

# THE EFFECTS OF SHORT-TERM PHYSICAL RESTRAINT AND ISOFLURANE ANESTHESIA ON HEMATOLOGY AND PLASMA BIOCHEMISTRY IN THE ISLAND FLYING FOX (*PTEROPUS HYPOMELANUS*)

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**Abstract:** The effects of short-term physical restraint and isoflurane anesthesia on hematologic and serum biochemistry parameters were evaluated in 12 island flying foxes (*Pteropus hypomelanus*). Physical restraint was associated with significantly decreased calcium, cholesterol, globulin, albumin, hemoglobin concentrations, red blood cell count, and hematocrit and increased glucose, phosphorus, and potassium concentrations. Isoflurane restraint was associated with significantly decreased calcium, cholesterol, albumin, globulin, and glucose concentrations, aspartate transaminase and alkaline phosphatase activities, white blood cell count, red blood cell count, hemoglobin, hematocrit, and total neutrophil and lymphocyte counts and significantly increased phosphorus and chloride concentrations. For those variables where both restraint forms produced an alteration (red blood cell count, hemoglobin, hematocrit, calcium, phosphorus, glucose), the magnitude of change was always significantly greater for the physically restrained animals. This study indicates that in adult flying foxes isoflurane anesthesia is preferable to physical restraint for collection of blood samples for hematologic and plasma biochemical analysis. Additionally, blood samples should be collected as soon as possible after capture, regardless of restraint technique.

**Key words:** Hematology, flying fox, restraint, anesthesia, *Pteropus hypomelanus*, bat.

## INTRODUCTION

Blood collection in flying foxes requires either physical and/or chemical restraint. Both forms of restraint are reported to alter mammalian hematologic and plasma biochemical values (e.g., in sheep,<sup>1,9</sup> cattle,<sup>4</sup> foxes,<sup>6</sup> horses,<sup>12</sup> and ferrets<sup>8</sup>), but there are no published data for bats. The aim of this study was to determine which restraint method has the least effect on hematologic and plasma biochemical values in bats.

## MATERIALS AND METHODS

### Animals

Twelve (seven male, five female) adult ( $\geq 2$  yr old) island flying foxes (*Pteropus hypomelanus*) were studied. All bats were housed in indoor/outdoor enclosures at the Lube Foundation, a private breeding and research facility in north central Florida. The bats were fed a mixed diet of fruits, vegetables, and a commercial primate chow and were given water ad lib. All bats were initially healthy, with normal physical exams and hematologic values within reference ranges established for the collection. This study was approved by the Institutional Animal Care and Use Committee (IACUC), University of Florida.

### Procedure

The bats were divided into two treatment groups of six animals each; group 1 bats received physical restraint alone, and group 2 bats also received isoflurane. Two weeks later the procedures were repeated with the treatment groups reversed. During capture, care was taken to minimize disturbance of other bats and to ensure that the capture sequence was random. Anesthesia was induced by mask with isoflurane (5%, then 2% for maintenance) and oxygen (2 L/min) administered through a nonrebreathing system. For all treatments, blood was collected as soon as possible after physical capture of the bats in their enclosure. Time interval 1 was defined as the time from capture until first blood sample collection. After time interval 1 blood collection, each bat was restrained for 15 min; then time interval 2 blood was collected.

### Sampling

Approximately 1.5 ml of blood was collected from the median vein on the lateral aspect of the humerus; 0.5 ml was placed into a lithium heparin microcontainer and the remainder was immediately centrifuged at 3,000 rpm for 5 min. The plasma was transferred to a cryotube for transport and storage. All samples were submitted to a commercial laboratory (LabCorp, Birmingham, Alabama 35233, USA) for determination of complete blood count and plasma biochemical analysis using an automated counter (Abbott Cell-Dyn 3500) and biochemical analyzer (Olympus AU5200, Olympus

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**Table 1.** Mean and range of times of initial (time interval 1) and second (time interval 2) blood collections from adult island flying foxes (*Pteropus hypomelanus*) either physically restrained or anesthetized with isoflurane.

Time interval	Physical restraint (sec)		Isoflurane (sec)	
	$\bar{x} \pm SD$	Range	$\bar{x} \pm SD$	Range
1	160 $\pm$ 40 <sup>a</sup>	96–150	171 $\pm$ 57	113–208
2	901 $\pm$ 65	788–926	903 $\pm$ 62	769–982

<sup>a</sup>Significantly different from isoflurane restraint ( $P \leq 0.05$ ).

America, Melville, New York 11747, USA), respectively. The differential white blood cell (WBC) count was performed manually.

### Statistical analysis

Statistical analyses were performed using a commercial statistical computer program (Systat for Windows, Systat, Evanston, Illinois 60201, USA). Time interval 1 and time interval 2 mean response variable values for each treatment were compared using Student's paired *t*-test. Time interval 1 collection times were compared between treatment groups using an independent *t*-test. This method was also used to compare the percentage of change in response variables between time intervals 1 and 2, where the differences were significant for both groups. A *P* value of  $\leq 0.05$  was considered significant.

**Table 2.** Hematologic values obtained from blood samples collected at the beginning (time interval 1) and the end (time interval 2) of either 15 min of physical restraint or 15 min of isoflurane anesthesia of adult island flying foxes (*Pteropus hypomelanus*).

Variable	<i>n</i>	Physical restraint		Isoflurane restraint	
		Time 1	Time 2	Time 1	Time 2
White blood count ( $10^6/\text{mm}^3$ )	10	16.8 $\pm$ 7.6	16.3 $\pm$ 8.4	11.7 $\pm$ 4.4	9.3 $\pm$ 4.0 <sup>a</sup>
Red blood count ( $10^6/\text{mm}^3$ )	11	8,100 $\pm$ 300 <sup>b</sup>	8.4 $\pm$ 0.7 <sup>a</sup>	8.9 $\pm$ 0.4	8.2 $\pm$ 0.4 <sup>a</sup>
Hemoglobin (g/dl)	11	16.8 $\pm$ 1.4 <sup>b</sup>	15.1 $\pm$ 1.3 <sup>a</sup>	15.8 $\pm$ 1.0	14.7 $\pm$ 1.0 <sup>a</sup>
Hematocrit (%)	11	49.2 $\pm$ 3.2	44.0 $\pm$ 3.1 <sup>a</sup>	47.2 $\pm$ 2.7	43.9 $\pm$ 2.4 <sup>a</sup>
Mean corpuscular volume (U <sup>3</sup> )	11	53 $\pm$ 3	53 $\pm$ 3	53 $\pm$ 3	53 $\pm$ 5
Mean corpuscular hemoglobin (UUG)	11	17.9 $\pm$ 1.3	18.0 $\pm$ 1.2	17.8 $\pm$ 0.8	17.9 $\pm$ 1.0
Mean corpuscular hemoglobin concentration (%)	11	34.2 $\pm$ 1.8	34.3 $\pm$ 2.1	33.5 $\pm$ 1.0	33.4 $\pm$ 0.8
Neutrophils (%)	10	30 $\pm$ 15	26 $\pm$ 15	38 $\pm$ 12	38 $\pm$ 12
Neutrophils ( $10^6/\text{mm}^3$ )	10	5.0 $\pm$ 3.5	4.4 $\pm$ 3.9	4.3 $\pm$ 1.7	3.2 $\pm$ 1.0 <sup>a</sup>
Lymphocytes (%)	10	64 $\pm$ 18	61 $\pm$ 25	59 $\pm$ 12	57 $\pm$ 13
Lymphocytes ( $10^6/\text{mm}^3$ )	10	10.3 $\pm$ 5.7	11.0 $\pm$ 5.1	7.2 $\pm$ 3.7	5.7 $\pm$ 3.5 <sup>a</sup>
Monoocytes (%)	10	2 $\pm$ 2	2 $\pm$ 2	1 $\pm$ 1	2 $\pm$ 2
Monoocytes ( $10^6/\text{mm}^3$ )	10	0.3 $\pm$ 0.5	0.3 $\pm$ 0.3	0.1 $\pm$ 0.1	0.2 $\pm$ 0.2
Eosinophils (%)	10	2 $\pm$ 3	3 $\pm$ 4	2 $\pm$ 2	3 $\pm$ 3
Eosinophils ( $10^6/\text{mm}^3$ )	10	0.5 $\pm$ 0.7	0.5 $\pm$ 0.8	0.3 $\pm$ 0.2	0.2 $\pm$ 0.1

<sup>a</sup>Significantly different ( $P < 0.05$ ) from baseline values.

<sup>b</sup>Significantly different ( $P < 0.05$ ) from isoflurane-restraint values.

## RESULTS

Some samples were not processed because of clotting, inadequate blood volume, or laboratory error. If a sample point for a given response variable was missing for an animal, the data from that animal were not included. Thus, the sample size for some response variables was  $< 12$ . Time interval 1 was significantly shorter during physical restraint ( $140 \pm 40$  sec) than during isoflurane ( $171 \pm 57$  sec) treatments because of the additional time required to anesthetize the isoflurane treatment group bats before venipuncture (Table 1). Physical restraint time interval 1 calcium, sodium, potassium, and hemoglobin values were significantly greater and glucose and red blood cell (RBC) count were significantly lower than those values for isoflurane-anesthetized bats.

Physical restraint was associated with significant increases between time interval 1 and time interval 2 in plasma phosphorus, glucose, and potassium concentrations and a significant decrease in calcium, cholesterol, globulin, albumin, RBC count, hemoglobin, and hematocrit (Tables 2–4). Isoflurane restraint was associated with a significant increase in phosphorus and chloride concentrations and a decrease in calcium, cholesterol, albumin, globulin, and glucose concentrations, a decrease in aspartate transaminase (AST) and alkaline phosphatase (ALP) activities and a decrease in WBC, RBC, he-

**Table 3.** Plasma biochemical values obtained from blood samples collected at the beginning (time interval 1) and the end (time interval 2) of either 15 min of physical restraint or 15 min of isoflurane anesthesia of adult island flying foxes (*Pteropus hypomelanus*).

Variable <sup>a</sup>	n	Physical restraint		Isoflurane restraint	
		Time 1	Time 2	Time 1	Time 2
Calcium (mg/dl)	11	10.2 ± 0.4 <sup>b</sup>	8.9 ± 0.4 <sup>c</sup>	9.4 ± 0.4	8.7 ± 0.4 <sup>c</sup>
Phosphorus (mg/dl)	11	6.4 ± 1.3	11.0 ± 1.5 <sup>c</sup>	5.7 ± 1.9	7.1 ± 1.7 <sup>c</sup>
Sodium (mEq/L)	11	145 ± 3 <sup>b</sup>	144 ± 3	143 ± 3	142 ± 2
Potassium (mEq/L)	11	5.0 ± 0.6 <sup>b</sup>	5.5 ± 0.5 <sup>c</sup>	3.8 ± 0.4	4.1 ± 0.3
Chloride (mEq/L)	10	109 ± 3	107 ± 2	109 ± 2	111 ± 2 <sup>c</sup>
Cholesterol (mEq/L)	7	17 ± 11	13 ± 8 <sup>c</sup>	15 ± 9	13 ± 8 <sup>c</sup>
Triglycerides (mg/dl)	8	32 ± 16	31 ± 13	27 ± 11	23 ± 9
AST (SGOT) (IU/L)	9	68 ± 36	63 ± 37	50 ± 13	40 ± 13 <sup>c</sup>
Bilirubin, total (mg/dl)	11	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
GGT (IU/L)	9	2 ± 2	1 ± 1	1 ± 0	1 ± 1
ALT (SGPT) (IU/L)	9	12 ± 6	11 ± 5	17 ± 17	12 ± 7
ALP (U/L)	6	718 ± 158	804 ± 238	892 ± 311	801 ± 034 <sup>c</sup>
Total protein (g/dl)	11	7.9 ± 1.3	6.4 ± 2.2	6.9 ± 1.9	6.6 ± 0.4
Globulin (G) (g/dl)	7	3.7 ± 0.5	3.1 ± 0.3 <sup>c</sup>	3.4 ± 0.3	2.9 ± 0.3 <sup>c</sup>
Albumin (A) (g/dl)	7	4.6 ± 0.4	4.0 ± 0.4 <sup>c</sup>	4.2 ± 0.3	3.7 ± 0.2 <sup>c</sup>
A/G ratio (g/dl)	7	1.2 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.3 ± 0.1
BUN (mg/dl)	11	4 ± 2	4 ± 2	4 ± 1	4.2 ± 1.2
Creatinine (mg/dl)	10	0.5 ± 0.03	0.7 ± 0.0	0.5 ± 0.0	0.5 ± 0.0
Glucose (mg/dl)	10	91 ± 12 <sup>b</sup>	175 ± 56 <sup>c</sup>	124 ± 22	78 ± 35 <sup>c</sup>
Amylase (U/L)	11	837 ± 562	959 ± 362	1,156 ± 1,156	764 ± 390

<sup>a</sup> AST = aspartate aminotransferase; GGT = gamma glutamyltranspeptidase; ALT = alanine aminotransferase; ALP = alkaline phosphatase; BUN = blood urea nitrogen.

<sup>b</sup> Significantly different from isoflurane-restraint time 1 values.

<sup>c</sup> Significantly different ( $P \leq 0.05$ ) from time 1 values.

**Table 4.** Hematologic and plasma biochemical values that differed significantly between the beginning (time interval 1) and end (time interval 2) of 15 min of both physical and isoflurane restraint in island flying foxes (*Pteropus hypomelanus*). Negative and positive values indicate a decrease and increase, respectively.

Variable	n	% increase or decrease	
		Physical restraint	Isoflurane restraint
Red blood cell count <sup>a</sup>	11	-10.5 ± 3.5	-7.3 ± 2.2
Hemoglobin <sup>a</sup>	11	-10.1 ± 2.7	-7.1 ± 2.3
Hematocrit <sup>a</sup>	11	-10.4 ± 4.2	-7.0 ± 1.7
Calcium <sup>a</sup>	11	-12.4 ± 3.0	-7.3 ± 5.0
Phosphorus <sup>a</sup>	11	78.6 ± 43.1	29.6 ± 23.0
Cholesterol	7	-20.6 ± 9.7	-16.9 ± 8.0
Globulin	7	-16.0 ± 4.8	-13.9 ± 4.0
Albumin	7	-13.2 ± 4.8	-11.9 ± 3.0
Glucose <sup>a</sup>	10	93.2 ± 54.6	-37.5 ± 25.0

<sup>a</sup> Significant difference in percent change between physical and isoflurane restraint.

moglobin, hematocrit, and absolute neutrophil and lymphocyte counts (Tables 2, 3). Where both forms of restraint produced changes in a response variable (RBC, hemoglobin, hematocrit, calcium, phosphorus, creatinine, glucose), the magnitude of alteration was always significantly greater for physical restraint (Table 4).

## DISCUSSION

Short-term physical restraint and isoflurane anesthesia were both associated with time-dependent alterations in plasma biochemical and hematologic variables. It was not possible in this study to obtain baseline (unrestrained) values; rather blood samples were collected as soon as possible after capture. Despite this limitation, the physical restraint time interval 1 values for calcium, sodium, potassium, and hemoglobin were higher than those for isoflurane anesthesia probably because of the differences in initial blood collection times between the two groups.

Many of the biochemical value differences between time intervals 1 and 2 and between physical restraint and isoflurane anesthesia may be explained by differences in skeletal muscle activity. Isoflurane

anesthesia, by producing unconsciousness, would be expected to reduce muscle contraction. Muscle contractile energy is derived from the breakdown of ATP and phosphocreatinine.<sup>5</sup> As expected, a by-product of this process, phosphorus, was highest in physically restrained bats at time 2. Similar elevations have been observed following short-term exercise in humans and sled dogs.<sup>7,10</sup> An associated moderate hyperkalemia is postulated to be related to metabolic acidemia.<sup>2,5</sup>

A stress leukogram, a response to physical restraint reported in many mammalian species (e.g., cats, cattle, horses),<sup>3</sup> was not observed with either restraint method. Its absence in these flying foxes may reflect the short restraint period or may indicate a real species difference. The hyperglycemia in physically restrained bats may have been due to increased cortisol secretion. In a study of three flying fox species restrained in small wire cages for 3 hr, cortisol and glucose concentrations were markedly elevated, with a steady increase over the time period in which they were observed.<sup>13</sup>

The decrease in RBC, hemoglobin, hematocrit, and albumin and globulin concentrations in the physically restrained bats is suggestive of splenic sequestration.<sup>3</sup> Decreased PCV has previously been observed after significant stress in flying foxes.<sup>13</sup> Similar decreases in RBC, hemoglobin, hematocrit, WBC, and total neutrophil and lymphocyte counts were observed in isoflurane-anesthetized bats. Isoflurane anesthesia in horses and ferrets also produces splenic vasodilation, with red and white blood cell sequestration.<sup>8,11</sup> These changes occur within 15 min and begin to return to normal by 45 min after induction. In the isoflurane-restrained bat plasma AST and ALP activities were decreased, in contrast to horses where 1 hr of isoflurane anesthesia produces elevations in AST and ALP levels; however, the mechanism for these changes is unknown.<sup>12</sup>

### CONCLUSIONS

Although short-term physical and isoflurane restraint were associated with significant changes in hematologic and plasma biochemical values, physical restraint resulted in greater alteration. Isoflurane anesthesia, therefore, appears preferable for blood collection for hematologic and plasma biochemical analyses in flying foxes. However, observed alterations were time dependent, which emphasizes the importance of collecting samples as

soon as possible after capture regardless of restraint technique.

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