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HEMATOLOGIC AND PLASMA BIOCHEMICAL REFERENCE VALUES FOR THREE FLYING FOX SPECIES (*PTEROPUS* SP.)

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Abstract: Reference hematologic and plasma biochemical values from island (*Pteropus hypomelanus*), Malaysian (*P. vampyrus*), and Rodriquez Island (*P. rodricensis*) flying foxes were determined. In comparison to other mammals, these bats had very low plasma cholesterol and urea levels, which may be related to diet. The predominant white blood cells observed in *P. hypomelanus* and *P. vampyrus* were lymphocytes, while in *P. rodricensis* they were neutrophils. Elevated plasma levels of calcium, phosphorus, and alkaline phosphatase observed in the juvenile *P. hypomelanus* were expected, given the greater osteogenic activity of growing animals. In *P. hypomelanus,* bilirubin levels were higher in juveniles than in adults, and cholesterol levels were higher in females than in males.

Key words: Hematology, Megachiroptera, *Pteropus sp.,* flying fox, bat.

INTRODUCTION

The mammalian order Chiroptera is subdivided into the Microchiroptera and Megachiroptera. Megachiroptera comprises 42 genera and 166 species confined to a single family, the Pteropodidae.[6] This family of fruit- and nectar-feeding bats is found in the tropical and subtropical regions of the Old World, east to Australia and the Caroline and Cook islands.[8] Most bat blood studies have focused on Microchiropterans.[9] Consequently, there is limited published research examining flying fox hematology,[7,10–12] and there are very few reports of reference values[7,10] that can be used for captive animal health evaluation.

MATERIALS AND METHODS

Animals

Blood samples were collected from Malaysian (*Pteropus vampyrus, n* = 13), island (*P. hypomelanus, n* = 67) and Rodriguez Island (*P. rodricensis, n* = 16) flying foxes as part of a preventive health care program at the Lubee Foundation, a private research and breeding facility in north central Florida. The animals included in the study were assessed as healthy on the basis of physical examination. All bats were housed in indoor/outdoor flight enclosures; were fed a mixture of fruits, vegetables, commercial primate chow, and a vitamin supplement; were exposed to a natural photoperiod; and received water *ad libitum.* All animals were either captive-born or imported from their countries of origin at least 2 years prior to the study.

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Blood collection and processing

To facilitate physical examination and blood collection, each bat was anesthetized with isoflurane (Aerrane, Anaquest, Madison, Wisconsin, USA) (5% decreased to 2.5%) in oxygen (2 L/min) supplied through a mask attached to a nonrebreathing system. All blood samples were collected within 3 min of physical capture and between 9 and 11 AM to minimize any effects of restraint and circadian rhythms, respectively. A 3-ml heparinized (sodium heparin 1,000 IU/ml, Elkins-Sinn, Inc., Cherry Hill, New Jersey 08003, USA) syringe and 25-gauge needle were used to collect blood from either the brachial vein or artery on the medial surface of the humerus. After collection, the needle was removed, and 0.5 ml of blood was transferred to a lithium heparin tube (Microtainer, Becton Dickinson and Co., Rutherford, New Jersey, USA) and transported to a commercial clinical pathology laboratory (LabCorp, Gainesville, Florida, USA) for hematologic analysis, using a manual technique for the white cell differential and an automated counter (Abbott Cell-Dyn 3500) for the white blood cell count, red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and platelet count. An additional 1.0 ml of blood was centrifuged at 3,000 rpm for 5 min, and the plasma was transferred to a 1.0-ml cryotube placed in crushed ice and transported to the same commercial laboratory for biochemical analysis using an automated analyzer (Olympus AU5200): glucose, sodium, potassium, chloride, carbon dioxide, blood urea nitrogen, creatinine, phosphorus, total protein, albumin, alkaline phosphatase, aspartate transaminase, alanine transaminase, total cholesterol, bilirubin, BUN/creatinine ratio, glob-

ulin, albumin/globulin ratio, gamma-glutamyl transferase, ion gap, and ionized calcium.

RESULTS AND DISCUSSION

Plasma biochemical and hematological reference values for each species are presented in Tables 1 and 2, respectively. Plasma, rather than serum, was used for biochemical analysis because serum had been observed previously by the authors to be associated with false values such as hyperkalemia, hyponatremia, hypochloremia, and hypoglycemia. Although sodium heparin was used for an anticoagulant, we assumed it would not affect sodium values because of the small volume used. Plasma calcium, phosphorus, sodium, potassium, chloride, total protein, albumin, globulin, and creatinine were within reported reference ranges from a comprehensive review of biochemical values for primates, carnivores, pinnipeds, proboscids, perissodactyls, and artiodactyls.[2] However, urea levels were low, especially when compared to carnivores and pinnipeds.[2] This is probably a reflection of the low protein content of the flying fox diet, urea being the primary nitrogen end-product of protein metabolism. The cholesterol levels for all three bat species were very low compared to values reported for other mammals.[5] These values were even lower than those observed in swine fed a low-fat diet.[5] Plasma cholesterol levels reflect a balance between dietary intake and synthesis, primarily in the liver.[5] The diet of these bats is low in cholesterol and may account for the low plasma levels.

Glucose levels were higher than those previously reported for *P. hypomelanus* (94 ± 11 mg/dl) and *P. vampyrus* (88 ± 9 mg/dl).[11] The reason for this is unknown, but it may reflect the differences in restraint technique, collection times, and sample handling. The glucose levels were higher than those reported in domestic monogastric mammals.[5] In captive flying foxes, glucose levels have been shown to vary significantly during a 24-hr period, with the lowest levels just before feeding in late afternoon.[11] In this study, all blood collections were performed at the same time, in the morning, to avoid this effect.

The hematocrits were higher than those reported in wild-caught Indian flying foxes (*P. giganteous*) (37 ± 6%),[7] similar to grey-headed flying foxes (*P. poliocephalus*) (47 ± 0.7%),[12] and lower than wild and captive Egyptian fruit bats (*Rousettus aegyptiacus*) (44 ± 2 to 58 ± 4%).[10] Hemoglobin concentrations paralleled hematocrits and were greater than those reported in *P. giganteous* (13.4 ± 2.2 g/dl),[7] but less than *P. poliocephalus* (17.9 ± 1.3 g/dl)[12] and *R. aegyptiacus* (14.4 ± 1.4 to 17.4 ± 2.0 g/dl).[10] The predominant white blood cell observed in *P. hypomelanus* and *P. vampyrus* was the lymphocyte, as compared to the neutropil in in *P. rodricensis.*

Plasma biochemical and hematological reference values for juvenile (≤1 yr old) and adult (>1 yr old) *P. hypomelanus* are presented in Tables 1 and 2, respectively. The higher absolute lymphocyte numbers observed in juvenile *P. hypomelanus* are a common finding in juvenile mammals of many species.[4] The elevated levels of plasma calcium, ionized calcium, phosphorus, and alkaline phosphatase in the juvenile bats are also expected, given the greater osteogenic activity in growing animals.[5] The average alkaline phosphatase levels of juveniles were almost twice levels in adults. Bilirubin levels were also higher in juveniles than in adults. Plasma biochemical and hematological reference values for male and female, and pregnant and nonpregnant female *P. hypomelanus* are presented in Tables 3 and 4, respectively. Plasma cholesterol levels in females were generally higher than in males.

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Table 1. Plasma biochemical reference values for captive adult Malaysian (*Pteropus vampyrus*), adult Rodriguez Island (*P. rodricensis*), and adult (>1 yr old) and juvenile (≤1 yr old) Island (*P. hypomelanus*) flying foxes. Mean ± 1 standard deviation (minimum–maximum).

Biochemical variable	Malaysian adults (n = 13)	Island: Adults (n = 43)	Island: Juveniles (n = 26)	Rodriguez Island adults (n = 16)
Glucose mg/dl	152 ± 22 (114–192)	154 ± 36 (84–244)	148 ± 32 (96–184)	117 ± 33 (80–195)
Sodium mEq/L	136 ± 2 (133–138)	140 ± 2 (136–145)	143 ± 4 (136–151)	139 ± 2 (134–141)
Potassium mEq/L	3.9 ± 0.4 (3.2–4.9)	3.3 ± 0.4 (2.8–4.2)	3.6 ± 0.3 (3.1–4.4)	4.3 ± 0.6 (3.5–5.9)
Chloride mEq/L	100 ± 3 (93–105)	106 ± 2 (100–112)	105 ± 1 (102–107)	108 ± 2 (104–112)
Calcium mg/dl	8.9 ± 0.4 (8.2–9.9)	8.3 ± 0.5 (7.7–9.0)	9.0 ± 0.5 (8.1–10.5)	8.8 ± 1.2 (5.8–10.9)
Phosphorus mg/dl	3.6 ± 1.5 (1.9–7.1)	4.5 ± 2.2 (1.7–9.4)	7.3 ± 1.9 (4.4–11.3)	5.5 ± 2.8 (2.5–10.3)
Ion gap	23 ± 3 (19–32)	18.8 ± 2.8 (15–24)	22.5 ± 4.1 (15–32)	18 ± 2 (14–23)
Carbon dioxide content mEq/L	13 ± 4 (7–21)	15 ± 3 (11–20)	15 ± 3 (11–21)	14 ± 2 (9–17)
Urea nitrogen (BUN) mg/dl	13 ± 25 (2–27)	5 ± 4 (1–20)	4 ± 2 (1–10)	12 ± 5 (7–18)
Creatinine mg/dl	1.0 ± 0.6 (0.7–1.9)	0.6 ± 0.1 (0.2–1.0)	0.6 ± 0.1 (0.3–0.8)	0.8 ± 0.2 (0.5–1.1)
Protein g/dl	7.3 ± 0.3 (6.6–8.0)	7.4 ± 0.7 (5.8–9.0)	6.9 ± 0.4 (6.5–7.8)	6.9 ± 0.4 (6.1–7.6)
Albumin g/dl	4.3 ± 0.3 (3.8–4.7)	4.2 ± 0.3 (3.6–5.2)	4.3 ± 0.2 (3.7–4.7)	3.2 ± 0.3 (2.8–3.8)
Globulin g/dl	3.0 ± 0.3 (2.4–3.4)	3.2 ± 0.5 (2.1–4.1)	2.7 ± 0.3 (2.2–3.0)	3.6 ± 0.3 (3.2–4.2)
Albumin/globulin ratio	1.5 ± 0.2 (1.1–1.9)	1.3 ± 0.2 (0.9–1.8)	1.6 ± 0.2 (1.1–2.0)	0.9 ± 0.1 (0.7–1.0)
Alkaline phosphatase U/L	615 ± 311 (261–1,467)	1,023 ± 810 (430–2,410)	2,238 ± 820 (806–4,940)	2,077 ± 2,538 (596–10,400)
Aspartate aminotransferase U/L	32 ± 11 (19–40)	42 ± 19 (18–88)	39 ± 27 (23–53)	66 ± 21 (36–119)
Alanine aminotransferase U/L	13 ± 13 (3–21)	10 ± 6 (2–21)	10 ± 9 (1–17)	11 ± 4 (5–20)
Gamma-glutamyltransferase U/L	18 ± 10 (6–32)	3 ± 3 (0–14)	5 ± 2 (2–11)	12.5 ± 4.7 (7–22)
Cholesterol mg/dl	30 ± 14 (11–57)	17 ± 10 (1–37)	17 ± 11 (1–42)	33 ± 40 (2–152)
Bilirubin mg/dl	0.07 ± 0.05 (0.00–0.10)	0.5 ± 0.6 (0.0–3.0)	1.5 ± 1.5 (0.3–3.8)	0.06 ± 0.11 (0.00–0.40)

Table 2. Hematological reference values for captive Malaysian (*Pteropus vampyrus*), island (*P. hypomelanus*) and Rodriguez Island (*P. rodricensis*) flying foxes. Mean ± standard deviation (minimum–maximum).

Hematological variable	Malaysian adults (n = 13)		Island: Adults (n = 43)		Island: Juveniles (n = 26)		Rodriguez Island adults (n = 16)	
Hematocrit %	44 ± 2	(42–47)	46 ± 9	(28–66)	49 ± 8	(38–69)	43 ± 5	(35–51)
Erythrocyte count 10^6/mm^3	8.88 ± 0.59	(7.4–9.8)	8.70 ± 1.32	(7.1–11.4)	9.56 ± 1.27	(6.6–10.6)	7.95 ± 0.75	(6.7–9.3)
Hemoglobin g/dl	14.6 ± 0.9	(12.9–15.7)	15.4 ± 2.8	(11.2–22.6)	15.4 ± 1.9	(11.2–19.7)	14.2 ± 1.6	(11.1–16.2)
MCV fl	49.1 ± 2.8	(43.4–53.5)	50.5 ± 3.9	(42.3–57.0)	51.5 ± 3.5	(42.5–56.6)	54.4 ± 2.6	(50.0–59.0)
MCH pg	16.4 ± 1.0	(14.5–18.0)	17.2 ± 2.7	(14.5–19.6)	16.2 ± 1.5	(14.3–20.1)	17.8 ± 0.9	(16.6–19.7)
MCHC g/dl	33.5 ± 1.6	(30.0–35.5)	32.8 ± 2.9	(25.0–38.6)	31.0 ± 4.3	(26.8–36.3)	32.7 ± 1.0	(31.3–34.5)
WBC 1,000/mm^3	12.55 ± 3.24	(7.8–18.6)	11.51 ± 4.36	(4.1–19.2)	16.30 ± 5.89	(8.1–22.0)	6.46 ± 1.71	(3.5–9.7)
Monocytes %	0.7 ± 0.7	(0.0–2.0)	2.0 ± 4.2	(0–8)	1.6 ± 5.4	(0–9)	0.8 ± 0.6	(0–2)
Monocytes/mm^3	97 ± 111	(0–372)	238 ± 105	(0–366)	235 ± 157	(0–351)	57 ± 46	(0–160)
Lymphocytes %	64.0 ± 11.8	(43–88)	64 ± 16	(33–86)	76 ± 20	(45–94)	19.1 ± 6.3	(7–29)
Lymphocytes/mm^3	8,023 ± 2,608	(4,840–13,200)	7,557 ± 3,929	(1,472–16,320)	12,839 ± 6,531	(5,192–16,700)	1,184 ± 395	(531–1,856)
Segmented neutrophils %	34.8 ± 12.1	(11–56)	32 ± 14	(9–74)	21 ± 16	(6–51)	79.6 ± 6.0	(70–92)
Segmented neutrophils/mm^3	4,369 ± 1,782	(858–7,616)	3,581 ± 1,623	(779–6,150)	3,107 ± 2,044	(702–6,380)	5,184 ± 1,578	(2,555–8,372)
Eosinophils %	0.5 ± 0.5	(0)	1.0 ± 1.6	(0–4)	1.9 ± 5.4	(0–3)	0.2 ± 0.4	(0–1)
Eosinophils/mm^3	65 ± 63	(0)	121 ± 210	(0–800)	238 ± 649	(0–414)	11 ± 23	(0–60)
Banded neutrophils %	0.0 ± 0.0	(0–1)	0.05 ± 0.21	(0–1)	0.12 ± 0.32	(0–1)	0.3 ± 0.5	(0–1)
Banded neutrophils/mm^3	0.0 ± 0.0	(0–164)	8.3 ± 37.3	(0–192)	20.4 ± 57.2	(0–220)	21 ± 32	(0–82)
Basophils %	0.0 ± 0.0	(0)	0.0 ± 0.0	(0.0–0.2)	0.0 ± 0.0	(0)	0.0 ± 0.0	(0)
Basophils/mm^3	0 ± 0	(0)	2 ± 5	(0–24)	0.0 ± 0.2	(0)	0 ± 0	(0)

Table 3. Comparison of the plasma biochemical values of males and females (pooled) and of nonpregnant and pregnant female flying foxes (*Pteropus hypomelanus*). Mean ± 1 standard deviation (minimum–maximum).

Biochemical variable	Males (n = 23)	Females (n = 46)	Nonpregnant females (n = 20)	Pregnant females (n = 11)
Glucose mg/dl	143 ± 30 (92–204)	156 ± 36 (82–244)	149 ± 40 (82–244)	170 ± 16 (142–200)
Sodium mEq/L	142 ± 3 (137–148)	141 ± 4 (136–151)	140 ± 3 (136–151)	139 ± 2 (136–142)
Potassium mEq/L	3.5 ± 0.4 (2.8–4.2)	3.4 ± 0.4 (2.8–4.4)	3.2 ± 0.3 (2.8–4.4)	3.4 ± 0.3 (2.8–4.2)
Chloride mEq/L	105 ± 2 (102–109)	106 ± 2 (100–112)	106 ± 2 (100–112)	106 ± 3 (101–110)
Calcium mg/dl	8.7 ± 0.5 (7.7–9.6)	8.4 ± 0.6 (7.4–10.5)	8.3 ± 0.5 (7.4–10.5)	8.0 ± 0.4 (6.9–8.5)
Phosphorus mg/dl	6.4 ± 2.2 (3.4–11.3)	5.2 ± 2.5 (1.7–10.3)	4.7 ± 1.9 (2.1–10.3)	3.2 ± 1.4 (1.7–5.6)
Ion gap	20 ± 2 (17–23)	20 ± 4 (15–32)	19 ± 3 (15–32)	18 ± 3 (14–23)
Carbon dioxide content mEq/L	17 ± 2 (13–21)	14 ± 3 (8–20)	15 ± 2 (11–20)	14 ± 3 (12–19)
Urea nitrogen (BUN) mg/dl	5 ± 4 (1–20)	4 ± 3 (1–18)	4 ± 4 (1–18)	3 ± 2 (2–6)
Creatinine mg/dl	0.6 ± 0.1 (0.5–0.8)	0.6 ± 0.1 (0.2–1.0)	0.6 ± 0.2 (0.2–1.0)	0.6 ± 0.05 (0.5–0.6)
Protein g/dl	7.1 ± 0.6 (6.1–8.6)	7.3 ± 0.6 (6.5–8.3)	7.5 ± 0.6 (6.5–9.0)	7.3 ± 0.6 (5.8–7.8)
Albumin g/dl	4.3 ± 0.4 (3.6–5.2)	4.2 ± 0.3 (3.7–4.7)	4.2 ± 0.3 (3.7–4.8)	4.1 ± 0.3 (3.7–4.5)
Globulin g/dl	2.9 ± 0.4 (2.4–3.6)	3.1 ± 0.5 (2.1–4.2)	3.3 ± 0.5 (2.2–4.2)	3.2 ± 0.4 (2.1–3.7)
Albumin/globulin ratio	1.5 ± 0.2 (1.0–1.9)	1.4 ± 0.2 (0.9–2.0)	1.3 ± 0.2 (0.9–2.0)	1.3 ± 0.2 (1.0–1.8)
Alkaline phosphatase U/L	1,558 ± 1,186 (438–3,680)	1,434 ± 755 (429–4,940)	1,158 ± 540 (430–4,940)	757 ± 169 (500–1,035)
Aspartate aminotransferase U/L	36 ± 11 (22–65)	44 ± 26 (19–106)	50 ± 23 (18–88)	32 ± 6 (24–43)
Alanine aminotransferase U/L	10 ± 4 (4–19)	10 ± 8 (1–21)	12 ± 8 (1–21)	8 ± 4 (2–16)
Gamma-glutamyltransferase U/L	3 ± 2 (0–11)	4 ± 3 (0–14)	4 ± 3 (0–14)	3 ± 3 (1–11)
Cholesterol mg/dl	7 ± 9 (1–18)	22 ± 9 (9–46)	23 ± 9 (10–46)	15 ± 5 (9–27)
Bilirubin mg/dl	0.52 ± 0.38 (0.0–1.6)	1.06 ± 1.33 (0.1–2.7)	0.6 ± 0.6 (0.1–3.8)	0.6 ± 0.7 (0.1–2.7)

Table 4. Comparison of the hematological values of males and females (pooled) and of nonpregnant and pregnant female island flying foxes (*Pteropus hypomelanus*). Mean ± 1 standard deviation (minimum–maximum).

Hematological variable	Males (n = 23)	Females (n = 46)	Nonpregnant females (n = 20)	Pregnant females (n = 11)
Hematocrit %	45 ± 6 (34–51)	48 ± 9 (30–62)	47 ± 10 (28–69)	43 ± 7 (42–56)
Erythrocyte count 10^6/mm^3	8.66 ± 0.88 (7.6–10.5)	9.20 ± 1.52 (7.1–11.6)	8.95 ± 1.60 (8.1–11.6)	8.33 ± 1.25 (7.1–11.4)
Hemoglobin g/dl	15.3 ± 2.5 (11.4–17.8)	15.5 ± 2.5 (11.2–19.9)	15.9 ± 3.0 (13.1–19.7)	14.5 ± 2.2 (11.2–18.3)
MCV fl	51.4 ± 3.2 (42.5–56.0)	50.6 ± 4.0 (42.3–56.6)	49.1 ± 3.3 (44.0–56.6)	51.6 ± 4.7 (42.3–57.0)
MCH pg	17.3 ± 1.5 (14.3–20.1)	16.6 ± 2.7 (14.5–19.6)	16.7 ± 3.7 (13.9–19.2)	18.0 ± 1.4 (14.5–19.6)
MCHC g/dl	33.7 ± 1.6 (29.9 ± 36.4)	31.4 ± 4.0 (25.0–38.6)	32.9 ± 3.6 (25.0–38.6)	32.1 ± 2.5 (27.1–34.4)
WBC 1,000/mm^3	13.92 ± 4.19 (7.2–19.2)	13.06 ± 5.99 (3.4–22.0)	11.26 ± 4.00 (4.1–22.0)	11.45 ± 4.00 (3.4–19.2)
Monocytes %	1.8 ± 4.7 (0–7)	1.9 ± 4.7 (0–8)	2.1 ± 3.6 (0–8)	0.7 ± 1.0 (0–3)
Monocytes/mm^3	216 ± 546 (0–313)	247 ± 578 (0–966)	255 ± 455 (0–867)	86 ± 114 (0–366)
Lymphocytes %	69.2 ± 13.8 (45–90)	69.0 ± 19.8 (45–94)	65.3 ± 18.0 (33–94)	66.8 ± 11.1 (49–86)
Lymphocytes/mm^3	10,021 ± 4,103 (3,296–16,700)	9,365 ± 6,224 (1,472–17,600)	7,341 ± 3,506 (1,472–17,600)	8,019 ± 3,664 (2,040–16,320)
Segmented neutrophils %	27.8 ± 11.9 (9–52)	28.2 ± 17.2 (8–62)	31.1 ± 16.3 (6–74)	31.9 ± 11.6 (12–50)
Segmented neutrophils/mm^3	3,533 ± 1,454 (1,260–6,150)	3,337 ± 1,950 (780–6,380)	3,477 ± 1,704 (702–7,006)	3,271 ± 1,442 (1,360–5,888)
Eosinophils %	1.0 ± 2.2 (0–4)	1.4 ± 4.1 (0–4)	1.4 ± 2.0 (0–4)	0.4 ± 0.5 (0–1)
Eosinophils/mm^3	133 ± 251 (0–480)	181 ± 503 (0–452)	180 ± 269 (0–800)	51 ± 59 (0–161)
Banded neutrophils %	0.1 ± 0.3 (0–1)	0.1 ± 0.2 (0–1)	0.0 ± 0.3 (0–1)	0.1 ± 0.0 (0–1)
Banded neutrophils/mm^3	14 ± 44 (0–163)	12 ± 47 (0–220)	7.80 ± 52.51 (0–220)	17.45 ± 0.00 (0–192)
Basophils %	0.0 ± 0.0 (0.0–0.2)	0.0 ± 0.0 (0.0–0.2)	0.02 ± 0.06 (0.0–0.2)	0.00 ± 0.00 (0)
Basophils/mm^3	1 ± 4 (0–15)	1 ± 4 (0–24)	2.19 ± 6.62 (0–20)	0.00 ± 0.00 (0)

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