Comparative Biochemistry and Physiology, Part A 183 (2015) 36–44

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

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

Food composition influences metabolism, heart rate and organ growth during digestion in *Python regius*

Poul Secher Henriksen, Sanne Enok *, Johannes Overgaard, Tobias Wang

Zoophysiology, Department of Bioscience, Aarhus University, Denmark

ARTICLE INFO

Article history:
Received 10 November 2014
Received in revised form 10 December 2014
Accepted 16 December 2014
Available online 29 December 2014

Keywords:
Cardiovascular
Digestion
Gastric work
Postprandial response
Organ growth
Python regius
Regulation
Specific dynamic action

ABSTRACT

Digestion in pythons is associated with a large increase in oxygen consumption (SDA), increased cardiac output and growth in visceral organs assisting in digestion. The processes leading to the large postprandial rise in metabolism in snakes is subject to opposing views. Gastric work, protein synthesis and organ growth have each been speculated to be major contributors to the SDA. To investigate the role of food composition on SDA, heart rate (HR) and organ growth, 48 ball pythons (*Python regius*) were fed meals of either fat, glucose, protein or protein combined with carbonate. Our study shows that protein, in the absence or presence of carbonate causes a large SDA response, while glucose caused a significantly smaller SDA response and digestion of fat failed to affect metabolism. Addition of carbonate to the diet to stimulate gastric acid secretion did not increase the SDA response. These results support protein synthesis as a major contributor to the SDA response and show that increased gastric acid secretion occurs at a low metabolic cost. The increase in metabolism was supported by tachycardia caused by altered autonomic regulation as well as an increased non-adrenergic, non-cholinergic (NANC) tone in response to all diets, except for the lipid meal. Organ growth only occurred in the small intestine and liver in snakes fed on a high protein diet.

1. Introduction

Animals acquire their energy from food, but to make the ingested chemical energy available for metabolism and growth, they must also expend energy on digestion and assimilation. The rise in energy expenditure associated with digestion and assimilation, termed specific dynamic action (SDA), is particularly profound in sit-and-wait predators, such as pythons, that are exquisitely adapted to consume large meals at irregular intervals (Andrade et al., 2005; McCue, 2006; Secor, 2009). The SDA response is an archetypical integrated physiological response that requires coordination of the digestive and cardiorespiratory systems to secure assimilation, tissue growth, acid-base balance and oxygen delivery. Thus, the more than five-fold increase in oxygen consumption ($\dot{V}O_2$), which may exceed that measured during exercise (Enok et al., 2013; Secor, 1997; Secor and Diamond, 1998; Secor et al., 2000; Wang et al., 2002), is supported by increased heart rate (HR) and stroke volume as well as a redistribution of blood flow to the gastrointestinal organs (Secor et al., 2000; Starck and Wimmer, 2005; Skovgaard et al., 2009; Secor and White, 2010; Enok et al., 2012). The postprandial period is also characterized by a relative hypoventilation to counter the alkaline tide resulting from gastric acid secretion (Andrade et al., 2004; Overgaard et al., 1999; Secor et al., 2000; Wang et al., 2001a), as well as profound changes in size and capacity of the intestine and other visceral organs (Secor, 1995; Starck and Beese, 2001; Ott and Secor, 2007; Cox and Secor, 2008; Helmstetter et al., 2009; Enok et al., 2013; Hansen et al., 2013).

The SDA response is directly proportional to meal size (Andrade et al., 1997; Secor, 1997; Secor and Diamond, 1997; Toledo et al., 2003), and although it is well-known that proteins stimulate postprandial metabolism, while lipid exerts little influence (Secor and Diamond, 1997; Wang et al., 2012), a number of more subtle aspects of food composition remain unknown. Also there continues to be considerable disagreement regarding which processes account for the SDA response in pythons (Enok et al., 2013; Overgaard and Wang, 2002; Secor, 1995; Secor and Diamond, 1998; Wang et al., 2006). Secor (2003) proposed that gastric processes account for more than half of the SDA response, but subsequent studies reached considerably lower estimates (Andrade et al., 2004; McCue et al., 2005; Enok et al., 2013). If gastric acid secretion is energetically expensive, elevated buffer capacity of the meal should increase the metabolic costs of digestion, and hence elevate the SDA response.

While the metabolic effects of protein and lipid have been studied, the influence of food composition on the cardiovascular response and postprandial organ growth remains unknown. The postprandial rise in HR primarily arises from non-adrenergic, non-cholinergic (NANC) stimulation as well as withdrawal of vagal tone (Enok et al., 2012; Skovgaard et al., 2009; Wang et al., 2001b), but it remains to be determined whether

---

* Corresponding author at: Zoophysiology, Department of Bioscience, Building 1131, Universitetsparken, Aarhus University, 8000 Aarhus C, Denmark. Tel.: +45 40626572.
E-mail address: sanneenok@gmail.com (S. Enok).

http://dx.doi.org/10.1016/j.cbpa.2014.12.031

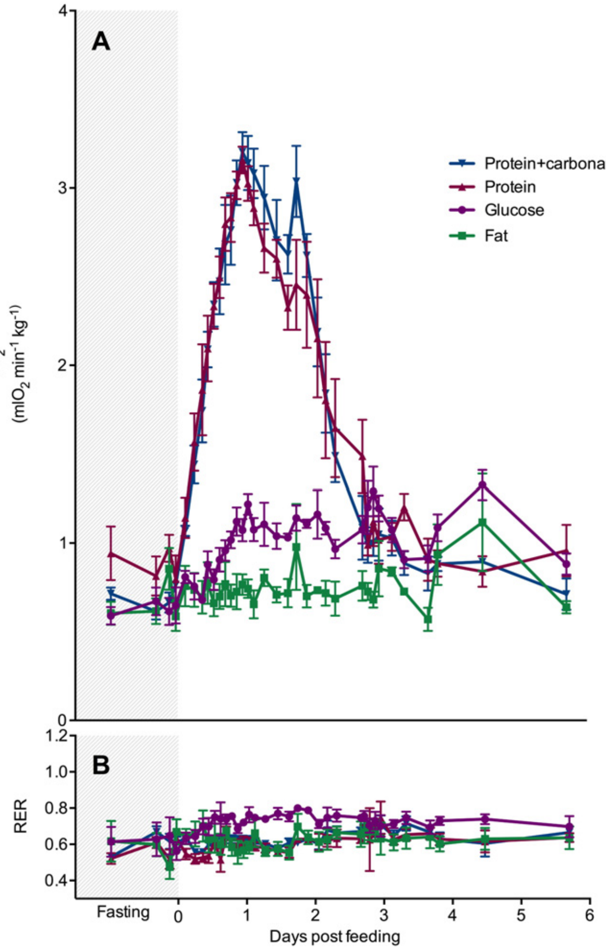

Fig. 1. Oxygen uptake rate ($\dot{V}$ O$_2$) and respiratory exchange ratio (RER) in four groups of *Python regius* during fasting and digestion of different meals. Green is snakes fed fat ($n = 4$), purple is snakes fed glucose (half the caloric content of the other meals) ($n = 4$), red is snakes fed protein ($n = 5$) and blue is snakes fed a protein + carbonate meal ($n = 5$). Data are shown for 2 days prefeeding until 6 days into the postprandial period. Values are mean ± S.E.M.

food composition affect the NANC contribution to the tachycardia in digesting snakes. Though, the influences of meal composition on the postprandial growth of the visceral organs have not been explored, it seems that the small intestine expands by incorporation of lipid droplets (Starck and Beese, 2001; Lignot et al., 2005; Enok et al., 2013) and it was recently suggested that free fatty acids stimulate cardiac growth (Jensen et al., 2011; Riquelme et al., 2011). Thus, it is also relevant to study the influence of meal composition on the postprandial growth of the visceral organs.

The primary objectives of this study are to i) address whether gastric acid secretion is a major contributor to the SDA response, ii) to investigate how food composition affects the NANC regulation of HR, specifically whether protein elicits a larger NANC response than fat and glucose, as is the case with the metabolic response, and iii) to examine the extent to which food composition affects postprandial organ growth, particularly whether lipid absorption alone triggers or causes intestinal growth. We address the contribution of acid secretion by increasing the buffer capacity of the meal by the addition of carbonate to stimulate acid secretion. If the costs of gastric acid secretion are indeed high, the addition must be expected to increase the SDA response. Secondly, we fed snakes with meals of glucose, fat or protein to characterize the autonomic regulation of the heart by injection of appropriate antagonists, and thirdly, we harvested visceral organs to examine the effects of food composition on organ growth.

2. Materials and methods

2.1. Experimental animals

48 ball pythons (*Python regius*, Shaw 1802) with a body mass between 259 and 734 g (442 ± 16 g) were purchased from a commercial supplier and maintained at Aarhus University for several months before onset of the experiments. 18 snakes (382 ± 17 g) were used for respirometry, while the other 30 snakes (479 ± 22 g) were used to measure heart rate and blood pressure before they were sacrificed to allow for weighing of the visceral organs. All snakes were held individually in boxes with a temperature gradient (25–32 °C) and a shelter. They had free access to water and were fasted at least 3 weeks prior to experiments. Experiments were performed in accordance with Danish Federal Regulations.

2.2. Measurement of gas exchange by closed respirometry

Metabolic rate was estimated indirectly by closed respirometry to measure O$_2$ consumption ($\dot{V}\,O_2$) and CO$_2$ production (VCO$_2$) (O$_2$ and CO$_2$ sensors and analyzers from Applied Electrochemistry with a 0.01% accuracy, N-22M, P61B, S-3A/I and CD-3A) and also allows for analysis of gas exchange ratio (RER). Snakes were placed individually in respirometers (3090 ml) and set in a temperature cabinet at 30 °C for one day in order to become accustomed to the respirometers. Resting metabolic rate (RMR) was determined from five measurements over the next two days. After determining RMR, snakes were allocated to one of 4 different feeding regimes. Three groups were fed iso-caloric meals (750 kJ per kg snake) of either lard ($N = 4$), chicken breast ($N = 5$) or chicken breast with carbonate added in the form of carbonate tablets (15 g CO$_3$

Table 1

Resting metabolic rate (RMR), max $\dot{V}$ O$_2$, Scope (max $\dot{V}$ O$_2$/RMR), specific dynamic action (SDA), SDA coefficient (SDA%), fasting and digesting respiratory exchange ratio (RER) presented for the four digesting groups. All values are means ± S.E.M.

		RMR (ml O$_2$ kg^{-1} min^{-1})	Max VO$_2$ (ml O$_2$ kg^{-1} min^{-1})	Scope	SDA (ml O$_2$ kg^{-1})	SDA coefficient (%)	RER fasting	RER digesting
Fat	N = 4	0.65 ± 0.08	1.19 ± 0.14	1.89 ± 0.32	1081 ± 597.1	2.8 ± 1.6	0.59 ± 0.08	0.63 ± 0.05
Glucose	N = 4	0.65 ± 0.03	1.49 ± 0.09	2.30 ± 0.20	2517 ± 389.1	13.2 ± 2.0*	0.61 ± 0.05	0.73 ± 0.02#
Protein	N = 5	0.89 ± 0.09	3.26 ± 0.05*	3.83 ± 0.41	5324 ± 213.6*†	14.0 ± 0.6*	0.57 ± 0.02	0.61 ± 0.02
Protein + carbonate	N = 5	0.73 ± 0.01	3.31 ± 0.14*	4.53 ± 0.16	6308 ± 399.0*†	16.5 ± 1.0*	0.61 ± 0.01	0.63 ± 0.02

* Significant difference from fat.
† Significant difference from glucose.
\# Significant difference from protein.