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Plasma and tissue enzyme activities of banded water snakes (*Nerodia fasciata*) and diamondback water snakes (*Nerodia rhombifer*)

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OBJECTIVE
To measure plasma and tissue activities of alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase (AST), creatine kinase, and γ-glutamyltransferase in 2 snake species.

ANIMALS
6 banded water snakes (*Nerodia fasciata*) and 6 diamondback water snakes (*Nerodia rhombifer*).

PROCEDURES
Blood was collected via the ventral tail vein to measure plasma enzyme activities. Animals were then euthanized, and samples of 9 tissues were collected from each snake: skeletal muscle, cardiac muscle, liver, spleen, lung, kidney, testicle, pancreas, and gallbladder. Tissues were frozen for 30 days, then homogenized and processed. Supernatants were collected and analyzed within 24 hours of processing. A linear mixed model was used to determine differences in enzyme activity between tissues and species and assess interactions between tissues and species.

RESULTS
Activities of all enzymes were found to differ significantly among tissues. There were also significant differences between species for all enzyme activities, except AST activity. The kidney had the highest alanine aminotransferase and γ-glutamyltransferase activities. Alkaline phosphatase activity was significantly highest in liver and kidney tissues than in other tissue. Creatine kinase activity was highest in skeletal muscle, followed by cardiac muscle and kidney. AST activity was present in all tissues evaluated, but was highest in liver, kidney, and cardiac muscle in both species.

CLINICAL RELEVANCE
Results reinforced the importance of characterizing the origin of tissue enzymes in reptiles to improve our understanding of biochemistry results and highlighted the differences that can exist in tissue enzyme activities between closely related species.

Snakes are popular household pets, with an estimated 555,000 being kept in the US,[1] and are also commonly maintained in zoological institutions. As snakes become more common in these settings, their caretakers have an expectation that veterinarians will provide the same high-quality care they have come to expect for domestic animals. However, with > 3,000 species of snakes around the world, there remain large gaps in our diagnostic and therapeutic knowledge regarding this group of reptiles. Therefore, it is imperative that we pursue evidence-based research focused on closing this informational gap to ensure best practices for these animals.

Plasma biochemistry testing is commonly performed as a component of baseline diagnostic testing for snakes. Enzyme activities are routinely measured in a plasma biochemistry profile and can aid in evaluating organ function and disease progression.[2] High plasma enzyme activities may be an indication of abnormal cellular leakage or enzyme induction secondary to a disease process, whereas low plasma enzyme activities may indicate decreased tissue or organ function.[3] Identifying the tissue of origin of each enzyme increases the clinical usefulness of plasma biochemistry testing, because the tissue of origin may be highly species specific.[4,5] Without an understanding of tissue specificity, evaluation of plasma enzyme activity is of limited value.[2]

Several studies[6–11] have attempted to characterize plasma enzyme activities of snakes, but most of these studies were conducted prior to 2016 and did not follow the guidelines of the American Society of Veterinary Clinical Pathologists.[12] Regardless, many clinicians still use results of these studies to interpret plasma enzyme activities in snake patients. Unfortunately, our current knowledge of

the tissue origin of enzymes in snakes is limited to a single study[13] involving yellow rat snakes (*Pantherophis alleghaniensis*), and our knowledge of the tissue origin of enzymes in other reptiles and amphibians is limited to 6 additional studies involving loggerhead sea turtles (*Caretta caretta*),[5] Kemp's ridley sea turtles (*Lepidochelys kempii*),[14] American alligators (*Alligator mississippiensis*),[15] green iguanas (*Iguana iguana*),[16] eastern box turtles (*Terrapene carolina*),[4] and Cuban tree frogs (*Osteopilus septentrionalis*).[2] To determine the diagnostic value of plasma enzyme testing in snakes, additional studies are needed that characterize the tissue origins of these enzymes and determine whether tissue activities are associated with plasma activities.

The purpose of the study reported here was to determine the tissues of origin for alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), and γ-glutamyltransferase (GGT) in banded water snakes (*Nerodia fasciata*) and diamondback water snakes (*Nerodia rhombifer*). The hypotheses tested in this study were that CK and AST activities would be highest in skeletal and cardiac muscles; GGT activity would be highest in the kidney, liver, and gallbladder; ALT and ALP would not be tissue specific; and tissue enzyme activities would differ between these 2 closely related species.

Materials and Methods

Animals

The study was designed as a cross-sectional study and was performed in accordance with rules and regulations established by Louisiana State University Institutional Animal Care and Use Committee (protocol No. 18-034). Twelve adult male wild-caught snakes (6 banded water snakes and 6 diamondback water snakes) obtained from a reptile distributor were used in the study. Snakes had originally been obtained for use in a separate reproduction study, and all tissue collection and processing for the present study were opportunistic. The sample size for the study was determined on the basis of the following assumptions: a minimum difference in tissue enzyme activities of 100 U/g between tissues, SD of 50 U/L, power = 0.9, and $\alpha = 0.05$.

Sample collection

Blood was collected with a nonheparinized syringe and 22-gauge needle from the ventral tail vein of each snake prior to euthanasia. Blood samples were immediately placed in evacuated tubes containing lithium heparin (BD microtainer; Becton, Dickinson and Co) and centrifuged at 3,000 X *g* for 5 minutes. Plasma was removed and frozen at –20 °C until tested.

Each snake was weighed and then euthanized with a 2-stage euthanasia protocol. All animals were sedated with alfaxalone (15 mg/kg, SC). Snakes were then given a single injection of pentobarbital (39 mg/kg, IV in the ventral tail vein), decapitated with a No. 10 scalpel blade at the atlantooccipital joint, and pithed.

A full gross necropsy was performed immediately following euthanasia. Samples of skeletal muscle (epaxial muscles), cardiac muscle (combined atria and ventricle), lung, liver, gallbladder (emptied of bile), pancreas, spleen, kidneys, and testicle were collected. Gross connective tissues and blood vessels were removed from the tissue samples. Tissues were separated as 0.5-g samples and frozen at –20 °C for 30 days.

Sample processing

Samples were processed in a fashion similar to that described in other studies.[2,15] Tissue samples were thawed at room temperature and, once thawed, were washed with ice-cold deionized water, minced with a No. 10 scalpel blade, and placed in 5 mL of ice-cold deionized water. Samples were then placed on ice and thoroughly homogenized with a variable-speed homogenizer (VWR 200; VWR International) and 7-mm-diameter generator probe (peripheral speed, 9 m/s) for 30 seconds at the 18,000 to 24,000 rpm setting. Samples were sonicated with a sonicator (Branson 1510; Branson Ultrasonics Corp) for 30 seconds and then centrifuged at 2,000 X *g* for 15 minutes at 4 °C. The supernatant was removed, and the pellet was suspended in 5 mL of ice-cold deionized water. The sample was vortexed for 10 seconds and then homogenized, sonicated, and centrifuged again as described. The second supernatant was removed and added to the previous supernatant. The pellet was once again suspended in 5 mL of ice-cold deionized water, thoroughly mixed, and processed for a third time as described, and the supernatant was collected and added to the pooled supernatant.

Enzyme activity determination

Plasma samples and tissue supernatants were analyzed immediately after processing at the Louisiana State University clinical pathology laboratory with a clinical chemistry analyzer (AU680; Beckman Coulter Inc). Tissue enzyme activities reported by the analyzer as units per liter were multiplied by the total volume of supernatant per gram of tissue (wet weight) to obtain activity as units per gram of tissue for statistical analysis.[2,15] Coefficients of variation for control samples processed at the same time as the study samples were 2.1%, 2.8%, 1.7%, 1.8%, and 1.1%, respectively, for ALP, ALT, AST, CK, and GGT activity.

Statistical analyses

Distributions of the data were evaluated for normality with the Shapiro-Wilk test and by examining skewness, kurtosis, and q-q plots. Body weights were normally distributed and are reported as mean ± SD and range. Plasma and tissue enzyme activities were not normally distributed and are reported as median, interquartile (25th to 75th percentile) range, and

range. Plasma and tissue enzyme activities were log transformed for parametric analysis.

An independent samples *t* test was used to determine whether body weight differed between species. A linear mixed model was used to test for differences in enzyme activity between tissues and species and evaluate the interaction of tissue and species. The random variable in the model was snake; tissue and species were included as fixed variables. The Mauchly test for sphericity was used to assess covariance. If the covariance assumption was violated, the Greenhouse-Geisser method was used to interpret model result. When differences in enzyme activities between tissues were identified, the least-squares method was used to characterize specific differences between tissues. Pearson correlation was used to determine whether plasma and tissue enzyme activities were correlated. For these comparisons, tissue enzyme activities were converted to units per kilogram to make a direct comparison to plasma activities in units per liter. All analyses were performed with commercial software.[17] Values of $P \leq 0.05$ were considered significant.

Results

The banded water snakes weighed significantly ($P = 0.033$) less (mean ± SD, 147.2 ± 38.2 g; range, 88.2 to 206.4 g) than the diamondback water snakes (259.6 ± 105.4 g; 155 to 1,425 g). Activities of all enzymes were found to differ significantly among tissues **(Table 1)**. There were also significant differences between species for all enzyme activities, except for AST; however, there was a significant effect of the interaction between species and tissue for AST activity. Because of the small sample sizes and in agreement with recommendations of the American Society of Veterinary Clinical Pathologists,[12] box-and-whisker plots were created for each enzyme by tissue and species **(Figure 1)**.

Liver and kidney ALP activities were significantly (all $P < 0.001$) higher than spleen, pancreas, skeletal muscle, gallbladder, and lung ALP activities **(Table 2)**. Pancreas ALP activity was significantly higher than spleen ($P < 0.001$), skeletal muscle ($P < 0.001$), gallbladder ($P < 0.001$), lung ($P < 0.001$), and testicle ($P = 0.023$) activities. Cardiac muscle ALP activity was significantly higher than spleen ($P < 0.001$), pancreas ($P = 0.008$), skeletal muscle ($P < 0.001$), gallbladder ($P < 0.001$), and lung ($P < 0.001$) activities. Testicle ALP activity was significantly higher than spleen ($P < 0.001$), pancreas ($P = 0.023$), skeletal muscle ($P < 0.001$), gallbladder ($P < 0.001$), and lung ($P < 0.001$) activities. There were no significant (all $P > 0.10$) differences in ALP activities among liver, kidney, cardiac muscle, or testicle. ALP activities were low and not significantly (all $P > 0.15$) different among lung, spleen, and gallbladder. ALP activities in diamondback water snakes were higher than activities in banded water snakes for all tissues, except skeletal muscle, pancreas, and lung.

Kidney ALT activity was significantly ($P < 0.001$) higher than activities in all other tissues **(Table 3)**. Liver ALT activity was also significantly ($P < 0.001$) higher than activities in all other tissues except kidney and pancreas ($P = 0.199$). Pancreas ALT activity was significantly ($P < 0.001$) higher than activities in all other tissues except kidney and liver. Testicle ALT activity was significantly higher than spleen ($P < 0.001$), heart ($P = 0.037$), skeletal muscle ($P = 0.001$), gallbladder ($P < 0.001$), and lung ($P = 0.001$) activities. There were no significant (all $P = 1.0$) differences in ALT activity among spleen, heart, skeletal muscle, and gallbladder. Banded water snakes had lower ALT activities than did diamondback water snakes, except for cardiac muscle ALT activity.

Liver AST activity was significantly (all $P < 0.001$) higher than spleen, pancreas, skeletal muscle, gallbladder, lung, and testicle activities **(Table 4)**. Kidney AST activity was significantly (all $P < 0.001$) higher than spleen, pancreas, skeletal muscle, gallbladder, lung, and testicle activities. Cardiac muscle AST activity was significantly higher than spleen ($P = 0.002$), pancreas ($P = 0.021$), skeletal muscle ($P = 0.003$), gallbladder ($P = 0.002$), lung ($P = 0.004$), and testicle ($P = 0.003$) activities. Skeletal muscle AST activity was significantly higher than spleen ($P = 0.026$), pancreas ($P < 0.001$), and gallbladder ($P = 0.021$) activities. Lung AST activity was significantly (all $P < 0.001$) higher than spleen, pancreas, and gallbladder activities. There were no significant differences in AST activity between liver and kidney ($P = 0.989$), liver and cardiac muscle ($P = 0.874$), kidney and cardiac muscle ($P = 0.880$), skeletal muscle and lung ($P = 0.352$), skeletal muscle and testicle ($P = 0.567$), gallbladder and testicle ($P = 1.0$), or lung and testicle ($P = 0.073$).

Table 1—Results of linear mixed modeling of tissue enzyme activities in 6 banded water snakes (*Nerodia fasciata*) and 6 diamondback water snakes (*Nerodia rhombifer*).

Enzyme	Variable	AIC	*F* statistic	*P* value
ALP		560.9		
	Species		6.4	0.029
	Tissue		35.9	< 0.001
	Tissue X species		2.0	0.053
ALT		692.9		
	Species		72.1	< 0.001
	Tissue		85.6	< 0.001
	Tissue X species		42.7	< 0.001
AST		821.6		
	Species		0.7	0.408
	Tissue		28.4	< 0.001
	Tissue X species		4.1	< 0.001
CK		1003.2		
	Species		53.1	< 0.001
	Tissue		127.6	< 0.001
	Tissue X species		94.5	< 0.001
GGT		65.7		
	Species		11.6	< 0.001
	Tissue		102.6	< 0.001
	Tissue x species		10.7	< 0.001

AIC = Akaike information criterion. ALP = Alkaline phosphatase. ALT = Alanine aminotransferase. AST = Aspartate aminotransferase. CK = Creatine kinase. GGT = α-Glutamyltransferase.

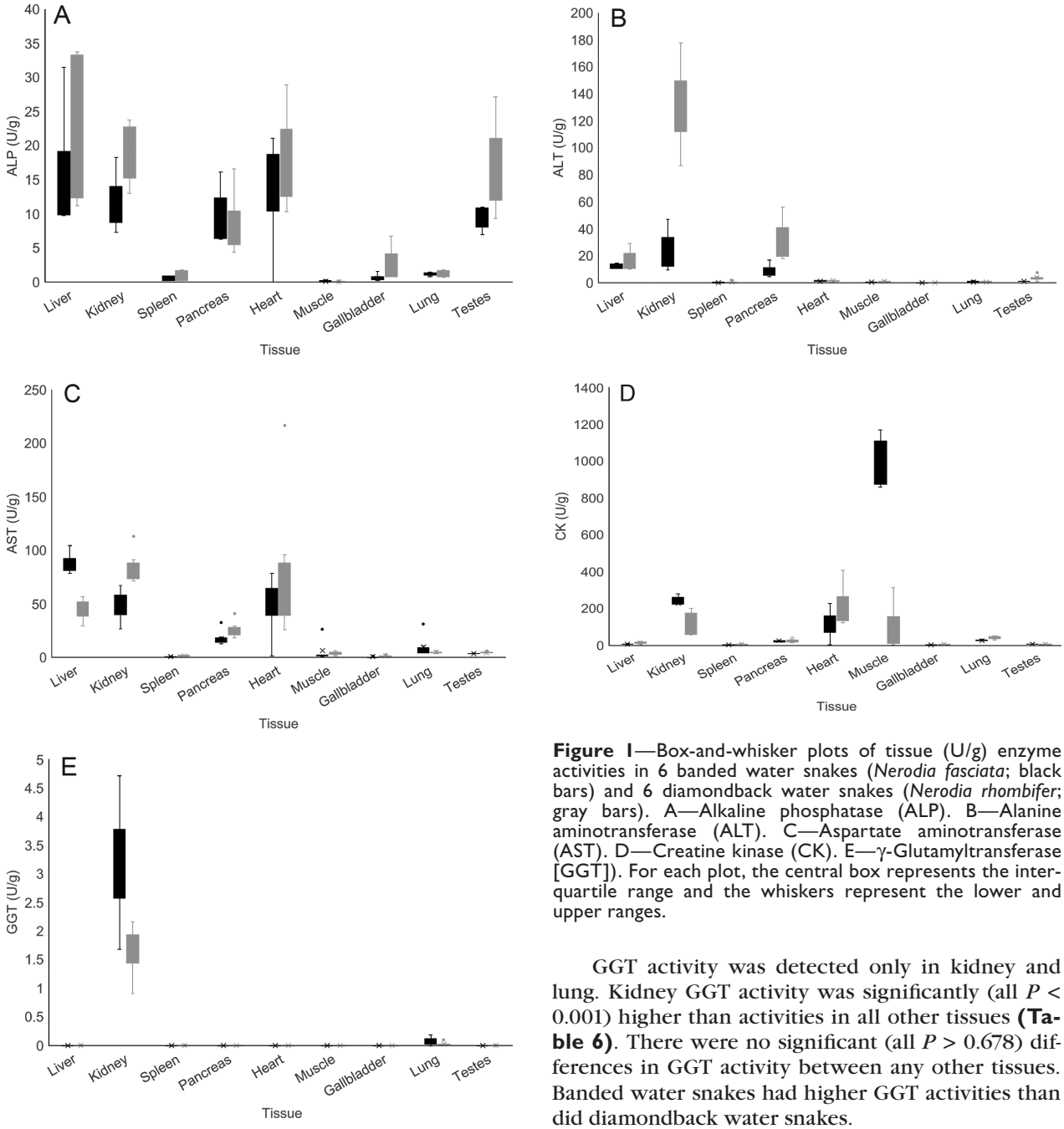

Figure 1—Box-and-whisker plots of tissue (U/g) enzyme activities in 6 banded water snakes (*Nerodia fasciata*; black bars) and 6 diamondback water snakes (*Nerodia rhombifer*; gray bars). A—Alkaline phosphatase (ALP). B—Alanine aminotransferase (ALT). C—Aspartate aminotransferase (AST). D—Creatine kinase (CK). E—γ-Glutamyltransferase [GGT]). For each plot, the central box represents the interquartile range and the whiskers represent the lower and upper ranges.

Skeletal muscle CK activity was significantly (all $P < 0.001$) higher than activities in all other tissues **(Table 5)**. CK activity was also significantly (all $P < 0.001$) higher in cardiac muscle and kidney than in all other tissues except skeletal muscle. There was no significant ($P = 0.763$) difference in CK activity between cardiac muscle and kidney. There were no significant (all $P > 0.242$) differences in CK activity between any other tissues. CK activities in kidney, skeletal muscle, and testicle were higher in banded water snakes than in diamondback water snakes, whereas CK activities in all other tissues were higher in diamondback water snakes than in banded water snakes.

GGT activity was detected only in kidney and lung. Kidney GGT activity was significantly (all $P < 0.001$) higher than activities in all other tissues **(Table 6)**. There were no significant (all $P > 0.678$) differences in GGT activity between any other tissues. Banded water snakes had higher GGT activities than did diamondback water snakes.

Plasma ALP activity was positively correlated with liver ($r = 0.823$; $P < 0.001$), kidney ($r = 0.903$; $P < 0.001$), pancreas ($r = 0.853$; $P = 0.001$), cardiac muscle ($r = 0.860$; $P < 0.001$), gallbladder ($r = 0.483$; $P = 0.017$), lung ($r = 0.910$; $P < 0.001$), and testicular ($r = 0.859$; $P < 0.001$) activities **(Table 7)**. Plasma ALP activity was not significantly correlated with skeletal muscle ($P = 0.872$) activity.

Plasma ALT activity was positively correlated with liver ($r = 0.873$; $P < 0.001$), kidney ($r = 0.681$; $P < 0.001$), pancreas ($r = 0.642$; $P = 0.001$), cardiac muscle ($r = 0.782$; $P = 0.001$), skeletal muscle ($r = 0.703$; $P < 0.001$), lung ($r = 0.660$; $P < 0.001$), and testicular ($r = 0.600$; $P < 0.001$) activities (Table 7). Plasma ALT

Table 2—ALP activity (U/g) in various tissues from 6 banded water snakes and 6 diamondback water snakes.

		ALP (U/g)		
Tissue	**Species**	**Median**	**IQR**	**Range**
Liver	Banded water snake	11.7	9.8–19.1	9.7–31.4
	Diamondback water snake	22.0	12.3–33.2	11.2–33.7
Kidney	Banded water snake	11.6	8.7–14	7.3–18.3
	Diamondback water snake	19.7	13.0–22.7	13.0–23.8
Cardiac muscle	Banded water snake	16.3	10.4–18.7	0–21
	Diamondback water snake	16.4	10.3–22.4	10.3–28.9
Skeletal muscle	Banded water snake	0.2	0–0.2	0–0.3
	Diamondback water snake	0	0–0.1	0–0.2
Gallbladder	Banded water snake	0.4	0.3–0.8	0.2–1.5
	Diamondback water snake	1.4	0.8–4.1	0.8–6.7
Spleen	Banded water snake	0.4	0.2–0.8	0.1–0.9
	Diamondback water snake	0.7	0–1.6	0–1.8
Pancreas	Banded water snake	8.2	6.3–12.3	6.3–16.2
	Diamondback water snake	6.9	4.3–10.4	4.3–16.6
Lung	Banded water snake	1.1	1–1.3	0.8–1.5
	Diamondback water snake	1.1	0.7–1.7	0.7–1.8
Testicle	Banded water snake	9.5	8.0–10.8	7.0–10.9
	Diamondback water snake	16	11.9–21	9.3–27.1

IQR = Interquartile (25th to 75th percentile) range.
See Table 1 for remainder of key.

Table 3—ALT activity (U/g) in various tissues from 6 banded water snakes and 6 diamondback water snakes.

		ALT (U/g)		
Tissue	**Species**	**Median**	**IQR**	**Range**
Liver	Banded water snake	12.2	10.6–14	10.6–14.7
	Diamondback water snake	14.4	10.2–24.7	9.9–29.3
Kidney	Banded water snake	20.6	12.4–33.6	9.5–46.8
	Diamondback water snake	136.1	100.1–159.6	86.9–177.4
Cardiac muscle	Banded water snake	1.3	0.9–1.6	0.1–1.6
	Diamondback water snake	1.2	0.8–1.8	0.5–2.9
Skeletal muscle	Banded water snake	0.4	0.3–0.5	0.3–0.5
	Diamondback water snake	0.7	0.5–1.3	0.2–1.5
Gall bladder	Banded water snake	0	—	0
	Diamondback water snake	0.1	0–0.2	0–0.3
Spleen	Banded water snake	0.1	0–0.3	0–0.4
	Diamondback water snake	0.4	0.1–0.9	0–2.1
Pancreas	Banded water snake	7.1	5.4–11.2	4.3–16.5
	Diamondback water snake	23.7	18.6–48.3	17.8–56
Lung	Banded water snake	0.4	0.3–1.1	0.2–1.5
	Diamondback water snake	0.6	0.3–1	0.3–1.4
Testicle	Banded water snake	0.8	0.7–1	0.7–1
	Diamondback water snake	3.1	2.2–5	0.8–7.6

See Tables 1 and 2 for key.

activity was not significantly correlated with splenic ($P = 0.322$), cardiac muscle ($P = 0.82$), or gallbladder ($P = 0.359$) activities.

Plasma AST activity was positively correlated with liver ($r = 0.873$; $P < 0.001$), kidney ($r = 0.681$; $P < 0.001$), pancreas ($r = 0.664$; $P < 0.001$), cardiac muscle ($r = 0.782$; $P < 0.001$), skeletal muscle ($r = 0.703$; $P < 0.001$), lung ($r = 0.660$; $P < 0.001$), and testicular ($r = 0.600$; $P = 0.002$) activities (Table 7). Plasma AST activity was not significantly correlated with splenic ($P = 0.125$) or gallbladder ($P = 0.359$) activities.

Plasma CK activity was positively correlated with liver ($r = 0.759$; $P < 0.001$), kidney ($r = 0.814$; $P < 0.001$), pancreas ($r = 0.829$; $P < 0.001$), cardiac muscle ($r = 0.756$; $P < 0.001$), skeletal muscle ($r = 0.615$; $P = 0.002$), lung ($r = 0.917$; $P < 0.001$), and testicular ($r = 0.871$; $P < 0.001$) activities (Table 7). Plasma CK activity was not significantly correlated with splenic ($P = 0.119$) or gallbladder ($P = 0.236$) activities (Table 7).

Plasma GGT activity was positively correlated with kidney ($r = 0.845$; $P < 0.001$) and lung ($r = 0.477$; $P = 0.018$) activities but was not significantly correlated with liver ($P = 0.09$), spleen ($P = 0.09$), pancreas ($P = 0.09$), cardiac muscle ($P = 0.09$), skeletal muscle ($P = 0.09$), gallbladder ($P = 0.09$), or lung ($P = 0.42$) activities (Table 7).

Table 4—AST activity (U/g) in various tissues from 6 banded water snakes and 6 diamondback water snakes.

		AST (U/g)		
Tissue	**Species**	**Median**	**IQR**	**Range**
Liver	Banded water snake	83.6	80.3–97.3	78.1–104.1
	Diamondback water snake	47.2	34.7–53.5	29.5–56.8
Kidney	Banded water snake	51.1	34.4–61.1	26.7–68.8
	Diamondback water snake	77.5	72.1–96.3	71.1–113.1
Cardiac muscle	Banded water snake	47.7	28.7–70.5	1.6–78.3
	Diamondback water snake	54.5	34.1–126	25.5–216.4
Skeletal muscle	Banded water snake	1.6	1–13.8	0.8–25.8
	Diamondback water snake	2.9	2.1–5.3	1.2–5.8
Gall bladder	Banded water snake	0.1	0.1–0.3	0.1–0.7
	Diamondback water snake	0.8	0.5–1.8	0.3–2.6
Spleen	Banded water snake	0.6	0.1–0.7	0.1–1
	Diamondback water snake	1.3	0.3–1.9	0.3–2.2
Pancreas	Banded water snake	14.1	13.5–22.4	12.5–32.6
	Diamondback water snake	21.6	19.6–32.3	18.1–40.6
Lung	Banded water snake	4.7	3.8–15.3	3.8–30.9
	Diamondback water snake	4.4	3.5–5.2	3.1–6.4
Testicle	Banded water snake	3.3	3.2–3.7	3.1–3.7
	Diamondback water snake	4.3	3.7–4.9	2.8–5.8

See Tables 1 and 2 for key.

Table 5—CK activity (U/g) in various tissues from 6 banded water snakes and 6 diamondback water snakes.

		CK (U/g)		
Tissue	**Species**	**Median**	**IQR**	**Range**
Liver	Banded water snake	6.1	4.8–7.5	3.8–8.3
	Diamondback water snake	10.9	8.4–16.6	7.4–20
Kidney	Banded water snake	241.5	195.9–267.4	115.6–279
	Diamondback water snake	71.6	58.6–173.1	56.6–200.1
Cardiac muscle	Banded water snake	106.6	50.9–182.2	4.1–227.4
	Diamondback water snake	174.5	133.2–264.5	121–407.2
Skeletal muscle	Banded water snake	959.9	868–1140.4	860.6–1171.2
	Diamondback water snake	60.6	10.6–155.2	0.7–311.8
Gall bladder	Banded water snake	0.6	0.4–1.4	0–2.9
	Diamondback water snake	3.5	2.4–6.1	1.5–12.2
Spleen	Banded water snake	1.5	0–2.3	0–2.7
	Diamondback water snake	3.6	2–7.7	1.8–8.6
Pancreas	Banded water snake	19.2	17.9–32.1	17.1–52.7
	Diamondback water snake	20.7	17.9–29.2	15.1–42.7
Lung	Banded water snake	27.9	22.6–31	15–33
	Diamondback water snake	43	36.7–47.8	29.5–51
Testicle	Banded water snake	4.9	4.6–5.8	4.6–6.9
	Diamondback water snake	4.3	3.6–6	2.7–7.2

See Tables 1 and 2 for key.

Table 6—GGT activity in various tissues from 6 banded water snakes and 6 diamondback water snakes.

		GGT (U/g)		
Tissue	**Species**	**Median**	**IQR**	**Range**
Kidney	Banded water snake	3.5	2.1–4	1.7–4.7
	Diamondback water snake	1.7	1.4–1.9	0.9–2.2
Lung	Banded water snake	0.1	0–0.1	0–0.2
	Diamondback water snake	0	0–0	0–0.1

No GGT activity was detected in any of the other 7 tissues tested.

Discussion

In the present study, we were able to characterize plasma and tissue activities of ALP, ALT, AST, CK, and GGT in 2 species of water snakes. Results supported our current understanding that tissue enzyme activities can vary among species, even closely related species, and reinforced the need to study tissue enzyme activities at a species level. Tissue ALP, ALT, CK, and

Table 7—Plasma (U/L) and tissue (U/g) enzyme activities in 6 banded water snakes and 6 diamondback water snakes.

Enzyme	Sample	Median	IQR	Range
ALP	Plasma	95.5	31.8–143.5	0–162
	Liver	14.2	10.4–29.2	9.7–33.7
	Kidney	14.5	11.3–20.8	7.3–23.8
	Spleen	0.5	0.2–0.9	0–1.8
	Pancreas	7.4	6.3–10.5	4.3–16.6
	Cardiac muscle	16.3	13.4–19.8	0–28.9
	Skeletal muscle	0	0–0.2	0–0.3
	Gall bladder	0.8	0.4–1.7	0.2–6.7
	Lung	1.1	0.8–1.4	0.7–1.8
	Testicle	10.9	9.3–16.1	7–27
ALT	Plasma	36	22–184.5	11–331
	Liver	12.2	10.6–16.8	9.9–29.3
	Kidney	66.8	19.9–136.5	9.5–177.4
	Spleen	0.2	0–0.5	0–2.1
	Pancreas	17.2	7.1–25.2	4.3–56
	Cardiac muscle	1.2	0.9–1.6	0.1–2.9
	Skeletal muscle	0.5	0.4–0.7	0.2–1.5
	Gall bladder	0	0–0.1	0–0.3
	Lung	0.4	0.3–0.9	0.2–1.5
	Testicle	1	0.8–3.3	0.7–7.6
AST	Plasma	188.5	129.7–262	44–1,106
	Liver	67.4	46.2–84.2	29.5–104.1
	Kidney	69	50–78.7	26.7–113
	Spleen	0.6	0.3–1.4	0.1–2.2
	Pancreas	19.6	14–27.6	12.5–40.7
	Cardiac muscle	49.2	37.2–75.7	1.6–216.4
	Skeletal muscle	2.4	1.3–5.2	0.8–25.8
	Gall bladder	0.4	0.1–0.8	0.1–2.6
	Lung	4.4	3.8–6.1	3.1–30.9
	Testicle	3.7	3.2–4.3	2.8–5.8
CK	Plasma	1,817	1,058.2–2,166.2	530–2,216
	Liver	7.9	5.8–11.7	3.8–20
	Kidney	182.1	71.6–246.6	56.6–279
	Spleen	2.1	1.4–3.7	0–8.6
	Pancreas	19.5	18.4–25.1	15.1–52.7
	Cardiac muscle	152.2	89.5–206.6	4.1–407.2
	Skeletal muscle	311.8	49.4–959.9	0.7–1,171.2
	Gall bladder	2.1	0.6–3.5	0–12.2
	Lung	31.7	27.9–44.6	15–51
	Testicle	4.6	4.3–5.5	2.7–7.2
GGT	Plasma	0	0–2.2	0–6
	Kidney	2	1.6–3.5	0.9–4.7
	Lung	0	0–0.1	0–0.2

See Tables 1 and 2 for key.

GGT activities were significantly different between species in this study, but tissue AST activities were not. However, a significant interaction between tissue AST activities and species was found, suggesting that species plays some role in tissue AST activity. In addition, results confirmed our hypotheses that ALT and ALP would not be tissue specific and that tissue enzyme activities would differ between these 2 closely related species. However, results differed from our hypothesis that AST activities would be highest in skeletal and cardiac muscles, in that activities were actually highest in liver, kidney, and cardiac muscle. Additionally, GGT activity was specific for the kidney in both species and was not detected in liver and gallbladder.

In patients, the magnitude of increase in plasma enzyme activity may relate to the severity of tissue damage, with slight damage resulting in values < 2 times the upper reference limit and severe damage resulting in values > 50 times the upper reference limit.[18] However, for enzymes that have a narrow reference interval or short half-life, any increase in plasma enzyme activity may be considered clinically important. Because of the small sample size (n = 6/species) in the present study, we can only report our observed values and were not able to calculate reference intervals. Reviewing tissue activities for the various enzymes in the present study, > 4-fold differences in median activity between species were found only for skeletal muscle ALP activity (banded water snakes,

0.21 U/g; diamondback water snakes, 0.00 U/g), kidney ALT activity (banded water snakes, 20.56 U/g; diamondback water snakes, 136.08 U/g), spleen ALT activity (banded water snakes, 0.10 U/g; diamondback water snakes, 0.45 U/g), gallbladder AST activity (banded water snakes, 0.13 U/g; diamondback water snakes, 0.81 U/g), skeletal muscle CK activity (banded water snakes, 959.88 U/g; diamondback water snakes, 60.6.00 U/g), and gallbladder CK activity (banded water snakes, 0.61 U/g; diamondback water snakes, 3.46 U/g). Although these differences were statistically significant, they did not appear to be biologically relevant, except for the difference in skeletal muscle CK activity. All of the CK activities in the banded water snakes were higher than those measured in the diamondback water snakes. The animals were all obtained from the same source and handled under similar conditions by the investigators; however, it is possible that something differed in their handling prior to our acquisition of the snakes. Increases in activities of this leakage enzyme have also been reported with iatrogenic muscle damage related to venipuncture,[18] and although the same technique was used to collect blood from the ventral tail vein in both species, it is possible that there was more muscle damage during collection of samples from the banded water snakes. CK is also directly related to muscle mass, and in humans, it is generally acknowledged that serum CK activity may be influenced by the amount of lean muscle mass.[19] Serum CK activities in females are typically lower than those in males as a direct result of lower lean body mass[20]; however, the difference in humans is not as large as the difference we found for these 2 species. In addition, the banded water snakes were significantly smaller than the diamondback water snakes and would be expected to have less overall muscle mass. Further study investigating a larger sample size that follows the guidelines recommended by the American Society of Veterinary Clinical Pathologists[12] is needed to determine whether the difference in CK activity between species is clinically relevant.

ALP activities were present in low levels in all of the tissues evaluated in the present study. ALP activity was highest in liver, kidney, cardiac muscle, and testicle, and plasma activity was positively correlated with activities in these tissues. The low levels of activity and general lack of similarity between these tissues suggests that ALP activity may be challenging to interpret. However, it may have value as a secondary enzyme for assessing certain tissues. For example, ALP activity was significantly higher in cardiac muscle compared with skeletal muscle, and plasma ALP activity was not correlated with skeletal muscle ALP activity. Thus, ALP may be used with CK to assess potential sources of muscle damage, with high CK and ALP activities suggesting cardiac muscle injury versus skeletal muscle injury. Studies evaluating ALP activity and histopathologic changes in snakes with cardiac disease are needed to further elucidate the value of this enzyme, as we have limited cardiac biomarkers for snakes. Higher ALP activities were also observed in cardiac tissue, compared with skeletal muscle, in Cuban tree frogs and American alligators.[2,15] The only other species of snake to be evaluated, yellow rat snakes,[13] had the highest ALP activity in the kidney. Eastern box turtles[4] and green iguanas[16] also have higher ALP activity in the kidney. The 2 species of snakes in the present study similarly had higher ALP activity in the kidney; thus, this enzyme may have some value as a marker for renal impairment, although urine, rather than plasma, may be a better sample for measuring ALP activity. Tissue ALP activities in loggerhead and Kemp's ridley sea turtles were highest in the spleen and lung, respectively, whereas ALP activity in these tissues for the water snakes in our study was negligible. In mammals, ALP activity is generally highest in the gastrointestinal tract and bone. Unfortunately, these tissues were not evaluated in the present study. Future studies should include these tissues to further elucidate the roles these tissues play in snakes.

ALT activity was present in all tissues except the gallbladder in the banded water snakes in the present study. The highest activities were found in the kidney, followed by the liver and pancreas, and plasma ALT activity was highly correlated with activities in these tissues. ALT activities in the 2 water snake species were most similar to activities in loggerhead sea turtles,[5] Cuban tree frogs,[2] and Eastern box turtles,[4] for which ALT activity is highest in the kidney and liver. These findings are in contrast to those for yellow rat snakes,[13] American alligators,[15] and green iguanas,[16] for which ALT activity is highest in kidney and muscle but low in other tissues, including liver. On the basis of kidney ALT activities reported for all groups of reptiles tested, this enzyme may be a useful indicator of renal disease in these animals. However, the authors suspect that ALT activity might be best measured in urine versus plasma, because enzyme released from damaged renal cells would more likely be found in the urine and not the blood.[3] Future studies measuring ALT activity in the urine should be performed to assess its role as an indicator of renal disease in reptiles.

AST activity was found to be present in all tissues evaluated in the present study, but was highest in the liver, kidney, and cardiac muscle in both species. There was no significant difference in AST activities between snake species, although the distribution between species varied slightly, with AST activity in diamondback water snakes being highest in the kidney followed by cardiac muscle and liver, whereas in banded water snakes, AST activity was highest in the liver followed by kidney and cardiac muscle. In yellow rat snakes[13] and green iguanas,[16] AST activity was found to be highest in cardiac muscle, with less activity in liver and kidney. Similar to the water snakes, AST activity was highest in liver, kidney, and cardiac muscle in loggerhead sea turtles,[5] with lower activity in skeletal muscle. In other species of reptiles and

a single amphibian species,[2,4,14,15] AST activity was found to be high in skeletal muscle. In many vertebrates, plasma AST activity is commonly used with CK activity to assess skeletal muscle damage.[18,21,22] For the water snakes in the present study, plasma AST activity was positively correlated with both cardiac and skeletal muscle, suggesting these species follow a similar pattern to other described reptiles.

CK activity was significantly higher in banded water snakes than in diamondback water snakes in the present study. In banded water snakes, CK activity was highest in skeletal muscle followed by kidney and cardiac muscle, but in diamondback water snakes, CK activity was highest in cardiac muscle followed by kidney and skeletal muscle. CK is an important enzyme in muscle metabolism, but in the present study, we also found high CK activity in the kidney. In other reptiles and an amphibian, CK was also found to originate from cardiac and skeletal muscles.[4,5,13,14] Additionally, the gastrointestinal tract was found to have CK activity attributable to the presence of smooth muscle.[4,5,13,14] The gastrointestinal tract wasn't evaluated in the present study, but the authors suspect CK activity would have been high in gastrointestinal tract tissues. The high CK activity in the kidney suggests CK could have value in assessing renal disease, and a study to measure urine CK activity in healthy snakes and snakes with renal impairment is warranted.

GGT activity was low in both species in the present study, although banded water snakes did have higher GGT activities, and was found to be specific for the kidney in both species. GGT activity was also found to be high in the kidneys of Cuban tree frogs and eastern box turtles, but negligible in other reptiles.[1,3,5,13–16] Measuring GGT activity in urine may prove valuable, and urine GGT activities should be evaluated along with urine activities of other enzymes (ALP, AST, and CK) in healthy reptiles and reptiles with renal impairment to determine their value.[23]

There were several limitations associated with the present study, including the limited number of tissues evaluated, freezing of samples prior to testing, and lack of testing for isoenzymes and the fact that we did not evaluate the effect of temperature on tissue enzyme activities and did not measure tissue enzyme activities in female snakes.

In the present study, we evaluated plasma enzyme activities and enzyme activities in 9 tissues from 12 male snakes. The gastrointestinal tract, brain, and bone were not sampled. The snakes used in the present study had originally been obtained for a reproductive study, and the collection of tissues was opportunistic. Thus, our study demonstrates how multiple studies can be completed with a single group of animals and should remind us of the need to consider such opportunities, especially for animal welfare reasons. Because of financial limitations, gastrointestinal tract, brain, and bone were specifically not selected because these tissues are not considered important sources of these enzymes, other than CK in the gastrointestinal tract and ALP in bone, on the basis of results of previous studies.[3–5,13,23]

Samples in the present study were collected and immediately frozen at –20 °C rather than analyzed. This was done because tissue samples were collected opportunistically. However, studies[24,25] in humans and rats suggest that for studies of enzyme activity, storing samples at –20 °C for up to 3 months will not impact sample quality or results. In our study, all samples were processed within 1 month after collection.

Measuring plasma isoenzyme activities in the present study could have proved useful, because isoenzyme activities can be used to more precisely determine the tissue of origin or determine which tissue of origin is the primary contributor to plasma activities. Additional studies focusing on characterizing isoenzymes may aid in further characterizing tissue of origin and organ function.

In the present study, we did not evaluate the role of temperature on tissue enzyme activities. Tissues were harvested immediately following euthanasia and were immediately placed on ice before being frozen. Metabolism in reptiles is known to vary with temperature; thus, future studies evaluating the effects of temperature on measured enzyme activities are warranted. Additionally, whether the euthanasia protocol used in the present study, which included alfaxalone and pentobarbital, could have an impact on measured enzyme activity is not known. In rats, an overdose of pentobarbital has been found to increase AST activity but not ALT activity.[26] Additional studies are warranted to determine the effects of pentobarbital overdose on tissue enzyme activities in reptiles.

Finally, in the present study, we evaluated tissue enzyme activities only in male snakes. Although it is thought that enzyme activities in male and female reptiles may be similar, specific studies are needed to confirm this. Several other studies that evaluated enzyme activities in reptiles did not disclose the sex of the reptiles used[13,14,16] or examine the enzyme activity of the gonads.[15] Of the studies that did evaluate the enzyme activity of the reproductive tract, most did not differentiate between ovaries and testicles and grouped the male and female reproductive tracts together as gonad.[2,4] Enzyme activities were measured separately for ovaries, oviducts, and testicles in loggerhead sea turtles, with none of these reproductive tissues having important enzyme activities.[5] On the basis of these results, we believe that sex does not appear to play an important role in enzyme activities; however, further studies are warranted.

In conclusion, the present study characterized the activity of 5 enzymes in the plasma and tissues of 2 species of water snakes. Results reinforced the current understanding that tissue enzyme activity can vary between even closely related species. Although the enzyme activities measured in the present study were similar to those reported in other studies, differences existed that warrant further study. Additional-

ly, although clinicians typically rely on blood samples to measure enzyme activities in their patients, future investigations into enzyme activities in urine should also be considered.

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