AGE-RELATED CHANGES IN HEMATOLOGY AND BLOOD BIOCHEMISTRY VALUES IN ENDANGERED, WILD RING-TAILED LEMURS (*LEMUR CATTA*) AT THE BEZÀ MAHAFALY SPECIAL RESERVE, MADAGASCAR

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Source: Journal of Zoo and Wildlife Medicine, 49(1):30-47.
Published By: American Association of Zoo Veterinarians
https://doi.org/10.1638/2017-0008R1.1
URL: http://www.bioone.org/doi/full/10.1638/2017-0008R1.1

Journal of Zoo and Wildlife Medicine 49(1): 30–47, 2018

AGE-RELATED CHANGES IN HEMATOLOGY AND BLOOD BIOCHEMISTRY VALUES IN ENDANGERED, WILD RING-TAILED LEMURS (*LEMUR CATTA*) AT THE BEZÀ MAHAFALY SPECIAL RESERVE, MADAGASCAR

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Abstract: The health of 44 wild ring-tailed lemurs (*Lemur catta*) at the Bezà Mahafaly Special Reserve was assessed across three age classes: <5 yr (young), 5–9 yr (adult), and ≥10 yr (old). Hematology and biochemistry tests were performed manually (leukocyte count and differential, packed cell volume, total protein) and using a point-of-care analyzer (hematocrit, hemoglobin, glucose, blood urea nitrogen, creatinine, sodium, potassium, chloride, ionized calcium, total carbon dioxide, anion gap), respectively. Urine specific gravity was measured via refractometry. Age- and sex-related differences were detected. Old lemurs had significantly lower lymphocyte count than adult and young lemurs, leading to markedly lower total leukocyte count and higher neutrophil-to-lymphocyte ratio. Decreased lymphocyte count with advanced age is consistent with immunosenescence. Young lemurs had significantly higher total protein, monocyte count, and potassium than adult and old lemurs but significantly lower ionized calcium than adult lemurs. Males had significantly higher leukocyte, neutrophil, and monocyte counts; lower percentage basophils; and higher blood urea nitrogen than females. Females had markedly higher glucose than males. Young females had the highest monocyte count and total protein, which were significantly lower in the adult and old age classes. Basophil count was stable in females across age but dropped precipitously in males in the adult and old age classes. Within adult and old age classes, males had significantly higher blood urea nitrogen and lower basophils than females. Glucose was significantly higher after α2 agonist administration. Identifying age-related hematologic and biochemical changes in apparently healthy wild ring-tailed lemurs will aid in clinical diagnosis and treatment of lemurs in human care, which is especially relevant for management of geriatric animals in zoo populations. Equally important, a better understanding of the ability of aging lemurs to tolerate environmental stressors will inform the capacity for this species to cope with ongoing and future habitat alteration.

Key words: Age effects, captive management, conservation, health, immunosenescence, lemur.

INTRODUCTION

According to the IUCN Primate Specialist Group, 91% of lemur species are threatened with extinction. Habitat degradation and loss remain major threats to primate survival, with Madagascar being a top hot spot in terms of biodiversity and ongoing habitat loss, and the endemic lemurs being the most endangered primate taxa.[54] The lemurs of Madagascar, including the iconic and endangered ring-tailed lemur (*Lemur catta*), face probable extinction within the next half century if adequate conservation programs are not developed.[50,54]

Since 2003, ring-tailed lemurs at the Bezà Mahafaly Special Reserve (BMSR) have been studied intensively to assess the effects of anthropogenic and climate factors on behavior, ecology, genetics, and health.[38,40,51] Recent surveys indicate massive declines in ring-tailed lemur populations in areas historically inhabited, with BMSR being one of the last remaining viable source populations.[16,29] Health examinations have been performed on a subset of the population annually between 2003 and 2012,[38,48,49,55] including collection of biometric measurements, data on tooth wear and dental health, dental impressions, and varied biological samples. Although studies have evaluated the health of this population,[38,51,52,55] the effect of aging on wild lemur health remains largely unexplored. The goal of this study is to assess age-related changes in hematology and biochemistry values, 1) to help better understand the ability of aging lemurs to tolerate environ-

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mental stressors and cope with ongoing and future habitat alteration and 2) to aid clinical diagnosis and treatment of geriatric lemurs in zoo populations.

MATERIALS AND METHODS

Research adhered to strict animal handling protocols and received Institutional Animal Care and Use Committee (IACUC) approval (University of North Dakota IACUC Protocol #0802-2 and animal assurance number A3917-01), as well as being permitted by the Convention on International Trade in Endangered Species (CITES Madagascar: 531C-EA10/MG10; CITES US: 11US040035/9) and Madagascar National Parks (086/12/MEF/SG/DGF/DCB.SAP/SCB).

Study site and species

The BMSR is located in southwestern Madagascar. Established in 1986 as part of the Madagascar National Parks system, the reserve has been the focus of socio-ecological studies of ring-tailed lemurs since 1987.[53,57] The study site within the reserve is 80 ha of fenced gallery forest, ranging from riverine forest along the seasonal Sakamena River to more open, dry forest within 1 km of the river margin. It also includes degraded habitat in all forest types outside the reserve fence.

This study site is home to nine troops of ring-tailed lemurs that are habituated to humans and have been studied intensively for the past three decades.[57] Additional troops live in and around the reserve, making the reserve population much larger than the nine intensively studied troops. Study lemurs are identified by colored nylon collars with numbered tags and transponders, placed upon their initial evaluation. Beginning in 2003 and routinely thereafter, lemurs in the study population have been captured at 2 yr of age. This results in a study population composed mostly of known-aged individuals. However, some lemurs were first collared as full adults ($\geq$5 yr), with their age grade determined by somatic measures, dental development and tooth wear (specifically maxillary canine wear, which begins after 5 yr in this population), and reproductive maturity.[2,5,52] Therefore, many of the oldest lemurs in the study population have an estimated age >10 yr. Lemurs in the current study ranged in age from 3 to >10 yr and were divided into three age classes: <5 yr (young; $n = 8$), 5-9 yr (adult; $n = 18$), and $\geq$10 yr (old; $n = 18$). With lemurs at BMSR rarely living beyond 15 yr,[6] the three age classes represent approximately equal segments of life.

Capture and data collection

During June 2011, 44 wild ring-tailed lemurs were anesthetized, captured, recovered, and released as previously described.[30,38,55] A complete physical examination, morphometric measurements, and dental impressions were performed on each lemur. Blood was collected and transferred into separate tubes containing ethylenediaminetetraacetic acid (EDTA) (Micro tube 1.3 ml K3E, Sarstedt AG & Co, 51588 Nümbrecht, Germany) and lithium heparin (Vacuette® 2 ml, Greiner Bio-One, Monroe, North Carolina 28110, USA, or BD Microtainer® 400 µl, Becton, Dickinson and Company, Franklin Lakes, New Jersey 07417-1880, USA) for hematology and biochemistry profiles, respectively. Tubes containing EDTA were filled first, with the remainder of the blood sample placed into either a 400-µl or a 2-ml lithium heparin tube. Micro tubes were filled to recommended levels, whereas 2 ml heparin tubes received variable amounts of blood (1–2 ml). Urine (mixed first void and midstream) was collected via manual expression of the urinary bladder. Samples were held in shade at ambient temperature (65–75°F) until processed within 2–8 hr of collection. Total leukocyte count was performed manually (Whi-pette®, Exotic Animal Solutions, Inc., Hueytown, Alabama 35223, USA). Microscopic determination of leukocyte differential count and hemoparasite examination was performed on blood smears. Neutrophil-to-lymphocyte ratio (NLR) was calculated from the leukocyte count. Packed cell volume (PCV) was determined by microcentrifugation, and hematocrit (HCT) and hemoglobin (Hb) were measured using a point-of-care analyzer (i-STAT®, Abbott Point of Care Inc., Princeton, New Jersey 08540, USA). Plasma total protein (TP) was measured by refractometry. Biochemistry parameters, obtained using a point-of-care analyzer, included sodium (Na), potassium (K), chloride (Cl), ionized calcium (iCa), total carbon dioxide, glucose (Glu), blood urea nitrogen (BUN), and creatinine (Cr). Urine specific gravity (USG) was measured by refractometry.

Statistical analyses

Data were analyzed in JMP Pro 13 (2016; SAS Institute Inc., Cary, North Carolina 27513, USA) either using a one-way analysis of variance and the Tukey–Kramer post hoc test or the Student's

Table 1. Hematology profiles of wild ring-tailed lemurs from three different age classes in the Bezà Mahafaly Special Reserve, Madagascar. Total leukocyte count and leukocyte differential count were performed manually. PCV was determined by microcentrifugation. Plasma TP was measured by refractometry. HCT and Hb were measured using a point-of-care analyzer. Tukey–Kramer Honest Significant Difference test or Student's *t*-test with Bonferroni adjustment. Significance set at $P \leq 0.05$. Min indicates minimum; Max, maximum; NS, not significant.

	All ages combined			<5 yr old		
Parameter	Mean ± SD	Min–Max	*n*	Mean ± SD	Min–Max	*n*
Leukocytes (10^3/μl)						
Both sexes	7,534 ± 2,364	3,520–12,100	44	8,669 ± 2,517[a]	4,895–12,100	8
Female	6,993 ± 2,421[d]	3,520–12,100	30	8,415 ± 3,241	4,895–12,100	5
Male	8,694 ± 1,812[d]	5,188–11,550	14	9,093 ± 863	8,415–10,065	3
Female vs male	0.0070[d]			NS		
Neutrophils (cells/μl)						
Both sexes	2,644 ± 1,085	490–5,518	44	2,377 ± 1,217	490–4,378	8
Female	2,374 ± 1,001[d]	490–4,689	30	2,133 ± 1,407	490–4,378	5
Male	3,221 ± 1,066[d]	1,875–5,518	14	2,783 ± 911	1,875–3,696	3
Female vs male	0.0098[d]			NS		
Lymphocytes (cells/μl)						
Both sexes	3,985 ± 1,948	1,319–9,196	44	5,132 ± 2,157[a]	2,081–9,196	8
Female	3,748 ± 1,958	1,319–9,196	30	5,183 ± 2,664[a]	2,081–9,196	5
Male	4,493 ± 1,897	1,349–7,508	14	5,047 ± 1,440	4,208–6,710	3
Female vs male	NS			NS		
Monocytes (cells/μl)						
Both sexes	423 ± 190	140–920	44	605 ± 143[ac]	395–876	8
Female	391 ± 190[d]	140–876	30	648 ± 150[ac]	484–876	5
Male	492 ± 178[d]	176–920	14	533 ± 121	395–616	3
Female vs male	0.0495[d]			NS		
Eosinophils (cells/μl)						
Both sexes	357 ± 290	0–1,200	44	363 ± 351	84–1,085	8
Female	338 ± 219	64–965	30	293 ± 240	98–616	5
Male	399 ± 411	0–1,200	14	478 ± 534	84–1,085	3
Female vs male	NS			NS		
Basophils (cells/μl)						
Both sexes	119 ± 130	0–673	44	176 ± 224	0–673	8
Female	138 ± 97	0–320	30	147 ± 97	88–320	5
Male	78 ± 179	0–673	14	224 ± 389	0–673	3
Female vs male	NS			NS		
Neutrophils (%)						
Both sexes	36.3 ± 12.7	10–62	44	27.5 ± 12.7[a]	10–42	8
Female	35.3 ± 12.4	10–62	30	25.2 ± 14.1[a]	10–40	5
Male	38.6 ± 13.6	19–62	14	31.3 ± 11.6	19–42	3
Female vs male	NS			NS		
Lymphocytes (%)						
Both sexes	51.0 ± 13.6	25–76	44	58.5 ± 13.6[a]	39–76	8
Female	51.5 ± 13.4	25–76	30	60.4 ± 15.8[a]	39–76	5
Male	50.1 ± 14.5	26–70	14	55.3 ± 11.0	48–68	3
Female vs male	NS			NS		
Monocytes (%)						
Both sexes	5.9 ± 2.7	2–13	44	7.6 ± 2.9[c]	4–12	8
Female	5.9 ± 3.1	2–13	30	8.6 ± 3.1[c]	4–12	5
Male	5.8 ± 1.9	2–8	14	6.0 ± 1.7	4–7	3
Female vs male	NS			NS		
Eosinophils (%)						
Both sexes	4.8 ± 3.4	0–16	44	4.0 ± 3.4	1–11	8
Female	4.9 ± 2.7	1–13	30	3.4 ± 2.3	1–7	5
Male	4.6 ± 4.6	0–8	14	5.0 ± 5.3	1–11	3
Female vs male	NS			NS		

Table 1. Extended.

5–9 yr old			≥10 yr old			Age class comparison		
Mean ± SD	Min–Max	*n*	Mean ± SD	Min–Max	*n*	<5 vs ≥10	<5 vs 5–9	5–9 vs ≥10
8,291 ± 2,230[b]	4,235–11,550	18	6,273 ± 1,912[ab]	3,520–9,185	18	0.0328[a]	NS	0.0205[b]
7,657 ± 2,131	4,235–10,890	12	5,832 ± 1,940	3,520–9,185	13	NS	NS	NS
9,558 ± 2,007	6,307–11,550	6	7,418 ± 1,408	5,188–9,075	5	NS	NS	NS
NS			NS					
2,752 ± 1,271	1,290–5,518	18	2,653 ± 841	1,167–4,434	18	NS	NS	NS
2,408 ± 1,025	1,290–4,689	12	2,435 ± 875[d]	1,167–4,434	13	NS	NS	NS
3,441 ± 1,526	2,183–5,518	6	3,220 ± 392[d]	2,813–3,692	5	NS	NS	NS
NS			0.0032[d]					
4,659 ± 1,777[b]	1,863–7,950	18	2,801 ± 1,393[ab]	1,319–5,808	18	0.0070[a]	NS	0.0061[b]
4,396 ± 1,664[b]	1,863–7,950	12	2,597 ± 1,282[ab]	1,319–5,235	13	0.0203[a]	NS	0.0356[b]
5,184 ± 2,038	2,867–7,508	6	3,331 ± 1,682	1,349–5,808	5	NS	NS	NS
NS			NS					
399 ± 190[c]	176–920	18	367 ± 165[a]	140–631	18	0.0064[a]	0.0208[c]	NS
341 ± 131[c]	191–568	12	339 ± 176[a]	140–631	13	0.0023[a]	0.0026[c]	NS
517 ± 246	176–920	6	437 ± 120	300–617	5	NS	NS	NS
NS			NS					
370 ± 296	0–1,040	18	342 ± 273	0–1,200	18	NS	NS	NS
365 ± 273	64–965	12	330 ± 163	120–631	13	NS	NS	NS
381 ± 365	0–1,040	6	374 ± 485	0–1,200	5	NS	NS	NS
NS			NS					
110 ± 108	0–318	18	102 ± 91	0–276	18	NS	NS	NS
147 ± 110[d]	0–318	12	126 ± 90[d]	0–276	13	NS	NS	NS
36 ± 67[d]	0–127	6	41 ± 66[d]	0–151	5	NS	NS	NS
0.0170[d]			0.0252[d]					
33.4 ± 11.9[b]	18–60	18	43.2 ± 10.3[ab]	25–62	18	0.0067[a]	NS	0.0375[b]
31.8 ± 10.1[b]	18–49	12	42.4 ± 10.1[ab]	25–62	13	0.0141[a]	NS	0.0533[b]
36.7 ± 15.4	21–60	6	45.2 ± 11.9	31–62	5	NS	NS	NS
NS			NS					
55.4 ± 12.1[b]	33–73	18	43.3 ± 11.7[ab]	25–64	18	0.0149[a]	NS	0.0130[b]
56.5 ± 10.8[b]	41–73	12	43.5 ± 10.8[ab]	25–61	13	0.0268[a]	NS	0.0249[b]
53.3 ± 15.2	33–70	6	43.0 ± 15.1	26–64	5	NS	NS	NS
NS			NS					
4.9 ± 1.9[c]	2–8	18	6.1 ± 3.0	2–13	18	NS	0.0462[c]	NS
4.6 ± 1.6[c]	2–8	12	6.2 ± 3.5	2–13	13	NS	0.0330[c]	NS
5.5 ± 2.5	2–8	6	6.0 ± 1.6	4–8	5	NS	NS	NS
NS			NS					
4.6 ± 3.3	0–13	18	5.4 ± 3.5	0–16	18	NS	NS	NS
4.9 ± 3.5	1–13	12	5.5 ± 1.9	3–8	13	NS	NS	NS
3.8 ± 3.2	0–9	6	5.2 ± 6.4	0–16	5	NS	NS	NS
NS			NS					

Table 1. Continued.

Parameter	All ages combined			<5 yr old		
	Mean ± SD	Min–Max	*n*	Mean ± SD	Min–Max	*n*
Basophils (%)						
Both sexes	1.8 ± 2.0	0–8	44	2.4 ± 3.0	0–8	8
Female	2.1 ± 1.8[d]	0–7	30	2.2 ± 2.2	1–6	5
Male	1.0 ± 2.1[d]	0–8	14	2.7 ± 4.6	0–8	3
Female vs male	0.0500[d]			NS		
Neutrophil-lymphocyte ratio						
Both sexes	0.84 ± 0.55	0.14–2.48	44	0.54 ± 0.34[a]	0.14–1.03	8
Female	0.80 ± 0.52	0.14–2.48	30	0.50 ± 0.39[a]	0.14–1.03	5
Male	0.93 ± 0.62	0.28–2.38	14	0.60 ± 0.30	0.28–0.88	3
Female vs male	NS			NS		
PCV (%)						
Both sexes	42.3 ± 4.0	29–52	44	44.3 ± 4.7	37–52	8
Female	42.5 ± 3.3	36–48	30	44.0 ± 3.2	40–48	5
Male	41.8 ± 5.4	29–52	14	44.7 ± 7.5	37–52	3
Female vs male	NS			NS		
HCT (%)						
Both sexes	35.3 ± 3.3	30–45	40	36.0 ± 4.0	32–45	8
Female	34.9 ± 2.7	30–40	26	36.0 ± 2.5	32–39	5
Male	36.0 ± 4.1	30–45	14	38.0 ± 6.2	33–45	3
Female vs male	NS			NS		
Hb (g/dl)						
Both sexes	12.0 ± 1.1	10.2–15.3	40	12.5 ± 1.4	10.9–15.3	8
Female	11.9 ± 0.9	10.2–13.6	26	12.2 ± 0.9	10.9–13.3	5
Male	12.2 ± 1.4	10.2–15.3	14	12.9 ± 2.1	11.2–15.3	3
Female vs male	NS			NS		

[a] Significant difference between young and old age classes.
[b] Significant difference between adult and old age classes.
[c] Significant difference between young and adult age classes.
[d] Significant difference between females and males.

t-test using a Bonferroni adjustment. Significance was set at $P \leq 0.05$.

RESULTS

All lemurs appeared healthy. Several lemurs showed evidence of recovery from previous injuries (torn pinnae, old canine tooth fistulas, presumed healed fractures). All individuals had Laelapidae mites. No lemurs had evidence of hemoparasites.

Age effects were detected in several blood values (Tables 1, 2). Old lemurs had significantly lower number of leukocytes, number of lymphocytes, and percentage lymphocytes and significantly higher percentage neutrophils and NLR than adult and young lemurs. Young lemurs had significantly higher monocyte count, K, and TP than adult and old lemurs. Young lemurs had significantly higher percentage monocytes and lower iCa than adult lemurs.

Sex effects were evident in several blood values (Tables 1, 2). Male lemurs had significantly higher number of leukocytes, neutrophils, and monocytes and significantly lower percentage basophils than females. Females had higher Glu than males. Males had higher BUN than females.

Sex differences were found for some parameters across age classes (Tables 1, 2). Young female lemurs had significantly higher monocyte count and TP than adult and old females and significantly higher percentage monocytes than adults. Old female lemurs had significantly higher percentage neutrophils and NLR and significantly lower number and percentage lymphocytes than adult and young females. Young male lemurs had significantly higher K than adult and old males.

Few sex differences were found within age classes (Tables 1, 2). Within the adult and old age classes, males had significantly higher BUN and significantly lower basophil count than females. Within the old age class, males had significantly more neutrophils than females.

USG ranged widely (1.006–1.080; $n = 37$). There was no difference in USG between sexes (mean ± SD: males 1.024 ± 0.016, $n = 13$; females 1.021 ±

Table 1. Continued. Extended.

5–9 yr old			≥10 yr old			Age class comparison		
Mean ± SD	Min–Max	*n*	Mean ± SD	Min–Max	*n*	<5 vs ≥10	<5 vs 5–9	5–9 vs ≥10
1.6 ± 1.8	0–7	18	1.7 ± 1.6	0–5	18	NS	NS	NS
2.1 ± 2.0	0–7	12	2.2 ± 1.6	0–5	13	NS	NS	NS
0.5 ± 0.8	0–2	6	0.6 ± 0.9	0–2	5	NS	NS	NS
NS			NS					
0.68 ± 0.41[b]	0.26–1.82	18	1.14 ± 0.61[ab]	0.41–2.48	18	0.0179[a]	NS	0.0213[b]
0.61 ± 0.29[b]	0.26–1.07	12	1.10 ± 0.59[ab]	0.41–2.48	13	0.0483[a]	NS	0.0324[b]
0.82 ± 0.59	0.30–1.82	6	1.25 ± 0.74	0.48–2.38	5	NS	NS	NS
NS			NS					
42.6 ± 3.0	36–48	18	41.1 ± 4.5	29–47	18	NS	NS	NS
42.7 ± 3.3	36–48	12	41.8 ± 3.5	38–47	13	NS	NS	NS
42.5 ± 2.5	40–46	6	39.2 ± 6.6	29–47	5	NS	NS	NS
NS			NS					
35.8 ± 3.0	31–43	17	33.9 ± 2.9	30–39	15	NS	NS	NS
35.3 ± 2.6	31–40	11	34.0 ± 2.9	30–39	10	NS	NS	NS
36.8 ± 3.5	34–43	6	33.8 ± 3.1	30–37	5	NS	NS	NS
NS			NS					
12.2 ± 1.0	10.5–14.6	17	11.6 ± 1.0	10.2–13.3	15	NS	NS	NS
12.0 ± 0.9	10.5–13.6	11	11.6 ± 1.0	10.2–13.3	10	NS	NS	NS
12.5 ± 1.2	11.6–14.6	6	11.5 ± 1.1	10.2–12.6	5	NS	NS	NS
NS			NS					

0.017, $n = 24$) or among age classes (young 1.029 ± 0.021, $n = 8$; adult 1.017 ± 0.008, $n = 16$; old 1.025 ± 0.021, $n = 13$).

DISCUSSION

Hematology and blood biochemistry parameters change with aging in many primate species.[20,39,44,56] However, few studies have focused on strepsirrhine species, and these studies compare only juveniles and adults.[12,14,21,28,36,43] Grouping all adults together precludes detection of advanced age changes, such as would reflect deteriorating organ function, immune competency, or nutritional status. The current study uniquely compares three age classes, in an attempt to detect physiological changes associated with aging in a wild lemur population.

Three levels of data analysis were performed, each providing greater resolution to questions of age and sex effects. First, data were analyzed across age classes and between sexes. Second, sex effects were evaluated across age classes. Third, sex effects were evaluated within age classes. However, the small number of young males may have limited statistical significance of some differences.

Total leukocytes, neutrophils, and lymphocytes

Leukocytes participate in body defense, and changes in the number and type of circulating leukocytes occur with infection, inflammation, stress, immune status, and neoplasia. Use of absolute leukocyte numbers allows more consistent evaluation of leukogram responses than using relative percentages.[31]

In this study, the leukogram showed significant age-related changes, with leukocyte and lymphocyte counts decreasing with increasing age in both sexes (Table 1, Fig. 1). This decrease in lymphocytes, coupled with stable to slightly increased neutrophils, results in a significantly increased NLR with age, to >1 in old lemurs (Table 1). Changes in the leukocyte differential reveal a similar pattern, with old lemurs having significantly lower percentage lymphocytes and higher percentage neutrophils than adult and young lemurs (Table 1, Fig. 2).

In the BMSR population, the primary driver of significantly lower leukocyte count and higher

Table 2. Blood biochemistry values in wild ring-tailed lemurs from three different age classes in the Bezà Mahafaly Special Reserve, Madagascar. All values obtained using a point-of-care analyzer. Tukey-Kramer Honest Significant Difference test or Student's *t*-test with Bonferroni adjustment. Significance set at $P \leq 0.05$. Min indicates minimum; Max, maximum; NS, not significant.

	All ages combined			<5 yr old		
Parameter	Mean ± SD	Min–Max	*n*	Mean ± SD	Min–Max	*n*
Glucose (mg/dl)						
Both sexes	167 ± 81	61–361	40	209 ± 109	74–361	8
Female	182 ± 92[d]	69–361	26	247 ± 115	74–361	5
Male	139 ± 49[d]	61–228	14	145 ± 75	84–228	3
Female vs male	0.0298[d]			NS		
Blood urea nitrogen (mg/dl)						
Both sexes	9 ± 8	3–35	40	7 ± 5	3–15	8
Female	7 ± 7[d]	3–35	26	6 ± 5	3–15	5
Male	13 ± 8[d]	3–28	14	8 ± 4	3–11	3
Female vs male	0.0170[d]			NS		
Creatinine (mg/dl)						
Both sexes	0.9 ± 0.2	0.5–1.4	40	0.9 ± 0.2	0.6–1.3	8
Female	0.9 ± 0.2	0.5–1.4	26	0.8 ± 0.2	0.6–1.1	5
Male	0.9 ± 0.2	0.6–1.3	14	1.0 ± 0.3	0.8–1.3	3
Female vs male	NS			NS		
Sodium (mEq/L)						
Both sexes	143 ± 3	139–148	40	143 ± 3	139–147	8
Female	143 ± 3	137–148	26	143 ± 2	140–146	5
Male	143 ± 3	136–148	14	143 ± 4	139–147	3
Female vs male	NS			NS		
Potassium (mEq/L)						
Both sexes	3.7 ± 0.6	2.5–5.1	40	4.2 ± 0.7[ac]	3.1–5.1	8
Female	3.7 ± 0.6	2.5–5.1	26	4.0 ± 0.8	3.1–5.1	5
Male	3.7 ± 0.6	2.9–4.8	14	4.5 ± 0.3[ac]	4.2–4.8	3
Female vs male	NS			NS		
Chloride (mEq/L)						
Both sexes	104 ± 4	97–114	40	103 ± 4	99–111	8
Female	104 ± 4	98–111	26	104 ± 4	101–111	5
Male	104 ± 5	97–114	14	102 ± 4	99–106	3
Female vs male	NS			NS		
Ionized calcium (mM/L)						
Both sexes	0.92 ± 0.08	0.62–1.04	40	0.86 ± 0.15[c]	0.62–1.03	8
Female	0.93 ± 0.07	0.76–1.04	26	0.89 ± 0.12	0.76–1.03	5
Male	0.90 ± 0.10	0.62–1.01	14	0.80 ± 0.20	0.62–1.01	3
Female vs male	NS			NS		
Total carbon dioxide (mM/L)						
Both sexes	25 ± 5	15–37	40	26 ± 4	20–32	8
Female	25 ± 5	15–37	26	24 ± 3	21–27	5
Male	25 ± 6	16–32	14	28 ± 7	20–32	3
Female vs male	NS			NS		
Anion gap (mM/L)						
Both sexes	19 ± 2	10–24	40	19 ± 1	17–21	8
Female	19 ± 2	13–24	26	19 ± 1	19–20	5
Male	18 ± 3	10–22	14	19 ± 2	17–21	3
Female vs male	NS			NS		
Total protein						
Both sexes	7.0 ± 0.5	6.0–8.4	44	7.4 ± 0.7[ac]	6.2–8.4	8
Female	7.0 ± 0.5	6.0–8.4	30	7.6 ± 0.6[ac]	7.0–8.4	5
Male	6.9 ± 0.6	6.2–8.0	14	7.1 ± 0.8	6.2–7.6	3
Female vs male	NS			NS		

[a] Significant difference between young and old age classes.
[b] Significant difference between adult and old age classes.
[c] Significant difference between young and adult age classes.
[d] Significant difference between females and males.

Table 2. Extended.

5–9 yr old			≥10 yr old			Age class comparison		
Mean ± SD	Min–Max	*n*	Mean ± SD	Min–Max	*n*	<5 vs ≥10	<5 vs 5–9	5–9 vs ≥10
162 ± 84	61–345	17	152 ± 57	69–264	15	NS	NS	NS
177 ± 96	76–345	11	156 ± 64	69–264	10	NS	NS	NS
134 ± 51	61–194	6	143 ± 42	98–187	5	NS	NS	NS
NS			NS					
9 ± 6	3–24	17	12 ± 10	3–35	15	NS	NS	NS
6 ± 3[d]	3–12	11	9 ± 10[d]	3–35	10	NS	NS	NS
13 ± 7[d]	3–24	6	16 ± 10[d]	3–28	5	NS	NS	NS
<0.013[d]			<0.04[d]					
0.8 ± 0.1	0.6–1.0	17	1.0 ± 0.2	0.5–1.4	15	NS	NS	NS
0.9 ± 0.1	0.7–1.0	11	1.1 ± 0.3	0.5–1.4	10	NS	NS	NS
0.8 ± 0.1	0.6–1.0	6	0.9 ± 0.1	0.7–1.0	5	NS	NS	NS
NS			NS					
143 ± 3	137–148	17	142 ± 3	136–148	15	NS	NS	NS
142 ± 2	137–145	11	143 ± 3	138–148	10	NS	NS	NS
144 ± 3	140–148	6	142 ± 4	136–146	5	NS	NS	NS
NS			NS					
3.6 ± 0.5[c]	2.5–4.3	17	3.5 ± 0.4[a]	2.9–4.3	15	0.0124[a]	0.0408[c]	NS
3.6 ± 0.6	2.5–4.3	11	3.5 ± 0.3	2.9–4.0	10	NS	NS	NS
3.6 ± 0.4[c]	2.9–4.0	6	3.4 ± 0.5[a]	2.9–4.3	5	0.0132[a]	0.0303[c]	NS
NS			NS					
105 ± 5	98–114	17	104 ± 4	97–112	15	NS	NS	NS
104 ± 5	98–110	11	104 ± 4	98–111	10	NS	NS	NS
108 ± 5	102–114	6	102 ± 6	97–112	5	NS	NS	NS
NS			NS					
0.94 ± 0.05[c]	0.87–1.04	17	0.93 ± 0.04	0.84–1.02	15	NS	0.0350[c]	NS
0.95 ± 0.06	0.87–1.04	11	0.94 ± 0.04	0.87–1.02	10	NS	NS	NS
0.93 ± 0.03	0.89–0.96	6	0.91 ± 0.05	0.84–0.96	5	NS	NS	NS
NS			NS					
23 ± 5	15–31	17	26 ± 6	17–37	15	NS	NS	NS
24 ± 5	15–31	11	25 ± 6	18–37	10	NS	NS	NS
22 ± 5	16–28	6	26 ± 6	17–31	5	NS	NS	NS
NS			NS					
19 ± 3	10–24	17	18 ± 2	13–20	15	NS	NS	NS
20 ± 2	17–24	11	18 ± 2	13–20	10	NS	NS	NS
18 ± 4	10–22	6	18 ± 2	16–20	5	NS	NS	NS
NS			NS					
6.9 ± 0.2[c]	6.6–7.2	18	6.8 ± 0.5[a]	6.0–8.0	18	0.0082[a]	0.0543[c]	NS
6.9 ± 0.2[c]	6.6–7.2	12	6.8 ± 0.5[a]	6.0–7.4	13	0.0032[a]	0.0095[c]	NS
7.0 ± 0.2	6.7–7.2	6	6.7 ± 0.8	6.2–8.0	5	NS	NS	NS
NS			NS					

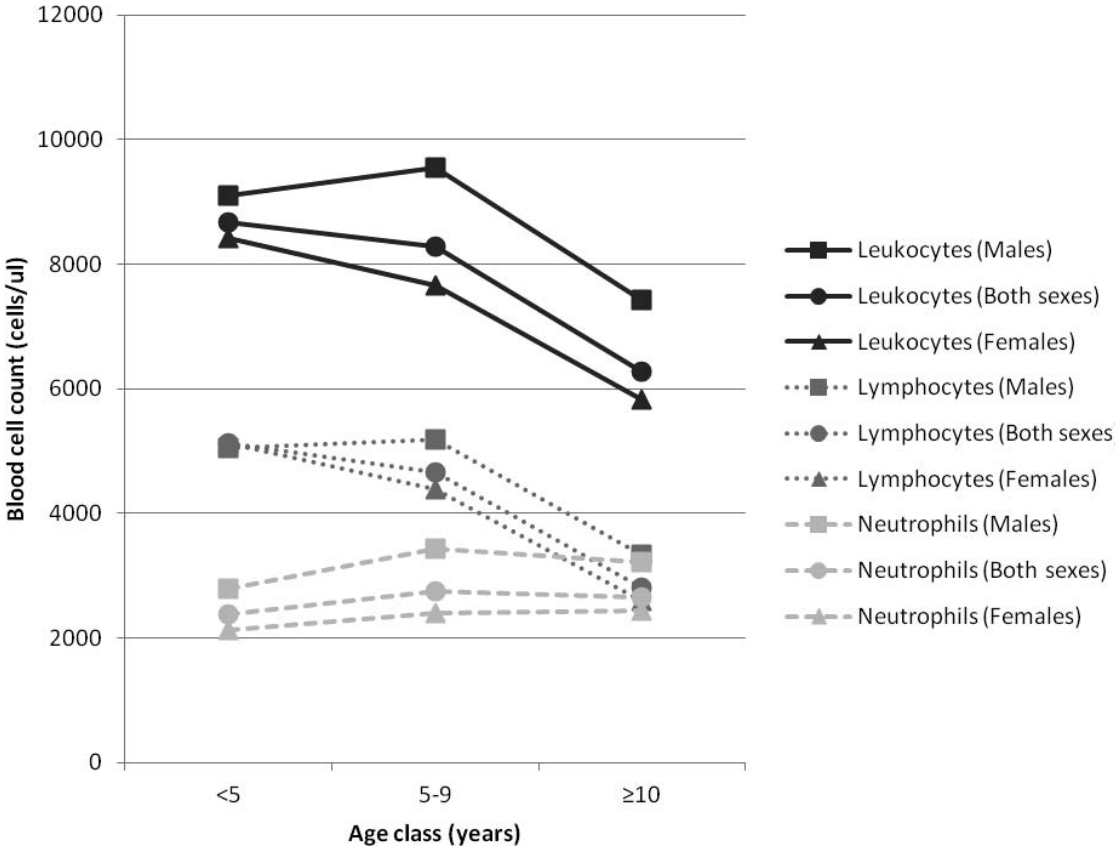

Figure 1. Mean total leukocyte count, neutrophil count, and lymphocyte count changes across age classes and between sexes.

NLR in old lemurs is a significant drop in lymphocytes in old lemurs, consistent with immunosenescence. Immunosenescence refers to attenuation of immune function associated with aging, characterized by changes in lymphocyte development and function.[34] Although declining production of B lymphocytes and reduced functional competence of memory B cells are apparent with aging, the primary driver of immunosenescence is decreased production and function of T lymphocytes.[34,35]

T lymphocytes differentiate and mature in the thymus, which begins to involute at puberty and continues to atrophy with advancing age.[35] This age-associated thymic atrophy results in decreased production of naïve T lymphocytes. Additionally, lymphocyte function decreases and the CD4-to-CD8 T lymphocyte ratio inverts (to <1), a hallmark of immunosenescence.[18,63]

The NLR can be an indicator of inflammatory and/or immune status. Increased NLR can be caused by either decreased lymphocyte count, increased neutrophil count, or both. Individuals with increased NLR may have less physiological reserve to survive inflammatory insults and thus increased risk of mortality.[46] The NLR increases with age and has been used to predict prognosis and survival in humans with various disorders.[32,46]

Stress-induced endogenous steroid release produces a stress leukogram, characterized by moderate leukocytosis with mature neutrophilia, lymphopenia, and elevated NLR.[31] External stressors such as transportation, exercise, and restraint have been shown to affect the NLR in various domestic and wild animals, with glucocorticoid release causing lymphocyte redistribution from blood to other body compartments.[43] Although old lemurs may suffer higher levels of physiological and/or nutritional stress, the leukogram dynamics in this population do not support this idea. Old lemurs have a significantly higher NLR than younger age classes, but this rise in NLR was driven by lymphopenia alone. Also, the lymphocyte decline in old lemurs was not accom-

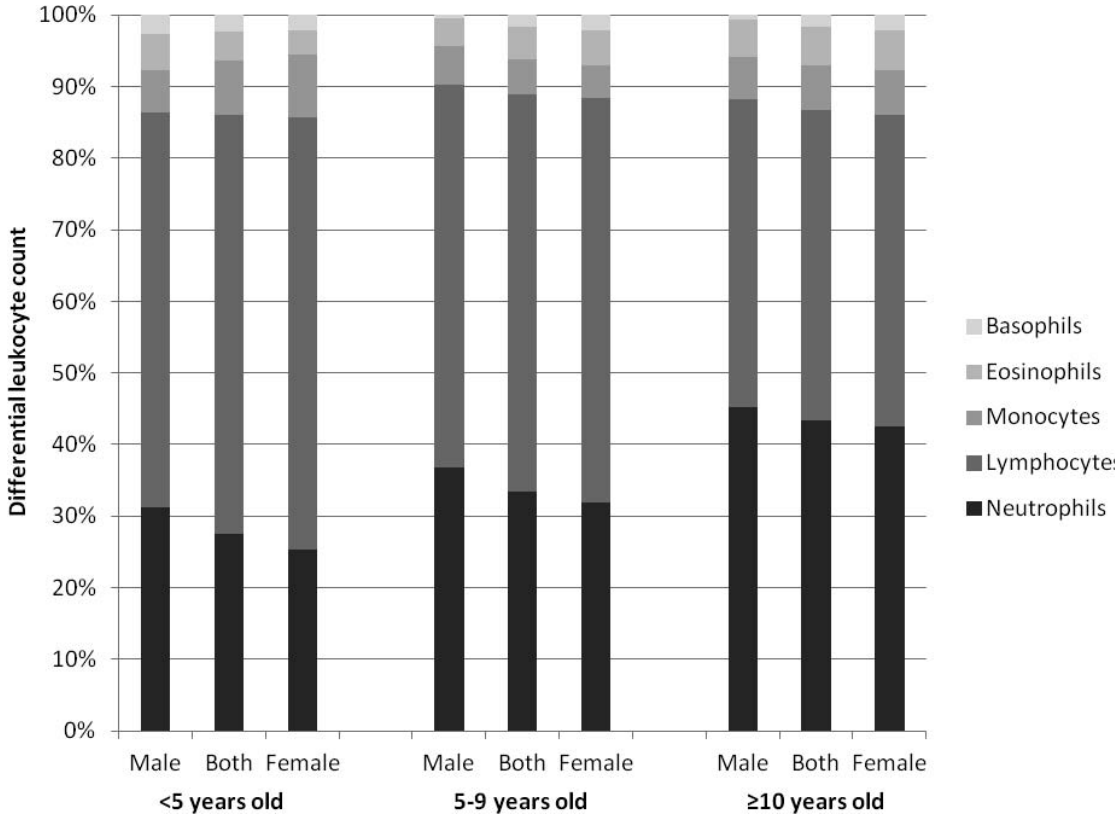

Figure 2. Differential leukocyte count across age classes and between sexes.

panied by increased neutrophil or leukocyte counts (Fig. 1), as seen with stress leukograms. In contrast, adult Verreaux's sifaka (*Propithecus verreauxi*) had higher NLR than immature sifaka, because of both increased neutrophils and decreased lymphocytes. This difference was thought to suggest that adult animals were more stressed during captures, rather than to be indicative of infection.[43]

These results compare to semicaptive free-ranging ring-tailed lemurs, with a similar trend of decreasing lymphocytes with increasing neutrophils and increasing NLR seen with aging through 12 yr. However, lemurs ≥13 yr of age showed an increased lymphocyte count compared to younger age classes (Norton, unpubl. data).

Though not specifically analyzed by authors, a review of published neutrophil and lymphocyte counts reveals a pattern of increased NLR in adults compared to juveniles in other strepsirrhine species.[14,21,43] In the current study, the most significant declines in leukocyte and lymphocyte counts occurred between the adult and old age classes (Fig. 1). This level of age-specific detail is lost in studies that group all adults into a single age class.

Monocytes

Monocytes participate in virtually all inflammatory and immune reactions within the body. Monocytosis can occur in response to chronic inflammatory conditions, including bacterial or viral infections, gastrointestinal disorders, autoimmune diseases, neoplasia, or pregnancy.[4,61]

Males had significantly higher monocyte count but similar percentage monocytes compared to females (Table 1). This contrasts with other studies of ring-tailed lemurs where females had higher percentage and/or number of monocytes than males (Norton, unpubl. data).[38,55]

Young lemurs had significantly higher monocyte counts than adult and old lemurs (Table 1, Fig. 3). Young females had the highest monocyte count, with significant decline between the young and adult age classes. In women, monocyte counts decrease when estrogen levels are high, compared to men and postmenopausal women.[60] Monocyte differences in the current study may reflect

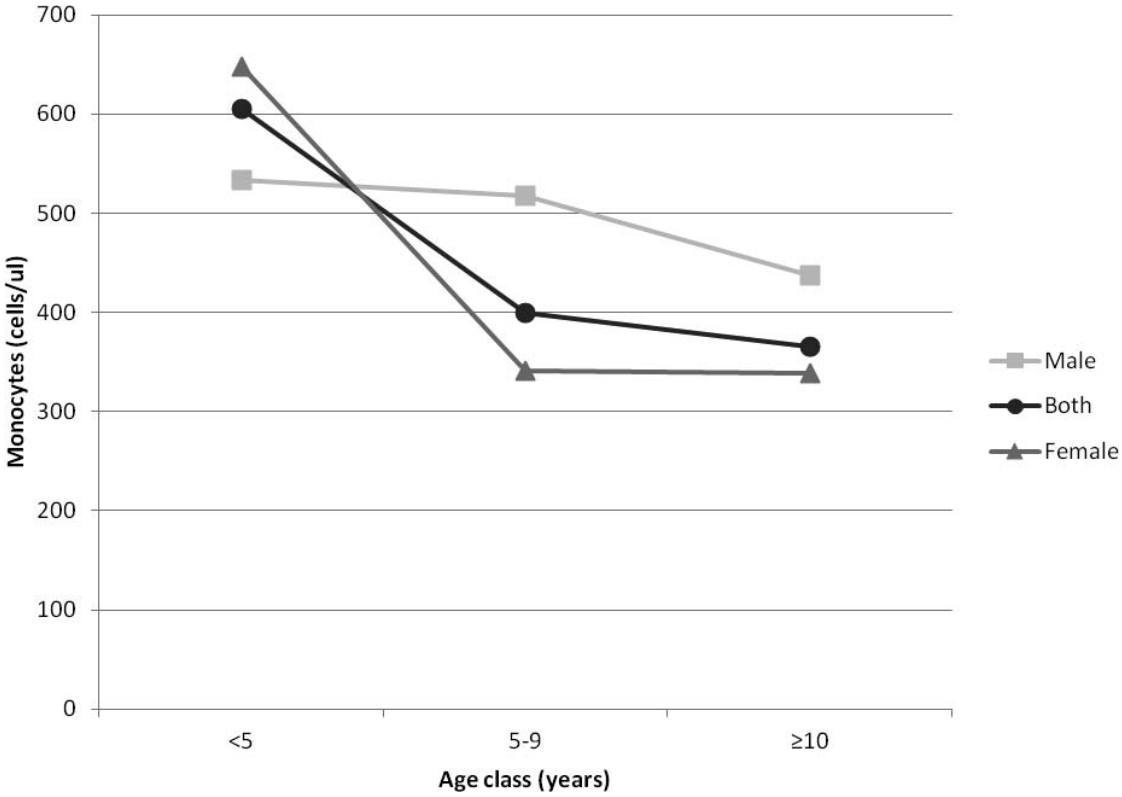

Figure 3. Mean monocyte count across age classes and between sexes.

differences in sex hormone levels in sexually mature females.

Basophils

Basophils participate in hypersensitivity reactions, hemostasis, lipid metabolism, parasite response, and neoplastic diseases.[31] Males had significantly lower percentage basophils than females (Table 1). However, young males had the highest basophil count, with a distinct drop between the young and adult age classes such that adult and old male lemurs had a significantly lower number of basophils than adult and old female lemurs (Table 1, Fig. 4). Many hormones, including thyroid hormone, corticosterones, sex steroids, and insulin, can depress the number of circulating basophils.[41]

Plasma TP

Plasma TP levels in this ring-tailed lemur population were associated with age and sex. Young lemurs had significantly higher TP than adult and old lemurs, because of the fact that young female lemurs had higher TP than all other age–sex classes (Table 1, Fig. 5). Age- and sex-related differences in TP are seen variably in other primate species. In contrast to the present study, other studies report TP lower in juveniles than in adults or no difference between ages (Norton, unpubl. data).[14,21,27,36,42–44,59] In a previous study of ring-tailed lemurs at BMSR[38] and in a semicaptive free-ranging colony (Norton, unpubl. data), females had higher TP than males (all ages combined). Similarly, female red ruffed lemurs (*Varecia rubra*) (adults) and female Hamadryas baboons (*Papio hamadryas*) (age 2–8 yr) had higher TP than males.[10,19] However, many primate species show no sex difference in TP.[14,22,27,33,39,43,44]

Increases in plasma TP can occur with a decrease in plasma water and relative increase in plasma proteins, as occurs with dehydration.[11] Hydration differences were not appreciated during examination, and there were no differences in other biomarkers of hydration (PCV, Cr, Na, Cl) in this study group.

Absolute increases in plasma proteins can occur with a variety of conditions. Increased albumin can result from increased protein intake.[58] Fibrinogen, α-globulins, and some β-glob-

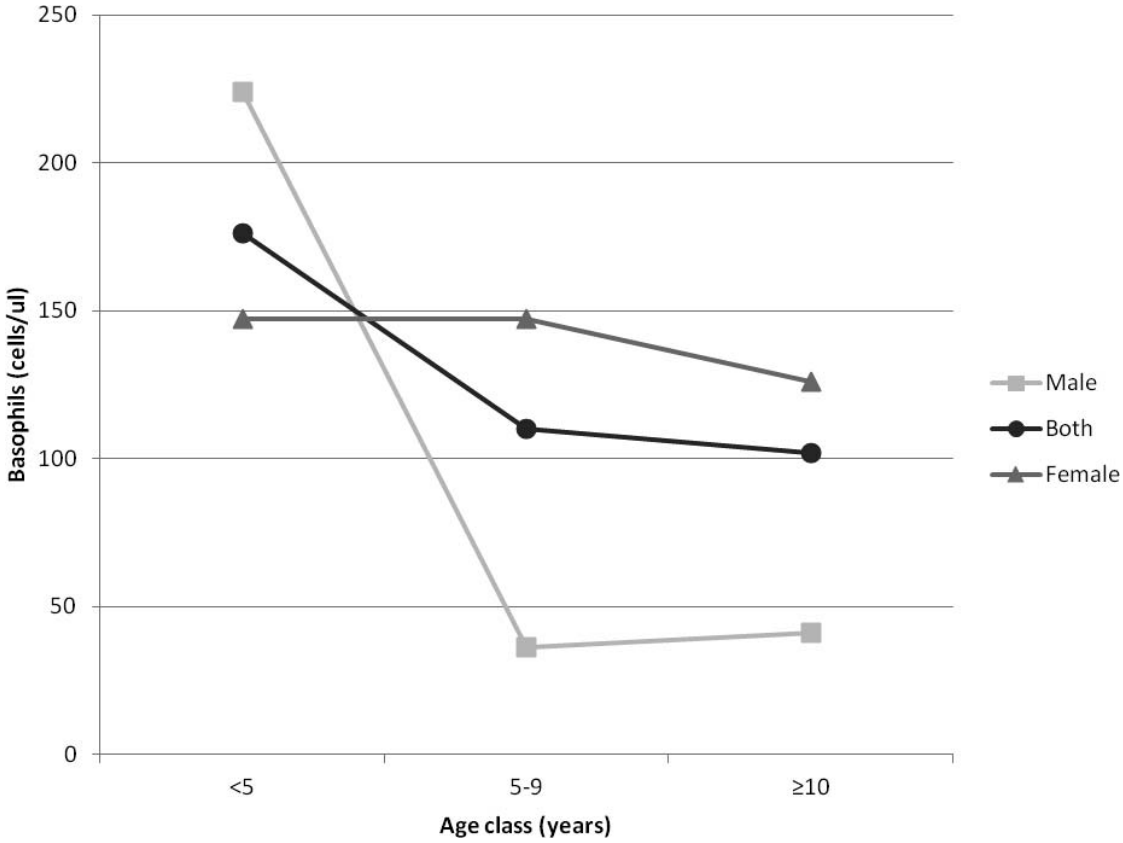

Figure 4. Mean basophil count across age classes and between sexes.

ulins can increase with infection, inflammation, neoplasia, and near-term pregnancy.[11] Gamma globulins can increase with inflammation and persistent antigenic stimulation.[11,62] Causes of artifactual increases in TP via refractometer include lipemia, hemolysis, hyperbilirubinemia, severe hyperglycemia, azotemia, hypernatremia, and hyperchloremia.[11,62] None of these were detected in study samples.

Although determination of protein fractions was not possible for this study, several other strepsirrhine studies have measured serum albumin and globulins. Four previous studies of lemurs documented that TP increases were due to increases in albumin, with globulin levels remaining relatively stable, resulting in an increase in the albumin-to-globulin ratio (AGR) with increasing age.[9,10,25,38] In contrast, semicaptive free-ranging ring-tailed lemurs show an increase in TP with age until 12 yr, characterized by steadily rising globulins leading to a steady decline in AGR (Norton, unpubl. data).

Increased TP and albumin in captive lemurs compared to wild lemurs has been attributed to higher nutritional planes in captivity.[9,10,25,38] A study in black-and-white ruffed lemurs confirmed that captive diets have higher crude protein levels than wild diets.[8] The TP and albumin increases in captive lemurs were accompanied by increases in BUN, as could be expected when a higher-protein diet is consumed.[9,10,25,38] However, wild female ring-tailed lemurs had higher TP and albumin than males but no difference in BUN.[38] Differences in protein intake in the wild may be small enough to increase albumin but not BUN.

Potassium

K levels were similar in male and female lemurs. Similarly, many other primate studies show no sex difference in K in adults.[14,22,27,33,36,38,43,44] In contrast, male Hamadryas baboons and Tonkean macaques (*Macaca tonkeana*) had significantly higher K than females.[19,59]

Young lemurs had significantly higher K than adult and old lemurs, with young males having significantly higher K than young females and all other age–sex classes (Table 2). Similarly, captive juvenile rhesus macaques (*Macaca mulatta*) had

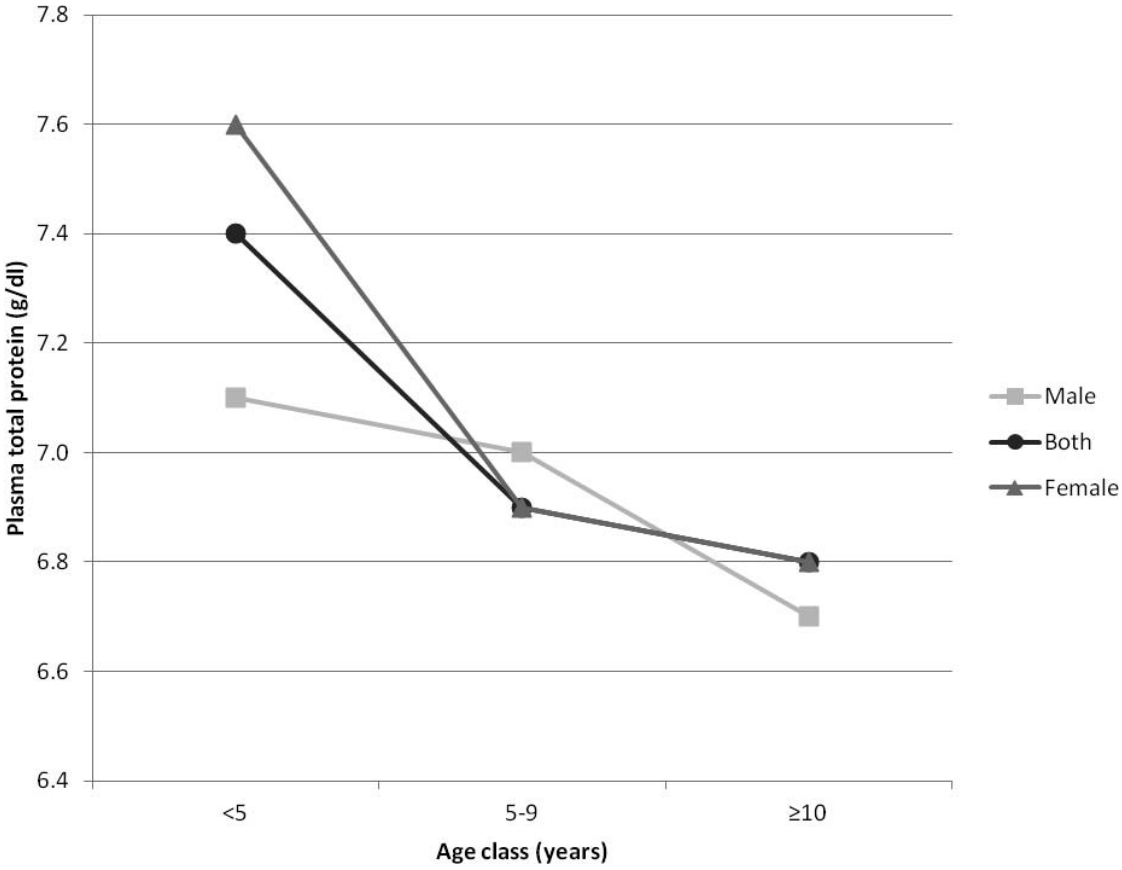

Figure 5. Mean plasma total protein across age classes and between sexes.

significantly higher K than adults,[45] and semi-captive free-ranging ring-tailed lemurs <1 yr had higher K than all older age classes (Norton, unpubl. data). However, studies in other strepsirrhine species have reported no age-related differences in K.[14,21,43]

The biological significance of these K differences is minimal because all values are within reported intervals for healthy wild and captive ring-tailed lemurs.[9,38] Common causes for increased plasma K include sample hemolysis, delayed separation of plasma from cells, acute exercise, muscle trauma, and decreased urinary K excretion.[7] In this study, there was no evidence of renal disease or severe sample hemolysis. Venipuncture difficulties, time to individual sample processing, and degree of hemolysis were not recorded. Variations in capture events and post-darting exercise were difficult to quantify.

Ionized calcium

Young lemurs had significantly lower iCa than adults, with no significant sex differences (Table 2). This contrasts with other studies in primates wherein total calcium levels were higher in younger/juvenile animals than in older animals (Norton, unpubl. data)[14,21,44,45,59] or the same as in older animals.[43]

Bone growth, calcium and vitamin D intake, and sunlight exposure can influence blood calcium levels. Vitamin D levels have been measured in several wild strepsirrhine species.[9,10,21,22,25–27,38,43] More in-depth evaluation of the calcium status of lemurs would require quantification of calcium and vitamin D intake as well as serum calcium, vitamin D, and parathyroid hormone testing.

Ionized calcium can be affected by several preanalytical factors, including sample pH changes, calcium binding by anticoagulant, sample dilution by anticoagulant solution, sample collection, and sample storage.[3] In this study, blood samples were not collected and handled anaerobically, possibly resulting in loss of CO_2, which increases pH and falsely decreases iCa. Larger heparin tubes were inconsistently filled to capacity, possibly resulting in calcium binding by excess

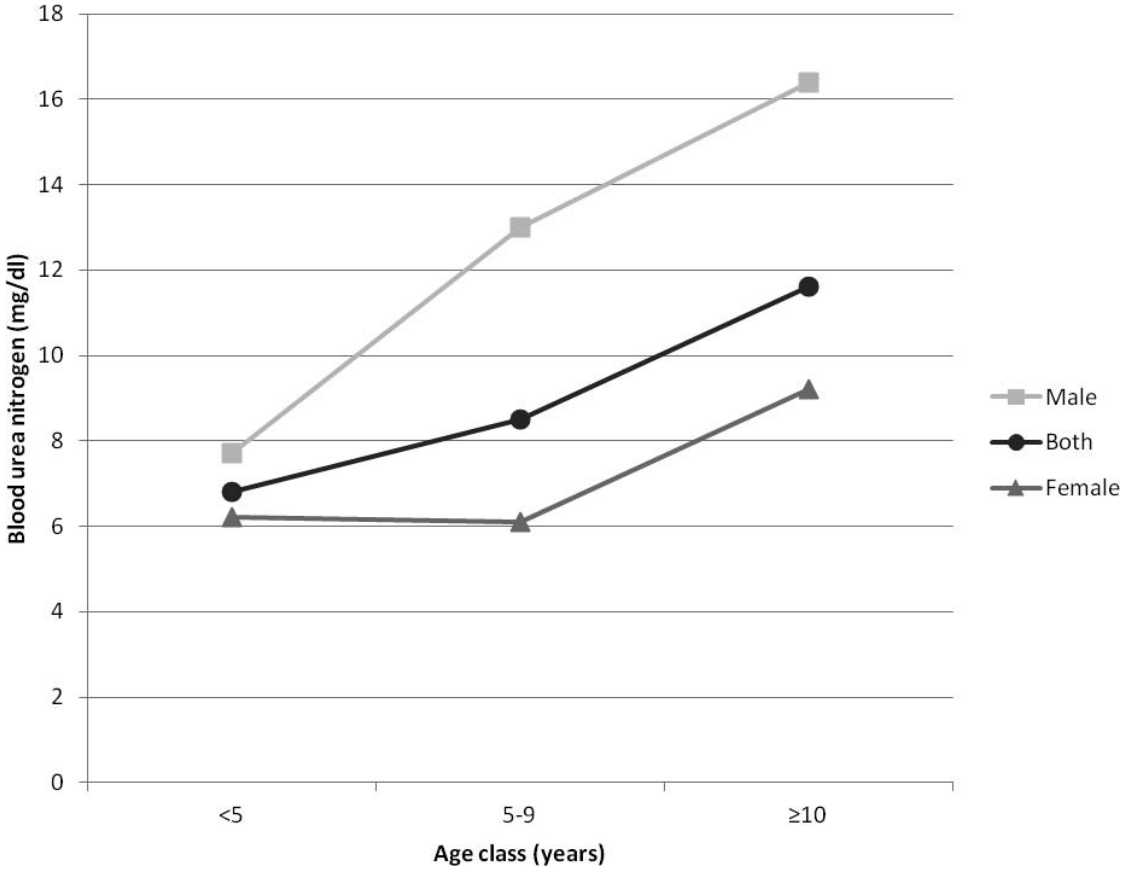

Figure 6. Mean blood urea nitrogen across age classes and between sexes.

heparin. Whole blood samples were stored at ambient temperature for 2–8 hr before analysis. Glycolysis in cells in whole blood can produce lactic acid, which decreases pH and falsely increases iCa. However, sample collection and storage were similar among the age classes. In fact, the four lemurs with lowest iCa values were captured late in each capture session; thus, their sample storage times were in the short end of the range. In the future, preanalytical variables can be eliminated by measuring iCa in whole, unheparinized blood at the time of sample collection.

Blood urea nitrogen

Males had significantly higher BUN than females, and BUN increased nonsignificantly with age in males. In the adult and old age classes, males had significantly higher BUN than females (Table 2, Fig. 6).

BUN is synthesized by the liver during protein catabolism and is excreted through the kidney. Elevations in BUN can reflect altered renal function, state of hydration, or protein metabolism.[13] In this study, Cr values were stable across ages and sexes (Table 2), and 78% of individual lemurs produced concentrated urine (≥1.012), suggesting normal renal function. Physiologic markers of dehydration can include elevated PCV/HCT, Hb, TP, BUN, Cr, and USG,[15] with BUN often rising more than Cr. The rise in BUN was not accompanied by similar increases in PCV, TP, Cr, or USG, so dehydration does not appear to be the cause for the changing BUN.

Because there is no evidence of renal compromise or dehydration, protein catabolism should be considered as a cause for the higher BUN in males. Protein catabolism results from breakdown of dietary protein, tissue protein turnover, or metabolism of gastrointestinal bleeding. High-protein diets may cause BUN to increase, but Cr is generally not increased unless occult renal disease is present.[17] Therefore, BUN is a potentially useful indicator of dietary protein intake.[13]

Several studies comparing wild and captive lemurs found that captive animals had higher

BUN, which was attributed to captive diets that were likely higher in protein than the diets of wild lemurs.[9,10,23–25] A study in black-and-white ruffed lemurs has confirmed that captive diets are higher in crude protein than diets consumed in the wild.[8] Among adult lemurs at BMSR, males feed more on leaves as compared to pregnant females during the dry season, which is when this study was performed.[47]

Sex- or age-related differences in protein metabolism are another potential cause for higher BUN in adult and old male lemurs. In humans, BUN rises with age, and men have higher BUN than women and children. This may be due to differences in protein metabolism, potentially linked to differences in sex hormones, muscle mass, and/or physical activity.[37]

Glucose

Blood Glu levels were variable and significantly higher in females than in males. Glu levels can be affected by food consumption, catecholamine release, drugs, prolonged sample storage time, and HCT. In this study, there was no association between Glu and morning time of capture, HCT, or estimated sample storage time, indicating that precapture feeding, HCT, and Glu loss through glycolysis do not explain the variation in Glu. Hemograms were inconsistent with stress response, but catecholamine levels were not measured. All lemurs were immobilized and supplemented with the same drugs.[30,38,55] Blood was collected quickly after capture. However, many lemurs required supplementation with an α_2 agonist prior to sample collection. The α_2 agonists inhibit insulin release and induce glucagon release from the pancreas, resulting in blood Glu elevation. Lemurs that had blood collected before supplementation with an α_2 agonist had significantly lower mean Glu (95 $\pm$ 31 mg/dl; $n =$ 12) than lemurs that received a single supplement (180 $\pm$ 78 mg/dl; $n = 18$; $P = 0.0075$) or two supplements (227 $\pm$ 75 mg/dl; $n = 5$; $P = 0.0033$).

PCV vs HCT

In this study and a previous study, mean PCV values obtained by centrifugation were approximately 7% higher than mean HCT values obtained using the point-of-care analyzer (Table 1).[55] Error in PCV determination is generally minimal. Falsely elevated PCV can occur if erythrocyte packing during centrifugation is incomplete, which can happen with PCV >50%, insufficient centrifugation, or overfilled microhematocrit tubes.[61] None of these variables were apparent in this study. The point-of-care analyzer uses conductivity to measure HCT based on human erythrocyte measurements; Hb is calculated from HCT. Plasma conducts electrical current, and blood cells act as insulators. Sample conductivity is affected by PCV, mean cell volume (MCV), sample temperature, plasma electrolyte concentration, and other nonconductive elements such as proteins, lipids, and leukocytes.[1] Species with lower MCV may have higher electrical conductivity, which is inversely related to HCT. Ring-tailed lemur MCV (59–72 fl) is lower than human MCV (80–95 fl),[64] theoretically leading to higher conductivity and lower HCT and Hb.

CONCLUSIONS

Age- and sex-related changes in hematology and biochemistry values were present in the BMSR ring-tailed lemur population. Old lemurs showed significant decline in leukocyte and lymphocyte count and resultant elevation of NLR. Age–sex interactions were present in monocyte and basophil counts, TP, K, and BUN. Young females had the highest monocyte count and TP, which dropped as they became adults. Basophil count was stable in females across age but declined precipitously in males in the adult and old age classes. Young males had the highest K of any age–sex group. Overall, males had higher BUN than females, with BUN increasing with age in males. Unexpectedly, iCa was lowest in the young age class. Glu levels were significantly higher in lemurs after α_2 agonist administration. PCV measured by microcentrifugation was uniformly higher than HCT as measured by the point-of-care analyzer.

Identifying age-related changes in blood values in apparently healthy wild ring-tailed lemurs will aid in clinical diagnosis and treatment of lemurs in human care, which is especially relevant for management of geriatric zoo populations. Additionally, better understanding of the ability of aging lemurs to tolerate environmental stressors will inform the capacity for the species to cope under ongoing and future habitat alteration. This is especially relevant given that new data on historical populations of wild ring-tailed lemurs indicate a precipitous drop in numbers.[16,29] Thus, these new health and biomedical data are vital for conservation efforts for this species.

Acknowledgments: Funding was provided by the National Science Foundation (BCS 0922475), Riverbanks Zoo Conservation Support

Fund, the University of Colorado, Primate Conservation Inc., the International Primatological Society, the American Society of Primatologists, and the St. Louis Zoo (FRC 06-1). Abaxis Animal Health provided the i-STAT® point-of-care analyzer. Many people contributed to the success of this project. Aimee Norris, L.V.T., provided capture and laboratory support. The ecological monitoring staff at the Bezà Mahafaly Special Reserve, especially Enafa, assisted with animal captures. Dr. Dave Miller, D.V.M., established the veterinary protocols for this project over 15 yr ago. Dr. Randy Junge, D.V.M., had the foresight to develop the Prosimian Biomedical Survey Program, which inspired decades-long work on lemur health and biology.

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Accepted for publication 22 October 2017