HHS Public Access

Author manuscript

J Muscle Res Cell Motil. Author manuscript; available in PMC 2024 June 01.

Published in final edited form as:

J Muscle Res Cell Motil. 2023 June ; 44(2): 95–106. doi:10.1007/s10974-022-09632-2.

Utility of the burmese Python as a model for studying plasticity of extreme physiological systems

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Abstract

Non-traditional animal models present an opportunity to discover novel biology that has evolved to allow such animals to survive in extreme environments. One striking example is the Burmese python (*Python molurus bivittatus*), which exhibits extreme physiological adaptation in various metabolic organs after consuming a large meal following long periods of fasting. The response to such a large meal in pythons involves a dramatic surge in metabolic rate, lipid overload in plasma, and massive but reversible organ growth through the course of digestion. Multiple studies have reported the physiological responses in post-prandial pythons, while the specific molecular control of these processes is less well-studied. Investigating the mechanisms that coordinate organ growth and adaptive responses offers the opportunity to gain novel insight that may be able to treat various pathologies in humans. Here, we summarize past research on the post-prandial physiological changes in the Burmese python with a focus on the gastrointestinal tract, heart, and liver. Specifically, we address our recent molecular discoveries in the post-prandial python liver which demonstrate transient adaptations that may reveal new therapeutic targets. Lastly, we explore new biology of the aquaporin 7 gene that is potently upregulated in mammalian cardiac myocytes by circulating factors in post-prandial python plasma.

Keywords

Post-prandial adaption; Extreme biology; Metabolism; Tissue growth; Molecular signaling

Background

The study of non-traditional animal models that have evolved extreme physiological adaptations to survive can uncover novel biology that might be relevant to human health and disease. Examples include humidity sensors developed based on mechanisms of moisture conservation in the nostrils of camels of the Sahara Desert (Li et al. 2021). Similarly, the discovery of a potent glucose-like peptide-1 (GLP-1) homologue in Gila Monster saliva

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Author contributions Y.T. and T.G.M. conceptualized and prepared the draft of the manuscript. L.A.L. edited the manuscript. T.G.M prepared Fig. 1. B.C.H performed the RT-qPCR research experiments and provided data for Fig. 3. Y.T. prepared the remaining figures and table. All authors contributed to literature search and to writing of the subsequent versions of the manuscript.

Conflict of interest There are no relevant conflicts of interest.

Ethical approval The authors declare that this paper complies with ethical standards in publishing.

was developed as a therapeutic for Type 2 diabetes because of its much longer half-life than human GLP-1 (Christel and DeNardo 2006). The Burmese python (*Python molurus bivittatus*) is another example of a model with extreme biology that has been employed by scientists over the past 20 years as a unique model organism to investigate the mechanisms underlying their rapid and massive responses to fasting and feeding (Secor and Diamond 1997a; Andersen et al. 2005; Riquelme et al. 2011).

Burmese pythons are among the largest snake species in the world. To conserve energy, pythons evolved as ambush predators but remain sedentary for most of their existence. When they do eat, pythons consume large meals equal to 25–100% of their body mass, though in some cases greatly exceeding it (Wall 1912; Secor and Diamond 1998). These large meals are typically followed by periods of fasting, which may extend over a year when prey is scarce (Benedict 1933; Secor and Diamond 1998). This feeding cycle of massive, irregular meals in ambush hunting reptiles is accompanied by periods of heightened and near-absent metabolic activity, the magnitude of which is unmatched in other vertebrates (Secor and Diamond 1997a). Due to the large size of the python's meal, most of its organs undergo substantial hyperplasia, hypertrophy or a combination of both in the days following a meal to help digest the contents of the meal (Secor and Diamond 1995; Starck and Beese 2001; Andersen et al. 2005; Lignot et al. 2005; Riquelme et al. 2011; Wang and Rindom 2021). Notably, this growth is completely reversible, and organs return to just above their fasting size after the meal is digested (Secor 2008). With its extreme physiology, the Burmese python is a valuable model to investigate questions in metabolic and organ plasticity.

In this review, we discuss the unique post-prandial changes in metabolism and organ structure/function in Burmese pythons at a global physiological level. While less is known regarding the molecular differences between fed and fasted pythons, we highlight our recent study investigating post-prandial python liver. We also provide new data from our group on translating python biology to mammals including the role of the transmembrane protein channel aquaporin-7 (AQP7) in rodent cardiomyocytes and hearts.

Extreme physiology of the burmese python

Post-prandial metabolism

To conserve energy and survive bouts of caloric deprivation, pythons maintain extremely low basal metabolic activity. This is accomplished, in part, by regression in the sizes of most python organs during fasting, accompanied by minimized cardiopulmonary activity to limit energy expenditure (Secor and Diamond 1997a; Hicks and Bennett 2004). However, upon consuming a meal, the python metabolic rate increases by as much as 40-fold and remains elevated for up to two weeks (Secor and Diamond 1997a). In comparison, this striking metabolic surge in a post-prandial python is of the same magnitude of a thoroughbred race-horse going from rest to a full sprint but this increase in the snake is sustained for 6–9 days (Weber et al. 1987; Secor and Diamond 1997a). The most common meal size in the wild, and that most often used experimentally, is 25% of the python's body weight preceded by a 28 day fast (Secor and Diamond 1998) (Fig. 1 A). The first 36 h following ingestion of a meal are characterized by a rapid increase in oxygen consumption, which matches the rate when the python moves at its maximum speed of 1.5 km/hour (Secor et al. 2000a; Hicks

and Bennett 2004). Oxygen consumption then gradually declines to fasted rates by one to two weeks post-feeding (Wang et al. 2002; Starck et al. 2004; McCue et al. 2005; Wang and Rindom 2021). In parallel with increased oxygen consumption, Burmese python organs (except for the brain) also undergo massive increases in size, including the intestines, liver, kidneys, pancreas, lungs and heart in the first 72 h following meal ingestion (Andersen et al. 2005; Lignot et al. 2005; Andrew et al. 2017). Organ sizes then regress at a slower rate beginning 4 days post-feeding (DPF), and following defecation around day 8–10DPF marks the end of digestion (Secor and Diamond 1995). The post-prandial increase in organ size and metabolic activity that occur periodically in the Burmese python are unparalleled in magnitude or timescale by any mammalian species.

The cost of digestion: specific dynamic action—The ingestion and digestion of large intact prey by Burmese pythons is associated with substantial energy cost as organ size and activity must be rapidly upregulated. The energy expenditure required to process a meal is termed specific dynamic action (SDA) (Rubner 1902; McCue 2006). The SDA for Burmese pythons eating meals equal to 25% of their body mass was measured at ~ 450 kJ/kg, approximately 25% of the ingested energy, though SDA increases with larger meals (Secor and Diamond 1997b; Ott and Secor 2007; Cox and Secor 2007). By comparison, the average SDA for frequently feeding species like mammals is ~ 10% (McCue 2006). While the energetic cost of a meal is considerable in pythons, their generally sedentary lifestyle involves little energy expenditure for foraging. In fact, studies estimate SDA in ambush hunting snakes accounts for ~ 30% of total annual expended energy (McCue and Lillywhite 2002).

Considering the high energetic payout to accomplish digestion, researchers coined the relationship as the ‘pay-before-pumping’ effect, an analogy to the common mechanism of automobile fueling in the United States (Secor and Diamond 1995). Several factors have been proposed as the primary consumers of energy during digestion, including gastric acid production, protein synthesis, and increased organ metabolic activity (Secor 2003; McCue et al. 2005). The exact contribution of gastric acid production to SDA is not clear. One study suggested it accounted for up to 55% of python SDA (Secor 2003). However, a study of the *Boa constrictor* snake, a close relative of pythons, found that SDA was not reduced when gastric acid production was blocked with the proton pump inhibitor omeprazole (Andrade et al. 2004). Work by McCue and colleagues suggests that increased *de novo* protein synthesis, instead of gastric acid production, is the most energetically demanding process, accounting for 70% of SDA (McCue et al. 2005). Regardless of which process is the primary energy sink, that such a large amount of energy is required to digest a meal in a predator that has just undergone an extended fasting period seems inefficient. Interestingly, a study of another closely related species, the Ball python (*Python regius*), found that these predators do not withdraw from their own energy stores to pay off the debt. Instead, Ball pythons use energy from their digesting prey to fuel metabolism (Starck et al. 2004). Given these findings, the analogy between python SDA and automobile fueling may be better revised to ‘pay-while-pumping’.

Factors influencing post-prandial metabolism—As alluded to above, several factors can impact the size and duration of the post-prandial metabolic response in pythons. Foremost among these is meal composition. McCue and colleagues compared the impact of lipid, carbohydrate, and protein-rich meals from multiple sources on SDA in Burmese pythons. They found that meals containing complete protein (containing all essential amino acids) elicited the largest SDA, while simple proteins like gelatin or starch and incomplete amino acid mixtures failed to elicit the same response (McCue et al. 2005). Others have also shown that the complete intestinal response is only activated by protein-heavy meals (Secor et al. 2002). The size of the meal also influences the metabolic response. Studies have shown that increasing meal size is associated with increased peak oxygen consumption and SDA (Secor and Diamond 1997b; Cox and Secor 2007). Of note, whether pythons fast for three days or 60, the same metabolic response to a meal is observed, and refeeding during an ongoing digestion triggers an even larger post-prandial SDA (Overgaard et al. 2002). The frequent feeding behavior of invasive pythons in the Florida Everglades has been posited to protect against die-off from freezing by maintaining a high metabolic rate (Card et al. 2018). Changes in environmental temperature do impact the length of digestion, with higher temperatures leading to shorter digestion times. However, temperature itself has no effect on SDA (Wang et al. 2002).

Post-prandial organ growth and functional remodeling

Due to the rapid timescale, extent of organ growth, and near-complete reversibility, the python represents an excellent animal model for deciphering the mechanisms of organ remodeling (Fig. 1B). In this section, we discuss the gastrointestinal and cardiopulmonary changes that occur during digestion, which have been the most extensively researched to date.

Post-prandial gastrointestinal response

In the fasted state, the pH of the python stomach is neutral, with gastric acid production inhibited to conserve energy. Once the feeding starts, the stomach is flooded with hydrochloric acid, and the pH quickly drops below 2, where it remains for over a week before climbing to neutral again by 15DPF (Secor 2003; Bessler and Secor 2012) (Fig. 1 C). The intestines undergo the greatest magnitude of remodeling of any organ during digestion, with the small intestine more than doubling in mass (Holmberg et al. 2002; Ott and Secor 2007). This organ growth is specifically driven by changes in the mucosal layer (Cox and Secor 2008). Along with massive growth, intestinal blood flow and oxygen consumption increase by 8 and 4-fold, respectively within 24 h following a meal (Secor 2005; Secor et al. 2012). Additionally, the intestinal microvilli, which almost completely atrophy during fasting, increase nearly 5-fold in length during digestion (Secor et al. 2000b; Starck and Beese 2001) (Fig. 1 C). By comparison, humans and other frequently feeding species display little or no change in microvillus morphology during or between meals (Secor 2005). This increased surface area paired with an up to 10-fold increase in intestinal enzyme activity (including maltase and aminopeptidase-N) synergize to maximize nutrient uptake (Ott and Secor 2007; Cox and Secor 2008; Secor 2008). For example, intestinal glucose and amino acid uptake increase up to 15-fold by 1DPF (Secor et al. 2000b). Like other features of digestion,

enzyme activities and nutrient uptake peak at 3DPF and return to baseline around 10DPF (Cox and Secor 2008) (Fig. 1 C). In addition to the remodeling of host cell morphology and activities, the python gut microbiome undergoes a bacterial community shift with feeding (Costello et al. 2010). The specific involvement of the microbiome in the post-prandial responses requires further investigation.

Post-prandial cardiopulmonary response

To meet the increased tissue demand for oxygen during digestion, the Burmese python must rapidly increase both respiration and cardiac output. During the first 8 h of digestion, python breathing frequency increases by almost 5-fold (Secor et al. 2000a). These ventilation rates are maintained during the subsequent 2 days of digestion (Secor et al. 2000a). Pulmonary tidal volume is decreased or unchanged at 1DPF and then climbs to a peak, ~ 40% higher than fasted levels at 2.5DPF (Secor et al. 2000a). Interestingly, despite the increased ventilation during the early phases of digestion, pythons experience a relative hypoventilation and respiratory acidosis due to the massively increased metabolic CO_2 production (Secor et al. 2000a; Hicks and Bennett 2004; Wang and Rindom 2021). Arterial PCO_2 levels increase by 20% to a peak of ~ 27 mmHg at 1DPF and then progressively decrease back to fasted levels by 4DPF (Overgaard et al. 1999). Unlike in mammals where increased arterial PCO_2 is associated with reduced PO_2, arterial levels of both gases increase in the fed python (Overgaard et al. 1999; Wang et al. 2001; Hicks and Bennett 2004). This anomalous relationship may occur through a combination of intraventricular shunting due to the unique anatomy of the reptile heart, which consists of two atria and a single anatomically undivided ventricle (Hicks et al. 1996; Starck et al. 2005; Jensen et al. 2010), and increased blood oxygen affinity in the fed state (Overgaard and Wang 2002). Increased blood oxygen is essential in the fed state with the elevated oxygen demands of organs working to digest the meal.

Accompanying elevated blood oxygen is an increase in cardiovascular function. Work by the Secor laboratory showed that python heart rate and stroke volume increase by 300–400% and 50%, respectively in the fed state, leading to a ~ 400% increase in cardiac output (Secor and Diamond 1995; Secor et al. 2000a; Secor and White 2010) (Fig. 1D). Increased heart rate arises due to both release of vagal tone on the heart and increased circulating histamine released by mast cells in the 24 h following a meal (Enok et al. 2012). This positive chronotropic effect of histamine is mediated through cardiac H2 receptors (Skovgaard et al. 2009). The mechanism of histamine release during digestion is unknown but is not associated with levels of the peptide hormones gastrin and cholecystokinin (Enok et al. 2012). Despite the massive increase in cardiac output, python blood pressure only marginally changes during digestion as total peripheral resistance decreases by nearly the same magnitude (Wang and Rindom 2021).

Like most other python organs, the heart undergoes reversible physiological hypertrophy during digestion (Fig. 1D). Multiple studies show that the python heart increases in mass by ~ 40% (Andersen et al. 2005; Secor 2008; Riquelme et al. 2011). Importantly, this growth is not due a shift in water between the intracellular and extracellular compartments as fed python dry ventricle mass displays the same magnitude of increase over fasted

(Andersen et al. 2005). Cardiac hypertrophy is associated with increased expression of genes of the sarcomere, the molecular unit of contraction in muscle cells (Andersen et al. 2005; Riquelme et al. 2011). This suggests that the cell growth occurs through increased synthesis of sarcomere proteins, which account for ~ 80% of myocyte mass (Hoppeler and Flück 2002). In contrast, other studies report only increased stroke volume, heart rate and VO_2, but no cardiac hypertrophy in post-prandial pythons (Enok et al. 2013, 2016a). As to what triggers cardiac growth in the python, one study showed that the hypertrophic response of the heart to digestion is linked to oxygen supply/demand mismatch (Slay et al. 2014). Work from our group and others over the last decade has begun to unravel the molecular mechanisms of post-prandial organ remodeling in the Burmese python, which we discuss in the next section.

Translating burmese python biology to mammals: post-prandial molecular signaling

Molecular signaling in post-prandial hepatic adaptation

Liver is the principal tissue in coordinating metabolic homeostasis, including regulating the synthesis, storage, and redistribution of nutrients such as carbohydrates, lipids, and vitamins. As metabolic diseases including obesity and Type II Diabetes disrupt the overall balance of metabolic homeostasis, these conditions pose severe metabolic perturbations and can trigger progressive pathologies and permanent damage in liver such as non-alcoholic fatty liver disease (Lonardo et al. 2005). In mammalian models, researchers have learned that promoting liver function by reversing hepatic steatosis and suppressing hepatic gluconeogenesis represents an effective therapeutic strategy for many metabolic diseases (Petersen et al. 2005; Wada et al. 2010).

After consuming a large meal, the Burmese python liver increases wet weight by 53% at 3DPF (Secor and Diamond 1995). By comparing the size of a single meal consumed by the Burmese python to frequently fed mammals, the post-prandial circulating nutrients in Burmese python plasma would represent a dramatic nutrient overload for any mammalian species. Thus, the Burmese python represents a fascinating model organism to identify naturally evolved solutions that may overcome pathological metabolic processes in mammals. However, only a few studies have investigated the post-prandial response of the python liver and the importance of this organ in metabolic homeostasis.

To study the integrated post-prandial metabolic responses of Burmese python tissues, an RNA-Seq analysis of 4 metabolic organs, including heart, kidney, liver, and small intestine, was reported. This study identified significantly differentially regulated genes: 722 genes in the heart, 750 genes in the kidney, 711 genes in the liver, and 1284 genes in the small intestine between fasted, 1DPF and 4DPF states. Specifically, bioinformatics analysis predicted involvement of NRF2, mTOR, Akt, LXR/RXR and PPAR pathways in the post-prandial response of the liver (Andrew et al. 2017). Another liver transcriptome analysis of the Burmese python liver identified upregulated expression of apolipoproteins and albumin, which indicate increased lipid metabolism (Duan et al. 2017). They also reported

increased gene expression associated with an antioxidant response, which is consistent with bioinformatic prediction of the NRF2 pathway activation (Andrew et al. 2017).

Recently, our group provided evidence for an adaptive response to post-prandial nutrient overload by the python liver and described the digestive molecular response in the python liver that is unparalleled in meal size or metabolite spike magnitude in mammals (Magida et al. 2022). Besides the dramatic > 100-fold increase in plasma triglyceride (TG) (Secor and Diamond 1998; Riquelme et al. 2011), there are many other metabolites circulating in plasma that likely facilitate post-prandial nutrient clearance. We defined the circulating metabolites profile in fasted versus post-prandial plasma and identified a massive increase of circulating bile acids and fatty acids which are responsible for a transient but potent activation of hepatic nuclear receptors including PPAR and FXR. They coordinate hepatic uptake, trafficking, and catabolism of fats, bile acids and cholesterol (Kersten et al. 2000). Burmese pythons have evolved to have well-conserved PPARα functional sites as have many other species, and this allows them to regulate fatty acid clearance and oxidation via canonical PPARα signaling. Indeed, PPARα target genes including SCD, ACAA2, CD36 and CPT2 are quickly and robustly activated at early digestion stages (1DPF and 3DPF) in response to the surging levels of circulating lipids in plasma, and then regress in the late digestion stages (Magida et al. 2022). In addition to transient metabolic gene activation in python liver, the p38 MAPK stress pathway is also transiently activated at 1DPF and then inactivated during late digestion.

Usually, activation of p38 is associated with apoptosis (Canovas and Nebreda 2021) whereas a protective role of p38 has been described in hepatocytes (Flach et al. 2011; Lee et al. 2011; Hwang et al. 2020). A genetic model of obesity (*ob/ob* mice) and high fat diet-fed obese mice showed reduced hepatic p38 MAPK phosphorylation. p38 activation by constitutively active MKK6 expression in obese and diabetic mice reduced endoplasmic reticulum (ER) stress and maintained euglycemia (Lee et al. 2011). Another study focusing on MKP-1, a phosphatase of p38, also demonstrated that MKP-1 negatively regulates p38 activation and TG metabolism in the liver (Flach et al. 2011). A more recent study further supported the beneficial role of p38 in hepatocytes. When hepatocytes undergo mild stress such as a high fat diet or early stages of hepatic steatosis, activation of p38 promotes fatty acid metabolism by facilitating β-oxidation, thereby reducing TG accumulation in the liver (Hwang et al. 2020).

Some responses that were seen in the heart were also observed in liver during the early stages of digestion. These include upregulated lipid oxidation and antioxidant responses (Riquelme et al. 2011; Andrew et al. 2017; Magida et al. 2022) (Table 1). Cardiac myocytes undergo cellular hypertrophy, while the post-prandial liver also undergoes a substantial increase in mass through hyperplasia (Magida et al. 2022). In contrast to the heart, the python liver also exhibits transient hepatic steatosis, hyperlipidemia-induced insulin resistance indicated by PEPCK activation and AKT deactivation, and de novo fatty acid synthesis via FASN activation (Magida et al. 2022). These hepatic conditions are consistent with what has been seen in diabetic mice and in patients with obesity and NAFLD that manifest selective hepatic insulin resistance: insulin fails to block gluconeogenesis (i.e., no PEPCK suppression), but continues to stimulate lipogenesis (i.e., enhanced FASN

activation) (Brown and Goldstein 2008; Li et al. 2010; Vatner et al. 2015). Interestingly, reversal of insulin resistance is often observed in patients who undergo bariatric surgery (Stenberg and Thorell 2020). This clinical observation suggests that, like the python, insulin resistance may be reversible in humans under some conditions. However, the molecular mechanisms for reversibility remain unknown.

Finally, the liver is only one of the many metabolic organs in the Burmese python that exhibits dramatic post-prandial responses. Adipose tissue is likely to play an essential role in plasma lipid clearance and storage. We observed increased amounts of visceral adipose tissues, otherwise known as fat bodies in the post-prandial python abdominal cavity compared to fasted pythons (unpublished). Apart from the visceral fat in the abdominal cavity, we found that the post-prandial python heart is surrounded by adipose tissue as well, and we define this fat cluster as cardiac adipose tissue (Fig. 2). Both cardiac and visceral adipose tissues are likely to secret crucial adipokines and lipokines that contribute to the metabolic homeostasis during feast in pythons. The NRF2-mediated oxidative stress response pathway is predicted to be strongly activated in different tissues including the small intestine, liver, heart, and kidney (Andrew et al. 2017). This common pathway may suggest potential factor(s) circulating in plasma is shared. Many more studies need to be conducted on inter-organ signaling.

Molecular signaling in post-prandial cardiac adaptation

The type of beneficial cardiac remodeling seen in the Burmese python can only be achieved in humans after long-term exercise or during pregnancy. In addition to the rapid cardiac growth in post-prandial Burmese python, the fast regression in heart size during late digestion is also very interesting to investigate as we lack efficient ways of inducing the regression of pathological cardiac hypertrophy. Identifying essential cellular pathways regulating beneficial cardiac growth and following regression in Burmese python may offer therapeutic targets to combat cardiac diseases.

Previous work in our group demonstrated that the post-prandial cardiac hypertrophy in Burmese python is achieved by promoting PI3K/AKT signaling. In addition, the post-prandial python heart potently activates the transport and metabolism of fatty acids through activation of oxidation, paired with a strong antioxidant response (Riquelme et al. 2011). A hallmark of heart failure in humans is a transition from fatty acid to glucose metabolism (Doenst et al. 2013). Therefore, understanding how to promote fatty acid metabolism in human heart failure could be of significant therapeutic benefit. A combination of 3 distinct fatty acids (FAs) were identified in the post-prandial python plasma, including myristic (C14:0), palmitic (C16:0) and palmitoleic (C16:1). When these FAs or fed python plasma are put into the culture media of rat cardiac myocytes, they promote cellular hypertrophy. When they are infused into either fasted pythons or mice, they promote beneficial cardiac hypertrophy without triggering signs of pathological changes such as cardiac fibrosis (Riquelme et al. 2011).This demonstrates that this aspect of python biology can be translated to mammals.

To understand how the 3 FAs promote beneficial cardiac remodeling in mammalian models, comparison of gene expression profiles from neonatal rat ventricular myocytes (NRVMs)

treated with post-prandial plasma showed increased cell size and revealed the gene encoding AQP7 to be one of the most potently upregulated gene compared to cells treated with plasma from fasted snakes (Fig. 3 A). AQP7 was also upregulated in mice infused with the 3 FAs (Riquelme et al. 2011). AQP7 is a member of the aqua-glyceroporin family, known to regulate organ homeostasis. Human loss of function AQP mutations can cause a variety of diseases (Verkerk et al. 2019). AQPs 1, 4, 6, 7, 9 and 11 are expressed in the mouse heart, and APQs 1, 4, 7 and 11 are commonly found in the hearts of different mammalian species (Egan et al. 2006; Rutkovskiy et al. 2013; Tie et al. 2017). However, AQP7 is the only AQP induced by FA treatment or post-prandial plasma in NRVMs (Fig. 3B). We have shown that this is specific to cardiac myocytes and there is no response observed in cardiac fibroblasts treated with FAs (Fig. 3 C). Studies have shown that AQP7 is involved in glycerol transport and metabolism in the heart (Gambert et al. 2005, 2007; Skowronski et al. 2007; Hibuse et al. 2009). Despite the rather high level of induction of AQP7 in the FA treated NRVMs, its function in the cardiomyocyte remains unknown.

Global null AQP7 mice are severely obese, diabetic and have a 50% lower survival rate in the face of a pathological cardiac stimulus (Hibuse et al. 2009). This suggests a potential cardioprotective role of AQP7. Indeed, we found increased AQP7 expression in two models of physiological cardiac hypertrophy: exercise and pregnancy (Fig. 3D, E), but decreased expression in two models of pathological cardiac hypertrophy: hypertrophic cardiomyopathy and thoracic aortic banding (Fig. 3 F, G). Similar induction of AQP7 has been observed in mice in response to exercise, a high-protein diet, and a combination of both (Palabiyik et al. 2016). Additionally, adult rat ventricular myocytes (ARVMs) show higher levels of AQP7 expression under basal conditions compared to NRVMs, which may indicate that the FA cocktail treatment may promote the maturity of NRVMs and promote fatty acid oxidation (Fig. 3 H). Unfortunately, due to the lack of a cardiomyocyte-specific AQP7 null mouse model, we have not been able to determine how the loss of AQP7 in cardiomyocytes specifically alters cardiac phenotypes and function.

Conclusions and future perspectives

Together, evolution and the environment have allowed Burmese pythons to develop extreme physiological adaptations to adapt to either fasting for up to a year or consuming a meal larger than their own body weight. Mammalian species have evolved differently to consume multiples meals and go through multiple digestion cycles per day. When this cycle is perturbed chronically, pathologies can appear in various metabolic tissues. Burmese pythons exhibit extreme metabolic responses when consuming a large meal which provides scientists the opportunity to study metabolism under extreme condition and on an expanded time scale.

Multiple metabolic essential organs experience reversible post-prandial growth in the Burmese python. Secor and Diamond led the way of studying their extreme physiology starting in the 1990s. They paved the way to investigate this non-traditional animal model. Our group and others have established that the Burmese python is a good animal model to study its organ growth at the molecular level including the liver and heart. We demonstrated the post-prandial growth in python liver is a result of hyperplasia, which is different

from the hypertrophic response in the python heart (Riquelme et al. 2011; Magida et al. 2022). Similar pathways such as AKT signaling have been investigated in both organs and exhibited distinct responses: the AKT signaling pathway is activated in the heart to promote post-prandial cardiac hypertrophy at 1DPF, whereas AKT signaling is deactivated at the same time as FASN activation in 1DPF python liver, which reflects transient selective hepatic insulin resistance in the liver (Riquelme et al. 2011; Magida et al. 2022).

Since post-prandial python tissues show some distinct responses, more studies are needed to unveil tissue-specific processes including adipose, kidney and skeletal muscle to understand the integrated post-prandial responses in pythons. Adipose tissue is a crucial site to store plasma lipids as an energy reservoir, which is likely to play a significant role in supporting the long period of famine and the energy burst in promoting post-prandial organ growth in pythons. Does the cardiac adipose tissue play a distinct role from the visceral adipose tissue during post-prandial metabolic homeostasis? Likewise, the kidney is the organ that participates in control of the homeostasis of body fluids, osmolality, acid-base balance, and electrolytes and is therefore likely to be uniquely regulated in post-prandial pythons. How does the python kidney manage the nutrient surge such as the circulating TG during the early digestion stage? Skeletal muscle metabolizes glucose in mammalian species, but we did not observe any post-prandial glucose surge in post-prandial python plasma. What role does python skeletal muscle play in glucose metabolism?

While mammalian species do not have the same signaling response to meals that is seen in post-prandial python, the FA induced physiological cardiac hypertrophy in mice and NRVMs indicates that mammalian species can respond to bioactive molecules from pythons. To identify unique cellular signaling molecules, various models have been developed both in vitro and in vivo for mammalian species. However, these traditional approaches such as genetic manipulation and drug interventions are very difficult to perform in snakes due the lack of pharmacological data. Primary adult cardiac myocytes can usually only be maintained for very limited time in cell culture. Even with these limitations, there is still much more to be learned from python cardiac tissue. For example, the fasted python heart is significantly more fibrotic than a healthy mammalian heart (~ 18% vs. ~1–2%) and it remains unchanged in the post-prandial state (Riquelme et al. 2011). This level of physiological fibrosis could potentially be used as a model to study how python cardiomyocytes have evolved to function in what would be pathological conditions for the mammalian heart. Additionally, generating iPSCs from Burmese pythons will offer a much better system to study cellular signaling responses. Snake venom gland organoids have been successfully developed from cape coral snakes (*Aspidelaps lubricus*) using a modified mammalian organoid protocol, and these “mini venom glands” can produce functionally active venom (Post et al. 2020). Therefore, conserved stem cell signaling in snakes holds the potential to generate iPSCs lines for Burmese pythons and will allow us to dissect their mechanisms underlying their cellular responses by allowing for interventions with small molecules. In conclusion, this review offers a summary of the post-prandial extreme biology in Burmese pythons and points to the potential to translate snake biology to mammalian species. This path is not without its obstacles, but we think it is important to continue studying Burmese pythons to discover new biology.

Acknowledgements

We acknowledge Kristen Bjorkman for analyzing microarray data and Adam Huckaby for RT-qPCR analysis at the BioFrontiers Institute, University of Colorado Boulder.

Funding

This work was supported by NIH R01 GM29090 (L.A.L) and NIH T32 HL007822 (T.G.M).

Data Availability

The dataset generated to support the findings of the current study are available from the corresponding author upon reasonable request.

List of abbreviations

ACAA2	acetyl-CoA acetyltransferase 2
AKT	AKT serine/threonine kinase 1
AQP7	aquaporin 7
CD36	cluster of differentiation 36
CPT2	carnitine palmitoyltransferase 2
FASN	fatty acid synthase
FXR	farnesoid X receptor
LXR	liver X receptor
MKP1	mitogen-activated protein kinase phosphatase 1
mTOR	mammalian target of rapamycin
NAFLD	non-alcoholic fatty liver disease
NRF2	nuclear factor erythroid 2-related factor 2
PEPCK	phosphoenolpyruvate carboxykinase
PI3K	phosphoinositide-3 kinase
PPAR	peroxisome proliferator-activated receptor
P38	p38 mitogen activated kinase
RXR	retinoid X receptor
SCD	stearoyl CoA desaturase

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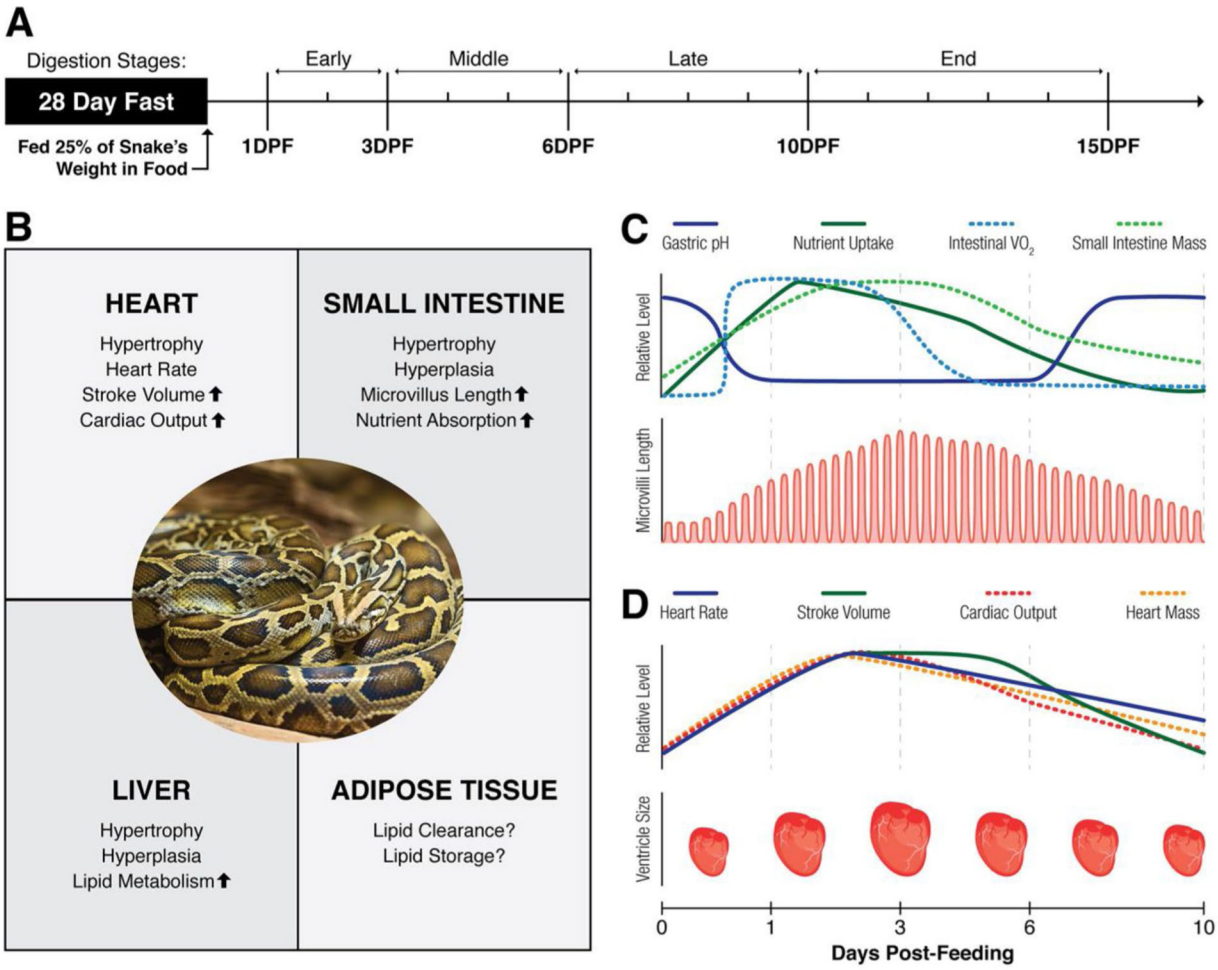

Fig. 1. Post-prandial changes in different Burmese python organs

(A) Typical experimental feeding paradigm and digestion stages in the post-prandial Burmese python. (B) Established post-prandial changes in the Burmese python heart, small intestine, and liver, whereas the function of adipose tissue requires further investigation. (C) Structural and functional changes in the gastrointestinal tract throughout digestion (Secor 2003, 2008; Cox and Secor 2008). (D) Structural and functional changes in the heart throughout digestion (Secor 2008; Secor and White 2010)

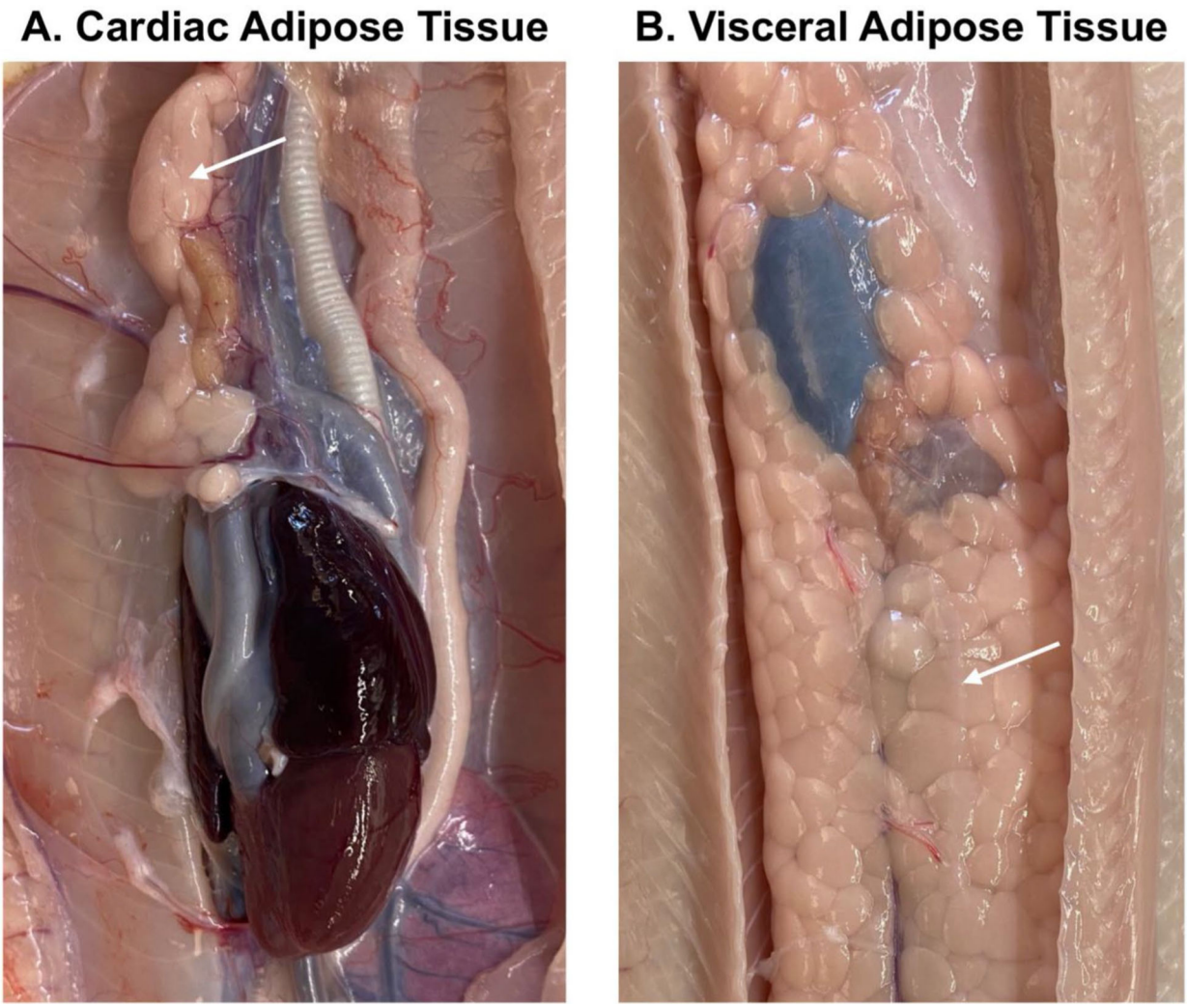

Fig. 2. Adipose tissues in the Burmese python

(A) Cardiac adipose tissue. (B) Visceral adipose tissue

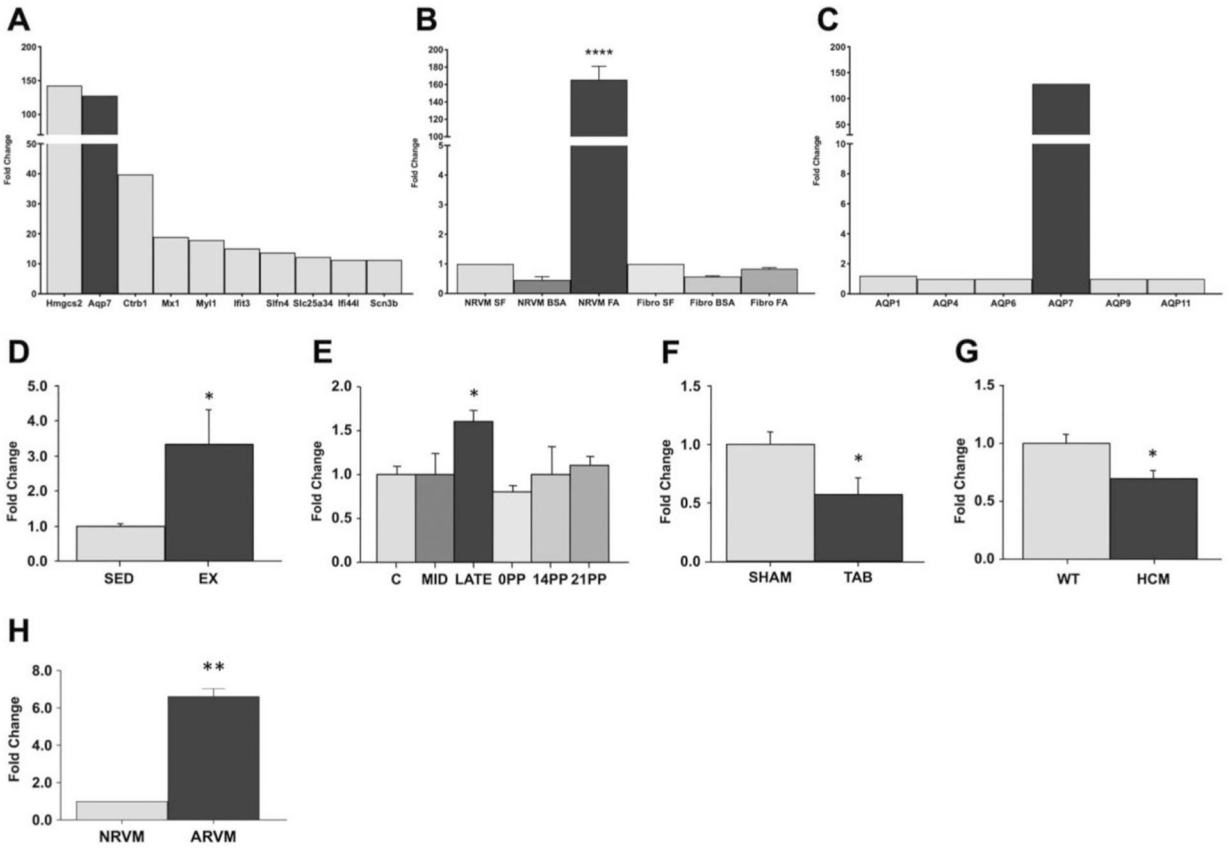

Fig. 3. AQP7 is induced in cardiac myocytes by python plasma and fatty acids and is oppositely regulated in physiologic and pathologic hypertrophy

(A) AQP7 is one of the tops differentially expressed genes in NRVMs treated with post-prandial python plasma. (B) AQP7 is the only upregulated AQP in NRVMs treated with post-prandial python plasma. (C) The response to FA/plasma cocktail treatment is specific to NRVMs and not cardiac fibroblasts. qPCR for AQP7 gene expression is normalized to 18s. D-E) AQP7 expression is upregulated in both physiological cardiac hypertrophy models: exercise and pregnancy qPCR for AQP7 gene expression is normalized to 18s. F-G) AQP7 expression is downregulated in both pathological cardiac hypertrophy models: TAB and HCM. qPCR for AQP7 gene expression is normalized to 18s. H) ARVM express higher levels of AQP7 compared to NRVM. qPCR for AQP7 gene expression is normalized to HPRT. Data are represented as mean ± SEM. n = 3/group (C, H). n = 6/group (D-G) *$p \leq 0.05$, **$p \leq 0.01$, ****$p \leq 0.0001$ versus serum free (SF) or wild type (WT). Neonatal rat ventricular myocytes (NRVM), adult rat ventricular myocytes (ARVM), bovine serum albumin (BSA), fatty acids (FA), fibroblast (fibro), sedentary (SED), exercise (EX), control (C), postpartum (PP), thoracic aortic banding (TAB), hypertrophic cardiomyopathy (HCM)

Table 1

post-prandial cellular signaling during early digestion stage in the python (Riquelme et al. 2011; Andrew et al. 2017; Magida et al. 2022)

	Growth	Metabolism	Others
Heart		CD36 ↑	
	p-Akt ↑	mFABP ↑	
	P-GSK3β ↑	CPT1B ↑	SOD2 ↑
	p-mTOR ↑	MCAD ↑	
		ECHD ↑	
		ACAA2 ↑	
Liver		FXR ↑	
		CD36 ↑	NRF2 ↑
	PCNA ↑	CPT1B ↑	pp38 ↓
	p-Akt ↓	MCAD ↑	
		ACAA2 ↑	
		FASN ↑	