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The potential influence of gastric acid secretion during fasting on digestion time in leopard sharks (*Triakis semifasciata*)

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Abstract

Vertebrates are known to differ in their response of gastric acid secretion during periods of fasting, yet the reasons for these differences remain unclear. Previously, continuous measurements of gastric pH in leopard sharks (*Triakis semifasciata*) had determined that acid secretion in this species is continuous. In order to determine if maintaining an empty acidic stomach may reduce digestion time of a subsequent meal, a simple descriptive model based on acid secretion rates was developed. In vivo gastric acid secretion rates were measured using an auto-titration technique. Acid secretion rates were pH dependent, with rates of 6.1±3.0 (±1 SD) mmol/h when gastric pH was >2.5, and 1.7±0.8 mmol/h when pH was 2.0–2.5. Analysis by Western blots suggests that pepsin secretion occurs within 1 h of feeding, and that there is a de-coupling of acid and pepsin secretion. The model estimates that digestion time can be reduced by 5.7±1.3 h and pepsin activity increased by 10–100% during that time if the stomach is acidic before feeding. Gastric acid secretion during fasting is hypothesized to reduce digestion time of a subsequent meal in frequently feeding sharks, which may be advantageous for exploiting resources that are spatially and temporally unpredictable.

Keywords: Digestion; Feeding ecology; Feeding frequency; Leopard sharks; Pepsin

1. Introduction

A paradox of gastric digestion in carnivorous vertebrates is the secretion of gastric acid during periods of fasting. During feeding, the stomach secretes hydrochloric acid (HCl) to convert the inactive zymogen pepsinogen into the protease enzyme pepsin, initiating digestion of proteins in prey items. The high density of mitochondria in acid secreting cells, across vertebrate lineages, is evidence of the energetic costs associated with acid secretion (Rebolledo and Vial, 1979; Sachs, 1994, Secor, 2003). Therefore it would appear unnecessary and energetically costly for acid to be secreted when the stomach is empty. It has been suggested that the secretion of gastric acid during fasting provides antiseptic conditions, limiting the growth of bacterial flora on the mucosa lining (Hunt, 1988; Joseph and Kirschner, 2004). However, an unexplained caveat to this interpretation is that some vertebrates do periodically cease acid secretion during fasting (Sachs, 1994; Secor, 2003; Papastamatiou and Lowe, 2005), and possible reasons behind the differences in the vertebrate fasting response have yet to be explored.

Elasmobranch fishes are one of the earliest known extant groups of vertebrates to have developed an acid secreting stomach (Smolka et al., 1994), making them a suitable subject for investigating the evolution of gastric acid secretion in carnivorous vertebrates. Furthermore, differences in the fasting response of gastric acid secretion are known to exist between elasmobranch species (Barrington, 1942; Papastamatiou and Lowe, 2004, 2005). It has been hypothesized that these differences may be related to the feeding frequency of the animal in the wild (Papastamatiou and Lowe, 2005).

The leopard shark (*Triakis semifasciata*) is a frequently feeding opportunistic predator found along the coast of California (Talent, 1976; Kao, 2000). Continuous measurements of gastric pH in captive leopard sharks have shown that this species maintains an acidic stomach during fasting (Papastamatiou and Lowe, 2004). Furthermore, it was suggested that by maintaining an acidic empty stomach, a leopard shark could reduce digestion time of a subsequent meal,

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as conditions in the stomach would be optimal for gastric breakdown of prey items (Papastamatiou and Lowe, 2004). However, presently no empirical or theoretical evidence exists to support this explanation for continual gastric acid secretion. In order to determine if maintenance of an acidic empty stomach reduces digestion time of a subsequent meal in leopard sharks, a simple descriptive model was developed to estimate the changes in gastric pH that would occur if the stomach was neutral prior to feeding. As input data for the model, pH-dependent acid secretion rates in leopard sharks were measured using a gastric auto-titration technique. Western blots were used to verify that leopard sharks do secrete pepsin in the stomach, and to examine time-series changes in the presence of pepsin in gastric fluids. Post-prandial changes in pepsin activity were predicted using previously published data on pepsin secretion rates, and pH-dependent activity of shark pepsin, in conjunction with basic physiological principles (e.g. gastric emptying time, conversion pH of pepsinogen to pepsin). The model was used to estimate differences in digestion time and pepsin activity, when the stomach is neutral before feeding as opposed to being acidic.

2. Methods

2.1. Acid secretion rates

In a previous study, continuous measurements of gastric pH were obtained from six free-swimming adult female leopard sharks (*T. semifasciata*, Total Length (TL) = 141.5 ± 11.9 cm, mean ± 1 SD), using an autonomous pH/temperature data-logger (Papastamatiou and Lowe, 2004). Free-swimming sharks were force-fed the data-loggers, which they retained from 5 to 16 d. During this period the sharks were fed meals of squid (*Loligo* spp.) at a variety of ration sizes (0.3–2% body weight (BW)). The pH of the empty stomach was 1.54 ± 1.42 (± 1 SD), with pH rising upon the ingestion of squid, followed by a gradual decline back down to baseline levels (Fig. 1, see Papastamatiou and Lowe, 2004). The initial increase in gastric pH following ingestion of prey is caused by seawater and the prey items themselves mixing with the small amounts of gastric acid present in the stomach before feeding. An increase in gastric acid secretion rate then causes the gradual decrease in pH back to baseline levels (Papastamatiou and Lowe, 2004). Using these data, meal-stimulated acid secretion rates were calculated in vivo using the auto-titration technique described by Gardner et al. (2002). The volume of 0.1 N HCl required to reduce the pH of 158 g of homogenized squid to a pH of 2.0 was determined using a manual titration. The homogenate had a pH of 6.5 and an approximate volume of 100 mL. The volume of 0.1 N HCl (mL) was then converted to mmol. The pH titration curve was best described by an exponential equation $\mathrm{pH} = 5.98e^{-0.021\ \mathrm{mmol\ HCl}}$ ($r^2 = 0.99$, $p < 0.0001$), and 55 mmol of HCl was required to lower the pH of the homogenized squid to 2.0. Due to the fact that leopard sharks ate meals of different masses, the mmol of HCl required to reduce the pH of each meal of squid was determined using a linear mass conversion (e.g. mmol HCl required to reduce pH to $2.0 = (x/158) * 55$, where x is meal size measured in (g)). In the previous study, titration times were determined for each meal of squid consumed by a leopard shark, with titration time defined as the time (in min) taken for pH to return to 2.0 after feeding (see Gardner et al., 2002; Papastamatiou and Lowe, 2004). Acid secretion rate could then be calculated in mmol/h as [mmol 0.1 N HCl required to lower pH of meal to $2.0 * 60$ / titration time (min)]. Mass specific acid secretion rates were also calculated in μmol/kg/h. Visual examination of the post-prandial change in gastric pH indicated that an inflection point existed in the rate of change of gastric pH following consumption of a meal of squid (Fig. 1, also see Papastamatiou and Lowe, 2004). Although the initial rapid decline may simply indicate the expulsion of seawater and other buffers from the stomach, a similar decline was not seen when sharks were fed meals of capelin (*Mallotus villosus*, Fig. 1b). Therefore, it is assumed that the change in the rate of pH decrease reflects a legitimate change in acid secretion rate. The mean inflection point for all six leopard sharks consuming squid was at pH = 2.5 ± 0.3. Consequently, acid secretion rates were calculated for each meal separately for gastric pH values >2.5 and for gastric pH values between 2.5 and 2.0 (see Fig. 1). The mmol HCl required to

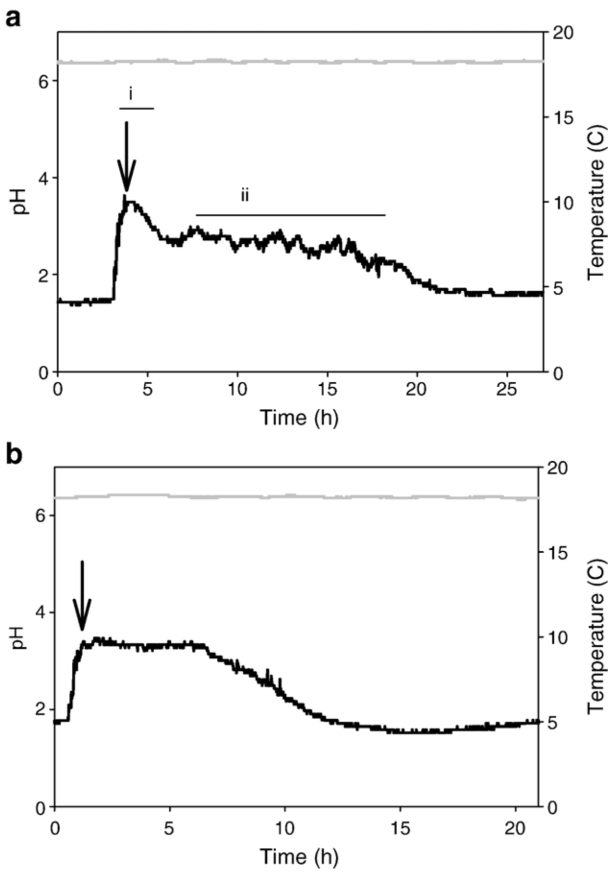

Fig. 1. Continuous measurements of gastric pH (black line) and temperature (gray line) in a free-swimming adult female leopard shark (*Triakis semifasciata*), TL = 150 cm. Data were obtained using an autonomous pH data-logger. a) Arrow points to time of feeding, when squid was consumed at 1% of the shark's body weight (% BW). Note the initial increase in gastric pH followed by a rapid decline in pH (i) and consequently a more gradual decrease (ii) indicative of a change in acid secretion rate. b) Arrow points to time of feeding when shark ate a meal of Capelin (*Mallotus villosus*) at 1% BW. Note the absence of a rapid decline in gastric pH. Data taken from Papastamatiou and Lowe (2004).