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BLOOD DONORS & BLOOD COLLECTION

TRANSFUSION

Impact of a single blood donation on hemoglobin mass, iron stores, and maximum oxygen uptake in pre-menopausal women—A pilot study

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Abstract

Background: During whole blood donation (BD), 500 mL of blood is drawn. The time interval between two BDs is at least 8–12 weeks. This period might be insufficient for restoring hemoglobin mass (Hbmass) and iron especially in women, who generally have lower Hbmass and iron availability. Since both variables influence physical performance, this pilot study aimed to monitor Hbmass, iron status, and maximum oxygen uptake ($\dot{V}O_{2max}$) recovery in women after a single BD.

Study Design and Methods: In 10 women (24.7 ± 1.7 years), Hbmass, hemoglobin concentration [Hb], iron status, and $\dot{V}O_{2max}$ were assessed before and up to 12 weeks after a single BD.

Results: BD reduced Hbmass from 562 ± 70 g to 499 ± 64 g ($p < .001$). Although after 8 weeks no significant mean difference was detected, 7 women had not returned to baseline after 12 weeks. [Hb] did not return to initial values (13.4 ± 0.7 g/dL) after 12 weeks (12.9 ± 0.7 g/dL, $p < .01$). Ferritin decreased from baseline until week 6 (40.9 ± 34.2 ng/mL vs. 12.1 ± 6.9 ng/mL, $p < .05$) and was not restored after 12 weeks (18.4 ± 12.7 ng/mL, $p < .05$), with 6 out of 10 women exhibiting iron deficiency (ferritin <15 ng/mL). $\dot{V}O_{2max}$ was reduced by 213 ± 47 mL/min (7.2 ± 1.2%; $p < .001$) and remained below baseline after 12 weeks (3.2 ± 1.4%, $p < .01$).

Discussion: For most pre-menopausal women, 12 weeks were not sufficient to recover from BD and achieve baseline Hbmass and iron stores resulting in prolonged reduction of aerobic capacity. A subsequent BD might lead to a severe anemia.

KEYWORDS

anemia; iron deficiency, blood volume, CO-rebreathing, hemoglobin

1 | INTRODUCTION

During whole blood donation (BD), ~500 mL of blood, containing approx. a total of 60–80 g hemoglobin is drawn regardless of body proportions when a minimum body weight of 50 kg is exceeded[1] and regardless of the sex of the donor. Women generally have a lower blood volume (BV) compared to men (approx. 78 mL/kg body mass or 4700 mL[2] vs. 85 mL/kg or 7100 mL[3]) and a hemoglobin concentration [Hb] that is 10% lower than in males (approx. 14.0 vs. 15.5 g/dL) and therefore considerably lower total body hemoglobin (Hbmass: 570 g[2] vs. 980 g[3]). Hence, the impact of BD on the hematological status is more pronounced in women, who lose ~12% of their Hbmass compared to ~7% in men.

Sufficient iron stores are essential to replace hemoglobin. One gram of hemoglobin contains 3.38 mg of iron[4] resulting in an iron demand of 240 mg after BD. However, women generally have lower iron stores than men,[5,6] exacerbated by menstruation.[7] Indeed, menstruating women often show iron deficiency (10%–30%) or iron deficiency anemia (1.5%–14%, in European countries).[7–9] In 2019, 29.9% of women aged 15–49 years worldwide were anemic, equivalent to over half a billion women.[10] To reduce the risk of iron deficiency after BD a minimum interval of 8 weeks,[11–13] or preferably 12–16 weeks[14] is advised between BDs. The total number of BDs per year is limited; in Germany, for instance, men and women are allowed to donate whole blood six and four times, respectively, in the United Kingdom, four and three times, and in the United States, six times without differences between sexes.[1,15,16]

To exclude anemia prior to BD, the only variable considered is [Hb], determined immediately before donation, whereby women with a concentration <12.5 g/dL and men <13.5 g/dL (Germany) are excluded from donation.[17] Inter-individual [Hb] is highly variable, indicated by the wide normal ranges of 12.0–16.0 g/dL for females and 13.5–17.5 g/dL for males.[18] [Hb] decreases when iron stores are nearly depleted, so donors whose [Hb] is slightly above the thresholds may already be affected by iron deficiency and the onset of anemia. After BD, Hbmass in men is restored after 36 ± 11 days, so 8 weeks until the next BDs are sufficient.[17] However, it is unknown whether this interval is sufficient for restoration in females.

Since the iron status of women has often not normalized 8 weeks after BD,[19] we hypothesize that an iron deficit is responsible for incomplete regeneration of the hemoglobin status in women, not detectable by determining [Hb] alone. [Hb] is influenced by plasma volume (PV) and therefore also by numerous external factors including hydration status,[20] body position,[21] circadian rhythm,[22] and exercise[23] and does not accurately represent Hbmass. The determination of Hbmass[17,24–26] excludes all these confounding factors and should therefore be used in the scientific field, but is yet to be investigated in female blood donors.

Endurance performance is closely related to the absolute amount of circulating hemoglobin.[27] In men, the BD-induced decline in Hbmass was associated with a reduction of $\dot{V}O_{2max}$[24,27–29] of approximately 250 mL/min,[27] which returned to baseline 5 weeks after BD. To the best of our knowledge, only a few studies exist on the recovery of aerobic performance after BD in women which have shown a decrease of 7.5 ± 1.1% in $\dot{V}O_{2peak}$ after BD which remained below baseline for 28 days.[30] Our hypothesis is that 8 weeks are not sufficient for complete recovery of performance in women with initially normal or low [Hb].

Therefore, the aim of this study was to determine Hbmass and iron status in women before and for 12 weeks after BD and to monitor their influence on performance ($\dot{V}O_{2max}$). The results are intended to inform the discussion on the maximum frequency of BDs and the necessity for iron supplementation after BD for women.

2 | METHODS

2.1 | Subjects

Thirteen female participants aged 19–28 years were recruited. Subject characteristics are presented in Table 1. According to the regulations of the local blood donating service (Bavarian Red Cross), 10 of them were allowed to donate blood. Iron supplementation was not permitted during the study. The study conformed to the standards set by the Declaration of Helsinki, except for registration in a database. Participation was voluntary, and the subjects were free to withdraw from the study at any time without reason. All subjects were informed of the requirements and risks of the study and provided written consent. The study was approved by the Ethics Committee of the University of Bayreuth/Germany (O 1305/1–GB).

2.2 | Study design

Blood components, iron status, and $\dot{V}O_{2max}$ were measured as described below, before, 1 day after, and regularly for 12 weeks following the donation of one unit of blood (~500 mL; see Figure 1). All participants completed the whole study except four volunteers who did

TABLE 1 Subject characteristics.

	Age (years)	Height (cm)	Body mass (kg)	BMI (kg/m^2)	rel. Hbmass (g/kg)	[Hb]* (g/dL)	Ferritin (ng/mL)	VO_2max (mL/min/kg)
Mean ± SD	24.7 ± 1.7	168 ± 5	63.5 ± 6.6	22.5 ± 1.9	8.9 ± 0.6	13.4 ± 0.7	40.9 ± 34.2	47.0 ± 4.4
Min	22	161	51.0	19.7	8.0	12.1	11.0	41.1
Max	28	177	72.0	25.5	9.8	14.9	130.0	55.3
Median	24.5	166	64.2	22.9	9.0	13.4	31.5	46.3

Note: Data are presented as mean values ± standard deviation (SD) as well as minimum and maximum.
Abbreviations: * venous [Hb]; Hbmass, total hemoglobin mass; rel., per body mass.

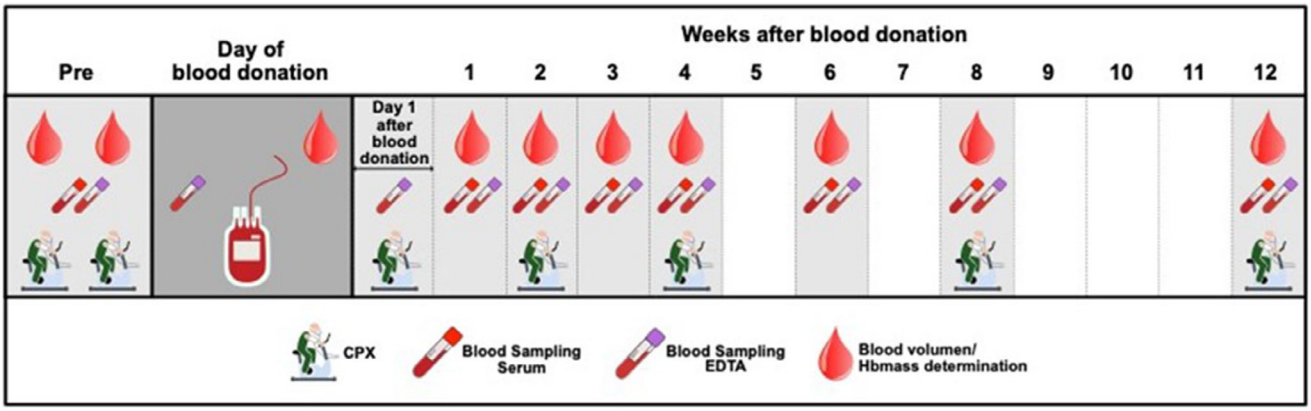

FIGURE 1 Study design. [Color figure can be viewed at wileyonlinelibrary.com]

not perform the cardio pulmonal exercise (CPX) test at week 12.

2.3 | Determination of hemoglobin mass and BV

A CO-bolus (0.8 mL/ kg body mass) was administered with 3 L of pure oxygen to the subject via a glass spirometer, which was then rebreathed for 2 min. Capillary blood samples taken from a hyperemized earlobe before and 6 and 8 min after the rebreathing procedure were analyzed for carboxyhaemoglobin (%COHb) using an OSM3 hemoximeter (Radiometer, Copenhagen, Denmark). For a detailed description of the method see.[26,31] The typical error for Hbmass determined from the duplicate measurements prior to the BD was 1.9% corresponding to 10.4 g of Hbmass. Hbmass was assumed to be completely restored after BD when baseline values were reached considering the typical error (baseline values—1.9%).

Subsequently, BV, erythrocyte volume (EV), and PV were calculated according to formulas 2–4:

$$\mathrm{BV\,(mL)} = (\mathrm{Hbmass\,(g)} \times 100)/(\mathrm{[Hb]\,(g/dL)} \times \mathrm{F}) \quad (1)$$

Hbmass = hemoglobin mass; [Hb] = venous hemoglobin concentration; F = cell factor (ratio peripheral/central [Hb]).[32]

$$\mathrm{EV\,(mL)} = \mathrm{Hbmass\,(g)}/\mathrm{MCHC} \times 100 \quad (2)$$

MCHC = Mean corpuscular hemoglobin concentration

$$\mathrm{PV\,(mL)} = \mathrm{BV} - \mathrm{EV} \quad (3)$$

The blood compartments immediately after BD were calculated using [Hb] of the blood sample taken immediately before BD.

2.4 | Incremental step test

An incremental step test on a cycle ergometer (Excalibur Sport, LODE®, Netherlands) was performed to determine $\dot{V}O_{2max}$. After a 3-min warm-up at 50 W, the load was continuously increased by 17, W every minute (in total

50 W / 3 min) until subjective exhaustion. Maximum respiratory exchange ratio (RER_{max}) ≥1.10 and cadence <80 rpm were applied as termination criteria that were achieved by all participants in all tests. Respiratory gases were analyzed breath-by-breath using the METALYZER® 3B (Cortex, Leipzig, Germany). The mean value of the last 30 s before exhaustion was used to determine the $\dot{V}O_{2max}$.

2.5 | Venous blood sampling

Venous blood samples were drawn before and for 12 weeks after BD (see Figure 1) in the morning from a cubital vein after 10 min in a seated position. A total of 10 mL of blood was centrifuged, and the serum was aliquoted and analyzed for ferritin, transferrin, transferrin saturation, C-reactive protein (CRP), erythropoietin concentration (EPO), and hepcidin. Additionally, 2.7 mL was drawn into ethylenediaminetetraacetate (EDTA) tubes to determine the [Hb], hematocrit (Hct), derived erythrocyte indices (MCH, MCHC, and MCV), and reticulocytes using a fully automated hematology system (Sysmex XN 1000-1-A, Sysmex, Norderstedt, Germany). Ferritin, transferrin, transferrin saturation, and CRP were analyzed by Roche Cobas C701 and E801 modules (Roche Holding AG, Basel Switzerland). Hepcidin was determined using a solid phase enzyme immunoassay (Hepcidin-25 (bioactive) HS ELISA [Enzyme-Linked Immuno Sorbent Assay] RE54261; IBL International GmbH, Germany). EPO was analyzed with a specific immunoassay (Erythropoetin ELISA NM56011, IBL International, Germany). An additional blood sample (EDTA) was taken before BD and was analyzed for [Hb] to calculate Hbmass in the transfusion bag. Furthermore, folic acid and vitamin B_{12} were measured using a Roche Cobas E801 module (Roche Holding AG, Basel Switzerland) once at the beginning of the study to exclude a deficit and a possibly associated, ineffective erythropoiesis.

2.6 | Blood donation

Volunteers underwent the general admission procedure at the local BD center before BD including questionnaires, medical check, and control of [Hb] through capillary blood sample taken from the earlobe (HemoCue®, HemoCue Holding AB, Ängelholm, Sweden). The [Hb] determined here is approx. 3%–4% above the concentration determined in venous blood using an automated system.[33]

All volunteers exceeded the [Hb] cutoff of 12.5 g/dL. Approximately, 500 mL of blood was drawn from a cubital vein. The volume of the blood drawn was determined by weighing the bag and tubing used, taking into account the specific gravity of the blood. A blood sample was taken immediately before the BD for analyzing [Hb]. Hbmass in the transfusion bag was then calculated according to the following formula:

$$\mathrm{Hbmass_{TB}\,(g)} = \mathrm{BV_{TB}\,(mL)} * \mathrm{[Hb]\,(g/dL)/100} \quad (4)$$

$\mathrm{Hbmass_{TB}}$ = hemoglobin mass in the transfusion bag; $\mathrm{BV_{TB}}$ = Blood volume in the transfusion bag.

2.7 | Statistics

Statistical analyses were performed with Graph Pad Prism 8.0 (GraphPad, La Jolla, CA, USA). Data are presented as mean ± standard deviation (SD). All data were assessed for normal distribution by means of the Kolmogorov–Smirnow test. A one-way ANOVA with a correction of multiple comparisons by controlling the false discovery rate or mixed effect model with multiple comparisons and two-stage linear step-up procedure of Benjamini, Krieger, and Yekutieli was performed to analyze the effects of BD on hematological parameters and $\dot{V}O_{2max}$ and to monitor the time course of the recovery process. To evaluate any causal relationship between the change in $\dot{V}O_{2max}$ and the change in Hbmass, a linear regression analysis was performed. The level of significance was set to $p < .05$.

3 | RESULTS

The total volume of blood drawn during BD including tubes and needles as well as the blood removed for the samples needed for tests for blood type and infectious diseases was 529 ± 11 mL, corresponding to a calculated loss in Hbmass of 70 ± 4 g. Due to blood sampling, subjects lost additionally 123 ± 5 mL blood over the course of the study corresponding to a loss in Hbmass of 16.3 ± 0.7 g in 12 weeks.

3.1 | Hematological parameters

Measured Hbmass decreased from 562 ± 70 g to 499 ± 64 g (−11.3 ± 1.6%, $p < .001$ compared to baseline), corresponding to a reduction of body mass

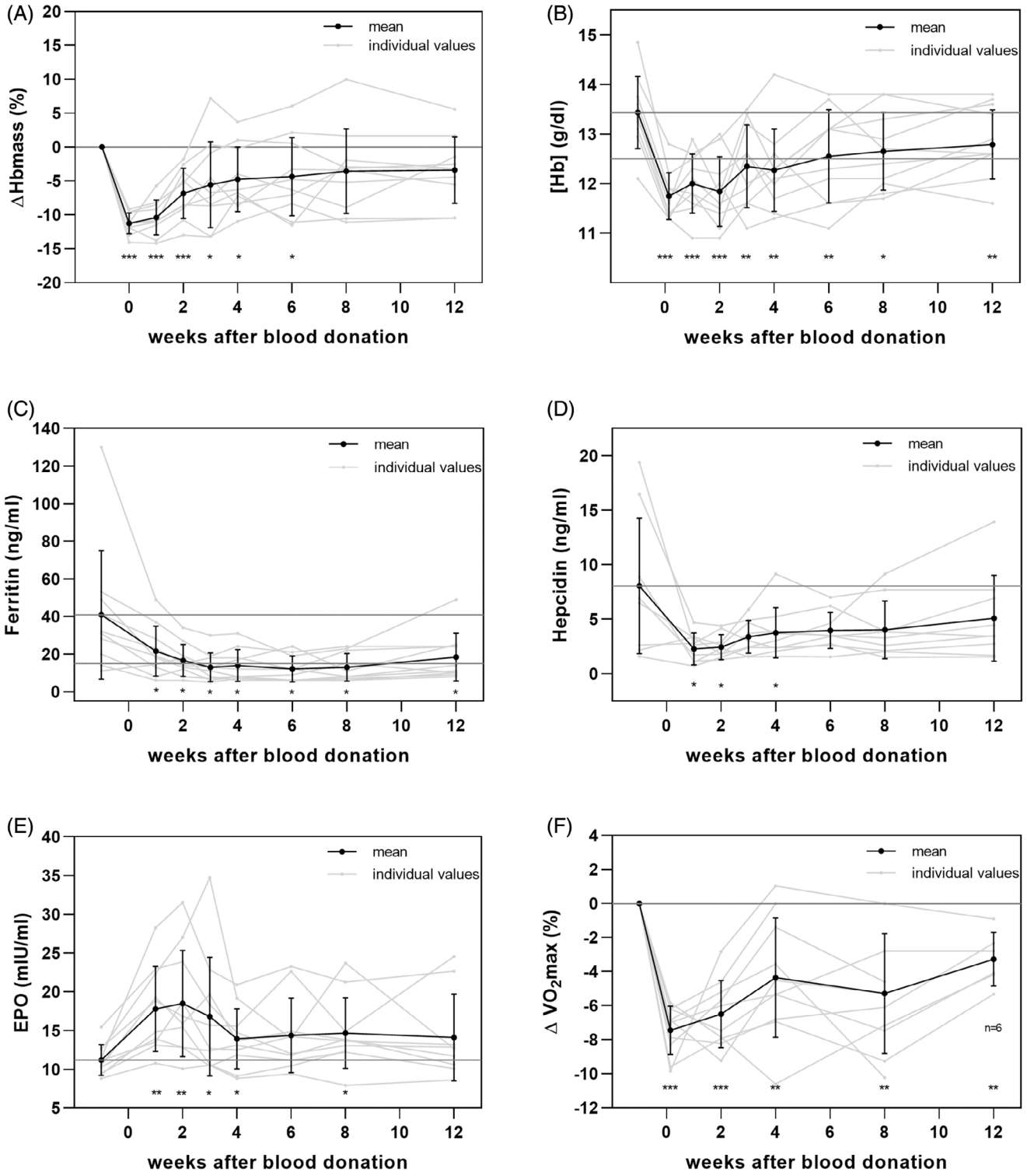

FIGURE 2 Time course of hematological parameters (A: hemoglobin mass (Hbmass); B: hemoglobin concentration ([Hb]); C: ferritin; D: hepcidin; E: erythropoietin (EPO)) and performance (F: maximum oxygen uptake ($\dot{V}O_{2max}$)) after a blood donation. Significant differences to baseline value: ***$p \leq .001$; **$p \leq 0.01$; *$p \leq .05$.

normalized Hbmass from 8.9 ± 0.6 g/kg to 7.9 ± 0.6 g/kg. Hbmass remained at a significantly lower level until week 6 after BD ($p < .05$). Accounting for the typical error (1.9%) of the CO rebreathing method, 7 out of 10 subjects did not reach their baseline Hbmass after 12 weeks (Figure 2A).

BV decreased by 483 ± 137 mL (EV: 175 ± 34 mL; PV: 308 ± 133 mL, $p < .001$) immediately after BD. The

TABLE 2 Time course of blood compartments after a blood donation.

	Weeks	Pre	BD	1	2	3	4	6	8	12[a]
Hbmass (g)	Mean	562.1	499.0	504.6	524.8	532.8	536.7	539.0	543.7	543.5
	SD	69.5	64.0***	70.5***	75.4***	88.2*	82.5*	84.4*	86.9	94.2
BV (mL)	Mean	4561.2	4078.5	4614.2	4872.3	4734.8	4818.6	4724.7	4717.0	4665.9
	SD	508.1	500.5***	537.4	654.2**	673.5	754.3	682.8	629.4	719.0
PV (mL)	Mean	2897.3	2589.4	3124.3	3322.8	3156.1	3211.9	3112.9	3095.3	3044.4
	SD	355.5	337.3***	338.2**	452.6***	423.8*	518.8*	461.7*	403.8	452.8
EV (mL)	Mean	1663.9	1489.1	1489.9	1549.5	1578.8	1606.7	1611.8	1621.7	1621.5
	SD	205.2	188.8***	217.9***	223.2***	267.3*	251.9	243.7	239.5	260.6

Note: Significant differences to baseline value: ***$p \leq .001$; **$p \leq .01$; *$p \leq .05$.
Abbreviations: BV, blood volume; EV, erythrocyte volume; Hbmass, total hemoglobin mass; PV, plasma volume.
[a]$n = 9$.

TABLE 3 Time course of hematological parameters after a blood donation.

	Weeks	Pre	1	2	3	4	6	8	12[a]
Transferrin (mg/dL)	Mean	298.1	291.4	319.1	333.4	335.1	323.3	328.9	324.4
	SD	62.7	44.7	62.8**	47.0**	58.1**	47.0*	54.3**	61.8*
Tsat (%)	Mean	28.0	16.5	13.6	16.7	14.0	15.1	15.8	17.5
	SD	10.5	13.6*	5.8***	8.4*	10.9**	9.0**	7.2**	8.7*
MCV (fl)	Mean	90.8	90.3	89.9	89.6	89.4	88.6	88.1	86.8
	SD	2.5	2.7	2.1*	2.3*	3.0	2.9**	2.4***	2.3***
MCH (pg)	Mean	30.4	30.6	30.5	30.3	29.9	29.6	29.5	29.1
	SD	0.8	1.0	0.9	1.3	1.1*	1.2*	1.2*	1.0**
MCHC (g/dL)	Mean	33.5	33.9	33.9	33.8	33.4	33.4	33.5	33.5
	SD	0.8	1.2	0.7	1.1	0.7	1.3	1.1	0.7
Hct (%)	Mean	40.2	35.4	34.9	36.6	36.7	37.5	37.8	38.2
	SD	1.9	1.7***	1.7***	1.9**	1.8**	1.9**	1.5**	1.8**
Reti (%)	Mean	1.4	1.8	1.6	1.5	1.4	1.1	1.1	1.0
	SD	0.2	0.5**	0.4	0.3	0.3	0.2*	0.3**	0.2***

Note: Significant differences to baseline value: ***$p \leq .001$; **$p \leq .01$; *$p \leq .05$.
Abbreviations: Hct, hematocrit; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; Reti, Reticulocytes; Tsat, transferrin saturation.
[a]$N = 9$.

day after BD, PV rose by 227 ± 205 mL above baseline and remained at a significantly higher level until the 6th week. BV had returned to baseline levels the day after BD and was unchanged after the second week (Table 2).

[Hb] decreased by 1.7 ± 0.5 g/dL ($p < .001$) 1 day after BD and was still significantly reduced after 12 weeks by 0.5 ± 0.5 g/dL ($p < .05$). At this time point, 8 out of 10 subjects exceeded the limit of 12.5 g/dL but did not reach their initial value (Figure 2B).

3.2 | Measures of iron deficiency

One week after BD, ferritin concentration decreased by $47.0 \pm 15.8\%$ reaching the lowest values (12.1 ± 6.9 ng/mL; $p < .05$) in week 6. Although a slight regeneration became apparent after 12 weeks, 6 of 10 participants were still below 15 ng/mL (Figure 2C). Two weeks after BD, serum transferrin significantly increased and remained at a higher level until week 12, while transferrin saturation showed the opposite time course (see Table 3).

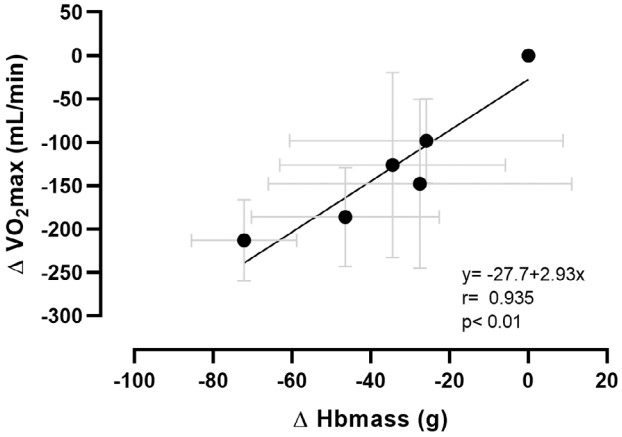

FIGURE 3 Relationship between changes in hemoglobin mass (ΔHbmass) and $\dot{V}O_{2max}$.

Hepcidin decreased by 51.2 ± 50.5% ($p < .05$) 1 week after BD and remained at a significantly lower level until week 4 (Figure 2C). EPO increased by 57.9 ± 37.6% 1 week after BD and remained at a significantly higher level up to week 8 (Figure 2D).

MCH and MCV decreased after BD, reaching significantly lower values compared to baseline after 4 and 2 weeks, respectively (Table 3).

3.3 | Aerobic capacity ($\dot{V}O_{2max}$)

After BD, $\dot{V}O_{2max}$ decreased by 213 ± 47 mL/min. Although $\dot{V}O_{2max}$ increased, thereafter, it remained significantly below baseline after 12 weeks (−98.3 ± 48.3 mL; $p < .05$; Figure 2F). Linear regression analysis between the mean change in $\dot{V}O_{2max}$ as dependent variable and mean change in Hbmass yielded a close relationship ($r = 0.93$; Figure 3).

4 | DISCUSSION

The major findings of this study indicate that 12 weeks after BD, 7 out of 10 women did not reach their initial Hbmass. This was accompanied by iron deficiency (ferritin <15 ng/mL) in five of them. Moreover, we observed a significant decrease in the $\dot{V}O_{2max}$ by 7.5%. $\dot{V}O_{2max}$ was still reduced up to 12 weeks after the BD.

4.1 | Hemoglobin mass

Similar to a previous study,[17] there was no significant difference between the calculated loss in Hbmass and the measured Hbmass, confirming the validity of the method used for monitoring the recovery of Hbmass.

One day after BD, BV loss was compensated by an increase in PV, so that declining [Hb] (−1.7 g/dL) reflected the reduced Hbmass. Thereafter, BV remained almost constant over the entire observation period due to the slowly increasing red cell volume and the accompanied gradual decrease in PV. This observation, which is known in principle,[34,35] proves the primary regulation of the BV, to which the red cell volume contributes in the long term and the PV in the short term.

To date, the recovery of Hbmass after BD has only been investigated in male blood donors.[17,24,25] In this study on female blood donors, Hbmass decreased by approx. 63 g corresponding to 13.6 ± 1.9% of baseline value, which is much higher than the 7.0%–8.8% decrease described in male donors.[17,24,25]

A recovery time of 36 days in men has previously been described,[17] with 72% of participants returning to baseline values, with the longest recovery lasting 8 weeks, and these findings have been replicated.[24] In the present study in women, Hbmass recovery was considerably delayed (Figure 2A). Up to week 6, there was a significant change from baseline, which was resolved by only 60% of the participants after 12 weeks. At this time point, approx. 75% of the hemoglobin lost through BD had been replaced.

4.2 | Iron status

The incomplete recovery of Hbmass 12 weeks after BD can partly be explained by insufficient iron availability. While the iron balance in men largely normalizes between two BDs,[36] incomplete compensation is often found in women.[7,37] A total of 25% of male first-time donors and 32% of regular donors failed to return to baseline ferritin concentration in 8 weeks after BD. Also, hepcidin, ferritin, and EPO had not returned to baseline by 180 days in first-time male donors.[38] In our study, all of the hematological and iron status parameters are similarly affected, and the time to complete recovery is substantially prolonged. During the first 6 weeks after BD, the ferritin concentration successively decreased by approx. 30 ng/mL, caused by increased erythropoietic activity.

Ferritin was already low (≤20 ng/mL) in three participants before BD and was still significantly decreased in the whole group 12 weeks later. The lowest mean ferritin value of 12 ng/mL was achieved in week 6 after BD, indicating iron deficiency (<15 ng/mL).[39] Even after 12 weeks, only four subjects recovered from this

deficiency and only one regained her baseline value. To reproduce the amount of hemoglobin removed, 270 mg of iron[4] is required, which corresponds to a ferritin level of about 34 ng/mL.[40,41] In fact, only 46 g of hemoglobin was restored, which corresponds to 205 mg iron and 26 ng/mL of ferritin, that is, almost exactly the difference from the baseline value in this study.

Sufficient iron supply for erythropoiesis is controlled via intestinal iron absorption from food and iron release from ferritin following a reduction[42,43] in the concentration of hepcidin.[44–46] We also found a significant drop of 51.2 ± 50.5% of hepcidin 1 week after BD. However, there were women who had low iron stores at baseline, and hence a low hepcidin concentration, which could not be further reduced following BD. We therefore conclude that in these women, intestinal iron absorption cannot be adequately adapted to the higher iron demand and may be one reason for the risk of the development of iron deficiency and anemia. A previous study[42] observed an increase in hepcidin after BD only when the [Hb] had returned to the individual normal value. In this study, neither parameter returned to baseline.

The other measures of the iron status determined here also indicate an iron deficiency that has not been compensated for up to 12 weeks after BD. Transferrin and transferrin saturation are still significantly increased or decreased, respectively, EPO was still increased until week 8, and the iron incorporation rate, characterized by MCH and MCV,[47] continuously decreased during the whole observation period proving the continuous iron deficiency.

4.3 | Maximum oxygen uptake

A meta-analysis[29] found a 7% reduction in $\dot{V}O_{2max}$ 1–2 days after BD of 400–500 mL, in line with our findings. Long-term effects on $\dot{V}O_{2max}$ have so far been investigated in only a few studies, almost exclusively in men. No significant changes in $\dot{V}O_{2max}$ were found just 14 days post-BD,[48] and baseline values were reached after 4 weeks.[24,27] To the best of our knowledge, aerobic capacity has not yet been studied in women over a period of several weeks. Our results clearly show that the initial $\dot{V}O_{2max}$ is not reached for at least 12 weeks when $\dot{V}O_{2max}$ was still 3.2% below baseline.

In general, the loss of hemoglobin is closely associated with a reduction in $\dot{V}O_{2max}$, and in our study, 1 g loss of Hbmass correlated with a 3 mL/min reduction in $\dot{V}O_{2max}$. This value is closely aligned with another study,[27] which described a decrease in $\dot{V}O_{2max}$ of 4 mL/min per 1 g of Hbmass after BD in trained subjects. The difference in the reduction of $\dot{V}O_{2max}$ may be due to the lower fitness level of our subjects. After BD, $\dot{V}O_{2max}$ increases as Hbmass recovers, suggesting a close and causal relationship.

The mechanism of performance degradation is mainly due to two factors in the transport of O_2 through the blood. Immediately after BD, the maximum stroke volume is reduced by a decrease in venous return because of diminished BV,[49] resulting in a cardiac output reduction of approx. 1.75 L/min. When the BV has normalized after day one, the accompanying hemodilution leads to diminished O_2 transport capacity and thus a lower maximum $avDO_2$.[50] In contrast to male blood donors, the period of hemodilution is prolonged in women due to lower iron stores and therefore less efficient erythropoiesis.

4.4 | Practical importance

The present study indicates that iron deficiency occurred after a single BD in the majority of the female blood donors despite them meeting all criteria for BD. Even 12 weeks after BD, neither iron stores nor Hbmass was completely compensated in most of the participants, resulting in a persistent decrease in performance. Nevertheless, according to the existing guidelines in Germany ([Hb] >12.5 g/dL[51]), 8 of our 10 women would have been allowed to donate blood again after 8 weeks, which would undoubtedly have exacerbated the iron deficiency and anemia. This problem is not unknown, but the topic is controversial. Earlier studies[52–54] also found lower [Hb] and borderline low iron stores after a single BD, which worsened significantly with further BDs. Since women are still needed as blood donors, they recommended offering long-term iron supplementation as a possible solution to prevent progression to iron deficiency anemia. Conversely, a study of 10,000 women[55] showed a significant deterioration in the iron status with increasing BD frequency, yet advocated more frequent donations by women, since critical ferritin and Hb values were not reached even after three donations within 16 weeks.

Applying mean group cutoffs for [Hb] as inclusion criteria for BDs increases the risk of individual blood donors slipping into iron deficiency anemia, which is of concern, especially with repeated donations. Our results suggest this after a single donation. Impaired performance may deter many women from BD.[30] We therefore recommend iron supplementation for women with low iron levels even before the first BD to prevent anemia and to be able to sustainably increase the number of BDs. With the CO rebreathing method used, the success of such a measure can be successfully evaluated.

Our study once again raises the issue of [Hb] in the diagnosis of anemia. Since the [Hb] is determined both by the amount of hemoglobin (Hbmass) and by the PV, a concentration alone can lead to misinterpretation, for example, in the case of dilutional anemia in heart failure.[56] Although women have a lower absolute and relative Hbmass than men, the PV in relation to lean body mass is not different.[57] This, in addition to regular iron loss during menstruation, could be another reason why women have lower [Hb] than men and thus lower cutoff values for anemia have been established in practice.[58] The varying frequency of adverse effects[59] and a higher tolerance rate for low [Hb] may also be related to this.[60] For suitability for a BD, a determination of Hbmass at low [Hb] (e.g. <13.0 g/dL) would be very helpful in order to be able to make an informed decision taking both values into account. We are aware that such a procedure cannot yet be used routinely. However, its effectiveness should be tested in pilot projects.

4.5 | Limitations

The data presented were derived from the small pilot study, so definitive conclusions cannot be drawn. The participants were all physically active and paid close attention to the quality of their diet, which in some cases was exclusively vegetarian. Therefore, their lean body mass and [Hb] were in the low range and did not correspond to that of the average population. However, as we wanted to draw attention to the potential risks of this particular group, we included these test subjects in the study. Furthermore, some confounding factors of daily life, such as blood loss through menstruation, were not considered, and dietary iron intake was not controlled in this study since test subjects were advised to maintain their usual diet. Nonetheless, our findings warrant further investigation of the important trends identified, particularly for vulnerable groups.

5 | CONCLUSIONS

For the first time, the Hbmass and $\dot{V}O_{2max}$ were measured in women after BD and the recovery process over 12 weeks. For many women, 12 weeks were not sufficient to recover from BD and to achieve baseline Hbmass and iron stores. A subsequent BD might lead to a severe anemia. $\dot{V}O_{2max}$ decreases immediately after BD, and recovery was closely related to the regain in Hbmass. Further studies should evaluate whether iron supplementation already before BD could accelerate recovery.

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CONFLICT OF INTEREST STATEMENT

WFJS is a managing partner of the company Blood tec GmbH, but he is unaware of any direct or indirect conflict of interest with the contents of this paper. The other authors have no conflicts of interest to declare.

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