BRIEF COMMUNICATION

Markers of iron metabolism in retired racing Greyhounds with and without osteosarcoma

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Key Words
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Background: Greyhounds have well-described clinicopathologic idiosyncrasies, including a high prevalence of osteosarcoma (OSA). Hematocrit, HGB, and HGB oxygen affinity are higher than in other dogs, while haptoglobin concentration is lower, so we hypothesized that Greyhounds have a different iron metabolism. To our knowledge, there are no reports on serum iron profiles in Greyhounds.
Objectives: To elucidate iron metabolism in Greyhounds, we wanted to compare serum iron concentration, total iron-binding capacity (TIBC), and percent transferrin saturation (%SAT) in healthy retired racing Greyhounds (RRGs) with OSA (RRGs – OSA), and also with non-Greyhounds (NGs), without and with OSA (NGs – OSA).
Methods: Serum iron concentration and unsaturated iron-binding capacity (UIBC) were measured by standard methods, and TIBC and %SAT were calculated in RRGs (n = 25), RRGs – OSA (n = 28), NGs (n = 30), and NGs – OSA (n = 32).
Results: TIBC was lower in RRGs than in NGs ($P < .0001$), and in RRGs – OSA than in NGs – OSA ($P < .0001$). NGs – OSA had lower TIBC than healthy NGs ($P = .003$). Percent SAT was higher in RRGs than in NGs ($P < .0001$) and in RRGs – OSA ($P = .008$), and %SAT was also lower in NGs than in NGs – OSA ($P = .004$). Percent SAT was also higher in RRGs – OSA than in NGs – OSA ($P = .001$). Both RRGs – OSA ($P = .02$) and NGs – OSA ($P < .0001$) had lower serum iron concentrations than their healthy counterparts.
Conclusion: Lower TIBC and higher %SAT may constitute another Greyhound idiosyncrasy compared with other dogs. In this study, all dogs with OSA had higher serum iron concentrations and %SAT than healthy dogs.

The adoption of Greyhounds after retirement from racing has markedly increased in the past few years, popularizing this breed as pets. Several clinicopathologic idiosyncrasies have been reported to date, such as higher hematocrit, HGB concentration, and whole blood viscosity; lower α- and β-globulin concentrations, and lower WBC and platelet counts, among others.[1–3] We recently also reported that the most common cause of death in retired racing Greyhounds (RRG) was cancer (66/114 dogs, 58%), and that osteosarcoma (OSA) was the most common cause of death in this group (28 dogs, 25%).[4] Therefore, novel treatment modalities for OSA are currently being evaluated. Interestingly, dihydroartemisinin, an extract from a Chinese plant, has been shown to be cytotoxic in canine OSA cell lines in vitro, which appears to be mediated by an interaction with intracellular iron producing reactive oxygen species (ROS).[5] Iron plays an important role in processes involving oxygen transport, energy metabolism, DNA expression, regulation of the cell cycle, and modulation of hydrogen peroxide levels.[6] When increased intracellular iron stores reach critical levels, mitochondrial damage through the formation of ROS can result, leading to subsequent tissue damage.[6–9] However, iron can also favor carcinogenesis, as neoplastic cells express high levels of transferrin receptors and internalize large amounts of iron from transferrin molecules.[7] In fact, one of the main reasons for the use of iron chelators in antitumor therapy is that rising blood iron levels appear to correlate with resistance to chemotherapeutic agents.[5] Thus, a good understanding of iron metabolism is fundamental to develop new cancer therapies. Currently, studies with dihydroartemisinin as part of a multidrug metronomic chemotherapy protocol in Greyhounds with OSA are ongoing.

To establish a base for a better understanding of iron metabolism, the goal of this study was to measure markers of iron metabolism such as serum iron concentration and unbound iron-binding capacity (UIBC), to calculate total iron-binding capacity (TIBC) (Figure 2) and % saturation (%SAT) in RRG and RRG–OSA, and to compare them with healthy non-Greyhound dogs (NGs), and NGs with osteosarcoma (NG–OSA).

A total of 115 dogs were assigned to one of 4 groups: (1) RRGs included 25 dogs, (2) RRGs – OSA 28 dogs, (3) NGs 30 dogs, and (4) NGs – OSA 32 dogs. NGs and NGs–OSA included several large dog breeds such as Labrador Retriever, Golden Retriever, German Shepherd, and Rottweiler. Dogs were included in the healthy groups based on their medical history and physical examination findings; none of the dogs were receiving any medication or served as blood donors.

Blood samples were obtained from fasted dogs by jugular venipuncture in the morning after physical examination or before surgery in dogs with OSA. Samples were collected in plain serum tubes (BD Vacutainer, Franklin Lakes, NJ, USA), allowed to clot, and then centrifuged at 1380*g* for 10 minutes. Sera were frozen at −30°C and analyzed in batch. Serum iron concentration and UIBC were analyzed by the standard colorimetric Ferrozine method (Roche Diagnostics, Cobas c501, Indianapolis, IN, USA) performed at the Ohio State University Clinical Diagnostic Laboratory. The color change in this assay is determined photometrically as a change in the absorbance and is directly proportional to unbound excess iron concentration and indirectly proportional to UIBC. It has been reported that some drugs such as acetaminophen, among others, lipemia, icterus, and/or hemolysis may interfere with this determination.[10] In this study, no hemolysis was observed in any of the samples. However, the presence of hemolysis, lipemia, or icterus was not documented for the study samples. TIBC and %SAT were calculated as follows: TIBC = UIBC + serum iron concentration, and %SAT = serum iron concentration/TIBC × 100.

A commercially available statistical software Prism version 4.0 (GraphPad Software Inc., San Diego, CA, USA) was used for analysis. Descriptive statistics were performed for all the variables measured. The D'Agostino & Pearson omnibus test was used to evaluate normality. Serum iron concentration, TIBC, and %SAT were compared using the nonparametric Mann–Whitney test. $P \leq .05$ was considered significant.

The TIBC was significantly lower in RRGs when compared with NGs ($P < .0001$), while % SAT was significantly higher in RRGs ($P < .0001$, Table 1) (Figures 1–3). There was no significant difference of TIBC between healthy RRGs and RRGs – OSA, but TIBC was significantly lower in RRGs – OSA than in NGs – OSA ($P < .0001$, Table 1). In contrast, NG – OSA

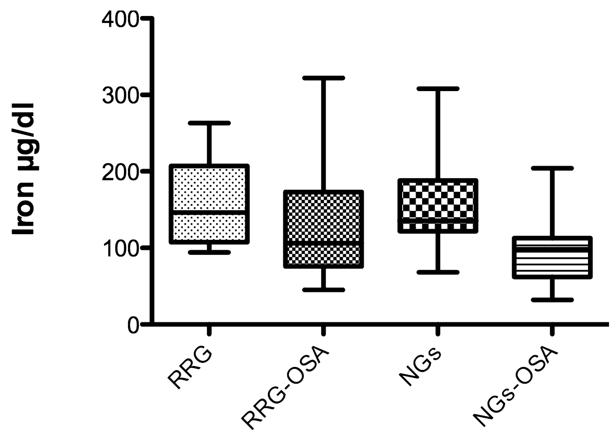

Figure 1. Box-and-whisker plot of serum iron concentration in healthy retired racing Greyhounds (RRGs), RRGs with osteosarcoma (RRG–OSA), healthy Non-Greyhounds (NGs), and NGs with OSA (NGs–OSA). The box represents 2.5–97.5 percentiles, the horizontal line in the middle of the box represents the median, and the whiskers indicate the standard error.

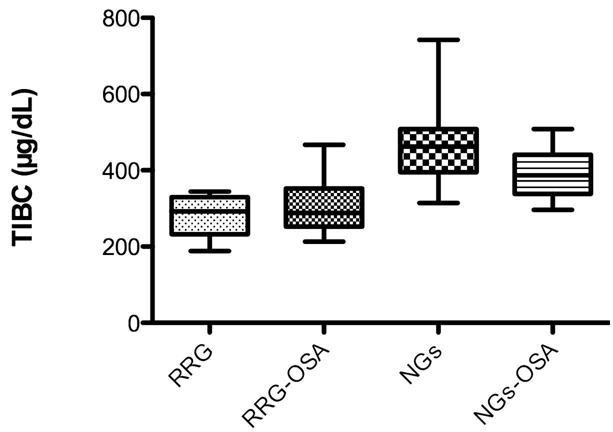

Figure 2. Box-and-whisker plot of total iron-binding capacity (TIBC) in healthy retired racing Greyhounds (RRGs), RRGs with osteosarcoma (RRG–OSA), healthy Non-Greyhounds (NGs) and NGs with OSA (NGs–OSA). The box represents 2.5–97.5 percentiles, the horizontal line in the middle of the box represents the median, and the whiskers indicate the standard error.

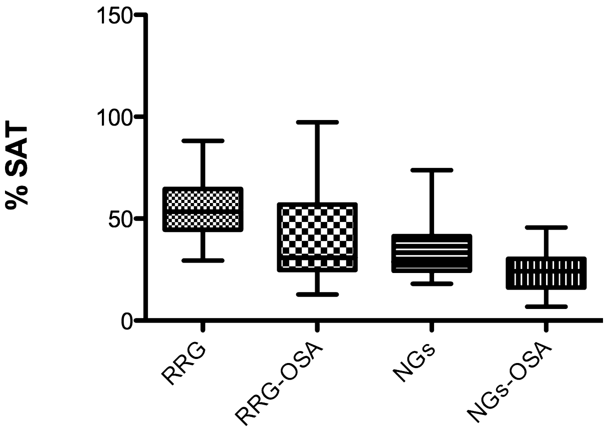

Figure 3. Box-and-whisker plots of percent transferrin saturation (%SAT) in healthy retired racing Greyhounds (RRGs), RRGs with osteosarcoma (RRG–OSA), healthy Non-Greyhounds (NGs), and NGs with OSA (NGs–OSA). The box represents 2.5–97.5 percentiles, the horizontal line in the middle of the box represents the median, and the whiskers indicate the standard error.

had significantly lower TIBC than NGs ($P = .003$). The %SAT was significantly lower in RRGs – OSA than in healthy RRGs ($P = .008$) and in NGs – OSA than in healthy NGs ($P = .004$), but was higher than in NGs – OSA ($P = .001$). Both RRGs-OSA and NGs – OSA had significantly lower serum iron concentrations than RRGs and NGs, respectively (Table 1).

During the past few years, numerous breed-specific clinicopathologic idiosyncrasies have been reported in Greyhounds and other Sighthounds. In a recent review, these included RBC variables in the high-end RI, WBC, and platelet counts in the low-end RI, lower total serum protein concentrations, higher creatinine concentration, higher HGB oxygen affinity, and lower haptoglobin (Hp) concentration, among others.[12]

Several mechanisms may explain the results of our study. The relative hypoferremia in dogs with OSA is likely due to iron sequestration by macrophages in response to cytokine release, thus resulting in lower serum iron concentration. Interestingly, some tumors have been shown to induce signals for iron sequestration.[13] In a study with 52 dogs with OSA and 50 healthy dogs, serum iron concentration and TIBC in dogs with OSA were significantly lower than in healthy dogs, confirming our results.[13]

Under normal conditions, 70% of the plasma transferrin is unsaturated providing protection against large amounts of free iron, a molecule with toxic oxidative potential.[6] If the transport capacity of transferrin is saturated, iron may bind to other ligands and result in ROS.[6] In people, high transferrin saturation has been associated with an increased mortality risk in certain cancer patients (Figure 3).[8] Increased transferrin saturation (> 45%) has also been associated with an increased cancer risk in people with a high daily iron intake (> 18 mg/day).[14]

A relevant finding in our study is the comparatively low TIBC probably indicating lower serum transferrin concentration, which, in light of similar serum iron concentrations in NGs, accounted for the high % SAT in the RRGs. This low transferrin concentration, possibly due to the same mechanisms associated with the previously reported hypo-haptoglobinemia and a low concentration of $\alpha-1$ acid glycoprotein, may be associated with a higher risk for OSA in RRG.[2]

Greyhounds have been reported to have shorter RBC life spans and macrocytic RBCs compared with other breeds, although reticulocyte counts, RBC morphology, and bone marrow analyses showed no obvious differences suggestive of hemolysis in those studies.[15] Hemolysis and consequent sequestration of HGB-Hp-complexes could have offered a potential explanation for the lower Hp levels. However, the short RBC life span theory has recently been refuted by a study that demonstrated no differences between Greyhounds and NGs.[16]

The function of transferrin in Greyhounds and the correlation with ferritin levels is not completely clear. One limitation of the present study is the lack of ferritin measurements, the only purportedly reliable variable providing relevant information regarding iron storage.[17] Quantification of serum transferrin

Table 1. Serum iron concentration, total iron-binding capacity (TIBC), and percent saturation (%SAT) in healthy retired racing Greyhounds (RRGs), RRGs with osteosarcoma (OSA), healthy Non-Greyhounds (NGs), and NGs with OSA.

	RRGs			NGs			*P* value comparison RRGs vs NGs	
	Healthy	OSA	*P* value	Healthy	OSA	*P* value	*P* value RRG vs NG	*P* value RRG–OSA vs NG–OSA
IRON μg/dl	146 ± 10.4	105 ± 13.4	.02*	135 ± 9.5	98 ± 7.3	.0001*	.84	.14
TIBC μg/dl	292 ± 9.5	288 ± 12.5	.42	462 ± 16.2	387 ± 11.5	.003*	.0001*	.0001*
% SAT	53.4 ± 3	31 ± 4	.008*	28.8 ± 2.7	24.2 ± 1.7	.0045*	.0001*	.001*

Data are median ± SE.
*$P < .05$ when comparing 2 groups.

receptors may have shed additional light on iron metabolism and its relationship with %SAT and serum iron concentration.[14]

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