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NOTE

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Repeated phlebotomies decrease body iron storage in adult dogs

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ABSTRACT. The aim of the present study was to determine changes in body iron storage in adult dogs following phlebotomy. We performed repeated phlebotomies by removing 1% body weight (approximately 10% of the total blood volume) weekly for a total of 12 times using adult beagle dogs without an iron-restricted diet. After treatment, stored iron was decreased, as demonstrated by gradual reductions in serum ferritin levels and hepatic iron contents. Anemia and abnormalities in blood chemistry analysis were not observed; therefore, this method was considered safe and useful for control of stored iron levels in adult dogs.

KEY WORDS: body iron storage, dog, phlebotomy

Iron is an essential metal element in the living body and is involved in various physiological roles, including hemoglobin formation, energy metabolism, DNA and RNA synthesis, and some enzyme systems, such as catalases and cytochromes [10]. However, because iron can facilitate the redox reaction, reactive oxygen species (ROS), such as hydroxyl radicals, can be generated under iron excess conditions [10]. ROS can damage DNA, lipids, and proteins and are produced in several diseases, such as cancer [9]. Moreover, increased iron loading in hepatocytes has been observed in humans with hepatitis C, and iron itself may be an important pathogenic factor. Phlebotomy can reduce alanine aminotransferase (ALT) levels in patients with hepatitis C owing to a reduction in nonspecific iron cytotoxicity [8, 16]. Thus, systemic or localized iron overload conditions can be an important health problem in human medicine.

In veterinary medicine, reports of diseases caused by iron overload are limited. Previous reports have shown that liver failure occurs secondary to iron overload in basenjis with pyruvate kinase deficiency [6], and repeated blood transfusions in a miniature schnauzer with pure red cell aplasia [13]. Although removal of hemoglobin-bound iron by phlebotomy is also an effective choice for controlling stored iron levels in veterinary patients, no studies have reported physiological data for phlebotomy targeting iron reduction. In the present study, we aimed to collect basic data for controlling body iron storage by evaluating changes in iron metabolism and storage following repeated phlebotomies in adult dogs.

Healthy male (n=2) and female (n=3) beagle dogs (mean body weight, 9.0 kg; Nihon Crea, Tokyo, Japan), ages 5–9 years, were used in this study. The dogs were maintained in a temperature- and light-controlled environment and were fed complete laboratory food (CD-5M; Nihon Crea) once a day, with free access to water. All procedures were approved by the Kitasato University Animal Committee (approval number: 14-085).

Phlebotomy treatments were performed once a week between 8 and 9 AM for 12 weeks, and whole blood was drawn from the jugular vein to a total volume corresponding to 1.0% body weight using an 18-gauge needle. We set the first day of phlebotomy as day 0. Blood and liver tissue samples were extracted according to the time course shown in Table 1. Blood samples were obtained from partial phlebotomized blood or separate 5 m*l* of blood samples. Liver biopsy was performed under general anesthesia using a 16-gauge Tru-cut biopsy needle (Super Core; Medical Device Technologies, Gainesville, FL, U.S.A.) with ultrasound guidance. The anesthesia protocol was conducted according to our previous report [3]. Complete blood count (CBC), absolute reticulocyte count, serological iron-related parameters (serum iron [SI], total iron binding capacity [TIBC], and serum ferritin concentration [sFt]), and hepatic nonheme iron content were evaluated [3]. Plasma biochemistry analysis was performed for total protein (TP), albumin (Alb), aspartate aminotransferase (AST), ALT, blood urea nitrogen (BUN), and creatinine (Cre) before (day 0) and after (day 84) repeated phlebotomies using an auto analyzer (Dimention RxL Max; Siemens, Munich, Germany).

All values are expressed as mean ± standard deviation (SD). The data at each time point were compared with those for day 0 using repeated measures analysis of variance and Dunnett's tests. Values were considered statistically significant when the P value was less than 0.05.

Changes in CBC are shown in Table 2. No significant changes were observed in packed cell volume (PCV) in this experiment.

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Table 1. Time table of treatment and sampling

Days	0	7	14	21	28	35	42	49	56	63	70	77	84
Phlebotomy	○	○	○	○	○	○	○	○	○	○	○	○	
CBC	○				○				○				○
Plasma biochemistry	○												○
Serum iron	○	○	○		○		○		○		○		○
TIBC	○	○	○		○		○		○		○		○
Serum ferritin	○	○	○		○		○		○		○		○
Hepatic non-heme iron	○		○		○				○				○

Table 2. Change of the CBC and reticulocyte count through phlebotomy treatment

Days	0	28	56	84
RBC ($\times 10^6/\mu l$)	6.2 ± 0.7	6.4 ± 0.6	6.5 ± 0.6	6.5 ± 0.5
Hb (g/d*l*)	14.7 ± 1.3	14.4 ± 1.8	14.9 ± 1.2	13.6 ± 1.6
PCV (%)	42.3 ± 4.9	43.6 ± 4.0	44.7 ± 3.9	42.6 ± 4.5
MCV (f*l*)	68.5 ± 1.3	68.3 ± 0.6	68.5 ± 2.1	65.5 ± 3.9[a)]
MCHC (g/d*l*)	34.7 ± 0.7	33.0 ± 1.6[a)]	33.4 ± 0.9[a)]	31.6 ± 0.5[a)]
PLT ($\times 10^3/\mu l$)	463 ± 113	538 ± 99	561 ± 100[a)]	642 ± 100[a)]
Absolute reticulocyte count ($\times 10^3$cells /μl)	28.1 ± 11.0	56.4 ± 6.8[a)]	54.2 ± 19.1[a)]	56.4 ± 10.5[a)]

a) $P<0.05$ vs Day 0

The mean corpuscular volume (MCV) was significantly decreased on days 28, 56 and 84, and the mean corpuscular hemoglobin concentration (MCHC) was significantly decreased on days 84. The number of platelets was significantly increased on days 56 and 84, and the absolute reticulocyte count was significantly increased on days 28, 56 and 84. Iron-related parameters and hepatic iron content are shown in Fig. 1. SI was decreased, and TIBC was increased significantly on days 14. Through repeated phlebotomies, hepatic iron content, which is one of the sensitive indicator of body iron storage [1], decreased significantly on days 28 from that on day 0. sFt, which is also an indicator of body iron storage [3], was significantly increased on days 14 and decreased on days 42 from that on day 0. Plasma biochemical analysis showed that there were no significant changes before and after repeated phlebotomy (Table 3).

Table 3. Plasma biochemical analysis before and after phlebotomy treatments

Days	0	84
TP (g/d*l*)	5.6 ± 0.7	5.7 ± 0.5
Alb (g/d*l*)	2.5 ± 0.8	2.8 ± 0.2
AST (U/*l*)	56.0 ± 48.7	42.2 ± 9.0
ALT (U/*l*)	49.6 ± 29.7	89.0 ± 52.1
BUN (mg/d*l*)	14.6 ± 6.3	13.9 ± 6.4
Cre (mg/d*l*)	0.4 ± 0.1	0.4 ± 0.1

Physiologically, iron absorption and release are suppressed to extremely low levels, and most necessary iron is provided from hemoglobin-bound iron contained in aged red blood cells in the semi-closed iron recycling system [2]. Since most of the body iron is present in red blood cells as hemoglobin-bound iron, phlebotomy is an efficient method to remove iron. Experimentally induced iron deficiency in dogs has been reported for dogs consuming a low-iron diet [5, 14] and in dogs consuming a low-iron diet combined with phlebotomy [12]; however, these previous studies used puppies. In growing dogs, the iron requirement is very high, and nutrient iron deficiency can be caused by decrease iron intake. Notably, stored iron restriction for adult dogs using nutritional methods is more difficult for the reasons described above and there are no reports describing a practical method for storage iron control in adult dogs. Repeated phlebotomies are somewhat invasive; however, we thought that this method may be suitable for decreasing stored iron quickly and effective. However, recent reports of iron overload in dogs have been associated with frequent blood transfusions due to pure red cell aplasia, and pyruvate kinase deficiency, both of which involve abnormalities in erythrocyte production [6, 13]. Iron utilization for erythrocyte production is considered effective only in the context of normal bone marrow erythropoiesis. Therefore, there is no target disease using phlebotomy treatment in current veterinary medicine. However, as with human medicine, iron is thought to be associated with the pathogenesis of some diseases; therefore, further studies are needed particularly to develop methods for evaluation for body iron storage in veterinary medicine.

With regard to the phlebotomy volume, Ooms *et al.* reported the safety of removal of a volume of blood equal to 1.5% of the body weight weekly for 4 weeks [12]. Therefore, we established our current method with reference to this report. Because no obvious abnormalities were observed in the dogs during the experiment, such as weight loss or changes in blood biochemical analysis, we assumed that the method used in the present study could be carried out safely.

Because hepatic iron content decreased with repeated phlebotomies, our method was thought to be effective for removing iron from the body in adult dogs. This was also supported by hypoferremia, elevated TIBC, and thrombocytosis, which has been reported in the iron-deficient state [15]. Unexpectedly, however, sFt was significantly elevated only on day 14. This was not consistent with the reduction of liver iron content. Although this may be a physiological response to body iron reduction

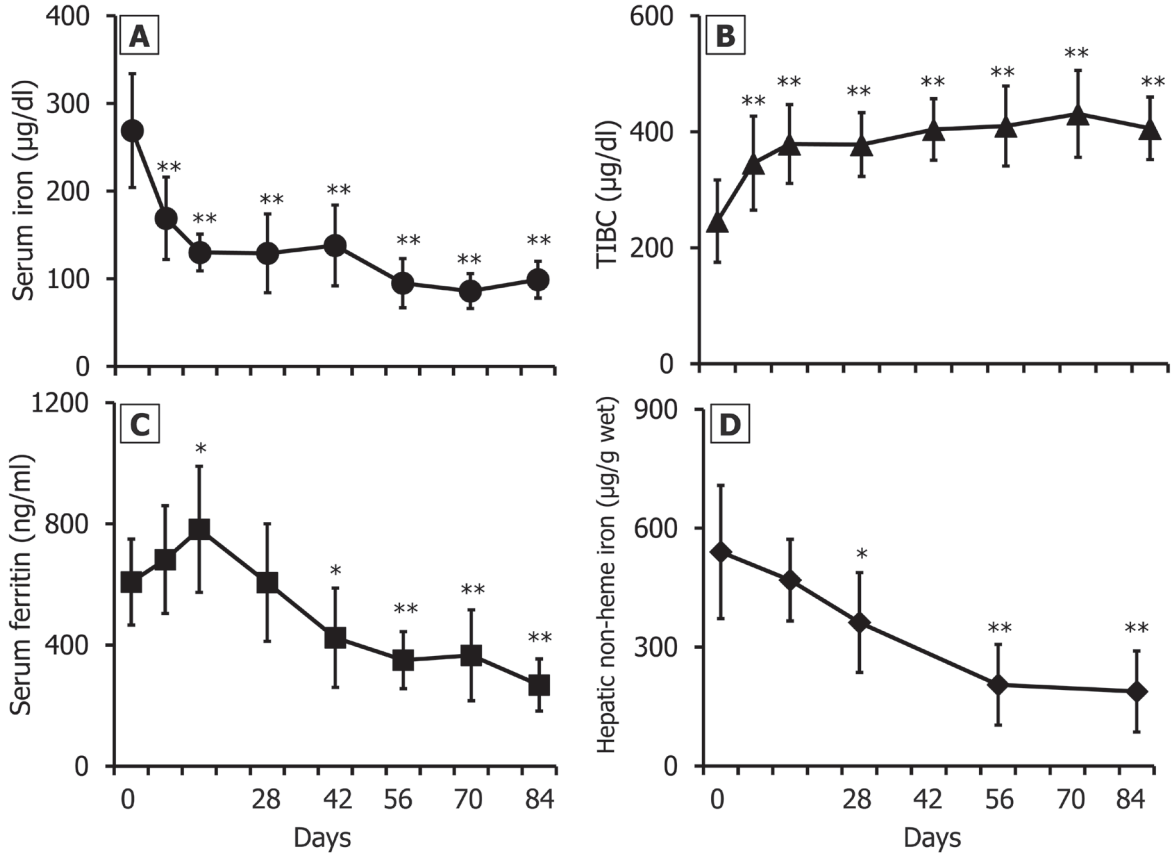

Fig. 1. Changes in iron-related parameters and hepatic iron contents during the study period. A: serum iron, B: total iron binding capacity, C: serum ferritin, and D: hepatic nonheme iron content. *: $P<0.05$ and **: $P<0.01$ versus before treatment (day 0).

by phlebotomy, the specific cause of this observation is still unknown. However, sFt is a noninvasive serological marker for determination of body iron storage in both human and veterinary medicine [7], and various diseases are associated with hyperferritinemia, which is independent of the amount of iron [2]. Therefore, further research is needed for interpretation of iron storage in dogs using sFt.

In the present study, anemia was not observed in dogs, and a significant increase in the number of reticulocytes, an indicator of erythropoiesis, was observed throughout the experiment. Because dogs have about 1 mg iron in 2 m*l* blood, dogs weighing 9.0 kg (mean weight in the present study) would have approximately 540 mg iron removed from the body during our experiment [10]. The body iron content of animals is approximately 19.8−48.5 mg/kg body weight; thus, dogs weighing 9.0 kg would be expected to have a whole-body iron content of 178.2–436.5 mg [10]. Although large amounts of iron were removed by repeated phlebotomies, iron deficiency anemia was not observed in the present study. This result could be explained by the observation that phlebotomy reduced iron storage, but that iron content did not decrease to anemic levels. We assumed that available iron for hematopoiesis was not depleted and recovered to a normal level by the next phlebotomy. These interpretations also suggested that the decrease in sFt and hepatic iron content reached to a plateau after day 56. Dietary iron is absorbed mainly in the duodenum, and iron uptake rate increases six-fold after induction of iron deficiency anemia in dogs [11]. The diet fed to the dogs in this study (CD-5M) contained 444.7 mg iron/kg dry matter (as per the product information), and this value is much higher than the reference value (80 mg iron/kg dry matter) provided by the Association of American Feed Control Officials (AAFCO) [4]. Thus, the high iron content in CD-5M and changes in iron absorption efficiency may have contributed to the maintenance of erythropoiesis; however, further studies are needed to evaluate iron removal and iron absorption.

In summary, our findings suggested that iron storage could be controlled by phlebotomy in dogs conveniently and safely without restricting dietary iron, and our data provide basic information for a method of controlling stored iron levels in veterinary medicine. To the best of our knowledge, this is the first report describing a method for reducing iron storage in adult dogs.

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