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Research Protocol

Reference Intervals of Fructosamine in Wild Florida Manatees (*Trichechus manatus latirostris*)

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Abstract

Florida manatees (*Trichechus manatus latirostris)* in captivity, both in rehabilitation centers or zoological institutions, are typically fed commercially grown lettuces and produce with a very limited amount of native forages. While a highly digestible, energy rich food is desirable for all critically ill animals in rehabilitation centers, prolonged feeding of such a diet in herbivores can lead to other metabolic conditions including metabolic syndrome, laminitis, and diabetes mellitus. Fructosamine reflects blood glucose concentrations over a two to three week time period in several domestic species and humans. Serum and or plasma samples from twenty-five wild Florida manatees were opportunistically analyzed for fructosamine and 90% confidence intervals (CI) were established. These values were then used to evaluate individual manatees in captivity in rehabilitation centers or long-term non-releasable manatees. Clinical suspicions of diabetes mellitus were confirmed in both rehabilitated and non-releasable manatees in zoological institutions using the CI for fructosamine from wild manatees. Clinical resolution of the diabetic state was achieved in two long term captive manatees by nutritional management and verified by reduction in fructosamine levels. Establishment of a CI for fructosamine will for enhanced health care of manatees in rehabilitation centers as well as those in long term care in zoological institutions.

Keywords: *Florida Manatee; Trichechus manatus latirostris; Fructosamine; Diabetes Mellitus; Foraging; Wildlife Rehabilitation*

Introduction

The Florida manatee (*Trichechus manatus latirostris*) is a member of the order Sirenia, comprising aquatic mammals including the dugong (*Dugong dugon*) and the three species of manatees - the West Indian manatee (*Trichechus manatus*), the West African manatee (*Trichechus senegalensis*), and the Amazonian manatee (*Trichechus inunguis*). There are two subspecies of West Indian manatee: the Antillean (*Trichechus manatus manatus*) and Florida manatee. Manatees and other sirenia are the only herbivorous marine mammals, with the Florida manatee being a generalist hind gut fermenting herbivore.

Florida manatees feed on over 60 species of aquatic plants for up to 8 hours daily, accumulating an estimated maximum as fed intake of approximately 10% of body weight [1]. Manatees in captivity, both in rehabilitation centers or long term permanent care in zoological institutions, are typically fed commercially grown lettuces and produce in additional to a very limited amount of native forages. These commercially grown feedstuffs contain from two to six times the caloric density of seagrasses and are very digestible with low fiber content [2]. While a highly digestible, energy rich food is desirable for critically ill manatees, prolonged feeding of such a diet can lead to other metabolic conditions [1,2].

Fructosamine reflects blood glucose concentrations over a two to three week time period in several animal species and elevations reflect persistent hyperglycemia and are strong indicators of Type 2 diabetes [3,4]. Diabetes has been diagnosed in a rehabilitated manatee that was released and did not survive, and other clinical cases are suspected (unpublished data). Establishing a reference interval (RI) for fructosamine in manatees will serve to enhance the care of these threatened species while in rehabilitation centers and guidance for those managed in zoological institutions. Fructosamine sampling may also provide energetic information on wildlife species due to the fact that a single sample will reflect two to three weeks of glucose status. This will eliminate the confounding effects of capture stress hyperglycemia in similar fashion that fructosamine measured at the same time in cats will discriminate between a transient stress hyperglycemia and true persistent metabolic hyperglycemia [4].

Materials and Methods

Blood was collected from the brachial vascular bundle during routine diagnostic health assessments of apparently healthy free ranging manatees (n = 25) during captures performed by the Florida Fish and Wildlife Conservation Commission and the U.S. Geological Survey, Sirenia Project http://cars.er.usgs.gov/Manatees/manatees.html) in December 2016 through February 2017 and December 2017 through February 2018. Manatees were captured in nets deployed either along shore or from a specialized net boat in open water at the Crystal River National Wildlife Refuge, Citrus County, Florida (28°53'01.6"N 82°35'33.1"W). Blood samples were processed on site and serum separated then refrigerated until they were placed in -80C freezer at ZooTampa at Lowry Park (ZT) later in the day of collection. Fructosamine levels of all collected samples were evaluated using an Idexx Catalyst™ biochemistry machine at ZooTampa's veterinary hospital clinical pathology laboratory. Mean, median, standard deviation, and maximum and minimum were calculated using untransformed data while 90% confidence intervals were calculated using Box-Cox transformed data. All statistical tests were conducted with Reference Value Advisor freeware™.

Results

Results are shown in table 1 below.

N	25	
Mean	235.2	umol/L
Median	232.0	umol/L
SD	39.3	umol/L
Minimum	154	umol/L
Maximum	311	umol/L
90% CI for lower limit	149	umol/L
90% CI for upper limit	317	umol/L

Table 1: *Descriptive statistics for fructosamine in healthy wild Florida manatees (Trichechus manatus latirostris) from Crystal River Florida. 90% confidence intervals were calculated using Box-Cox transformed data.*

Discussion

Fructosamine consists of glycosylated plasma protein and its concentration is believed to reflect average blood glucose concentrations over the preceding two to three week period in man [3] and some domestic species [4]. Its measurement has been advocated in other species as a measure of glycemic control that is not affected by transient factors such as recent feeding or stress [4]. This feature makes the

use of fructosamine as an indicator of glycemic state potentially useful in wildlife studies and eliminates the confounding influence of capture stress. In normal horses, mean fructosamine has been reported to be 248.7 µmol/l with the normal range (mean ± 2 s.d.) of 195.5 - 301.9 µmol/l [5] while mean values of fructosamine in cattle on day 28 postpartum are reported 253 +/- 20 umol/l [6]. Both herbivorous domestic species have values similar to those reported here in wild Florida manatees (mean 235.2 umol/l, 90% CI 149 - 317 umol/l).

Albumin is one of the dominant serum proteins that fructosamine binds with [4] and some authors have suggested that correction factors be applied to calculating the true fructosamine concentration, but this is controversial [3]. When corrections were applied to horses undergoing fructosamine measurements, this correction was found to not be significant [5] and therefore was not applied to the calculations of the CI for Florida manatee fructosamine concentrations in this report.

Pancreatic interstitial fibrosis and diabetes mellitus have been diagnosed in a closely related species, the rock hyrax (*Procavia caensis*) [7,8]. In the hyrax, a disparity of fiber content between wild and captive diets was believed to be responsible for the pathology seen. This disparity has strong parallels to what has been noted in manatee diets in captive situations compared to forage consumed by wild manatees [2]. Pancreatic interstitial fibrosis has been diagnosed in a long-term captive manatee that was unsuitable for release [unpublished data].

Manatees kept for extended stays in rehabilitation centers or long-term captive manatees, with their prolonged consumption of a relatively high energy low fiber diet, may be at risk of developing elevated levels of fructosamine. This elevation along with clinical signs such as obesity, supports a diagnosis of diabetes mellitus. Retrospective evaluation of serum stored at -80°C in both rehabilitated and long-term captive manatees shows several individuals with values significantly and persistently over the range report here for wild manatees with values as high as 900 to 1000 umol/l recorded.

Captive obese long-term manatees (n = 2) with persistently elevated levels of fructosamine have had their fructosamine levels return to the CI intervals determined by this study with simple dietary changes to enhance fiber intake. This was accomplished by using grass hay for the majority of their diet (~90% as fed). This change did not alter the pathology already caused by the diabetic state, and renal and cardiac disease consistent with diabetes mellitus has been seen consistently on pathology in this group of long term obese manatees on necropsy and histopathology, including one with documented changes in fructosamine as noted. Closer evaluation of the length of time in managed care, feeding alternatives, and even changes in release criteria may all minimize the effects of a prolonged captive diet on rehabilitating manatees.

Conclusion

Fructosamine levels in wild Florida manatees are similar to those in domesticated herbivores, cattle and horses. Manatees in captive settings may be at increased risk from the undesired effects of high energy, low fiber diets on domestic herbivores, including metabolic syndrome and diabetes mellitus. Long-term captive manatees with elevated fructosamine may have their hyperglycemic state reduced by the substitution of lower energy density higher fiber foodstuff for the high energy low fiber foods typically used. Manatees with repeated fructosamine levels over 300 umol/L should be suspected of developing diabetes mellitus and managed appropriately. Fructosamine measurements may be applicable to other wildlife species as a measure of energy status as it requires only a single sample and reflects glycemic state of an extended period of time.

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