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Serum triglycerides and ß-hydroxybutyrate predict feeding status in green turtles (*Chelonia mydas*): Evaluating a single blood sample method for assessing feeding/fasting in reptiles

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ABSTRACT

The foraging success or feeding rate of an animal is important information for ecologists and wildlife managers but can be difficult to assess, particularly in marine vertebrates that are hard to follow at sea. Here we evaluate a method for determining recent feeding history using a single blood sample by measuring the concentration of relevant serum metabolites. Five captive green turtles were either fed a maintenance diet or subjected to fasting periods ranging from 5–15 days. Serial serum samples were collected during both fed and fasted periods, and we determined triglyceride, ß-hydroxybutyrate, and glycerol concentrations using spectrophotometric assays. Serum triglyceride and glycerol concentrations decreased during fasting periods, while serum ß-hydroxybutyrate concentration increased during fasts. For triglyceride and glycerol, this decrease apparently occurred in the first 5 days of fasting and was unaltered by further fasting. ß-hydroxybutyrate concentration continued to increase during longer fasting periods. The decrease in serum glycerol was unexpected and may be due to metabolic down-regulation in fasted turtles. Serum triglyceride and ß-hydroxybutyrate appear to be good indicators of feeding state and can be applied to wild turtles.

1. Introduction

The ability to assess feeding rates of animals in wild populations is often critical to ecological studies and to conservation and management activities (Bailey et al., 2012). Understanding how individuals differ in foraging success can help explain tradeoffs in life history variables such as reproductive output and the decision to migrate (Price et al., 2004). Foraging success can also be used as an integrated measure of habitat quality, to prioritize areas for conservation, and to assess the effects of environmental contamination (Artacho et al., 2007; Guglielmo et al., 2005). Nonetheless, measuring foraging success can be difficult and time-consuming, often requiring tracking or recapture of individuals. This is particularly problematic for marine animals because of the difficulty of following and/or recapturing animals at sea. For this reason, we sought to find blood metabolites that vary predictably with feeding/fasting state in green turtles (*Chelonia mydas* L.), such that foraging success, or its proxy fattening rate, could be assessed with a single blood sample.

A plasma metabolite approach for evaluating feeding rate has been tested extensively in birds (Anteau and Afton, 2008; Guglielmo et al., 2005; Jenni-Eiermann and Jenni, 1994; Williams et al., 1999; Zajac et al., 2006), and is increasingly being used to assess feeding rate during avian migration (Lyons et al., 2008; Schaub and Jenni, 2001). It has been used to measure habitat quality (Artacho et al., 2007; Guglielmo et al., 2005; Seewagen et al., 2011), assess the effects of exotic vegetation on migrant refueling ability (Cerasale and Guglielmo, 2010), examine the effects of parasites on refueling (DeGroote and Rodewald, 2010), and to assess fattening rates of individuals with varied migration strategies (Boyle et al., 2010; Hays, 2008). Recent work has also validated the use of plasma metabolites to predict feeding status in bats (McGuire et al., 2009a) and subsequently applied them to field research in that group (McGuire et al., 2009b).

The basis of the plasma metabolite technique lies in the change in concentration of triglycerides, glycerol, ß-hydroxybutyrate, and other metabolites during different metabolic states. During the fed state, macronutrients are absorbed at the intestine and transported to the

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liver for repackaging as triglycerides. These triglycerides are then transported in very low density lipoproteins (VLDLs) via the blood to adipose tissue for storage. Thus, the concentration of triglycerides in the blood rises during feeding. During times of energy demand and low foraging activity, stored triglycerides are hydrolyzed within adipocytes to produce glycerol and free fatty acids, both of which are then exported to the blood for transport to other tissues. When exogenous glucose is limited, stored triglycerides can also be used to produce ketone bodies such as ß-hydroxybutyrate, which is used as a glucose substitute by tissues that ordinarily require glucose (e.g., brain). Thus, during fasting, the concentration of triglycerides in the blood drops while the concentrations of glycerol and ß-hydroxybutyrate increase. While this description applies generally to birds and mammals, these processes have not been investigated extensively in reptiles, although some studies have reported that triglyceride levels in the blood rise after feeding in squamates (Christel and DeNardo, 2007; Secor and Diamond, 1995). Green turtles are also interesting models for investigating plasma metabolites because they are hindgut fermenters with long digesta retention times (Bjorndal, 1980; Brand et al., 1999). In this paper, we describe the response of serum triglycerides, glycerol, and ß-hydroxybutyrate to fasting in captive green turtles, and evaluate their use in assessing feeding status.

2. Methods

2.1. Animals and dietary manipulation

Green turtles (*C. mydas* L.) were imported from the Cayman Turtle Farm, British West Indies, to the Zoology Animal Care Center, Department of Zoology, University of British Columbia (CITES Export Permit 2002/ky/000112; CITES Import Permit CA02CWIM0129). Protocols were approved by the UBC Animal Care Committee (A03-0255).

The turtles ranged in mass from 19.04–26.20 kg and were kept in a large oval fiberglass tank (10 m by 3 m by 1.5 m) filled with seawater. The turtles were fed 1–2% of body mass every other day on a diet of Purina Trout Chow R5D-VO5 (Purina Mills, LLC, St. Louis, MO, USA) mixed with an aqueous solution of flavorless gelatin, Reptavite® and Reptamin® (vitamin and mineral supplements). Food quantities were based on presumed daily calorific intake of wild green turtles (Bjorndal, 1997). For complete husbandry details see Jones et al. (2009).

Three turtles underwent a fed/fasted/fed scheme described previously (Jones et al., 2009). Briefly, during trials the turtles were moved to smaller isolation tanks (1 m diameter by 1.5 m deep) and maintained on their feeding schedule for 6 days, during which blood was drawn daily from the cervical venous sinus. The turtles were then fasted 15 days, and blood was drawn daily in the last 5 days of this fast. Finally, the turtles were returned to their normal feeding regime, and blood was drawn daily for 5 days (Fig. 1). In separate experiments, we collected data from these and another 2 turtles in the fed and fasted states; however, the fasting period was 10 days in length and blood was drawn on days 5, 8, and 10 of the fast.

2.2. Measurement of metabolites

Blood samples (2–5 ml) were drawn from the cervical venous sinus using 21-gauge by 1.5 inch BD needles and BD SST Gel and Clot Activator Vacutainers® (BD; Becton Dickinson and Co., Franklin Lakes, NJ, USA). All blood samples were left to clot for 30 min before centrifuging for 30 min at 1509 ×*g*. Serum was removed and transferred to Nalgene™ cryo-safe plastic tubes and frozen at −10 °C, samples were then transferred to −35 °C. Triglycerides were measured using a commercial kit supplied by Sigma-Aldrich (products F6428, T2449, and G7793; St. Louis, MO, USA) and modified to be used with 5 µl of serum (Guglielmo et al., 2005) and read using a microplate spectrophotometer (Wallac Victor^2 1420 multilabel counter, PerkinElmer, Waltham, MA, USA). Free glycerol was determined first; then after lipoprotein lipase hydrolysis, triglycerides were determined by subtracting free glycerol values. ß-hydroxybutyrate was determined using a commercial kinetic assay kit (R-Biopharm, Marshall, MI) that was similarly modified for 5 µl serum on a microplate reader. When values fell outside of the linear range of our standard curve (ß-hydroxybutyrate linearity standards, Stanbio, Boerne, TX, USA), those serum samples were diluted 5× with water and rerun, and true concentration was determined by multiplying the resulting values by 5.

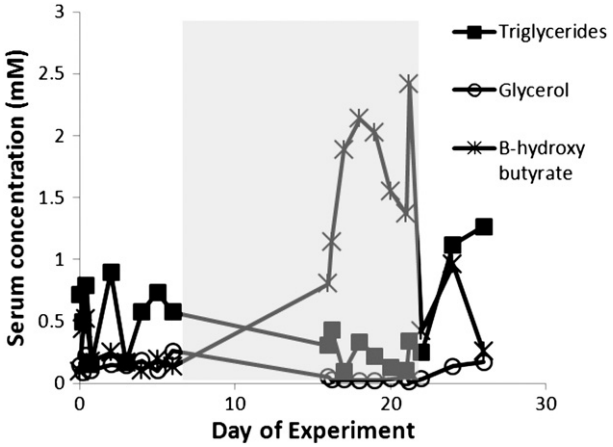

Fig. 1. Representative trace of serum triglycerides, glycerol, and ß-hydroxybutyrate of a green turtle on a normal feeding regime (fed every other day) followed by 15 days fasting and then returned to normal feeding. Shaded area represents the period of fasting.

2.3. Statistics

Metabolite concentrations were compared between turtles in fed and fasted states using a mixed model in the R statistical software (Pinheiro et al., 2011; R Development Core Team, 2011) to account for repeated sampling on individuals. Similarly, the effect of fasting duration on metabolite concentration was evaluated using a mixed model approach in R. For presentation purposes in figures, a least squares regression is fitted and r^2 is presented based on all data without accounting for repeated measures.

3. Results

We ran a standard curve on every plate, and the r^2 values of all standard curves were greater than 0.98. Intra- and inter-plate coefficients of variation were: 14.0% and 11.7%, respectively for ß-hydroxybutyrate; 3.14% and 5.82%, respectively, for glycerol; and 8.30% and 8.29%, respectively, for triglycerides.

Fig. 1 presents a typical time course for one of the 3 turtles that was fed, fasted for 15 days, and then refed. When data from all turtles and experiments were combined, turtles that had been fasted (for any length of time, minimum = 5 days) had 32% lower triglycerides than turtles that were in the fed state ($P = 0.0064$, Fig. 2). Glycerol concentration also decreased 50% in fasted turtles compared to those that had been recently fed ($P < 0.0001$, Fig. 2). ß-hydroxybutyrate increased 372% during fasting ($P < 0.0001$, Fig. 2).

There was a significant linear relationship between all 3 metabolites and the length of fast ($P < 0.0124$ for all; Fig. 3). However, when examining only turtles in the fasted state (i.e., removing turtles that were fasted zero days), there was only a significant relationship between ß-hydroxybutyrate and fasting duration ($P = 0.0082$), while the relationships for glycerol ($P = 0.2861$) and triglycerides ($P = 0.3855$) were not statistically significant.