.2	31.7±0.2	31.5±0.3	31.8±0.3

Values are means ± standard error of mean (n=7 in the placebo (P) group and n=13 in the donation (D) groups for test series 1; n=5 in the P group and n=13 in the D groups for test series 2 and 3). Hct, haematocrit; Hb, haemoglobin; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; MPV, mean platelet volume; RBC, red blood cell count; RDW, red blood cell distribution width; Tf, transferrin; sTfR, soluble transferrin receptor; Fer, ferritin; WBC, white blood cell count; P, placebo; D, donation. *p<0.05 vs placebo, same time; **p<0.01 vs -1w of test series 1, same group; ***p<0.001 vs -1w of test series 1, same group; -1w: one week before the test series; 0d: day of donation; 2d: two days post-donation; 1w: one week post-donation; 2w: two weeks post-donation; 4w: four weeks post-donation.

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Table II - Effect of repeated blood donation on haematological parameters (continued from previous page)

Test series 1															Test series 2															Test series 2														
	-1w	2d	1w	2w	4w	-1w	2d	1w	2w	4w	-1w	2d	1w	2w	4w	-1w	2d	1w	2w	4w	-1w	2d	1w	2w	4w																			
MCHC (g/dL)	P	36.6±0.2	37.1±0.2	36.6±0.3	36.8±0.2	37.1±0.2	36.9±0.2	37.0±0.3	37.1±0.3	36.8±0.2	36.3±0.2	36.8±0.4	36.3±0.1	36.6±0.2	35.7±0.2																													
	D0	36.4±0.1	37.0±0.2	37.1±0.2	36.5±0.1	36.4±0.2	36.7±0.2	36.9±0.2	36.7±0.2	37.1±0.1	36.8±0.2	36.1±0.2	36.8±0.2	36.2±0.2	35.8±0.2																													
	D20	36.9±0.1	37.2±0.1	36.9±0.1	36.7±0.1	36.9±0.1	36.8±0.2	37.2±0.2	37.0±0.1	37.0±0.2	36.9±0.1	36.3±0.3	36.5±0.2	36.2±0.2	35.8±0.1																													
	D80	36.0±0.1	36.6±0.2	36.5±0.2	36.1±0.2	36.2±0.2	36.4±0.3	37.0±0.2	36.7±0.2	36.6±0.3	36.7±0.3	36.0±0.2	36.2±0.2	36.1±0.2	35.4±0.1																													
MCV (fL)	P	86.2±1.3	86.2±1.4	86.2±1.2	86.4±1.2	86.2±1.3	87.2±1.3	86.6±1.5	86.8±1.4	86.5±1.4	86.0±1.5	88.2±1.5	86.7±1.4	87.5±1.5	88.8±1.6***																													
	D0	87.1±0.8	86.9±0.9	87.1±0.8	87.2±0.9	86.8±1.0	87.1±0.9	86.1±0.9	86.5±0.9	86.7±1.0	86.3±1.0	85.8±1.0	85.1±1.0	85.9±1.0	86.9±1.1***																													
	D20	86.0±0.9	85.6±0.9	86.0±0.9	86.1±0.9	86.3±0.8	86.9±0.7	85.8±0.8	86.2±0.8	86.3±0.9	95.9±0.9	85.2±1.0	86.1±0.9	85.8±0.9	86.9±0.9**	88.0±0.9***																												
	D80	85.9±0.4	86.0±0.5	85.9±0.5	86.1±0.5	86.5±0.5	86.6±0.7	85.9±0.5	86.4±0.5	86.1±0.7	86.4±0.5	84.8±0.7	85.4±0.6	85.3±0.6	86.5±0.6**	87.5±0.6***																												
MPV (fL)	P	7.8±0.3	7.3±0.2	7.4±0.2	7.3±0.2	7.3±0.2	7.6±0.3	7.3±0.3	7.0±0.2	7.3±0.2	7.2±0.2	7.1±0.2	7.5±0.2	7.5±0.3	7.6±0.2																													
	D0	7.1±0.2	7.2±0.2	6.8±0.1	6.8±0.1	6.9±0.2	6.8±0.1	6.8±0.2	7.0±0.1	6.8±0.1	7.1±0.2	7.2±0.2	7.3±0.2	7.3±0.1	7.5±0.2																													
	D20	7.2±0.2	7.1±0.3	6.8±0.2	6.9±0.2	7.0±0.2	6.8±0.2	7.0±0.2	6.8±0.2	6.9±0.2	6.8±0.2	7.2±0.2	7.2±0.2	7.1±0.2	7.4±0.2																													
	D80	7.4±0.2	7.5±0.3	7.2±0.2	7.1±0.1	7.2±0.2	7.1±0.1	7.2±0.1	7.1±0.2	7.2±0.2	7.2±0.2	7.3±0.2	7.3±0.2	7.3±0.1	7.5±0.2																													
RBC (10 ¹² /L)	P	4.9±0.1	4.7±0.1	4.8±0.1	4.7±0.1	4.5±0.1*	4.6±0.1	4.6±0.1	4.6±0.1	4.6±0.1	4.6±0.1	4.7±0.2	4.6±0.1	4.7±0.2	4.6±0.1																													
	D0	5.0±0.1	4.5±0.1***s	4.5±0.1***s	4.6±0.1***	4.8±0.1**	4.9±0.1	4.5±0.0***	4.6±0.1**	4.5±0.1***	4.7±0.1	4.9±0.1	4.5±0.1***	4.6±0.1	4.7±0.1																													
	D20	4.9±0.1	4.4±0.1***	4.4±0.1***	4.5±0.1***	4.7±0.1*	4.6±0.1**	4.3±0.1***	4.4±0.1**	4.5±0.1	4.6±0.1	4.7±0.1	4.4±0.1***	4.4±0.1**	4.6±0.1																													
	D80	5.0±0.1	4.5±0.1***	4.6±0.1***	4.7±0.1**	4.8±0.1	4.9±0.1	4.5±0.1***	4.5±0.1***	4.6±0.1	4.6±0.1	4.7±0.1	4.5±0.1*	4.6±0.1	4.6±0.1																													
RDW (%)	P	12.3±0.3	12.2±0.3	12.2±0.2	12.0±0.3	11.9±0.2	12.2±0.4	12.4±0.3	12.5±0.3	12.3±0.2	12.6±0.2	12.2±0.3	11.8±0.1	12.2±0.3	11.7±0.2																													
	D0	12.2±0.1	12.1±0.1	12.2±0.1	12.1±0.1	11.9±0.1	12.2±0.1	12.2±0.1	12.4±0.2	12.4±0.2	12.3±0.1	11.8±0.1	11.7±0.2	11.9±0.2	11.7±0.1																													
	D20	12.4±0.2	12.1±0.2	12.4±0.2	12.3±0.2	12.2±0.2	12.0±0.1	12.2±0.1	12.2±0.1	12.1±0.2	12.4±0.1	11.8±0.1	11.6±0.1	11.7±0.2	11.8±0.1																													
	D80	12.6±0.2	12.3±0.2	12.3±0.1	12.5±0.2	12.3±0.1	12.2±0.1	12.2±0.1	12.3±0.1	12.4±0.1	12.5±0.1	12.0±0.1	11.8±0.1	12.0±0.1	11.9±0.1																													
WBC (10 ⁹ /L)	P	6.3±0.4	6.3±0.3	6.4±0.4	6.6±0.5	6.3±0.4	6.4±0.3	6.3±0.3	6.2±0.3	6.4±0.5	6.6±1.0	5.9±0.5	6.5±1.2	6.5±0.6	6.2±0.4																													
	D0	6.5±0.3	6.3±0.5	6.7±0.5	6.7±0.4	7.1±0.5	6.5±0.5	6.5±0.5	6.7±0.5	7.2±0.5	7.4±0.5	6.7±0.5	6.4±0.2	6.9±0.4	6.0±0.2																													
	D20	7.0±0.6	6.7±0.3	6.6±0.5	6.2±0.3	6.6±0.3	6.7±0.5	6.8±0.6	6.3±0.4	6.3±0.5	6.4±0.3	6.4±0.8	6.0±0.5	5.7±0.4	5.9±0.4																													
	D80	6.2±0.4	6.1±0.3	6.6±0.4	7.9±1.7	6.4±0.4	5.6±0.3	6.0±0.4	6.0±0.4	5.5±0.3	5.2±0.4	5.6±0.4	5.9±0.3	5.6±0.3	5.2±0.3																													
Platelets (10 ⁹ /L)	P	233±13	220±15	205±13	213±10	221±13	215±11	219±15	229±15	228±19	219±23	214±21	196±14	203±14	197±14																													
	D0	250±13	233±10	243±12	252±9	260±18	214±10	250±18	264±13	242±15	223±16*	232±19	218±16	223±10	207±14																													
	D20	264±17	257±16	271±13	277±15	256±13	246±11	262±13	271±15	268±13	249±14	251±17	256±14	256±14	231±15																													
	D80	232±18	227±16	242±18	248±14	234±18	226±14	237±16	236±20	227±14	221±19	212±9	223±20	223±16	201±15																													

Values are means ± standard error of mean (n=7 in the placebo [P] group and n=13 in the donation [D] groups for test series 1; n=5 in the P group and n=13 in the D groups for test series 2 and 3). Hct, haematocrit; Hb, haemoglobin; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MPV, mean platelet volume; RBC, red blood cell count; RDW, red blood cell distribution width; Tf, transferrin; sTfR, soluble transferrin receptor; Fer, ferritin; WBC, white blood cell count; P, placebo; D, donation. Sp=0.05 vs placebo, same time; *p<0.05; **p<0.01; ***p<0.001 vs -1w of test series 1, same group. -1w: one week before the test series; 0d: day of donation; 2d: two days post-donation 1w: one week post-donation; 2w: two weeks post-donation; 4w: four weeks post-donation.

Repeated blood donation alters maximal power output in the no iron group but not in the two groups given iron supplementation

Maximal power (P_{max}) evolved differently between the four groups after repeated blood donation (main group effect, $p=0.0198$) (Table I and Figure 3A). The evolution of P_{max} after repeated blood donation was different between the no iron group and the placebo group ($p=0.0193$) but not between the 20 mg ($p=0.7841$) and 80 mg iron groups ($p=0.8811$) and the placebo group. VO_2 peak tended to evolve differently between the four groups after repeated blood donation (main group effect, $p=0.0907$) (Table I and Figure 3B). The evolution of VO_2 peak after repeated blood donation tended to be different between the no iron group and the placebo group ($p=0.0819$) and was not different between the 20 mg ($p=0.6340$) and 80 mg iron group ($p=0.3163$) and the placebo group. These results show that iron supplementation, whatever the dose, limits the

effects of repeated blood donation on P_{max} and VO_2 peak. Lactate 190W (main group effect, $p=0.0806$) and total time (main group effect, $p=0.0753$) tended to evolve differently between the four groups after repeated blood donation (Tables I and III). No difference between groups was detected for lactate post ($p=0.3731$), maximal heart rate ($p=0.8017$), maximal ventilation ($p=0.9508$) and for oxygen pulse ($p=0.1949$).

DISCUSSION

The present study is the first to look prospectively at the effect of iron supplementation on functional and physiological parameters after repeated blood donation instead of one single donation, which better mimics donors' real-life behaviour. In addition, we included a placebo group which the earlier studies lacked, in order to evaluate the effect of iron supplementation after blood donation more rigorously.

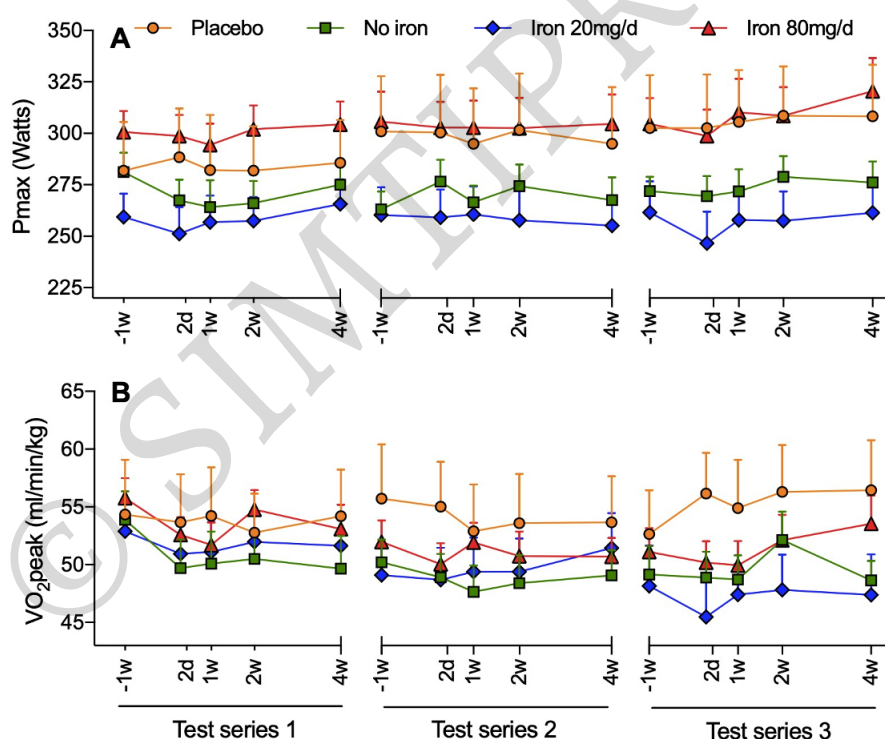


Figure 3 - Maximal power output and peak oxygen consumption

Evolution of the maximal power output (P_{max} , A) and peak oxygen consumption (VO_2 peak, B) before and after each of the three test series in the placebo and donation groups. levels before and after each of the three test series in the placebo and donation groups. Data are expressed as means $\pm$ standard error of mean. $n=8$ in the placebo group, $n=12$ in each the three donation groups for test series 1; $n=5$ in the placebo group, $n=12$ in the donation 20 mg group and $n=11$ in the donation no iron and donation 80 mg groups for test series 2; and $n=5$ in the placebo group, $n=11$ in each of the three donation groups for test series 3. P_{max} : maximal power output; VO_2 peak: peak oxygen consumption; -1w: one week before the test series; 0d: day of donation; 2d: two days post-donation; 1w: one week post-donation; 2w: two weeks post-donation; 4w: four weeks post-donation.

Table III - Effect of repeated blood donation on exercise parameters

		Test series 1						Test series 2						Test series 2					
		-1w	2d	1w	2w	4w	-1w	2d	1w	2w	4w	-1w	2d	1w	2w	4w			
Lactate 190W (mmol/L)	P	4.9±1.9	4.5±1.5	5.0±1.8	4.5±1.7	4.1±1.4	4.1±1.8	3.7±1.4	3.5±1.3	4.3±1.9	4.0±1.7	3.8±1.7	3.2±1.1	2.7±0.8	2.7±1.0	3.2±1.2			
	D0	3.2±0.3	3.5±0.4	3.1±0.4	3.2±0.4	2.9±0.2	2.5±0.3	2.8±0.3	2.7±0.3	2.6±0.3	2.6±0.3	2.5±0.3	2.8±0.4	2.8±0.3	2.7±0.3	2.7±0.2			
	D20	4.7±0.7	5.7±0.7	5.3±0.7	4.7±0.5	4.3±0.5	4.6±0.6	5.0±0.6	4.9±0.6	4.1±0.5	4.3±0.6	4.1±0.5	4.4±0.6	4.7±0.5	3.6±0.4	4.1±0.5			
	D80	2.7±0.4	3.1±0.5	2.6±0.4	2.9±0.5	2.2±0.3	2.5±0.4	2.8±0.5	2.7±0.6	2.3±0.4	1.9±0.3	2.1±0.4	2.3±0.3	2.5±0.5	2.3±0.4	2.1±0.3			
Lactate post (mmol/L)	P	11.7±1.4	11.7±1.6	11.0±1.6	11.5±1.5	11.8±1.3	11.0±1.3	10.5±0.8	9.1±1.0	11.8±0.9	9.9±1.7	10.8±1.0	10.1±1.4	10.4±1.8	9.7±1.2	8.8±1.5			
	D0	10.4±0.9	10.3±0.9	11.4±0.9	9.8±1.0	9.1±0.9	7.6±0.8	10.1±0.9	9.3±0.8	9.8±1.0	8.1±0.9	7.8±1.1	9.0±1.2	8.9±1.2	9.2±1.2	7.4±1.1			
	D20	12.5±1.3	12.6±1.1	12.3±1.3	11.4±1.1	12.7±1.0	11.4±1.1	12.3±0.8	12.8±1.0	11.5±1.0	10.8±1.0	10.4±0.7	9.7±1.2	11.2±1.3	9.8±1.0	11.1±1.1			
	D80	10.5±1.0	11.5±1.3	10.1±1.2	11.8±1.3	9.7±1.1	9.4±1.0	10.1±1.0	9.5±0.8	8.9±0.9	9.4±1.1	8.4±0.7	9.3±0.6	9.9±0.9	8.8±1.1	9.1±0.9			
HR max (bpm)	P	189±4	191±3	191±4	187±3	186±3	188±4	186±4	184±4	183±4	182±4	186±4	184±4	184±5	184±4	180±4			
	D0	193±3	190±2	190±3	187±3	186±4	186±4	191±3	185±4	188±4	184±4	181±5 ^{***}	187±3	185±3	186±4	184±3			
	D20	194±2	192±3	193±2	192±2	191±2	189±2	191±2	189±3	187±3	187±3	188±3	185±4	187±2	185±3	191±3			
	D80	185±3	188±3	185±3	186±3	182±3	183±3	184±3	182±3	180±3	180±3	180±2	181±3	181±3	179±3	180±3			
VE max (l/min)	P	153±12	152±14	156±13	147±17	155±13	170±14	164±14	155±13	166±16	155±15	161±16	159±14	168±16	166±14	167±15			
	D0	173±6	153±7	153±9	154±5	156±7	151±6	167±7	151±5	158±8	152±8	152±7	159±7	157±6	161±9	160±8			
	D20	163±5	158±8	157±6	158±6	158±6	149±8	150±8	150±5	153±7	143±9	153±9	141±9	156±8	145±6	154±11			
	D80	166±8	168±9	160±9	167±9	164±9	153±9	157±9	159±9	157±11	154±11	162±9	153±8	159±8	169±11	169±11			
Total time (min)	P	16.1±0.6	16.6±0.6	15.9±0.6	16.2±0.4	16.6±0.4	17.4±0.8	17.4±0.9	17.0±0.8	17.4±0.8	17.0±0.8	17.5±0.7	17.5±0.7	17.7±0.7	17.9±0.6	17.9±0.7			
	D0	16.1±0.6	15.2±0.7	15.5±0.7	15.2±0.7	15.5±0.7	14.5±0.6	15.8±0.7	15.1±0.5	15.6±0.7	15.0±0.9	15.5±0.5	15.3±0.7	15.5±0.7	15.9±0.7	15.5±0.7			
	D20	14.6±0.8	14.1±0.9	14.5±0.9	14.7±0.8	15.2±0.1	14.7±0.9	14.6±0.9	14.7±0.9	14.5±1.0	14.2±0.8	14.8±1.0	14.8±1.0	14.5±0.9	14.5±1.0	14.8±1.0			
	D80	17.4±0.7	17.3±0.7	17.0±0.7	17.6±0.8	17.6±0.7	17.5±0.9	17.5±0.8	17.5±0.9	17.5±1.0	17.6±1.0	17.6±0.8	17.3±0.9	18.0±1.1	17.9±0.9	18.0±1.9			

Values are means ± standard error of mean (n=8 in placebo, n=12 in each of the three donation groups for test series 1; n=5 in the placebo group, n=12 in the donation 20 mg group and n=11 in the donation no iron and donation 80 mg groups for test series 2; n=5 in placebo, n=11 in each of the three donation groups for test series 3). HR: heart rate; VE: ventilation; P: placebo; D0: donation + no iron; D20: donation + 20 mg iron; D80: donation + 80 mg iron; -1w: one week before the test series; 0d: day of donation; 2d: two days post-donation; 1w: one week post-donation; 2w: two weeks post-donation; 4w: four weeks post-donation. ***p<0.001 vs -1w of test series 1, same group.

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Table III - Effect of repeated blood donation on exercise parameters (continued from previous page)

	Test series 1					Test series 2					Test series 2				
	-1w	2d	1w	2w	4w	-1w	2d	1w	2w	4w	-1w	2d	1w	2w	4w
P	0.29±0.03	0.28±0.02	0.28±0.02	0.28±0.02	0.29±0.02	0.30±0.03	0.30±0.02	0.29±0.02	0.29±0.02	0.30±0.02	0.28±0.02	0.31±0.02	0.30±0.02	0.31±0.02	0.31±0.03
D0	0.28±0.01	0.26±0.01	0.26±0.01	0.27±0.01	0.27±0.01	0.27±0.01	0.26±0.01	0.26±0.01	0.26±0.01	0.27±0.01	0.27±0.01	0.26±0.01	0.26±0.01	0.28±0.01	0.26±0.01
D20	0.27±0.02	0.27±0.01	0.27±0.01	0.27±0.02	0.27±0.01	0.26±0.02	0.26±0.02	0.26±0.02	0.27±0.02	0.28±0.02	0.26±0.02	0.25±0.02	0.25±0.02	0.26±0.02	0.25±0.02
D80	0.30±0.01	0.28±0.01	0.28±0.01	0.30±0.01	0.29±0.01	0.28±0.01	0.27±0.01	0.29±0.01	0.28±0.01	0.28±0.01	0.29±0.01	0.28±0.01	0.28±0.01	0.29±0.01	0.30±0.02

Values are means ± standard error of mean (n=8 in placebo, n=12 in each of the three donation groups for test series 1; n=5 in the placebo group, n=12 in the donation 20 mg group and n=11 in the donation no iron and donation 80 mg groups for test series 2; n=5 in placebo, n=11 in each of the three donation groups for test series 3). HR: heart rate; VE: ventilation; P: placebo; D0: donation + no iron; D20: donation + 20 mg iron; D80: donation + 80 mg iron; -1w: one week before the test series; 0d: day of donation; 2d: two days post-donation; 1w: one week post-donation; 2w: two weeks post-donation; 4w: four weeks post-donation. ##p<0.001 vs -1w of test series 1, same group.

Currently, there are no international guidelines concerning iron supplementation after blood donation. Different formulations, amounts and durations of use are prescribed according to local policies. Prospective interventional studies are scarce and primarily performed on iron-deficient donors (ferritin <30 µg/L)^{8,11,12,39}, with two studies comparing individuals with higher (>26 µg/L) vs lower (<26 µg/L) ferritin levels^{7,9}. Of note, the threshold below which ferritin levels reflect iron deficiency remains itself unclear and has been proposed to be between 10 and 40 µg/L⁴. Here, in all donation groups, ferritin levels dropped to about 30 µg/L 1 month after the third blood donation. Whether this value reflects true iron deficiency depends on the threshold chosen. At a minimum, repeated blood donation favours a state of iron insufficiency. Beyond this debate around threshold, it has never been tested before whether iron supplementation could prevent the fall in ferritin levels with repeated blood donation in non-iron-deficient donors and thereby prevent their deferral later on. In the present study, neither 20 mg nor 80 mg iron per day for 28 days post-donation limited the fall in ferritin levels from 100 µg/L to 30 µg/L after three blood donations. The same observation was made for Hbmass, the post-donation loss of which could not be prevented by either dose of iron. These results indicate that the strategy of iron supplementation used here was not sufficient either to prevent or to limit the drop in ferritin levels and Hbmass in individuals with normal iron status pre-donation. It was previously shown that ferritin levels took about 100 days to return to pre-donation levels of about 50 µg/L when 37.5 mg iron per day was administered⁷. This strategy seems difficult to apply in real-life as it implies that donors would have to take iron supplements continuously from one donation to the next. In addition, such a supplementation scheme raises issues of compliance and habituation. In addition to causing stomach discomfort and constipation, daily iron intake is known to be less effective over time because of, among other factors, an increase in hepcidin levels⁴⁰. Hepcidin levels have been positively associated with ferritin levels¹¹. Blood donation was found to cause a rapid drop in hepcidin levels, which persisted for several weeks⁴¹. We confirmed those results here and found that 80 mg, but not 20 mg, iron supplementation

limits the effect of repeated blood donation somewhat as the hepcidin levels in the 80 mg group were not different from those of the placebo group over time.

In addition to Hbmass, ferritin and hepcidin, sTfr can be used as a valuable marker for determining iron status⁴. Increased sTfr levels reflect the functional iron compartment and have been shown to correlate with depleted iron stores³⁸. Accordingly, here, blood donation increased erythropoietin levels within 2 days while it took about 2 weeks for soluble transferrin levels to increase. Both 20 mg and 80 mg iron supplementation strategies seemed to dampen the effects of blood donation on the levels of sTfr and erythropoietin as neither of these strategies was found to be different from the placebo.

Taken together, our results show that iron supplementation, at least at the highest dose of 80 mg, was able to limit the effect of repeated blood donation on erythropoietin, hepcidin and sTfr levels, all markers of functional iron and/or iron metabolism regulation. However, beneficial effects were not seen on iron storage markers such as Hbmass and ferritin levels. In summary, the different markers for iron status indicate that repeated blood donation induced iron insufficiency and that iron supplementation had very selective corrective effects on iron status. A very important point to mention is that all volunteers were iron-replete young men and that the present conclusions only apply to this population. Indeed, iron-deficient subjects absorb more oral iron than iron-replete subjects, with potentially more beneficial effects of oral supplementation in iron-deficient volunteers than those observed here.

Alongside determining the effect of iron supplementation on iron status after repeated blood donation, the second aim of the study was to investigate the functional and physiological impact of supplementation. As poor iron status can indirectly affect endurance exercise performance by reducing oxygen transport capacity, the functional outcome chosen here was the maximal power output, which is highly dependent on peak oxygen consumption. The amelioration of maximal power output observed in the placebo group was blunted in the donation group not receiving iron. In the latter group, the peak oxygen consumption tended to be lower than in the placebo group over the whole experiment. For both

functional outcome measures, iron supplementation appeared to be beneficial as their evolution over time was not different from that of the placebo group, indicating that the negative effects of repeated blood donation on maximal power output and peak oxygen consumption were limited by iron intake. While no statistical difference was found between the 20 mg and the 80 mg groups, it is interesting to mention that the training effect we found on the maximal power output was gradually larger from the donation no iron group to the placebo group. From the first to the last test, maximal power output remained constant in the no iron group, increased by 3% in the 20 mg iron group, by 6% in the 80 mg iron group and by 7% in the placebo group. We hereby confirm the results we obtained after repeated blood donation in the placebo and donation group in our first study²¹ and extended them to the effect of iron supplementation.

Based on our results, the protective role of iron on peak oxygen consumption as well as on maximal power output is probably attributable to a preservation of enzymatic activity in the mitochondria within skeletal muscle rather than a preservation of oxygen transport capacity. Iron is a critical component of enzymes in the respiratory chain such as succinate dehydrogenase and cytochrome oxidase⁴². Further investigation is needed to determine the molecular mechanisms by which skeletal muscle function and metabolism benefits from iron supplementation to limit the deleterious effects of repeated blood donation on peak oxygen consumption and maximal power output.

An important finding of the present study is that restoration of physiological effects precedes the normalisation of Hbmass. If other physiological effects are also restored prior to Hbmass, haemoglobin measures, as currently conducted in Europe, may be sufficient as a reference to guide whether a donor should be deferred from donation. Ferritin levels should perhaps only be used to optimise/individualise iron supplementation strategy.

CONCLUSIONS

Iron supplementation limited the deleterious effects of blood donation on endurance sport performance but not the decline in iron status after repeated blood donation

in iron-replete young men. This beneficial effect of iron supplementation on endurance performance was probably mediated by the preservation of the activity of key enzymes involved in skeletal muscle function and metabolism rather than preservation of oxygen transport capacity as Hb_{mass} was not regulated by iron supplementation, whatever the dose used. These results underline the importance of iron supplementation to minimise the deleterious effects of blood donation on physiological functions, and the necessity to optimise the supplementation strategy to preserve iron status.

ACKNOWLEDGEMENTS

We thank Lutgarde Barrez for technical assistance during blood donations, Stéphanie Ernoux and Jean Cremer for technical assistance during exercise testing, and Céline Bugli for the statistical analyses. The study was funded by the Science Foundation of the Belgian Red Cross.

AUTHORSHIP CONTRIBUTIONS

Protocol design: BP, PV, LD; data acquisition: BP, DN, NB, RB, CVA, LD; data interpretation: CVA, VC, PV, LD; manuscript drafting: LD; manuscript revision and approval: BP, DN, NB, RB, CVA, VC, PV, LD.

DISCLOSURE OF CONFLICT OF INTEREST

The Authors have no conflict of interest to disclose. The results of the present study do not constitute endorsement by ACSM. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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