Evaluation of iron status in lemurs by analysis of serum iron and ferritin concentrations, total iron-binding capacity, and transferrin saturation

Cathy V. Williams, DVM; Randall E. Junge, MS, DVM, DACZM; Ilse H. Stalis, DVM, DACVP

Objective—To assess serum iron and ferritin concentrations, total iron-binding capacity, and transferrin saturation as indicators of iron metabolic status in 3 genera of lemurs and determine whether these variables are useful for screening for iron overload.

Design—Cross-sectional study.

Animals—11 ring-tailed lemurs (Lemur catta), 11 black lemurs (Eulemur macaco macaco), and 11 red-ruffed lemurs (Varecia rubra).

Procedures—Blood samples were collected weekly for 3 weeks and assayed for serum iron and ferritin concentrations and total iron-binding capacity. Liver biopsy specimens were evaluated histologically and assayed for total iron, nonheme iron, and trace mineral concentrations. Deposition of iron was scored on Prussian blue–stained slides.

Results—Hepatic iron content ranged from 497 to 12,800 µg/g dry weight (median, 2,165 µg/g). Differences were seen in mean hepatic iron content across genera, with ruffed lemurs having the highest concentrations and ring-tailed lemurs having the lowest. Iron accumulation in the liver was mild, and cellular pathologic changes associated with iron storage disease were not detected in any lemur. Ferritin concentration was the only variable that correlated significantly with hepatic iron content in all 3 genera of lemurs; however, both transferrin saturation and serum iron concentration were correlated with hepatic iron concentration in ring-tailed and ruffed lemurs.

Conclusions and Clinical Relevance—Serum ferritin concentration was the only variable that was consistently correlated with hepatic iron content in all 3 genera. Mean hepatic iron content varied across genera, suggesting that the propensity for lemurs to develop iron overload in captivity may vary across taxa. (J Am Vet Med Assoc 2008;232:578–585)

Iron is an essential trace element for most organisms. It is involved in many processes vital for survival, including oxygen transport, electron transport, and DNA synthesis. In excessive quantities, however, iron is toxic. Accumulation of iron in tissues is associated with disease, largely resulting from the potential of ferrous iron (Fe²⁺) to act as a catalyst in reactions that potentiate oxygen toxicity by generation of free radicals.¹ In humans, iron toxicosis affects multiple tissues and can lead to liver disease; heart disease; diabetes mellitus; neurodegenerative disorders; and an increased risk of cancer, particularly hepatic carcinomas. When iron absorbed by the body exceeds amounts needed for normal physiologic functions, the excess is either stored in combination with ferritin or deposited as hemosiderin in tissues. When hemosiderin is detected in tissues with no evidence of toxicosis, the condition is termed hemosiderosis. The term hemochromatosis is reserved for conditions in which there is functional or morphologic evidence of iron toxicosis.

<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>SI</th>
<th>TIBC</th>
<th>TIC</th>
<th>NIC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum iron</td>
<td>Total iron-binding capacity</td>
<td>Total iron concentration</td>
<td>Nonheme iron concentration</td>
</tr>
</tbody>
</table>

Hemosiderosis has been detected in several nondomestic species, including birds, reindeer, rhinoceroses, and primates.²⁻⁹ Among primates, lemurs are thought to be particularly susceptible to iron overload, and reports¹⁰⁻¹² of hemosiderosis in captive lemurs published in the 1980s described excessive iron deposits in tissues at necropsy in 67% to 100% of lemurs examined, whereas wild lemurs dying within a month of importation had no detectable hemosiderin deposits. A link to diet has been proposed, and some researchers have recommended altering the diets of captive lemurs to more closely mimic the presumed diet of wild lemurs.¹⁰⁻¹³ In contrast, a 2006 report by Glenn et al¹⁴ revealed hemosiderin deposition in tissues in only 32% of 153 lemurs dying of natural causes at the Duke Lemur Center, a prevalence that was substantially lower than percentages reported in earlier surveys. Authors of that study¹⁴ noticed that there appeared to be variation among species in the amount of iron detected in the liver, suggesting that some taxa may be more predisposed to developing iron overload in captivity. The discrepancy between

From the Duke Lemur Center, Duke University, Durham, NC 27705 (Williams); the St Louis Zoological Park, 1 Government Dr, St Louis, MO 63110 (Junge); and the Zoological Society of San Diego, 1334 Old Globe Way, San Diego, CA 92101 (Stalis). Supported by Purina Mills LLC and the National Science Foundation, grant No. DBI-0200748. Anatomic pathology work was fully supported by the Zoological Society of San Diego. Address correspondence to Dr. Williams.

reports suggests that much remains to be learned about iron accumulation and its clinical relevance in lemurs. Evaluating the iron status of lemurs is difficult, and hemosiderosis is most consistently diagnosed at post-mortem examination. Obtaining an antemortem diagnosis of iron overload requires liver biopsy, which may not be practical or possible in all situations. In addition, clinicians may be reluctant to perform the procedure to screen animals in which there are no clinical signs.

Measurement of various iron metabolic variables in blood is useful for screening humans and some domestic animals for iron overload, but the reliability of using serum tests to evaluate iron status in lemurs has not been determined. The process is complicated by the fact that lemurs are an extremely diverse group of primates, consisting of 71 species and subspecies grouped into 15 genera and 5 families. To assess the reliability of SI indices for use as a measure of iron status in lemurs, SI concentration, TIBC, and ferritin concentration were measured and transferrin saturation was calculated, and these values were compared with hepatic iron content in 3 genera of lemurs commonly kept in captivity. Serum and liver concentrations of copper and zinc, trace minerals known to affect transport and cellular uptake of iron, were also measured to determine whether these micronutrients were associated with differences in liver iron content or liver pathologic changes.

Materials and Methods

Animals—The study group consisted of 33 lemurs representing 3 genera: ring-tailed lemurs (*Lemur catta*), black lemurs (*Eulemur macaco macaco*), and red-ruffed lemurs (*Varecia rubra*). Eleven lemurs of each genus were tested. Lemurs were housed at the Duke Lemur Center in Durham, NC (n = 19), or the St Louis Zoological Park in St Louis, Mo (14). Studies were performed in accordance with current animal welfare regulations for the use of animals in research, and the study protocol was approved by the Institutional Animal Care and Use Committee at both Duke University and the St Louis Zoo. Animals ranged from 2 to 20 years of age and included 24 males and 9 females. Prior to entering the project, each lemur underwent a thorough physical examination. A CBC and serum biochemical analyses were performed to rule out underlying medical conditions that could affect test results. Despite having normal presurgical screening test results, 1 ruffed lemur was found to have bacterial hepatitis at surgery and was removed from the study, leaving 10 ruffled lemurs to be included in the study.

Diet—At the Duke Lemur Center, diets consisted of primate biscuit supplemented with fruits and vegetables. At the St Louis Zoo, ring-tailed and black lemurs were fed a 50:50 mixture of 2 brands of high-fiber primate biscuits supplemented with fruits and vegetables; ruffed lemurs received a 50:50 mixture of high-fiber primate biscuits and an extruded avian pellet supplemented with fruits and vegetables. Water was available ad libitum. Diet composition on a dry-matter basis was approximately 75% to 80% primate biscuit and 20% to 25% fruits and vegetables at both institutions. Lemurs did not receive any additional vitamin or mineral supplements. The approximate nutrient content of diets fed at both institutions is summarized (Table 1).

Sample collection and analysis—Blood samples were collected at weekly intervals for 3 weeks. All samples were drawn from 8:00 to 10:00 AM after feed had been withheld for 8 to 10 hours to control for variation in iron analyte values arising from normal diurnal variation or recent ingestion of a meal. Blood was drawn in a 6-mL syringe with a 22-gauge needle. The needle was removed from the syringe prior to transferring the blood into a 3.5-mL glass serum separator tube. Serum was separated from RBCs within 30 minutes of collection by centrifugation at 1,163 × g for 6 to 8 minutes. The degree of hemolysis in the sample was recorded. In all samples, hemolysis was either absent or minimal (graded 1+) on visual inspection. Immediately after collection of the last blood sample, liver biopsy was performed via laparotomy for histologic analysis, measurement of iron content, and trace mineral analysis. Serum and liver specimens for biochemical analysis were frozen at −80°C in 2-mL polypropylene cryogenic vials until analysis. Length of storage time for frozen samples ranged from 92 to 324 days. A portion of liver tissue was preserved in neutral-buffered 10% formalin solution for histologic analysis.

Serum samples were assayed for SI concentration, TIBC, and ferritin concentration at the Kansas State College of Veterinary Medicine Diagnostic Laboratory. Serum was analyzed for SI concentration and TIBC colorimetrically. Ferritin measurements were performed by use of a quantitative ELISA developed at that laboratory for detection of lemur ferritin. Percent transferrin saturation was calculated as SI concentration/TIBC × 100. All samples were analyzed in a single-batch assay to minimize variability.

The copper and zinc concentrations in serum and liver tissue were measured in samples collected during the third week of the project and analyzed at the Michigan State University Animal Disease Diagnostic Laboratory. Quantitative analysis of the iron content in liver tissue included analysis of both TIC (analyses

<table>
<thead>
<tr>
<th>Facility</th>
<th>Crude fat (%)</th>
<th>Crude protein (%)</th>
<th>NDF (%)</th>
<th>ADF (%)</th>
<th>Iron (ppm)</th>
<th>Vitamin C (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duke Lemur Center (all species)</td>
<td>4.6</td>
<td>14.8</td>
<td>17.1</td>
<td>12.4</td>
<td>159</td>
<td>580</td>
</tr>
<tr>
<td>St Louis Zoo (ring-tailed and black lemurs)</td>
<td>4.3</td>
<td>20.6</td>
<td>25</td>
<td>12.5</td>
<td>191</td>
<td>658</td>
</tr>
<tr>
<td>St Louis Zoo (ruffed lemurs)</td>
<td>4.4</td>
<td>20.6</td>
<td>18.1</td>
<td>9.8</td>
<td>167</td>
<td>1,120</td>
</tr>
</tbody>
</table>

Table 1—Summary of approximate fat, protein, neutral detergent fiber (NDF), acid detergent fiber (ADF), iron, and vitamin C content of diets fed to 32 captive lemurs of 3 genera in a study in which indices of iron metabolism were evaluated. Dietary content is reported on a dry-matter basis for quantities fed per lemur, and all diets were formulated to meet minimum National Research Council requirements for nonhuman primates. Information is derived from the Duke Lemur Center and St Louis Zoo in-house records, product specifications from commercial diet manufacturers, and the USDA Nutrient Database for produce.
performed at Michigan State University Animal Disease Diagnostic Laboratory) and NIC (analyses performed at the Comparative Hematology Laboratory, College of Veterinary Medicine, Kansas State University).

Sections of liver were stained with H&E for general examination and with Prussian blue (potassium ferrocyanate) stain to evaluate the presence and distribution of iron. The slides were evaluated in a single-blind study by a board-certified veterinary pathologist (IHS). Deposition of iron was scored in Prussian blue–stained slides with a numeric grading system loosely based on that described by Deugnier et al in which iron is evaluated on the basis of location (hepatocytes, sinusoids, or portal tracts) and amount. With the modified grading system, the amount of iron in each tissue location received a grade of 0 to 4 (Appendix). The total iron score is the sum of the hepatocyte iron score, sinusoidal iron score, and portal iron score, with a maximum possible total iron score of 12.

**Statistical analysis**—Statistical analysis was performed by use of commercially available software. Values for SI concentration, TIBC, and transferrin saturation were approximately normally distributed, whereas the distributions of ferritin, TIC, and NIC were logarithmic. Therefore, values for those 3 measurements were natural-log transformed prior to further statistical evaluation.

Group means for serum analytes were compared via ANOVA by use of the Tukey-Kramer honestly significant difference method. For comparison of values within individuals by regression analysis, NIC was evaluated against serum analytes, age, trace minerals, and histologic grading scores. Pairwise associations were evaluated with the Pearson correlation method. The serially sampled variables (SI concentration, TIBC, ferritin concentration, and transferrin saturation) were evaluated according to sampling order (week 1, 2, or 3) and as mean values for all 3 weeks. There was no significant difference in final results or conclusions drawn between the 2 approaches, and mean values for the serial samples are reported. Values of P < 0.05 were considered significant for both ANOVA and regression analyses.

**Results**

Results of this study confirmed the existence of a strong linear relationship between TIC and NIC measurements of tissue iron content (r = 0.987; P < 0.001), with the equation for the line of best fit equal to TIC (µg/g) = 581 + 2.2 NIC (µg/g). The mean, SD, and range for measurements of liver iron content and SI analytes by species are summarized (Table 2). Values for NIC ranged from 123 to 5,024 µg/g, and values for TIC ranged from 497 to 12,800 µg/g of dry weight. There was no difference in mean liver iron content by sex or institution. Mean values for TIC and NIC tended to be lowest in ring-tailed lemurs and highest in ruffed lemurs, with black lemurs having intermediate values; however, the differences were only significant between ring-tailed lemurs and ruffed lemurs (P = 0.002 for both variables). Mean values of serum iron analytes also varied by species. Despite large overlap of individual values among genera, mean values for SI concentration, ferritin concentration, and transferrin saturation were significantly higher for ruffed lemurs than for black lemurs (P = 0.048, P = 0.004, and P = 0.006, respectively), whereas mean TIBC was higher in ring-tailed lemurs than in ruffed lemurs (P = 0.026). Ruffed lemurs had lower serum ferritin concentrations for a given NIC than either of the other 2 species tested (Figure 1).

The relationship between mean liver NIC and the 4 SI analytes was evaluated to determine whether 1 or more analytes varied predictably in relation to iron

---

### Table 2—Summary of values for liver iron content and SI analytes by species in the same 32 lemurs as in Table 1.

<table>
<thead>
<tr>
<th>Lemur species</th>
<th>Age (y)</th>
<th>NIC (µg/g)</th>
<th>TIC (µg/g)</th>
<th>SI concentration (µg/g)</th>
<th>TIBC (µg/g)</th>
<th>Ferritin concentration (ng/dL)</th>
<th>TS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ring-tailed</td>
<td>8.6 ± 5.9</td>
<td>349 ± 140</td>
<td>1,300 ± 467</td>
<td>231 ± 43</td>
<td>433 ± 47</td>
<td>104 ± 84</td>
<td>55 ± 15</td>
</tr>
<tr>
<td>Black</td>
<td>15.1 ± 4.3</td>
<td>1,105 ± 458</td>
<td>3,123 ± 790</td>
<td>198 ± 41</td>
<td>422 ± 47</td>
<td>177 ± 81</td>
<td>47 ± 11</td>
</tr>
<tr>
<td>Ruffed</td>
<td>11.5 ± 6.8</td>
<td>1,938 ± 1,536</td>
<td>4,845 ± 3,547</td>
<td>262 ± 67</td>
<td>377 ± 47</td>
<td>58 ± 57</td>
<td>69 ± 17</td>
</tr>
</tbody>
</table>

TS = Transferrin saturation.

*Values with different superscripts (A, B) within a column are significantly (P < 0.05) different.
stores in the liver. Because iron stores are known to increase with age in humans, the relationship between age and NIC was also evaluated. Correlation coefficients for the pairwise comparisons were summarized (Table 3). When evaluated as a group, positive correlations were detected between NIC and age, SI concentration, ferritin concentration, and transferrin saturation, and a negative correlation was seen between NIC and TIBC. Examination of the relationship between variables on the basis of genus indicated that the strength of the correlation between a given variable and NIC varied substantially among lemurs of different genera. Significant associations between increasing liver NIC and increasing ferritin concentration were seen in all 3 genera, whereas associations between NIC and SI concentration, and between NIC and transferrin saturation, were observed in ring-tailed and ruffed lemurs but not in black lemurs. Similarly, a relationship between increasing age and increasing liver NIC was detected only in ruffed lemurs.

Mean liver content of zinc and copper were 242 ± 9 ppm and 30 ± 22 ppm, respectively. Mean serum measurements for zinc and copper were 1.4 ± 0.4 and 1.6 ± 0.4 ppm, respectively. Positive correlations were observed between liver iron content and liver zinc content (for NIC, r = 0.553 and P < 0.001; for TIC, r = 0.659 and P = 0.001). No correlations were detected between tissue or serum copper and liver iron content.

The semiquantitative scoring of iron in Prussian blue–stained slides correlated well with quantitative hepatic iron measurements. Total iron score correlated positively with both TIC (r = 0.866; P < 0.001) and NIC (r = 0.854; P < 0.001; Figure 2) and with age (r = 0.573; P = 0.006). Values for total iron score ranged from 0 to 8 (of a maximum value of 12), with values generally being lowest in ring-tailed lemurs (mean ± SD total iron score, 1.5 ± 1.3; range, 0 to 4), intermediate in black lemurs (2.9 ± 1.0; range, 0 to 7), and highest in ruffed lemurs (4.2 ± 3.1; range, 0 to 8). Overall, the amount of iron in liver tissue was minimal to mild, with 24 of 32 (75%) lemurs having total iron scores of 3 or less. One black lemur and 2 ruffed lemurs had total iron scores of 7, and 2 ruffed lemurs had scores of 8. Liver iron was predominately located in sinusoidal and portal areas, with the mesenchymal iron score (sinusoidal iron score + portal iron score) accounting for 60% to 100% of iron detected in all instances. Iron deposition in hepatocytes was generally minimal. Thirty of 32 (94%) lemurs had a hepatocyte iron score of 0 or 1, and 2 of 32 (6%) had a hepatocyte iron score of 2.

Although microscopic evaluation focused primarily on iron accumulation, both H&E and Prussian blue–stained sections were also assessed for other changes. Seven of the 32 (22%) lemurs had no notable changes in liver morphology. Twenty-five (78%) lemurs had minimal to mild changes, including inflammatory cell infiltrates, bile duct proliferation, fibrosis, or cestodes. Changes seen were mild and were not correlated with iron accumulation; therefore, they were not considered further.

**Discussion**

Publications reporting liver iron content in humans and animals variably report content as either TIC or NIC. Total iron concentration includes all forms of iron contained within a sample, whereas NIC measures only storage forms of iron and is not confounded by iron contained in hemoglobin or myoglobin. For purposes of comparing the results of this study with data published for other species, both measurements were obtained. A strong linear relationship was confirmed between the 2 methods. No significant differences were detected between the 2 measurements and correlations with other variables. However, given that NIC is considered a more reliable measurement of tissue iron stores, NIC is used in the following discussion of the relationships between liver iron content and other variables.

Although no data are available on what constitutes toxic concentrations of iron in lemurs, the concentrations measured in the present study are generally low relative to those known to be problematic in other animals and humans. The limited quantity of iron in hepatocytes, the ab-

---

**Table 3—Summary of correlation coefficients and P values for comparisons of liver NIC and SI analytes by species in the same lemurs as in the preceding tables.**

<table>
<thead>
<tr>
<th>Variable pair</th>
<th>Ring-tailed lemur (n = 11)</th>
<th>Black lemur (11)</th>
<th>Ruffed lemur (10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r value</td>
<td>P value</td>
<td>r value</td>
</tr>
<tr>
<td>LogNIC/age</td>
<td>0.45</td>
<td>0.17</td>
<td>0.45</td>
</tr>
<tr>
<td>LogNIC/SI</td>
<td>0.77*</td>
<td>&lt; 0.01</td>
<td>0.07</td>
</tr>
<tr>
<td>LogNIC/TIBC</td>
<td>-0.62*</td>
<td>0.04</td>
<td>-0.33</td>
</tr>
<tr>
<td>LogNIC/Ferritin</td>
<td>0.67*</td>
<td>0.02</td>
<td>0.68*</td>
</tr>
<tr>
<td>LogNIC/TS</td>
<td>0.74*</td>
<td>&lt; 0.01</td>
<td>0.22</td>
</tr>
</tbody>
</table>

*Denotes significance at P < 0.05. See Table 2 for remainder of key.
sence of morphologic histologic changes, and the generally low total iron scores indicate that iron accumulation in our study population was mild overall. These findings suggest that, although hemosiderosis may be common in captive lemurs, the frequency of lemur developing iron overload severe enough to cause clinical manifestations and hemochromatosis may be lower than previously thought.

Among nonhuman primates, excessive iron accumulation has been reported in captive new world primates (Saimiri, Callithrix, Sararinus, Cebuella, and Lagothrix spp), lowland gorillas (Gorilla gorilla graueri), and colobine monkeys (Colobus spp) in addition to lemurs.6,10,11 The wide range of hepatic iron concentrations seen in the present study is in agreement with results of other studies involving both anthropoid and prosimian primates. Hepatic iron concentrations in the literature are commonly reported in units of micrograms per gram or micromoles per gram and as wet weight or dry weight measurements. Values of 3.3 to 3.5 g of wet tissue are estimated to be equivalent to 1 g of dried liver tissue.6 For the purposes of comparing values contained in previous reports and the present study, a multiplication value of 3.4 was used to convert wet weight to dry weight values. Moles were converted to grams according to the atomic weight of iron (55.85 g).

Our observations that hepatic iron content varied by genus were consistent with the findings of Glenn et al4 in a 2006 study in which liver iron content was measured in lemurs of 15 species that died of natural causes at the Duke Lemur Center. Similar to our findings, when hepatic iron concentrations in ruffed, black, and ring-tailed lemurs were compared, the ruffed lemurs tended to have the highest concentrations, ring-tailed lemurs had the lowest concentrations, and black lemurs had intermediate concentrations. In the present study, a strong positive association between age and hepatic iron content was seen in ruffed lemurs. Although it is possible that diet may have contributed to the variability observed in iron accumulation, it is unlikely that diet alone can explain the variation. The gross composition and iron content of diets offered to each species at both institutions were similar. It is possible that different lemur taxa have different capacities for binding or regulating absorption of dietary iron and thus for developing iron overload. Additional studies are needed to determine whether there are differences in membrane transporter affinity or intestinal transporter expression among lemur species of different taxa.

Data are limited on what constitutes a toxic concentration of iron in various nonhuman primate species. Marmosets are particularly prone to developing iron overload in captivity.6 In a study6 in which marmosets were fed high-iron diets, those with liver iron concentrations exceeding 10,000 µg/g died prematurely, suggesting that concentrations above this are associated with a high mortality rate in that species. In humans with genetic hemochromatosis (an inherited disorder of iron regulation), the threshold concentration above which fibrosis develops is approximately 22,000 µg/g.23 In vivo evidence of increased lipid peroxidation, decreased mitochondrial respiratory control, and abnormal hepatic microsomal function has been seen in mice when hepatic iron concentrations exceed 10,000 to 12,000 µg/g of dry weight.24,25 In the present study, only 1 animal, a ruffed lemur with a TIC of 12,800 µg/g, had a hepatic iron concentration above 10,000 µg/g, and none had concentrations approaching those known to induce fibrosis in humans. Of the 32 lemur tested, 22 (69%) had a TIC < 3,500 µg/g, a concentration considered insignificant in humans, whereas 9 of 32 (28%) had hepatic iron concentrations from 3,500 to 6,500 µg/g, consistent with mild accumulation.21

With dietary iron overload, iron first accumulates in the nonparenchymal (Kupffer) liver cells and then is deposited in parenchymal cells (hepatocytes) when overload becomes more pronounced. Hepatic cell necrosis and periportal fibrosis follow.11,12 In the present study, 2 lemur, 1 with a TIC of 3,860 µg/g and 1 with a TIC of 6,390 µg/g, had slight fibrosis on histologic analysis, but bridging fibrosis and cirrhosis were not detected in any lemur. Lemurs with higher liver iron concentrations appeared to have minimal to mild bile duct proliferation, individual hepatocyte necrosis, or both; however, both findings were also seen in lemur with low iron concentrations. This is not surprising, given that these changes are nonspecific and can be caused by multiple etiologies unrelated to iron accumulation. Although it is tempting to suspect iron toxicosis as the cause of liver damage in lemur when stainable iron is detected, it is important to look extensively for alternate explanations and rule out other possible causes.

The strong relationship detected between semi-quantitative assessment of liver iron content in Prussian blue–stained slides and direct measurements of liver iron content suggests that use of a standardized histopathologic scoring system by trained pathologists can provide a reasonable approximation of liver iron stores in lemur. Such an approach may be helpful when it is not possible to perform direct biochemical analysis of tissue iron concentrations.

Measurements of SI concentration, TIBC, ferritin concentration, and transferrin saturation are routinely used to evaluate iron status in humans and various domestic animals.15–17 Use of blood parameters to evaluate iron status in exotic species is more problematic because normal values for the various parameters have not been established for most exotic animals. As a further complication, the usual test to measure serum ferritin concentration is an immunologic assay and is species specific. Thus, assay reagents must be developed and validated for each species. In the present study, the ferritin assay used was one developed for ruffed lemur. However, when sera from other lemur, including black and ring-tailed lemur, were tested with ferritin purified from ruffed lemur to establish a standard curve, test results obtained were of the same order of magnitude as seen in sera from ruffed lemur. Additionally, the capture and indicator antibodies used in the assay are polyclonal and should, therefore, recognize multiple epitopes.20 For these reasons, the developers of the assay are confident that it reliably measures ferritin in a variety of lemur taxa. Nonetheless, it is prudent to interpret the ferritin measurements obtained in taxa other than ruffed lemur with some caution until the assay can be independently validated in each species.

Among domestic animals, ferritin assays are available for horses, pigs, cats, dogs, and cows. In most species tested except for rats, ferritin concentration is closely correlated to total body iron stores.27–33 The positive association between ferritin and liver iron concentrations observed in the present study suggests that this relation-
ship likely holds true in lemurs as well, although our data indicate that there may be differences in the precise nature of the relationship at the genus level. Although ruffed lemurs tended to have higher hepatic iron content than lemurs of the other 2 genera, they had the lowest serum ferritin concentrations. We had predicted that lemurs with the highest quantities of hepatic iron would have the highest serum ferritin concentrations. Although this was true within a single species, it was not consistent across genera. Ruffed lemurs had much lower serum ferritin concentrations for a given NIC value than did either of the other 2 species tested. One possible explanation is that ferritin in ruffed lemurs is more heavily tissue-bound than in ring-tailed or black lemurs, resulting in less circulating ferritin in the bloodstream available for measurement. Another alternative is that in ruffed lemurs, conversion of ferritin to hemosiderin occurs more readily and a greater proportion of storage exists as hemosiderin and less as ferritin. Regardless of the explanation, establishment of reference ranges in lemurs should be developed on the basis of genus at minimum and, possibly, at the species level. Reference ranges for ferritin concentration established in humans and other primates cannot reliably be extrapolated to lemurs.

Transferrin saturation is an assessment of tissue iron supply. The calculation uses SI expressed as a percentage of TIBC. Transferrin saturation decreases in iron deficiency and in chronic disease states associated with infection, inflammation, and malignant disease. Values increase in response to increased iron absorption from the gastrointestinal tract and increased demand for hematopoiesis. Increases in total body iron stores are typically associated with increases in SI concentration and decreases in TIBC and, as a result, increases in transferrin saturation. In the present study, transferrin saturation was positively correlated with hepatic iron concentrations in ruffed and ring-tailed lemurs, but not in black lemurs. The inability to establish a clear relationship between SI concentration, TIBC, or transferrin saturation and hepatic iron content in black lemurs may be a result of either the small number of animals available for study or the presence of subclinical illness not detected by our screening protocol. It also cannot be ruled out that some aspects of iron kinetics in black lemurs may differ from those of the other 2 genera. In dogs and cats, tissue NIC and serum ferritin concentrations are correlated, but there is no correlation between NIC and SI concentration or TIBC measurements. Thus, data in domestic animals support the premise that the usefulness of SI values as estimates of iron stores must be evaluated independently in different taxa.

There was a great deal of individual variation among values in the weekly samples for each of the SI indices evaluated (data not given). No constant pattern or direction of change was detected in any analyte, and the fact that no values consistently increased or decreased during the sampling period suggested that blood loss from venipuncture did not influence results. Therefore, a single test result from a single lemur likely has limited predictive value. It is generally recommended that humans screened for iron overload have persistently high values of transferrin saturation, ferritin concentration, or both before they are considered candidates for further diagnostic testing to rule out iron overload. Our data suggest that, in lemurs as in humans, repeated measurement of multiple analytes yields more reliable assessment of iron status in a given lemur.

Mean values of serum iron indices obtained in this study are generally higher than those reported in other studies37,41 in similar lemur species in both captive and natural environments. However, it is difficult to make direct comparisons between reports. Previous reports contain no measurements of hepatic iron content, making it impossible to determine how closely serum values for the various tests reflect the iron status of the animals studied. Differences in sample collection techniques, study populations, and statistical analyses varied among studies. It is also possible that other physiologic or dietary factors known to influence iron indices, such as recent ingestion of a meal, hormonal fluctuations associated with pregnancy or contraception, recent blood loss through phlebotomy or injury, or presence of subclinical illness in some animals, may have influenced results. Closely controlling these variables in future studies would improve investigators’ ability to make valid comparisons between reports.

It is well established that metabolic pathways for copper, zinc, and iron are closely linked.22,43 In the present study, copper and zinc concentrations in serum and liver were similar to normal values reported for other primates, and obvious imbalances were not detected.22,44,45 The increase in hepatic zinc content seen in association with increasing hepatic iron content in the present study is consistent with findings in rats and humans with iron overload.46-49 The finding can be explained by the fact that zinc and iron share transport and storage proteins.48-50 The likelihood that lemurs in our study cohort had nutritional imbalances of either copper or zinc is low, but the possibility that abnormal trace mineral metabolism contributes to iron accumulation in captive lemurs cannot be ruled out entirely.

Hepatic iron contents in lemurs in the present study were generally low and were not associated with histologic changes in the liver. However, studies of lemurs housed at multiple institutions are needed to gain a better understanding of the prevalence, severity, and clinical impact of iron overload in captive lemurs. The finding that lemurs of different genera accumulate iron at different rates suggests that there may be subtle differences in iron absorption or regulation across taxa. Measurement of serum ferritin concentration in conjunction with transferrin saturation may constitute a noninvasive approach for evaluating iron status in lemurs; however, more information is needed regarding the predictive value of the tests, and reference ranges will likely need to be established independently for the various taxa.

a. Purina Monkey Diet 5038, PMI Nutrition International LLC, Brentwood, Mo.
b. HMS Hi-Fiber Primate Diet, HMS ZOO DIETS, Bluffton, Ind.
c. Marion Leaf Eater (primate) Food, Marion Zoological, Plymouth, Minn.
d. Tropical Bits, Marion Zoological, Plymouth, Minn.
e. Becton-Dickinson, Franklin Lakes, NJ.
f. VWR International LLC, Westchester, Penn.
g. Sigma Diagnostics Inc, St Louis, Mo.
h. SAS Institute Inc, Cary, NC.
Appendix

Summary of grading system used to obtain semiquantitative measurements of iron in Prussian blue–stained liver tissue specimens from 32 captive lemurs.

<table>
<thead>
<tr>
<th>Hepatocyte iron score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Granules absent or barely discernible at 10X magnification</td>
</tr>
<tr>
<td>1</td>
<td>Granules barely discernible at 10X but easily discernible at 40X</td>
</tr>
<tr>
<td>2</td>
<td>Discrete granules visible at 10X</td>
</tr>
<tr>
<td>3</td>
<td>Discrete granules visible at 20X</td>
</tr>
<tr>
<td>4</td>
<td>Masses visible at 20X or by the naked eye</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sinusoidal iron score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Granules absent or barely discernible at 10X magnification</td>
</tr>
<tr>
<td>1</td>
<td>Scattered cells contain few granules—barely visible at 10X, easily discernible at 40X</td>
</tr>
<tr>
<td>2</td>
<td>Moderate number of cells contain granules, visible at 40X</td>
</tr>
<tr>
<td>3</td>
<td>Numerous cells contain granules, visible at 40X, few clusters of more than 3 macrophages with intracellular iron</td>
</tr>
<tr>
<td>4</td>
<td>Numerous cells contain granules, visible at 40X, multiple clusters of more than 3 macrophages with intracellular iron</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Portal iron score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Granules absent or barely discernible at 10X magnification</td>
</tr>
<tr>
<td>1</td>
<td>Scattered cells contain few granules—barely seen at 10X, easily discernible at 40X</td>
</tr>
<tr>
<td>2</td>
<td>Moderate number of cells contain granules, visible at 40X</td>
</tr>
<tr>
<td>3</td>
<td>Numerous cells contain granules, visible at 40X, few clusters of more than 3 macrophages with intracellular iron</td>
</tr>
<tr>
<td>4</td>
<td>Numerous cells contain granules, visible at 40X, multiple clusters of more than 3 macrophages with intracellular iron</td>
</tr>
</tbody>
</table>

References

35. Ritchie RF, Palomaki GE, Neveux LM, et al. Reference distri-
Selected abstract for **JAVMA readers from the American Journal of Veterinary Research**

Minimum anesthetic concentration of isoflurane in captive thick-billed parrots (*Rhynchopsitta pachyrhyncha*)

**Julio A. Mercado et al**

**Objective**—To determine the minimum anesthetic concentration (MAC) of isoflurane in thick-billed parrots (*Rhynchopsitta pachyrhyncha*).

**Animals**—15 healthy thick-billed parrots.

**Procedures**—Anesthesia was induced and maintained with isoflurane in oxygen. In the first bird that was anesthetized, end-tidal isoflurane concentration was maintained at 1.6% for 15 minutes. After this period of anesthetic equilibration, an end-tidal gas sample was obtained for verification of isoflurane concentration. A toe was pinched to determine the bird’s response to pain, and the bird was then allowed to recover from anesthesia. To determine MAC, a so-called up-and-down approach was subsequently used in all 15 birds. Compared with the isoflurane concentration used for MAC determination in the first bird, maintenance isoflurane concentration for the second bird was increased by approximately 10% if the first bird reacted and decreased by approximately 10% if the first bird did not react to a toe pinch. These steps were then followed until all 15 birds had been anesthetized. Crossover events occurred when birds in sequence had discordant results (ie, 1 reactor and 1 nonreactor). The MAC was defined as the mean of the isoflurane concentrations measured during these crossover events.

**Results**—Mean MAC of isoflurane in thick-billed parrots was estimated to be 1.07% (95% confidence interval, 0.97% to 1.16%).

**Conclusions and Clinical Relevance**—Isoflurane MAC appeared to be lower in thick-billed parrots than the MAC determined for other bird species. Determination of the species-specific requirements of thick-billed parrots should allow isoflurane anesthesia to be performed more safely in this endangered species. (*Am J Vet Res* 2008;69:189–194)