Research Article

The Effects of Feeding on Hematological and Plasma Biochemical Profiles in Green (Chelonia mydas) and Kemp’s Ridley (Lepidochelys kempii) Sea Turtles

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Received 12 February 2011; Accepted 26 April 2011

Academic Editor: Pedro J. Ginel

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In mammals, lipemic blood from sampling too soon after an animal feeds can have substantial effects on biochemical values. Plasma biochemical values in reptiles may be affected by species, age, season, and nutritional state. However, fasting status is not routinely considered when sampling reptile blood. In this paper, we evaluated 2-hour postprandial blood collection in two sea turtle species to investigate the effects of feeding on hematological and plasma biochemical values. Feeding had no significant effects on hematological values in either species, nor did it have an effect on plasma biochemistry values in Kemp’s ridley sea turtles. In postprandial green turtles, total protein, albumin, ALP, AST, ALT, amylase, and cholesterol increased significantly, and chloride decreased significantly. Although statistically significant changes were observed, the median percent differences between pre- and postprandial values did not exceed 10% for any of these analytes and would not likely alter the clinical interpretation.

1. Introduction

Green sea turtles (Chelonia mydas) are found in tropical to semitropical waters around the world and are the second most abundant sea turtle found off the Eastern United States [1, 2]. Juvenile green turtles found in coastal waters are in a transition phase from a more carnivorous diet (shrimp, snails, and ctenophores) to a primarily herbivorous diet (sea grasses), which creates a physiological status unique from other sea turtles [3]. Kemp’s ridley sea turtles (Lepidochelys kempii) are the smallest and most endangered of all sea turtles. They are found in waters along the eastern coast of North America, ranging from Mexico to as far north as New York and Massachusetts [2]. Kemp’s ridley turtles are carnivorous and opportunistic, feeding primarily on crabs, mollusks, and fish [3]. Juvenile green and Kemp’s ridley turtles use shallow coastal waters for foraging grounds, making them susceptible to a number of human-induced traumas (e.g., boat strike and fishing interactions) and natural disease processes (e.g., cold-stunning). Hundreds of juvenile turtles are found dead or severely debilitated each year, with many being brought into rehabilitation centers for treatments.

Hematology and plasma biochemistries are valuable for monitoring animal health; however, environmental and procedural factors can have variable affects on reported values. Postprandial blood collection can yield lipemic samples due to increases in serum triglycerides in the form of chylomicrons. Several biochemical analytes change with diet, feeding, and lipemia in a wide range of species, including mammals [4–13], birds [14–19], sharks [20], and reptiles [21, 22]. The observed alterations in biochemical values have led to recommendations on prephlebotomy fasting times for many species [4, 6, 17, 19]. However, few recommendations have been made for reptiles.
2. Materials and Methods

2.1. Animals and Environment. Ten juvenile green sea turtles and ten juvenile Kemp’s ridley sea turtles undergoing rehabilitation for various conditions at the Karen Beasley Sea Turtle Rescue and Rehabilitation Center, Topsail Island, North Carolina, were used for this study. Median and range of weights, straight carapace lengths (nuchal notch to pygal notch), and days in rehabilitation are shown in Table 1. Only turtles that were active, eating well, and not on any major treatments were included. Not all were considered ready for release, as some were still being treated for minor shell lesions (topical treatment) and for being under the desired body condition for release. Turtles were individually housed in variably sized plastic or fiberglass tanks containing filtered saltwater. Larger tanks were plumbed into a communal biological filtration; smaller tanks were on a daily dump routine. Most of the turtles remained in indoor tanks, but a few were placed in temporary outdoor enclosures during the afternoons to facilitate daily operations of the rehabilitation facility and provide UV light exposure. Water temperatures ranged from 25°C–29°C.

Table 1: Size data and time in rehabilitation for green (n = 10) and Kemp’s ridley (n = 10) sea turtles investigated for the effects of feeding on hematology and plasma biochemical profiles.

| Analyte            | Green sea turtles | Kemp’s ridley sea turtles |
|--------------------|-------------------|---------------------------|
| Weight (kg)        | Median 6.0        | Median 6.4                |
| SCL-N (cm)         | 35.8              | 33.3                      |
| Days in rehabilitation | 284            | 117                       |

Straight carapace length (nuchal notch to pygal notch).

2.2. Sampling and Feeding. Blood was collected in conjunction with routine health monitoring and prerelease examinations. Initial blood samples were taken first thing in the morning following an approximately 24 hr fast prior to any food items being offered. Turtles were manually restrained on an exam table, and the head was restrained in ventral flexion over the side of the table to facilitate blood collection. A 22 ga × 2.54 cm needle and 3 mL syringe were heparinized and used to collect 2-3 mL of blood from the external jugular vein. After initial sampling, the turtles were returned to their holding tanks and offered food. A combination of frozen thawed capelin (Mallotus villosus) with lesser amounts of squid (Loligo sp.), and/or blue crab (Callinectes sapidus) was offered at 1.5%–9.0% body weight. Based on proximate analyses of diet items performed previously at this facility (squid and blue crab; Microbac Laboratories, Inc., Southern Testing and Research Division, Wilson, NC, USA) and previously published values for capelin and squid [38], capelin contains a moderate amount of lipid (7.0%–23.3% dry matter basis), while squid and blue crab contain a lesser amount of lipid (squid 8.3%–11.4%, blue crab 2.4% dry matter basis). All turtles were given 30 min to feed, and all consumed a minimum of 50% or more of the diet offered. Two hours after feeding, a second blood sample was collected. For turtles that took longer than 10 min to consume the offered diet, the time for the postprandial blood sample was calculated from the time the turtle stopped eating or excess food was removed from the tank. The median (range) time from feeding to the postprandial blood draw was 126 min (113–142 min).

2.3. Blood Processing and Analysis. For each turtle, two blood smears were made, two hematocrit tubes filled, and the remainder of the blood was placed in a sterile 1.5 mL conical vial with no additive (Fisher Scientific Company, Pittsburgh, Pa, USA) on wet ice until centrifugation. All samples were centrifuged within 67 min of collection. Hematocrit tubes were centrifuged at 3900 × g for 5 min, and packed cell volume (PCV) determined. Total solids (TS) were determined by refractometer. The conical vials were centrifuged at 1000 × g for 10 min, and the plasma harvested. Plasma samples were submitted along with two corresponding blood samples to a commercial diagnostic laboratory (Antech Diagnostics, Memphis, Tenn, USA) for biochemistry analysis and differential cell counts within one day of collection. Plasma biochemistry panels were performed on a Hitachi 717 Chemistry Autoanalyzer (Hitachi Instruments Inc., San Jose, Calif, USA). Details on plasma biochemistry assay methodology are summarized elsewhere [39].

2.4. Statistical Analysis. All analyses were performed with JMP 7 (SAS Institute, Cary, NC, USA). Median and range were determined for all hematological and plasma biochemical data. The Wilcoxon matched-pairs signed rank test was used to compare differences between preprandial and postprandial values. The data were then grouped by hematologic values (PCV, % heterophils, % lymphocytes, % monocytes, % eosinophils, and % basophils), plasma enzymes (ALP, AST, ALT, CPK, amylase, and lipase), metabolic indicators (total protein, albumin, globulins, glucose, BUN, creatinine, uric acid, cholesterol, triglycerides, and total bilirubin), and ions (sodium, potassium, chloride, calcium, inorganic phosphate,
| Analyte         | Median Preprandial | Range | Median Postprandial | Range |
|----------------|--------------------|-------|--------------------|-------|
| Total protein  | 40 (4.0)           | 34–45 (3.4–4.5) | 40 (4.0)           | 35–48 (3.5–4.8) |
| Albumin        | 15 (1.5)           | 13–18 (1.3–1.8) | 16 (1.6)           | 13–19 (1.3–1.9) |
| Globulin       | 24 (2.4)           | 20–30 (2.0–3.0) | 24 (2.4)           | 22–31 (2.2–3.1) |
| Glucose        | 6.8 (122)          | 5.3–7.3 (95–131) | 6.4 (116)          | 5.4–8.1 (97–145) |
| Urea nitrogen  | 43.6 (122)         | 26.4–60.0 (74–168) | 43.6 (122)         | 27.5–58.9 (77–165) |
| Creatinine     | 18 (0.2)           | 18–26 (0.2–0.3) | 18 (0.2)           | 18–35 (0.2–0.4) |
| Cholesterol    | 5.1 (198)          | 2.1–8.5 (80–328) | 5.2 (202)          | 2.3–8.5 (88–329) |
| Triglyceride   | 3.8 (348)          | 0.3–7.3 (23–662) | 3.9 (354)          | 0.3–8.6 (25–779) |
| Uric Acid      | 36 (0.6)           | 6–71 (0.1–1.2)  | 71 (1.2)           | 6–107 (0.1–1.8)  |
| Calcium        | 1.8 (7.4)          | 1.6–2.0 (6.5–7.8) | 1.8 (7.0)          | 1.6–1.9 (6.5–7.7) |
| Phosphate      | 2.4 (7.6)          | 1.5–2.9 (4.7–8.9) | 2.5 (7.8)          | 1.5–2.8 (4.8–8.8) |
| Sodium         | 154                | 150–158 | 155                | 153–164 |
| Potassium      | 4.1                | 3.9–4.4  | 4.4                | 3.6–4.7 |
| Chloride       | 127                | 120–131 | 124                | 120–127 |
| Magnesium      | 1.7 (4.2)          | 1.2–2.5 (3.0–6.1) | 1.7 (4.2)          | 1.2–2.3 (2.9–5.7) |
| ALP*           | U/L                | 98      | 40–197             | 96     | 41–211 |
| ALT*           | U/L                | 6       | 1–35               | 6      | 0–45  |
| AST*           | U/L                | 491     | 66–3607            | 523    | 69–4128 |
| CK*            | U/L                | 786     | 370–8289           | 812    | 361–8166 |
| Amylase        | U/L                | 528     | 457–699            | 564    | 462–740 |
| Lipase         | U/L                | 8       | 0–16               | 10     | 0–16  |

* ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase.

and magnesium), and the sequential Bonferroni technique was applied within each group to reduce the chance of type I error [40]. A P value of <.05 after sequential Bonferroni correction was considered statistically significant. A median difference of 10% or greater for an analyte was arbitrarily chosen as a point at which a statistical difference may become clinically relevant and, therefore, may lead to an alteration in the clinical interpretation or therapeutic course.

3. Results

There were no significant differences between pre- and postprandial PCV and differentials for either the green turtle or the Kemp’s ridley sea turtle (data not shown). There were no significant differences between preprandial and postprandial values in the Kemp’s ridley sea turtles (Table 2).

In postprandial samples in the green turtles (Table 3), there were significant increases in total protein ($P = .0390$), albumin ($P = .0351$), alkaline phosphatase (ALP; $P = .0156$), alanine aminotransferase (ALT; $P = .0351$), aspartate aminotransferase (AST; $P = .0120$), amylase ($P = .0100$), and cholesterol ($P = .0312$). In addition, there was a significant decrease in chloride ($P = .0234$). Of these analytes that were statistically different after feeding, none had a median percent difference of greater than 10% between pre- and postprandial samples (Table 4). Although median percent differences were greater than 10% for globulin and creatine kinase (CK) in green turtles, and for lipase and uric acid in Kemp’s ridley turtles, the variability of data was high, and those differences were not statistically significant.

4. Discussion

All hematological and biochemical values for both species of sea turtles were consistent with values previously reported for these species [25, 30, 31, 35, 41–43] except for inorganic phosphate, AST, and ALT in the green turtles. Inorganic phosphate was at the upper range or slightly higher than reported values [25, 30, 35, 41, 43], which is likely related to diet. The marked elevation in AST and ALT of most of the green turtles, as compared to reported values, is a phenomenon that has previously been observed in this population (unpublished data). The underlying cause is uncertain, but it may also be of dietary origin related to a more carnivorous diet in captivity. Because postprandial values were compared with preprandial values for each turtle, the initial values were not a factor in the analysis.

No significant differences were found between preprandial and postprandial hematological values for either the green turtle or the Kemp’s ridley sea turtle. This finding was expected, because postprandial sampling is linked with lipemia and interference with analytes that utilize photometric analyses [4, 5, 12, 44]. Hematological values are obtained through direct microscopic evaluation of cell numbers and types; therefore, alterations due to postprandial sampling would not be expected. Lipemia has also been linked with
increased hemolysis due to red cell fragility [4]; however, little to no hemolysis was observed in any of the samples used.

The green sea turtle showed more postprandial changes in plasma chemistry values than were observed in the Kemp’s ridley sea turtle. In the green turtle, total protein, albumin, ALT, AST, ALP, cholesterol, and amylase significantly increased following feeding, but none changed to a degree that would likely lead to alterations in medical management. In the Kemp’s ridley sea turtle, there were no significant differences between pre- and postprandial samples. These differences between the two species could relate to differences in their natural diets. Juvenile Kemp’s ridley turtles are carnivorous, consuming primarily crustaceans [3], whereas the juvenile green turtles shift from a carnivorous to a more herbivorous lifestyle [3, 45]. However, in the rehabilitation setting of the current study, both species are primarily fed capelin with some squid, and with many Kemp’s ridleys also being fed soft-shell blue crab. The difference observed might then be due to each species’ specialized physiology for their natural diet (i.e., inclusion of vegetation in the diet of the juvenile green turtles of this size). Research on the effects of natural diets on plasma biochemical profiles at different life stages is warranted.

The increased values observed in green turtles contrast with what has been observed in the red-eared slider (Trachemys scripta elegans), where no differences in any of these analytes were found in 24 hr and 48 hr postprandial samples [46]. Variable ALP, AST, and ALT activities have been measured in multiple tissues in reptiles, resulting in indeterminate degrees of clinical utility [47–49]. Despite statistically significant increases, none of these enzymes changed substantially (i.e., no median percent differences >10%) in the current study, and both pre- and postprandial ALP values were comparable to published ranges for green sea turtles [25, 30, 41–43]. The initial plasma activity levels for AST and ALT were higher than in previous reports [25, 30, 41–43], but the median percent postprandial changes were minor (<10%). Apurateaminotransferase and ALT have been found in skeletal muscle to some extent [48, 49]. It is possible that the initial phlebotomy caused mild muscle damage and subsequent leakage of the enzymes from the muscle cells. This leakage of the enzyme into the peripheral circulation could account for mild increases in enzyme activities observed in the postprandial samples. Changes in CK, however, were highly variable and not consistently supportive of the interpretation of muscle damage. These same studies found these three enzymes in liver, kidney and variable concentrations in other organ tissues [48, 49], and postprandial elevations may be indicative of organs gearing up for digestion (e.g., liver, pancreas, and gastrointestinal tract). There are several isoenzymes of these tissue enzymes that may contribute to the overall increase in plasma enzyme activity. Although outside the scope of this paper, evaluation of the isoenzymes would help to better characterize the observed enzyme plasma activities.

**Table 3**: Medians and ranges for preprandial and postprandial plasma biochemical values from juvenile green sea turtles (n = 10). System International and conventional units (in parentheses) are provided.

| Analyte             | Preprandial | Postprandial |
|---------------------|-------------|--------------|
|                     | Median      | Range        | Median      | Range       |
| Total protein       | 46 (4.6)    | 26–55 (2.6–5.5) | 49 (4.9)    | 25–60 (2.5–6.0) |
| Albumin             | 19 (1.9)    | 10–25 (1.0–2.5) | 21 (2.1)    | 10–26 (1.0–2.6) |
| Globulin            | 26 (2.6)    | 16–33 (1.6–3.3) | 29 (2.9)    | 15–35 (1.5–3.5) |
| Glucose             | 8.2 (146)   | 5.4–10.3 (97–184) | 8.3 (149)   | 6.8–9.6 (122–171) |
| Urea nitrogen       | 30.0 (84)   | 19.3–51.4 (54–144) | 29.6 (83)   | 19.3–49.6 (54–139) |
| Creatinine          | 18 (0.2)    | 9–18 (0.1–0.2) | 18 (0.2)    | 18–26 (0.2–0.3) |
| Cholesterol         | 5.4 (207)   | 2.4–8.1 (93–311) | 5.5 (216)   | 2.4–9.2 (93–352) |
| Triglyceride        | 1.7 (151)   | 0.9–4.4 (83–401) | 1.7 (154)   | 0.8–4.8 (72–441) |
| Uric Acid           | 59 (1.0)    | 48–125 (0.8–2.1) | 89 (1.5)    | 54–137 (0.9–2.3) |
| Calcium             | 1.7 (6.8)   | 1.4–2.5 (5.7–7.8) | 1.8 (7.3)   | 1.4–2.2 (5.5–8.6) |
| Phosphate           | 2.9 (9.0)   | 2.3–13.2 (6.1–13.2) | 3.0 (9.3)   | 2.3–13.7 (7.2–13.7) |
| Sodium              | 154         | 148–162       | 154         | 148–159       |
| Potassium           | 4.3         | 3.5–6.7       | 4.1         | 3.2–5.3       |
| Chloride            | 121         | 116–131       | 116         | 109–122       |
| Magnesium           | 3.2 (6.3)   | 1.5–3.3 (3.7–8.1) | 3.1 (6.2)   | 1.7–3.0 (4.1–7.4) |
| ALP                 | 54          | 31–63         | 60          | 32–68         |
| ALT                 | 90          | 14–237        | 108         | 15–285        |
| AST                 | 1486        | 396–3175      | 1684        | 438–5491      |
| CK                  | 1072        | 145–1802      | 994         | 208–2540      |
| Amylase             | 1086        | 163–1470      | 1154        | 194–1531      |
| Lipase              | 20          | 2–48          | 20          | 2–53          |

*Indicates postprandial values that differ significantly from preprandial values (P < 0.039).

b ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase.
Cholesterol and triglycerides may be increased in reptiles due to hepatic lipolysis, vitellogenesis, and prehibernation [50]. Pythons exhibited a 50-fold increase in triglycerides and an 80% increase in cholesterol following a meal [51]; however, no time frame was provided. In red-edared slider turtles fed a high-fat diet, triglycerides increased significantly within 2 days and did not return to normal until 4 days after feeding [52]. There was no evaluation of a normal diet or cholesterol concentrations. In the current study, with a moderate-to-low-fat diet, triglycerides did not increase significantly in either species, and cholesterol significantly increased 2 hr after feeding in the green turtle but not in the Kemp’s ridley sea turtles. The postprandial cholesterol concentrations were, however, increased by less than 10%, and were at or minimally above published reference intervals for cholesterol in green turtles [25, 41, 42, 50].

Although sea turtles have a long gastrointestinal transit time (up to 7 or 8 days in the Kemp’s ridley turtle [53] and can tolerate a prolonged fast (possibly months for adults of some green turtle populations during reproductive migration) [54]), the 24-hr fast used in this study of juvenile sea turtles is ecologically and physiologically relevant. In the wild, green turtles on foraging grounds feed once to twice daily, and primarily during the daylight hours [3, 55, 56]. In Hawaii, green turtles have been found to feed primarily at night in some areas, where they foraged from evening to around sunrise [57]. Green turtles in areas of poor food availability, highly disturbed grass beds, fed more continuously (approximately 9 hr) during daylight hours [58]. Kemp’s ridley sea turtles feed primarily on more active and mobile prey such as crabs [3, 53, 59–61] and, therefore likely feed more sporadically throughout the day [61]. As a result of their natural feeding behaviors, a 24-hr period without food constitutes a valid fast for these two species.

None of the changes observed in hematologic and plasma chemistry values between pre- and 2 hr postprandial blood samples were considered sufficient to revise a clinical interpretation or trigger a change in an animal’s course of treatment. It is possible that a longer preprandial fast, or following biochemical profiles for a longer period of time after feeding, would yield greater alterations. However, for a juvenile green or Kemp’s ridley sea turtle feeding once daily, there is little consequence to sampling 2 hr postprandial versus after a 24-hr fast. Timing of blood collection in regularly feeding sea turtles appears to be less critical for interpretation of clinical pathology values than in some other species.

Acknowledgments

The authors thank all the volunteers at the Karen Beasley Sea Turtle Rescue and Rehabilitation Center for their dedication and work with sick and injured sea turtles and for their help with the turtles during this study. They also thank two classes of veterinary students for technical assistance with sample collection and processing.

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