Comparative Biochemistry and Physiology, Part A 194 (2016) 62–66

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Low cost of gastric acid secretion during digestion in ball pythons

Simon Nørgaard, Kim Andreassen, Christian Lind Malte, Sanne Enok, Tobias Wang *

Zoophysiology, Department of Bioscience, Aarhus University, Denmark

ARTICLE INFO

Article history:
Received 12 October 2015
Received in revised form 5 January 2016
Accepted 7 January 2016
Available online 21 January 2016

Keywords:
Acid-base
Metabolism
Reptile
Snake
Specific dynamic action

ABSTRACT

Due to their large metabolic responses to digestion (specific dynamic action, SDA), snakes represent an interesting animal group to identify the underlying mechanisms for the postprandial rise in metabolism. The SDA response results from the energetic costs of many different processes ranging over prey handling, secretions by the digestive system, synthesis of enzymes, plasticity of most visceral organs, as well as protein synthesis and nitrogen excretion. The contribution of the individual mechanisms, however, remains elusive. Gastric acid secretion has been proposed to account for more than half of the SDA response, while other studies report much lower contributions of the gastric processes. To investigate the energetic cost of gastric acid secretion, ball pythons (*Python regius*) were fed meals with added amounts of bone meal (up to 25 g bone meal kg^{-1} snake) to achieve a five-fold rise in the buffer capacity of the meals. Direct measurements within the stomach lumen showed similar reduction in gastric pH when buffer capacity was increased, but we found no effects on the rise in oxygen consumption over the first three days of digestion. There was, however, a slower return of oxygen consumption to resting baseline. We conclude that gastric acid secretion only contributes modestly to the SDA response and propose that post-absorptive processes, such as increased protein synthesis, are likely to underlie the SDA response.

1. Introduction

As a ubiquitous response, animals increase metabolism during digestion (Andrade et al., 2005; McCue, 2006; Secor, 2009). This metabolic response, denoted specific dynamic action (SDA), stems from a variety of physiological and biochemical processes starting from stimulation of oral secretions immediately after ingestion to increased protein synthesis, nutrient deposition and costs associated with nitrogen excretion upon intestinal absorption (McCue, 2006). In all animals studied, the SDA response is both larger and prolonged when meal size is increased, and the factorial rise in metabolism is particularly pronounced in ectothermic vertebrates having a low resting metabolic rate. Many snakes, including pythons and rattlesnakes, ingest large meals that may exceed 50% of their own body mass (*e.g.* Greene, 1983), and will, in such cases, exhibit SDA responses where oxygen consumption ($\dot{V}O_2$) increases even more than during maximal physical activity (Andrade et al., 1997; Secor et al., 2000). In these snakes, the postprandial period is also characterised by pronounced growth of the visceral organs and particularly an impressive doubling of the mass of the small intestine already within the first 12–24 h (Secor and Diamond, 1995; Hansen et al., 2013). Hence, snakes form ideal animals for studying physiological and biochemical processes underlying the SDA response (Secor and Diamond, 1998; Barboza et al., 2010).

It is now generally accepted that the intestinal remodelling occurs at low energetic expenditure (Starck and Beese, 2001; Overgaard et al., 2002; Secor, 2003), but the costs of the gastric functions, including acid and enzyme secretion, epithelial growth and motility have remained more difficult to resolve (Enok et al., 2016). By bypassing the stomach through infusion of mechanical degraded rodent meals directly into the intestines of Burmese pythons, Secor (2003) concluded that gastric acid secretion accounts for more than half of the total SDA response, and thus represents the single largest contributor to this impressive metabolic feat. In boas, Andrade et al. (2004) however, demonstrated that pharmacological inhibition of gastric acid secretion by the specific H^+ ATPase inhibitor omeprazole had no influence on the SDA response. A low cost of gastric processes was supported by the subsequent observation that ligation of the pyloric sphincter in ball pythons, to prevent the chyme from entering the intestines, greatly attenuated the SDA response (Wang et al., 2006; Enok et al., 2013).

As an alternative to blocking gastric function, Henriksen et al. (2015) added carbonate to the meals in ball pythons (15 g kg^{-1} snake) and did not observe any further increment in the SDA response. In the present study, we follow up on this approach and added two dosages of bone meal to double and triple the normal amount of bone buffering in the

* Corresponding author at: Zoophysiology, Department of Bioscience, Aarhus University, C.F. Møllers Alle 3, Building 1131, Universitetsparken, 8000 Aarhus C, Denmark.
E-mail address: tobias.wang@bios.au.dk (T. Wang).

http://dx.doi.org/10.1016/j.cbpa.2016.01.003
1095-6433/

prey. To verify the intended effects of the bone meal, we determined the buffer capacity of the meals *in vitro* and we confirmed that gastric pH was indeed lowered when the snakes digested meals with increased buffer capacity.

2. Materials and methods

2.1. Experimental animals

Python regius (227–1182 g) were purchased from a commercial supplier and kept at Aarhus University in vivaria equipped with a local heat source to provide temperatures from 25 to 32 °C and a photoperiod of 12 h/12 h. The snakes were fed on mice once a week and had free access to water, but were fasted at least three weeks before experimentation to ensure they were in a post-absorptive state. All snakes grew during captivity and appeared healthy. Experiments were performed according to Danish Federal Regulations.

2.2. Surgery and instrumentation

Anaesthesia was induced by placing the snake in a zip-lock bag containing swaps saturated with isoflurane, which resulted in the loss of reflexes within 5–10 min (Eatwell, 2010). The trachea was intubated for mechanical ventilation (Harvard Apparatus mechanical ventilator, Cambridge, MA, USA) of the lungs with 1–5% isoflurane prepared by a Fluotec 3 vaporizer (Simonsen & Weel; Vallensbæk, Denmark) at 5 breaths min^{-1} and a tidal volume of 30 ml kg^{-1}. Upon subcutaneous application of lidocaine for local anaesthesia, the vertebral artery was assessed through a ventrolateral incision and occlusively cannulated with a polyethylene (PE-50) catheter containing heparinized saline (50 IU/ml). The catheters were exteriorized through the skin and fastened with 2–3 sutures. Snakes were allowed to recover overnight at 30 °C within a climatic chamber; a period that suffices for heart rate and blood pressure to reach resting values (Olesen et al., 2008).

2.3. Meal types and addition of bone meal

Three meals with varying amounts of bone meal were prepared; a control meal of homogenized mice, and two meals of homogenized mice with either 12.5 or 25.0 g bone meal kg body $mass^{-1}$ (Naturreines Knochenmehl; Grau, Isselburg, Germany). Two different meal sizes were given. Meals of 10% of body mass were administered with two different amounts of bone; controls with no additional bone (total of 5.4 g bone kg^{-1} snake; Martin and Fuhrman, 1955), and meals added 25.0 g bone meal kg^{-1} (total of 30.4 g bone kg^{-1} snake). Meals constituting 15% of body mass were added no bone meal (total of 8.0 g bone kg^{-1} snake) as well as 12.5 or 25.0 g bone meal kg^{-1} (total of 20.5 and 33.0 g bone kg^{-1} snake, respectively). As bone meal is added to the homogenized meals the relative protein content will be reduced. Since protein-rich meals are related to large SDA responses (Henriksen et al., 2015) it could be argued that the effect of bone-rich meals would be masked by the lower protein content. With the assumption that mice contain around 47% protein (McCue et al., 2015), the relative reduction in protein will be less than 1.1% for the meals, which most likely would not have any effect on the SDA.

The snakes were fed a homogenized mixture of blended mice and bone meal by inserting an oesophageal rubber tube into the stomach lumen through the mouth, so the meal could be injected into the stomach.

To quantify the influence of the added bone meal on the buffer capacities of the meals, we suspended the meals in 100 ml deionized water and measured pH (Mettler Toledo pH combination electrode connected to a Radiometer PHM 62 Standard pH Meter) during titration with 0.2 M HCl.

2.4. Measurements of blood pressure, heart rate and arterial blood samples

Arterial catheters were connected to pressure transducers (PX600; Baxter Edwards, Irvine, CA, USA) that were calibrated daily against a static water column and connected to an in-house built preamplifier. Signals were recorded at 100 Hz using Biopac MP100 data acquisition system (Goleta, CA, USA) and heart rate was derived from the pulsatile arterial pressure.

Arterial blood samples were taken from undisturbed fasted snakes at 12 h intervals for 72 h after ingestion to obtain measurements of pH, PaO_2, $PaCO_2$ as well as Na^+ and K^+ concentrations using a GEM Premier 3500 Blood Gas Analyser (Instrumentation Laboratory; Bedford, MA, USA). Based on direct comparison to Radiometer electrodes, the blood gases were corrected as described by Malte et al., (2014). Haematocrit was determined by centrifuging capillary tubes at 14,700 *g* for 3 min. Plasma [HCO_3^-] was calculated using the Henderson–Hasselbach equation:

$$\left[HCO_3^-\right] = P_aCO_2\left(\alpha CO_2 \cdot 10^{pH-pK'}\right) - (\alpha CO_2 \cdot PCO_2)$$

where αCO_2 is the plasma CO_2 solubility (Heisler, 1984) and *pK'* is derived from a relationship between *pK'* and *pH* in pythons (Overgaard and Wang, 2002).

2.5. Measurement of metabolic rate

The rate of oxygen uptake ($\dot{V}O_2$) was measured using closed-system respirometry. Gas inlets and outlets enabled sampling using a syringe, where fractional oxygen content of dried gas was analysed using a S-3A oxygen analyser and an oxygen sensor N-22M (Ametek; Oak Ridge, TN, USA). The oxygen consumption was calculated by the following equation from Vleck (1987):

$$\dot{V}O_2 = \frac{V_{chamber}\left(F'iO_2 - F'eO_2\right)}{1 - F'eO_2(1 - RQ)}\Delta t^{-1}$$

where $F'iO_2$ and $F'eO_2$ are the initial and final fractions of oxygen in the chamber measured at the start and end of the 30 min interval (Δt) where the chamber was sealed. Gas samples were taken at an interval of 3–8 h for 8 days, with the highest frequency at the expected peak of oxygen consumption. By using a respiratory quotient value of 0.85 the maximal possible error of the calculated oxygen consumption is restricted to ±3.2% (Vleck, 1987). $V_{chamber}$ is the dry air volume of the respirometer when assuming an animal density of 1 kg l^{-1}. $\dot{V}O_2$ was expressed relative to mass and corrected to STPD.

2.6. Measurements of gastrointestinal pH

In 18 snakes (six for each meal type) we measured pH in the gastrointestinal tract at 24 and 48 h after feeding. The snakes were force-fed homogenized meals of 15% body mass with no additional bone or the highest additional bone content as described above and euthanized at either 24 or 48 h. The oesophagus and stomach of the snakes were emptied of chyme and pH in the gastrointestinal fluid was measured using universal pH indicator paper, which allowed us to differentiate 0.5 pH units (Merck; Hellerup, Denmark), placed in the posterior part of the oesophagus, the proximal and distal parts of the stomach as well as the anterior end of the small intestine.

2.7. Data analysis and statistics

Repeated measures were evaluated by one- or two-way ANOVA with a 95% level of confidence interval for statistical significance ($p < 0.05$). Values were compared between treatment groups (control and snakes fed bone enriched meals) and they were compared