Iron Status in Blood Donor Dogs

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Background: Despite the popularity of canine blood donor (BD) programs, there is scarce scientific information regarding iron status in this canine population of dogs.

Objective: To assess iron status in dogs used in a blood donor program.

Animals: A total of 130 healthy dogs (75 BD, 55 controls [C]) were included. A subset of dogs (n = 12) were used to evaluate the effects of repetitive donations by having a second and more recent sample analyzed.

Methods: Serum iron concentration (SI), unsaturated iron-binding capacity (UIBC), total iron-binding capacity (TIBC), and percentage transferrin saturation (%SAT) were obtained. Values were compared using a 2-way ANOVA (factors: BD status, breed). For the subset of BD, the first sample (less frequent donors -LD-, after a mean of 3.8 donations) was compared to a second sample (experienced donors -ED-, mean 13.6 donations) using a paired *t*-test.

Results: SI (183.7 ± 55.3 μg/dL) and %SAT (55.7 ± 17.4%) were higher and UIBC (152.6 ± 73.3 μg/dL) was lower in BD dogs than in C (153.9 ± 51.7 μg/dL, 43.8 ± 17.8%, and 224.1 ± 120.6 μg/dL, respectively). Also, UIBC and TIBC were lower, and %SAT higher in Greyhounds when compared with non-Greyhounds. ED had decreased %SAT and increased UIBC and TIBC when compared with LD.

Conclusions and Clinical Importance: Our canine BD population did not have iron deficiency and had higher SI concentration than C. However, ED (~14 consecutive blood donations every ~8 weeks) developed a mild iron deficiency, although values were still within canine reference intervals. Greyhounds have higher %SAT than non-Greyhounds, which might be a breed-specific peculiarity.

Key words: Greyhound; Iron deficiency; Iron-binding capacity; Transferrin saturation.

Iron is necessary for multiple biological functions, but likely the most important in red blood cell (RBC) production. Functional iron can be found as hemoglobin (Hb), myoglobin, storage iron (ferritin or hemosiderin), bound to the transferrin plasma carrier, or forming enzymes.[1] As there are no effective iron excretion mechanisms, the maintenance of an appropriate iron balance is regulated via intestinal absorption, erythropoiesis, recycling from senescent RBCs, and storage.[2]

Iron deficiency caused by inadequate intake is rare in dogs because most foods have adequate iron content. The major cause of iron deficiency anemia in dogs is chronic blood loss, usually in the gastrointestinal tract (eg, tumors, gastric ulcers, inflammatory bowel disease, and parasites).[2]

In people, iron deficiency anemia is common among blood donors (BD). Because of the importance of the welfare of BD, iron status and safety of blood donation frequency have been recently assessed through a large study called RISE (REDS-II Donor Iron Status Evaluation).[3] This study concluded that there is a high prevalence of iron deficiency among the BD population, particularly in young women, and that there is a strong association between iron depletion and prior donation intensity/time since last donation. Many first-time donors developed iron deficiency within 15–24 months, but iron status changed little among long-time donors. People that donated blood every <14 weeks had higher risk of iron depletion than people who donated every >14–20 weeks.[3]

Iron status in BD dogs is not reported. Additionally, and because of the common use of Greyhounds (G) as BD, we investigated iron homeostasis in this breed. Therefore, the objectives of this study were to assess iron status in the canine BD population, and to investigate serum iron profiles in G.

Abbreviations:

%SAT	percentage transferrin saturation
BD	blood donors
C	controls
ED	experienced donors
G	Greyhounds
Hb	hemoglobin
LD	less frequent donors
NG	non-Greyhounds
OSUABB	Ohio State University Animal Blood Bank
OSU-VMC	Ohio State University Veterinary Medical Center
RBC	red blood cell
SI	serum iron concentration
TIBC	total iron-binding capacity
UIBC	unsaturated iron-binding capacity

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Materials and Methods

We evaluated iron parameters from 130 healthy dogs: 75 were BD and 55 were healthy controls (C). Dogs were also classified as G and non-Greyhounds (NG; Table 1). Iron panel included serum iron concentration (SI), unsaturated iron-binding capacity (UIBC), total iron-binding capacity (TIBC), and percentage transferrin saturation (%SAT). Values were compared using a 2-way ANOVA (factors: BD status -BD/C-, breed -G/NG-). BD were part of the BD program at the Animal Blood Bank (OSUABB) at The Ohio State University Veterinary Medical Center (OSU-VMC); 450 mL was collected from each dog per blood donation, and the median number of donations at the time of sampling was 5.9 (range 0–19). Control dogs were patients admitted to OSU-VMC for either wellness checks or before elective surgeries (i.e. spay/neuter). All dogs from the OSUABB get complimentary food from the program (high-quality commercial diets), and none of them were on iron supplements. Blood samples in nondonor dogs were collected after signed owner's consent. The OSUABB has a current animal use protocol approved by the Institutional Animal Care and Use Committee (IACUC). Blood samples in nondonor dogs were collected after signed owner's consent. All dogs were healthy based on normal physical examination by a veterinarian and PCV/total solids.

For a subset of BD (n = 16), additional serum samples were available and used for evaluating the effects of repeated blood donations on iron homeostasis; the initial sample was referred to as less frequent donors (LD), and the more recent sample as experienced donors (ED), and only dogs which had donated more than 5 times between the 2 blood collection times were considered for analysis (n = 12). The mean time difference between LD and ED sampling times was 87 weeks (range 38–213 weeks). The number of donations for LD was 3.8 (range 0–7) and that for ED was 13.6 (range 6–23), and the number of donations between LD and ED was 10.5 (range 5–21); the time between 2 consecutive donations (calculated as [ED-LD/number of donation in between]) was 8.5 weeks (median; range 4.8–15.2). For this part of the study, we also measured packed cell volume (PCV, by microhematocrit tube centrifugation) and total protein concentration (TP, by refractometry), in addition to the iron panel (SI, UIBC, TIBC and%SAT); we found that neither PCV nor TP changed significantly from LD to ED (means; 59–57% and 5.9–6.0 g/dL, respectively).

Blood samples were collected from the jugular vein, placed into tubes without anticoagulant, and allowed to clot before centrifugation at 1380 × *g* for 10 min. In BD, all blood samples were collected before blood donation. Sera were frozen at −30°C and batched for analysis. SI was analyzed by a colorimetric assay (based on the FerroZine method without deproteinization), and UIBC was determined using FerroZine at the OSU-VMC Chemistry Laboratory (COBAS c501 analyzer; Roche Diagnostics, Indianapolis, IN); our laboratory is not equipped to measure ferritin in dogs. TIBC was calculated by adding UIBC and SI; meanwhile. % SAT was calculated using the formula: serum iron/TIBC × 100.

Normality test (D'Agostino & Pearson omnibus test) and descriptive statistics were performed. All parameters passed the normality test, and results are presented as mean or median (depending on data distribution) and ranges. Two-way ANOVA was used to compare BD and C, establishing BD status (BD/C) and breed (G/NG) as factors; Sidak posthoc test was selected to compare main effects and interactions between groups. For the second part of the study (over-time evaluation of a subset of BD), a paired *t*-test was performed to compare the 2 sampling times, LD and ED. Statistical significance was set at $P < .05$. Statistical software tools used for the analysis were SPSS 19 (descriptive and 2-way ANOVA) and GraphPad Prism® 5.0 (paired *t*-test and graphs).

Results

Iron Metabolism in BD Dogs

Results from the descriptive statistics of the serum iron panel are shown in Table 1. SIs were significantly higher ($P = .004$) in BD when compared with C, but not different between G and NG; no interaction between the 2 factors (BD status and breed) was

Table 1. Descriptive statistics for iron parameters in blood donors and control dogs.

	Blood Donor			Control			Total	Total
	All	Greyhound	Non-Greyhound	All	Greyhound	Non-Greyhound	Greyhounds	Non-Greyhounds
(A) Serum iron (SI)								
Mean	183.7*	182.1	192.3	153.9*	156.2	152.0	174.7	163.5
SD	55.3	56.9	47.5	51.7	51.9	52.4	56.4	53.7
N	75	63	12	55	25	30	88	42
(B) Unsaturated iron-binding capacity (UIBC)								
Mean	152.6*,§	146.6	184.2	224.1*,§	127.7	304.5	141.2#	270.1#
SD	73.3	74.3	61.4	120.6	47.8	102.5	68.1	107.1
N	75	63	12	55	25	30	88	42
(C) Total iron-binding capacity (TIBC)								
Mean	336.3§	328.7	376.5	378.0§	283.9	456.5	316.0#	433.6#
SD	52.7	49.8	51.0	112.9	47.4	88.8	52.9	87.3
N	75	63	12	55	25	30	88	42
(D) Transferrin saturation (%SAT)								
Mean	55.7*,§	56.5	51.6	43.8*,§	54.9	34.5	56.0#	39.4#
SD	17.4	18.0	13.9	17.8	14.9	14.6	17.1	16.2
N	75	63	12	55	25	30	88	42

SD, standard deviation; N, number of animals. Units: SI, UIBC and TIBC expressed in μg/dL; %SAT is expressed as %.
*Statistical difference: blood donor/control.
#Statistical difference: Greyhound/non-Greyhound.
§Interaction between the 2 factors (blood donor status and breed).

detected (Table 1A). The UIBC was lower ($P = .002$) in BD than in C and also lower ($P < .001$) in G compared with NG; there was interaction between the 2 factors ($P < .001$; Table 1B). Although there was no statistical difference in TIBC between BD and C, it was lower ($P < .001$) in G than in NG and there was also interaction between the 2 factors ($P < .001$; Table 1C). The BD group had higher ($P = .007$) %SAT than C, and it was also higher ($P < .001$) in G than in NG, with interaction between the 2 factors ($P = .024$; Table 1D).

Effects of Blood Donation over Time

All dogs in this part of the study (n = 12) were G.The results from the comparison between the first (LD) and the second (ED) samples revealed statistically significant differences in some of the iron parameters measured. Although SI did not change over time, UIBC and TIBC increased ($P = .005$ and $P < .001$, respectively) from 138.9 ± 68.1 μg/dL to 233.3 ± 95.5 μg/dL (UIBC), and from 326.9 ± 37.8 μg/dL to 387.3 ± 57.8 μg/dL (TIBC), and %SAT decreased ($P = .04$) over time from $58.5 \pm 18.8\%$ to $41.3 \pm 21.1\%$).

Discussion

Iron concentration in serum and %SAT (percentage of available sites bound to iron) were higher in the BD canine population when compared with C. These results differ from those in human transfusion medicine studies, where iron deficiency anemia is common in regular donors (defined by low SI and %SAT, along with low iron stores).[3] %SAT in a healthy individual should be ~33% (range 20–60%),[4] and all values in our study were within normal reference intervals.

When iron parameters of regular BD were studied at 2 different times over time (1 sample shortly after enrollment in the BD program -LD-, and a second sample when they were experienced donors -ED-), we found that dogs that had donated blood a mean of ~14 times (ED) had no changes in PCV/TP, but they did have lower %SAT and higher UIBC and TIBC thank LD. Average time between each blood donation was 8.5 weeks (2.1 months), meaning that each dog donated a standard unit of blood (~450 mL, same as humans) ~6 times per year; this resulted in statistically different, but clinically irrelevant, changes in the iron panel studied, as all values remained within the reference intervals.

Human high-intensity BD (also called "superdonors") are BD that despite donating blood 4–6 times per year do not develop iron deficiency anemia; however, they have lower ferritin and hepcidin levels, meaning that they have iron deficiency (low iron stores) compensated by increasing intestinal absorption of dietary iron.[5] Overall, our data suggest that canine BD, which donate blood ~6 times per year, have an iron metabolism comparable to that of superdonors. In the second part of our study (based on a limited number of dogs), we noted that long-term repetitive blood donations may cause mild iron deficiency in canine BD.

Greyhounds are becoming popular as pets because of the increasing number of adoptions after they retire from the racing career. They are excellent BD because of their size, easily accessible veins, and high frequency of DEA 1.1-negative status[6]; therefore, many animal blood banks have G among their BD population. When comparing G with NG in this study, no statistical difference was found in SI concentration, but we found that this breed has lower UIBC and TIBC, as well as higher %SAT when compared with NG. Although decreases in UIBC/TIBC can occur in inflammation or neoplasia, all the dogs included in this study were clinically healthy. These results in G (decreased TIBC and increased %SAT) are in agreement with a previous study[7]; thus, we suggest that these features could be breed-specific. G have higher hematocrit and Hb concentration than other canine breeds; as iron is necessary for Hb formation, we hypothesize that a need of RBC production in G (eg, because of exercise oxygen demand) may lead to mobilization of iron from stores and subsequent increase in SI and %SAT.

In conclusion, evaluation of our canine BD population indicates that standard collection does not lead to iron deficiency; in fact, when compared with healthy control dogs, BD have higher circulating serum iron and transferrin saturation. However, regular BD may develop a mild iron deficiency after ~14 consecutive donations. Further studies using a larger number of dogs, specific time points (eg, baseline before enrollment in the BD program, after 10 donations and after 20 donations) and study of iron stores (ie, ferritin levels) are warranted to better assess these changes.

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