Reference intervals and relationships between health status, carapace length, body mass, and water temperature and concentrations of plasma total protein and protein electrophoretogram fractions in Atlantic loggerhead sea turtles and green turtles

Ann G. Osborne, MS, DVM; Elliott R. Jacobson, DVM, PhD, DACZM; Michael J. Bresette; Dave A. Singewald; Russell A. Scarpino, MS; Alan B. Bolten, PhD

Objective—To determine reference intervals for concentrations of plasma total protein (TP) and electrophoretogram fractions (ELFs) for healthy, wild loggerhead sea turtles (*Caretta caretta*) and green turtles (*Chelonia mydas*) and to assess relationships between TP and ELF concentrations and health status, body size, body mass, and water temperature.

Design—Evaluation study.

Animals—437 healthy and 35 ill Atlantic loggerhead sea turtles and 152 healthy and 3 ill Atlantic green turtles.

Procedures—Free-ranging turtles were captured from a nuclear power plant intake canal in southern Florida. Plasma samples were obtained from all turtles. Plasma TP and ELF concentrations were measured, and reference intervals were calculated. Wilcoxon rank-sum tests were used to compare TP and ELF values between healthy and ill loggerhead sea turtles. Spearman rank correlations were evaluated between concentrations of TP and ELFs and carapace length, body mass, and water temperature.

Results—Reference intervals for TP concentrations were 2.2 to 5.2 g/dL and 2.0 to 5.4 g/dL for loggerhead sea turtles and green turtles, respectively. Except for γ-globulin, concentrations of ELFs were significantly higher in healthy than in ill loggerhead sea turtles. There was a positive correlation between TP, α-globulin, β-globulin, and γ-globulin concentrations and water temperature in loggerhead sea turtles and between only TP and α-globulin concentrations and water temperature in green turtles.

Conclusions and Clinical Relevance—Reference intervals for concentrations of TP and ELFs for healthy, free-ranging loggerhead sea turtles and green turtles can be used in combination with other diagnostic tools to assess health status of sea turtles. (*J Am Vet Med Assoc* 2010;237:561–567)

AQUATIC ANIMALS

Five of the 6 species of sea turtles that inhabit the Atlantic Ocean are found along the coasts of Florida. Loggerhead sea turtles (*Caretta caretta*) are found throughout the world in temperate and tropical waters and currently are listed as a threatened species by the US Federal Endangered Species Act.[1] Green turtles (*Chelonia mydas*) are also found throughout the world, typically along coastlines and in near-shore habitats. Green turtles are currently listed as an endangered species by the US Federal Endangered Species Act.[2] Loggerhead sea turtles and green turtles are frequently rescued along US coasts. Rescued turtles often have injuries and illnesses and are taken to facilities to be rehabilitated. Although blood samples are often collected for use in evaluating health status and to assist in mak-

Abbreviations

A:G	Albumin-to-globulin ratio
ELF	Electrophoretogram fraction
SCL	Straight carapace length
TP	Total protein

From the Department of Small Animal Clinical Sciences, College of Veterinary Medicine (Osborne, Jacobson), and the Department of Biology and Archie Carr Center for Sea Turtle Research, College of Liberal Arts and Sciences (Bolten), University of Florida, Gainesville, FL 32610; and Quantum Resources Incorporated, 6451 S Ocean Dr, Jensen Beach, FL 34949 (Bresette, Singewald, Scarpino). Dr. Osborne's present address is 6781 Veronica Ct, St Augustine, FL 32086.

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Address correspondence to Dr. Jacobson (JacobsonE@ufl.edu).

ing decisions regarding rehabilitation and release, there currently are few reports[3–9] available for use in assessing blood variables in sea turtles.

Protein electrophoresis is a useful ancillary diagnostic tool in mammals and birds and has the potential to be a useful tool in reptiles and amphibians.[10] In avian medicine, protein electrophoresis has been used to detect nephritis, mycotic diseases, chronic inflammatory diseases, and chlamydiosis.[11,12] Protein electrophoresis allows for the determination of the percentages of protein fractions; on the basis of the TP concentration, the concentration of the various protein fractions can be determined. The major protein fractions include albumin, α-globulin, β-globulin, and γ-globulin. In birds and mammals, there is a prealbumin fraction composed of a thyroid carrier protein, transthyretin.[13] In another study,[3] a prealbumin fraction was detected in protein electrophoretograms of only 1 of 41 loggerhead sea turtles. Albumin, the most abundant plasma protein, serves as a carrier and transport protein and functions in regulating blood volume by maintaining oncotic pressure in body fluids.[14] The acute-phase proteins are in the α-globulin and β-globulin fractions. Fibrinogen, a specific acute-phase protein, is in the β-globulin fraction. Circulating immunoglobulins and complement comprise the γ-globulin fraction. Relative proportions of albumin and globulins can be assessed by use of the A:G, such as in animals with hyperproteinemia, to more closely identify the dysproteinemia.[14] Objectives of the study reported here were to determine reference intervals for concentrations of plasma TP and ELFs for a population of loggerhead sea turtles and green turtles and to assess relationships between TP and ELF concentrations and health status, body size, body mass, and water temperatures at the time of capture.

Materials and Methods

Animals—Healthy and ill loggerhead sea turtles and green turtles were included in the study. All turtles were captured after they had entered the intake canal of the nuclear power plant at St Lucie, Fla. Blood protein concentrations and associated metadata used in the study were acquired as part of a multidisciplinary collaborative project that included the College of Veterinary Medicine and the Archie Carr Center for Sea Turtle Research at the University of Florida, Florida Power and Light Corp, the St Lucie Power Plant, the Marine Life Center of Juno Beach, and the Clearwater Marine Aquarium. The project was approved by the University of Florida Institutional Animal Care and Use Committee (protocol D370). All samples were collected by authorization of the Florida Fish and Wildlife Conservation Commission (marine turtle permit No. 086).

Assessment and collection of data and blood samples—The study was conducted through field capture of sea turtles during the period from July 2004 through April 2007. Each turtle found within the intake canal system of the nuclear power plant was captured and visually assessed by numerous personnel. Injuries, external grossly observable conditions (eg, fibropapillomatosis), alertness, barnacle coverage, and other epibiont loads were recorded. The most common injuries were those related to fishery interactions, boating interactions, and entrapment in the intake pipes.

Depending on the size of the turtle, an appropriately sized needle (20 to 23 gauge) was used to obtain a single blood sample from the dorsal cervical sinus of each turtle. Blood (up to 6 mL/turtle) was collected into evacuated tubes that contained lithium heparin as an anticoagulant. A portion of the blood from each turtle was used immediately to determine the PCV. Data recorded for each turtle included the date, body weight, minimum SCL, straight carapace width, overall health status, PCV, and water temperature.

Evaluation of health status and PCV was used to determine whether each turtle could immediately be released into the nearby Atlantic Ocean (healthy group) or required treatment and care at a rehabilitation facility (ill group). The study group consisted of 472 loggerhead sea turtles (437 healthy and 35 ill) and 155 green turtles (152 healthy and 3 ill). In the healthy turtles, 1 loggerhead sea turtle and 8 green turtles had fibropapillomas; none of these 9 turtles was categorized as severely affected, and all were released. However, data for these 9 turtles were not included for subsequent analysis. The 35 ill loggerhead sea turtles and 3 ill green turtles were sent to a rehabilitation facility within 24 hours after capture. Injuries or illnesses of this group of turtles were categorized, and outcome was subsequently reported as released, permanent resident of the rehabilitation facility, or died.

The remaining blood obtained from each turtle was centrifuged, and the plasma was harvested and stored in a liquid nitrogen tank. Once each month during the study, plasma samples were removed from the liquid nitrogen tank and shipped frozen on dry ice to the University of Florida for measurement of TP concentrations and for plasma protein electrophoresis. Results of plasma biochemical analysis and PCV values have been reported elsewhere.[15]

Plasma protein analyses and reference intervals—Detailed description of methods for determination of plasma protein fractions has been provided elsewhere.[3] Briefly, plasma TP concentration was determined by use of an automated analyzer[a] via the biuret method.[16] The protein fractions (prealbumin, albumin, α-globulin, β-globulin, and γ-globulin) were measured in plasma samples; fractions were quantified via agarose gel electrophoresis by use of commercially available kits[b] and a laser densitometer.[c] Relative percentages of protein fractions were determined by calculating the area under the curve of each of the protein bands. The A:G values were calculated by use of the following equation: albumin concentration/(α-globulin concentration + β-globulin concentration + γ-globulin concentration). Reference intervals and median, minimum, and maximum concentrations for plasma TP and ELFs (albumin, α-globulin, β-globulin, and γ-globulin) and the A:G were calculated for both species of healthy turtles. Some variables were not normally distributed; therefore, the central 95% of the values (ie, 2.5th to 97.5th percentiles) was calculated as the reference interval. Median, minimum, and maximum concentrations for plasma TP and ELFs were calculated for the ill loggerhead sea turtles.

Statistical analysis—Data for plasma protein concentrations and concentrations for each of the ELFs were evaluated by use of Q-Q plots[17,18] to determine whether the data were normally distributed. Nonparametric analyses were used. Wilcoxon rank-sum tests were used to compare findings between healthy and ill loggerhead sea turtles. In addition, the proportion of ill loggerhead sea turtles that had values outside the reference interval for the healthy loggerhead sea turtles was determined.

Spearman rank correlations were evaluated between concentrations of TP and ELFs and minimum SCL, body mass, and water temperature. Commercially available software[d] was used for statistical analyses. For all analyses, values of $P < 0.05$ were considered significant.

Results

Plasma TP and ELF concentrations—Median, minimum, and maximum values and reference intervals for plasma TP and ELF concentrations were determined **(Table 1)**. Data for 437 loggerhead sea turtles and 152 green turtles were used in the calculations. Body size (ie, minimum SCL) of the loggerhead sea turtles ranged from 34.0 to 104.5 cm (13.4 to 41.1 inches), with a mean ± SD of 67.8 ± 9.4 cm (26.7 ± 3.7 inches) and a median of 66.2 cm (26.1 inches). Body weight of the loggerhead sea turtles ranged from 5.2 to 184.6 kg (11.4 to 406.1 lb), with a mean ± SD of 46.5 ± 21.6 kg (102.3 ± 47.5 lb) and a median of 41.1 kg (90.4 lb). Minimum SCL of the green turtles ranged from 25.4 to 86.9 cm (10 to 34.2 inches), with a mean ± SD of 42.5 ± 11.4 cm (16.7 ± 4.5 inches) and a median of 40.4 cm (15.9 inches). Body weight of the green turtles ranged from 1.9 to 89.2 kg (4.2 to 196.2 lb), with a mean ± SD of 12.2 ± 12.1 kg (26.8 ± 26.6 lb) and a median of 8.1 kg (17.8 lb). Water temperature ranged from 16.6°to 29.6°C (61.9° to 85.3°F). Prealbumin fractions were detected in 41 of 437 (9.4%) healthy loggerhead sea turtles and in 2 of 152 (1.3%) healthy green turtles. However, concentrations of the prealbumin fractions were too low and detected in too few turtles for statistical analysis.

Comparison of healthy and ill turtles—Wilcoxon rank-sum tests were used to compare concentrations of plasma TP and ELFs between healthy and ill loggerhead sea turtles. Compared with results for the healthy turtles, significantly ($P < 0.001$) lower concentrations of TP, albumin, α-globulin, and β-globulin and the A:G were detected for the ill turtles **(Table 2)**. The γ-globulin concentrations did not differ significantly ($P = 0.98$) between the 2 groups. Because of an inadequate number of ill green turtles (n = 3), a statistical comparison could not be made for concentrations of TP and ELFs between healthy and ill green turtles.

Outcome for ill turtles—Ill turtles had 1 or more problems, which included traumatic wounds on the carapace, head, flippers, or a combination of these anatomic locations as a result of a propeller or shark bite; heavy epibiont (barnacle or leech) load; emaciation; lethargy; entanglement in monofilament fishing nets; and impacted cloaca. On the basis of this information, ill turtles were categorized into 4 problem groups: trauma (14 loggerhead sea turtle and 2 green turtles), emaciation and lethargy (16 loggerhead sea turtles), other (ie, impacted cloaca, heavy epibiont load, and entanglement in monofilament [3 loggerhead sea turtles and 1 green turtle]), and unknown (2 loggerhead sea turtles).

Table 1—Concentrations of plasma protein fractions and associated reference intervals for healthy loggerhead sea turtles (*Caretta caretta*) and green turtles (*Chelonia mydas*).

Plasma protein fraction	Loggerhead sea turtles (n = 437)				Green turtles (n = 152)			
	Median	Minimum	Maximum	Reference interval*	Median	Minimum	Maximum	Reference interval*
TP (g/dL)	3.3	1.4	6.3	2.2–5.2	3.6	1.7	6.2	2.0–5.4
Albumin (g/dL)	0.99	0.19	2.01	0.48–1.48	1.48	0.71	2.31	0.75–2.13
α-Globulin (g/dL)	0.46	0.12	1.41	0.23–0.85	0.52	0.25	0.86	0.29–0.82
β-Globulin (g/dL)	0.84	0.30	1.84	0.43–1.32	0.68	0.22	1.30	0.30–1.05
γ-Globulin (g/dL)	0.97	0.29	3.28	0.48–2.38	0.86	0.19	2.27	0.33–1.92
A:G	0.43	0	0.89	0.03–0.71	0.69	0.42	1.19	0.47–1.00

*Reference interval represents the central 95% of the values (ie, 2.5th to 97.5th percentiles).

Table 2—Concentrations of plasma protein fractions for 35 ill loggerhead sea turtles.

Plasma protein fraction	Median	Minimum	Maximum	Reference interval*	Values outside reference interval (%)†
TP (g/dL)‡	2.6	0.8	4.5	2.2–5.2	25.7
Albumin (g/dL)‡	0.58	0.13	1.23	0.48–1.48	31.4
α-Globulin (g/dL)‡	0.30	0.09	0.85	0.23–0.85	14.3
β-Globulin (g/dL)‡	0.51	0.29	1.66	0.43–1.32	37.1
γ-Globulin (g/dL)	0.95	0.22	2.01	0.48–2.38	5.7
A:G‡	0.27	0.14	0.73	0.03–0.71	2.9

*Reference interval calculated for 437 healthy loggerhead sea turtles. †Percentage of values for ill turtles that are outside the reference interval for the 437 healthy turtles. ‡Values differ significantly ($P < 0.001$) from the values for the 437 healthy turtles.
See Table 1 for remainder of key.

Of the 35 ill loggerhead sea turtles, 6 died, 23 were released, 2 became permanent residents at the rehabilitation facility, and 4 had an unknown outcome. Two turtles died the day of arrival at the rehabilitation facility, 1 died the day after arrival, 1 died 3 days after arrival, 1 died 10 days after arrival, and 1 died 22 days after arrival. Of the 6 ill loggerhead sea turtles that died, 3 had abnormal concentrations of TP or ELFs (or both). Albumin was the ELF most commonly detected with low concentrations in the ill loggerhead sea turtles (11/35 [31.4%]).

Correlation analysis—A significant ($P = 0.007$) interaction was detected between water temperature and body size (ie, minimum SCL) for loggerhead sea turtles because the larger, breeding adults were present only during the warmer summer nesting season. To analyze correlations between plasma protein fractions and water temperature and body size, data for the larger turtles (minimum SCL > 81 cm [31.9 inches]; n = 40) were removed. This value for minimum SCL was used because it represented the estimated minimum SCL of first-time nesters.[19] Removal of data for the larger turtles resulted in nonsignificant correlations between water temperature and minimum SCL ($P = 0.36$) or between water temperature and body mass ($P = 0.63$); however, there was still a sufficient range of minimum SCL (34.0 to 80.5 cm [13.4 to 31.7 inches]) and body weight (5.2 to 72.7 kg [11.4 to 159.9 lb]) in the remaining 395 loggerhead sea turtles to enable us to evaluate the effects of body size, body mass, and water temperature on plasma protein fractions. For green turtles, there was not a significant correlation between water temperature and minimum SCL ($P = 0.12$) or between water temperature and body mass ($P = 0.12$); thus, adjustments in the data were not needed.

Correlations between minimum SCL, body mass, and water temperature and concentrations of TP and ELFs for loggerhead sea turtles with a minimum SCL ≤ 81 cm and for green turtles were determined (**Table 3**).

Table 3—Spearman rank correlations (ρ values) between concentrations of plasma protein fractions and body size, body mass, and water temperature for loggerhead sea turtles and green turtles.

Variable	Minimum SCL*	Body mass	Water temperature
Loggerhead sea turtles (n = 377)†			
TP	0.070	0.099	0.187‡
Albumin	–0.151§	–0.018	0.011
α-Globulin	–0.086	0.020	0.110‖
β-Globulin	–0.003	0.043	0.125‖
γ-Globulin	0.191‡	0.123‖	0.157¶
A:G	–0.228‡	–0.110‖	–0.136#
Green turtles (n = 157)†			
TP	0.666‡	0.669‡	0.180‖
Albumin	0.557‡	0.559‡	0.115
α-Globulin	0.383‡	0.387‡	0.334‡
β-Globulin	0.413‡	0.413‡	0.071
γ-Globulin	0.696‡	0.698‡	0.142
A:G	–0.268‡	–0.269‡	–0.126

*Represents data for turtles with a minimum SCL of ≤ 81 cm (31.9 inches). †Fewer loggerhead sea turtles and green turtles are included here than in Table 1 because loggerheads with a minimum SCL > 81 cm were excluded and data were missing for some variables. ‡§‖¶#Represents a significant (‡$P < 0.001$, §$P = 0.003$, ‖$P < 0.05$, ¶$P = 0.002$, and #$P = 0.008$) correlation.

In loggerhead sea turtles, no significant correlations were found between TP concentration and minimum SCL or body mass, between albumin concentration and body mass, between α-globulin concentration and minimum SCL or body mass, and between β-globulin concentration and minimum SCL or body mass. Albumin concentration was significantly ($P = 0.003$) negatively correlated with minimum SCL, and γ-globulin concentration was significantly positively correlated with minimum SCL ($P < 0.001$) and body mass ($P = 0.017$). There was a significant positive correlation between water temperature and concentrations of TP ($P < 0.001$), α-globulin ($P = 0.034$), β-globulin ($P = 0.016$), and γ-globulin ($P = 0.002$).

Data for juvenile green turtles were available only for correlation analysis (Table 3). There were significant ($P < 0.001$) positive correlations between minimum SCL and body mass and concentrations of TP, albumin, α-globulin, β-globulin, and γ-globulin. There was a significant ($P < 0.001$) negative correlation between minimum SCL and body mass. Significant positive correlations were detected between water temperature and TP concentration ($P = 0.024$) and α-globulin concentration ($P < 0.001$).

Discussion

Evaluation of results of plasma biochemical analyses, including concentrations of plasma proteins and protein fractions, for healthy, wild sea turtles may aid in determining health status, which in turn could assist in making decisions regarding rehabilitation and release. Reference intervals for plasma protein ELFs determined by use of a sufficient sample size for various seasons of the year have not yet been established for sea turtles. Studies in which investigators quantified proteins by use of electrophoresis in sea turtles are limited to 29 loggerhead sea turtles in Florida Bay[3]; 39 foraging, 31 nesting, and 13 stranded loggerhead sea turtles along the coast of Georgia[20]; and 12 leatherback sea turtles (*Dermochelys coriacea*) in the Republic of Gabon.[21]

Establishing reference intervals requires a clinically normal population that is most representative of the target population with regard to health.[22] In the study reported here, investigators responsible for removing sea turtles from the intake canal of the nuclear power plant did not use a detailed health assessment protocol, although they did use previously developed criteria for categorizing turtles as healthy (ie, could be released immediately) or ill (ie, required treatment or rehabilitation).

Median concentrations of TP (3.3 g/dL) and albumin (0.99 g/dL) for the loggerhead turtles in the present study were lower than mean values for a migratory group of 42 loggerhead sea turtles (TP concentration, 3.6 g/dL; albumin concentration, 1.3 g/dL) and a residential group of 15 loggerhead sea turtles (TP concentration, 4.0 g/dL; albumin concentration, 1.1 g/dL) in North Carolina.[5] However, results for both of those groups of turtles in the North Carolina study were within the reference interval for TP concentration determined in the study reported here. Similarly, although loggerhead sea turtles captured at St Lucie, Fla, in the present study had a median TP concentration less than and an albumin concentration higher than those for a population of 23 loggerhead sea turtles near Cape Canaveral, Fla (TP concentration, 4.1 g/dL; albumin concentration, 0.6 g/dL),[4] values for the turtles near Cape Canaveral were within the reference interval for the loggerhead sea turtles in our study. Mean values for foraging (TP concentration, 3.7 mg/dL; albumin concentration, 0.79 mg/dL [n = 39 turtles]) and nesting (TP concentration, 5.2 mg/dL; albumin concentration, 1.15 mg/dL [25]) loggerhead sea turtles from the coast of Georgia[21] were within the reference interval for loggerhead sea turtles reported in our study. Compared with the median plasma TP concentration (4.3 g/dL) for 29 healthy loggerhead sea turtles in Florida Bay,[2] the median TP concentration for loggerhead sea turtles in our study (3.3 g/dL) was lower. However, the median albumin concentrations (0.99 g/dL) for these populations of turtles were similar, which suggested that the difference was attributable to the concentrations of the globulin fractions.

Findings for green turtles in the present report are similar to those reported for green turtles in other studies.[6,7] Concentrations for TP and albumin for 2 foraging groups (n = 53 and 37, respectively) of green turtles in Hawaii (TP concentration, 4.2 and 5.0 g/dL, respectively; albumin concentration, 1.7 and 1.7 g/dL, respectively)[7] and for 2 foraging groups (59 and 51, respectively) of green turtles in Australia (TP concentration, 4.3 and 3.4 g/dL, respectively; albumin concentration, 1.4 and 1.1 g/dL, respectively)[6] were within reference intervals for green turtles in our study (TP concentration, 2.0 to 5.4 g/dL; albumin concentration, 0.73 to 2.13 g/dL). Juvenile green turtles (n = 100) in the southern Bahamas[8] had a mean TP concentration (5.1 g/dL) that was higher than the median concentration of the green turtles in our study; however, the TP concentration for the green turtles in the southern Bahamas was within the reference interval for the green turtles in the present study. Environmental factors, such as diet and water temperature, as well as other spatial-temporal factors may be responsible for differences in plasma TP concentrations among populations.

Prealbumin fractions were identified in a small percentage of the loggerhead sea turtles (41/437 [9.4%]) and green turtles (2/152 [1.3%]), with concentrations too low to be statistically evaluated. Only a few loggerhead sea turtles from the coast of Georgia had a prealbumin fraction,[20] and no prealbumin fractions were detected in electrophoretograms of leatherback sea turtles in another study.[21] Further examination and identification of this fraction may help researchers and clinicians to determine its prevalence.

For sea turtles, there is limited information available on the proteins in each globulin fraction. Similar to the situation for mammalian α-globulin, ceruloplasm and haptoglobin have been identified in the plasma of loggerhead sea turtles.[23] Transferrin, a major iron-carrier protein, has been identified in the β-globulin fraction of mammals[13] but in the γ-globulin fraction of loggerhead sea turtles.[23] Immunoglobulin has been identified primarily in the γ-globulin fraction of loggerhead seas turtles; however, in the experience of one of the authors (ERJ), other turtles have a small portion of the immunoglobulin in the β-globulin fraction.

The median concentrations of the α-globulin (0.46 g/dL) and β-globulin (0.84 g/dL) fractions in loggerhead sea turtles of the study reported here were similar to the values reported in loggerhead sea turtles of another study.[3] However, the α-globulin fraction was evident as a single fraction, whereas in turtles from the coast of Georgia, α-globulin was subdivided into α-1 and α-2 fractions.[20] Although the combined mean concentration for the α-globulin fractions for foraging (0.26 g/dL) and nesting (3.8 g/dl) loggerhead turtles in the Georgia study[20] were lower than the mean concentration for the single α-globulin fraction in our study (0.46 g/dL), mean concentrations of β-globulin for foraging (0.99 g/dL) and nesting (1.7 mg/dL) loggerhead sea turtles from the coast of Georgia[20] were higher than the median values for the loggerhead sea turtles in our study (0.84 g/dL). The median concentration for the α-globulin fraction of green turtles in our study was similar to the value reported for loggerhead sea turtles[3] but lower than the value reported for leatherback sea turtles.[21] Median concentration of the β-globulin fraction of green turtles in our study was lower than that for both loggerhead sea turtles[3] and leatherback sea turtles.[21] Median concentration of the γ-globulin fraction of loggerhead sea turtles in our study (0.97 g/dL) was lower than that in loggerhead sea turtles in Florida Bay (1.94 g/dL).[3] The differences in γ-globulin concentrations between the 2 populations suggest differences in antigenic stimulation. It is possible that burdens of parasites and other infectious agents were greater in the Florida Bay population. The loggerhead sea turtles and green turtles of our study had higher median γ-globulin concentrations (0.97 and 0.86 g/dL, respectively), compared with the concentration of γ-globulin in leatherback sea turtles[21] but lower mean concentrations, compared with the concentration for foraging (1.57 mg/dL) and nesting (1.18 g/dL) loggerhead sea turtles from the coast of Georgia.[20]

Significant differences between healthy and ill loggerhead sea turtles for median concentrations of TP, albumin, α-globulin, and β-globulin and the A:G suggest that protein electrophoresis may be useful as part of an overall health assessment protocol for sea turtles. Median TP, albumin, α-globulin, and β-globulin concentrations and the A:G were all significantly lower in the ill turtles. The lower TP and albumin concentrations in the ill turtles may have been related to a decrease in food intake, nutritional status, or a decrease in protein assimilation, such as in various disease states (eg, protein-losing enteropathy, chronic hepatitis, and severe malnutrition). Albumin is considered a negative acute-phase protein in that decreases in concentrations can be associated with inflammatory conditions.[21] An increase in concentrations of α-globulin or β-globulin (or both) can result from an increase in acute-phase proteins in response to infection, inflammation, and tissue injury.[21] However, in the study reported here, the α-globulin and β-globulin concentrations were lower in the ill turtles than in the healthy turtles. The lower α-globulin and β-globulin concentrations in the ill turtles may have reflected a relative change to a low overall protein concentration, but the reason for such a change was not clear. Concentrations of γ-globulin did not differ significantly between healthy and ill loggerhead sea turtles. The small number of ill green turtles was insufficient to enable us to determine a significant difference in concentrations of plasma protein fractions between ill and healthy green turtles.

Eight green turtles and 1 loggerhead sea turtle in our study had fibropapilomas; because none of these turtles was categorized as severely affected, all were released. Still, we decided not to include the data of these turtles with those of the other healthy turtles. In a study[7] of green turtles in Hawaii in which plasma biochemical values were compared between healthy turtles and those with various categories of fibropapillomatosis, concentrations of TP, albumin, and globulin were significantly reduced in the turtles with advanced fibropapillomatosis, whereas turtles with mild and moderate fibropapillomatosis did not have significant changes in concentrations of plasma protein fractions.

Further evaluation of the ill loggerhead sea turtles in the study reported here revealed that more than half (20/35 [57.1%]) had 1 or more analytes (TP or ELFs) outside the reference range calculated for the healthy loggerhead sea turtles. This suggests that evaluating protein concentrations is not helpful as a singular determinant of general health. The change in TP and ELF concentrations depends greatly on the type of problem or disease process, chronicity of the condition, and other factors as discussed for each protein fraction. Unfortunately, in the study reported here, there was limited clinical information available for each turtle evaluated. Thus, a small number of broad health problems were used for the categories.

Sea turtles in the present study underwent a cursory physical examination, and those considered healthy at the time of capture at the intake of the nuclear power plant were returned to the Atlantic Ocean. Certain abnormalities, such as healed wounds on the flippers and carapace, missing portions of flippers, and increased epibiont load, were recorded for turtles that were considered healthy and released. Despite these findings, the reference intervals reported here are valid for the general sea turtle population, especially because this represents the largest data set for a sea turtle population. Similar findings would be expected for any sea turtle population. Similarly, the number of turtles in our study likely diluted the effect of the inclusion of potentially misclassified turtles.

Correlations between water temperature and concentrations of plasma proteins and protein fractions were variable for the loggerhead sea turtles. Although there was no significant correlation between albumin concentration and water temperature, concentrations of other analytes (ie, TP, α-globulin, β-globulin, and γ-globulin) were positively correlated with water temperature and the A:G was negatively correlated with water temperature. This suggested a pattern for the winter and spring months (cooler water) for loggerhead turtles in which TP, α-globulin, β-globulin, and γ-globulin concentrations were lower than concentrations in late summer or fall (warmer water). For green turtles, positive correlations were found between water temperature and concentrations of plasma proteins and α-globulin. Seasonal variation should be taken into account when comparing results for samples obtained across time.

The turtles captured in the canal system of the nuclear power plant at St Lucie are part of a unique, reliable, and consistent sample of the sea turtle population found in the Atlantic Ocean near the shore of south Florida. However, the group of turtles captured in the canal system may not have been a completely representative sample of the population of wild turtles for all ages and sexes. Potential sampling bias is important when considering the data reported here and using the reference intervals generated by our study. A survey of the sex and estimated age of the turtles was not part of this study; however, use of body size of the turtles as a proxy representation of age classes yielded an apparent overrepresentation of certain classes of body size. All green turtles in the study were sexually immature and were considered juveniles. A single class for body size (60 to 69.9 cm [23.6 to 27.5 inches]) comprised most of the loggerhead sea turtles in the study. Loggerhead sea turtles of this body size also would be considered juveniles on the basis of a body-size classification (50 to 70 cm [19.7 to 27.6 inches]) reported in another study.[5]

Examination of the effects of body size and body mass on TP and ELF concentrations in loggerhead sea turtles revealed that only γ-globulin concentrations increased as minimum SCL and body mass increased. In green turtles, TP, albumin, α-globulin, β-globulin, and γ-globulin concentrations increased with increasing minimum SCL and body mass. In other studies of loggerhead seas turtles[9] and green turtles,[8] TP, albumin, and γ-globulin concentrations increased with increases in body size (ie, minimum SCL). These findings may indicate geographic differences and species differences.

Analysis of plasma proteins via protein electrophoresis has long been used as a diagnostic tool in mammalian medicine and recently has been expanded for use in avian medicine. In the study reported here, we analyzed protein data from a large number of 2 protected species of sea turtles to enhance baseline health data by establishing reference intervals of TP and ELF concentrations and to investigate differences in health, body size, and seasonal effects on loggerhead sea turtles and green turtles that entered the intake canal of a power plant located on the coast of east central Florida. Because sea turtles have complex life histories, with turtles of a certain body size found in a given area, most of the turtles in our study were juveniles of unknown sex, with adults captured during only the warmer nesting season of the year. Although age and sex were not differentiated, we believe the reference intervals for concentrations of TP and ELFs in the study reported here are the most robust data set available for use as an adjunct diagnostic test in health assessment of populations of similarly sized loggerhead sea turtles and green turtles and for making clinical decisions regarding treatment of ill sea turtles brought into rehabilitation facilities. These data should be of value to sea turtle biologists and rehabilitators throughout the world.

a. Hitachi 911, Roche Diagnostics Corp, Indianapolis, Ind.
b. Beckman Paragon Protein SPE-II electrophoresis kit, Beckman Coulter Inc, Fullerton, Calif.
c. Beckman Appraise densitometer, Beckman Coulter Inc, Fullerton, Calif.
d. TIBCO Spotfire S+ 8.1 for Windows, TIBCO, Seattle, Wash.

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