The Journal of Experimental Biology 213, 1266-1271

doi:10.1242/jeb.033662

Fuel switching and energy partitioning during the postprandial metabolic response in the ball python (*Python regius*)

Stefan Waas[1], Roland A. Werner[2] and J. Matthias Starck[1,*]

[1]Department of Biology–Zoology, University of Munich (LMU), Großhadernerstr. 2, D-82152, Planegg-Martinsried, Germany and [2]Institut für Pflanzenwissenschaften, ETH Zürich, LFW C48.1, Universitätsstrasse 2, CH-8092, Zürich, Switzerland

*Author for correspondence (starck@uni-muenchen.de)

Accepted 6 January 2010

SUMMARY

Digestion, absorption and assimilation of the meal are active processes that require start-up energy before the energy contained in a meal can be utilized. The energetic costs associated with feeding (specific dynamic action, SDA) are high in sit-and-wait foraging snakes that tolerate long fasting periods. We used ^{13}C-labelled prey to partition between endogenous energy sources (i.e. snakes own resources) and exogenous energy sources (i.e. prey). A linear mixing model was then applied to determine the portion of ^{13}C originating from the different sources. The snakes showed a normal and typical postprandial response. By four hours after feeding, the $\delta^{13}C$-values indicated fuel switching from endogenous to exogenous. From then on, fuel mixing continuously increased until, at 20 h after feeding, 75% of fuel was exogenous. Resource partitioning showed that throughout SDA, the amount of exogenous energy increased to ~60% of SDA, which was equivalent to ~4.5% of the energy contained in a meal.

Key words: specific dynamic action, stable isotope ratio analysis, sit-and-wait foraging snake.

INTRODUCTION

Animals feed to acquire energy. However, before the energy contained in a meal becomes accessible to fuel the metabolism and the activities of an animal, the food must be digested, and nutrients must be absorbed and assimilated. Digestion, absorption and assimilation are active processes that require energy, initially from the animal's endogenous energy resources. This energy investment manifests itself as the specific dynamic action (SDA) and is measured as the increase in metabolic rate after the ingestion of a meal (Benedict, 1932). In endothermic mammals and birds, SDA is a relatively small portion of their daily energy budget but in ectothermic sauropsids, amphibians and fish it can amount to a remarkable portion of the daily energy budget (McCue, 2006; Overgaard et al., 2002; Wang et al., 2006). Measured SDA coefficients [i.e. the ratio of SDA (kJ) to the energy ingested] range between 4% and 17% of the energy content of a consumed meal in mammals (Gerth et al., 2009; McCue, 2006; Westerterp, 2001) and between 10% and 32% in ectothermic sauropsids (McCue, 2006; Wang et al., 2006). At the extreme, maximum values of 44 times standard metabolic rate (SMR) have been reported for SDA in Burmese python (Secor and Diamond, 1997; Secor and Diamond, 1998). However, the amplitude and the duration of SDA are modulated by the amount of food, the temperature, the time since last feeding and the food composition (Dorcas et al., 1997; McConnachie and Alexander, 2004; Secor and Diamond, 1997; Tattersall et al., 2004; Toeldo et al., 2003; Zaidan and Beaupre, 2003) and, generally, SDA reaches peak values of 10–17 times SMR (SMR at 30°C) maintained for 2–10 days (Großmann and Starck, 2006; Ott and Secor, 2007; Secor and Diamond, 2000).

Ambush-hunting snakes like pythons have become a model system for studies of SDA in ectothermic sauropsids because of their marked responses to feeding, in particular with respect to energy expenditure, enzyme activity and changes in organ size (McCue, 2006; Secor et al., 1994; Starck, 2005; Starck and Beese, 2001). Associated with this rise in metabolic rate, a significant increase in enzyme activities, membrane-bound transporter systems (Secor and Diamond, 1998), organ size (mainly gut and liver) and blood flow volume has been reported (Starck and Wimmer, 2005). As an evolutionary perspective, Secor noted that sit-and-wait foraging animals with long fasting intervals have proportionally higher SDA than frequent-feeding animals (Secor, 2001). Little is known about the source of the energy that fuels SDA. Previous studies on snakes suggested that SDA is dominated by preabsorptive costs and fuelled from endogenous sources (Secor, 2003; Secor and Diamond, 1995; Secor and Nagy, 2000; Secor et al., 1994). However, for ball python, we showed that SDA is partially fuelled from the animals' endogenous energy sources and partially from external sources, i.e. the meal (Starck et al., 2004). However, in our earlier study, we could not quantify the degree of energy partitioning from different sources, the time course of energy allocation to SDA and the moment of fuel switching.

For all animals that mount a proportionally large SDA, such as sit-and-wait foraging snakes, the source of energy for SDA is of key importance in feeding ecology and survival. If SDA were to be fuelled exclusively from the snakes' endogenous sources, it would need to save that amount of energy from a previous meal to be able to digest, absorb and assimilate the next meal. The necessary energy saved would actually be higher because capturing and ingesting the meal also require energy investment. For intermittent-feeding animals, the safety margins for fasting tolerance would therefore be limited by the energetic costs of SDA. Once a critical line has been crossed, an animal may potentially starve to death with a full stomach when it still has enough energy to swallow a meal but not enough energy to digest, absorb and assimilate the energy contained in it. Therefore, we hypothesize that reducing the amount of energy that is invested from own

resources and fuelling SDA from the energy contained in the meal potentially affects fitness and survival. In particular, in sit-and-wait foraging snakes with long fasting intervals and high SDA coefficients, reducing the start-up costs for digestion is considered beneficial because it would allow extending the safety margins of fasting to minimal energy reserves. In the present study, we measure energy partitioning, fuel switching, and timing of fuel use during SDA in a sit-and-wait foraging snake. We predict that, after initial energy investment from the snake's endogenous energy sources, the prey will be metabolized and serve as the main source to fuel SDA. In particular, we aim to: (1) identify the sources of energy for SDA; (2) detect the time when the snake switches from endogenous to exogenous sources; and (3) partition total SDA into a component that is fuelled from endogenous sources and a component that originates from exogenous sources. This includes an estimate of the preabsorptive energy investment.

MATERIALS AND METHODS

To differentiate between the fuel sources, we experimentally changed the isotopic composition of prey items by feeding prey with a diet enriched in ^{13}C. If, after feeding, the snake switches from oxidizing endogenous to exogenous energy sources, this will be manifested in a change of the isotopic signature in the CO_2 from the snakes' exhaled breath. A continuous and quantitative assessment of the isotopic composition of the snakes' exhaled breath before and after feeding allows us to document the timing and dynamic change in fuel utilization by the snake.

Animals

Eleven adult ball pythons (*Python regius*, Shaw 1802) from the institute's stock (Department of Biology, University of Munich, Germany) were used in this experiment. The snakes originated from a commercial snake farm but had been maintained in captivity for several years. Body mass ranged between 457 g and 1361 g (mean ± s.d. 868±261.7 g). The snakes were housed in groups of two or three in 120×80×60 cm (height × width × depth) glass cages with water *ad libitum* and shelter. Cage temperature ranged from 25 to 30°C, air humidity was 70% and the light was 12 h:12 h L:D. Snakes were fed once per month with live prey (different rodent species). At the beginning of the measurements, the snakes had been fasting for at least one month to assure that they were in postabsorptive state. The meal size was, on average, 71.2±30.4 g or 8.2±3.5% of the snakes own body mass. This equals 570±240 kJ per meal assuming an energy equivalent of 8.0 kJ g^{-1} (Secor, 2003).

Respirometry

We measured rates of oxygen consumption ($\dot{V}_{O_2}$) in an open flow system at 30°C chamber temperature. We used a FC-1B oxygen analyser and a Field Oxygen Analyser (FOX; both Sable Systems, Inc., Las Vegas, NV, USA). The system was calibrated once per week using industrial calibration gases. Before and after each session we took baseline values to account for potential drift of the measurement. The volume of the metabolic chamber was 3 litres. The air stream (50 ml min^{-1} for fasting snakes; 75 ml min^{-1} for digesting snakes) was bubbled through a solution of concentrated KOH to scrub off CO_2 and dried (silica gel blue; Roth GmbH, Germany) before entering the metabolic chamber. Oxygen consumption was measured continuously for 43 h. We calculated mass-specific $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}), corrected for standard temperature and pressure (Withers, 1977), by taking the lowest 10-min value that did not change by more than 0.1% O_2 concentration. Because of the relatively long equilibrium time, the lowest 10 min value was taken at least 3 h after the beginning of the trial. Oxygen data were analyzed with Data Can software (Sable Systems, Inc.).

Breath samples

During metabolic measurements, we took samples of the exhaled breath from each snake. Breath samples were taken directly from the air stream that left the oxygen analyzer. Breath samples were preserved in 12 ml glass exetainers (Labco, High Wycombe, UK). During the first 12.5 h after feeding, breath samples were taken every 30 min; between 13.5 h and 32.5 h after feeding, breath samples were taken at 60 min intervals; and between 33.5 h and 43.5 h after feeding, breath samples were taken every 120 min. We also took breath samples of seven ^{13}C-enriched mice using the same method.

Isotopic enrichment of prey items

Mice were obtained from the university's breeding stock (Department of Biology, University of Munich, Germany) and breeding-pairs were formed. For ^{13}C-enrichment of mice, breeding mice and their offspring were fed on corn, supplemented with vitamins (Vitakraft, Bremen, Germany), rodent pellets (Sniff, Soest, Germany) and bacon as a protein source. Measurement of exhaled CO_2 from mice reared on this diet showed that they were clearly enriched in ^{13}C ($\delta^{13}C$=–22.72±1.66‰) compared with the snakes (exhaled breath before feeding $\delta^{13}C$=–28.26±0.55‰).

Isotope ratio analyses

The $\delta^{13}C$-values of breath gas samples were measured as previously (Starck et al., 2004). Gas samples from exetainers were vented over a nafion dryer and flushed into a liquid nitrogen cold trap, where CO_2 and N_2O were collected. After removing other gases, the cold trap was defrosted and its contents were injected into a gas chromatography column (Paraplot Q; Varian, Walnut Creek, CA, USA) to separate CO_2 from NO_2. The isotopic composition of the CO_2 in the exhaled air of snakes was quantified using a modified Gasbench II (Finnigan MAT; Bremen, Germany) coupled to an isotope ratio mass spectrometer (Delta^{plus}XP; Finnigan MAT). The isotopic composition of the CO_2 is expressed in ‰ as the relative difference of its isotope abundance ratio relative to that of an international standard (Vienna PeeDee Belemnite; VPDB). Post-run off-line calculation and drift correction for assigning the final $\delta^{13}C$ values on the VPDB scale were done following the 'IT-principle' as described by Werner and Brand (Werner and Brand, 2001). $\delta^{13}C$-values that diverged more than 2‰ from the values measured directly before and after were not considered for the calculations.

Mixing model

We used a linear mass-balance mixing model in the form of:

$$\delta X_{\text{exhaled breath}} = p\delta X_{\text{A}} + (1 - p)\,\delta X_{\text{B}}, \quad (1)$$

where p equals the fraction of prey, and δX_{A} and δX_{B} are the isotopic composition of the prey and the snake's own fuel reserve, respectively (Martinez del Rio and Wolf, 2005). We solved the equation for p to calculate the fraction of the exhaled breath that originated from the prey. The model was applied to the $\delta^{13}C$ curves of each individual snake so that p was expressed as the mean and the standard deviation of N=7 individual snakes. Applying the results of the mixing model to the original $\dot{V}_{O_2}$ data, we partitioned SDA into one part that was fuelled from the snake's own sources and another part that was fuelled from oxidation of the prey. By calculating the area under each curve and recalculating $\dot{V}_{O_2}$ in units of energy (kJ), we estimated total energy contributions of each fuel source to SDA.

Experimental protocol and statistics

During a four-week pre-experimental period, all snakes were fasted and acclimatized to the following handling procedures. Following the pre-experimental acclimation period, we measured SMR of each snake in fasting condition. Each snake was measured four times for 24 h. To quantify SDA, seven snakes were fed with ^{13}C-enriched mice. Meal size averaged 8.2% of the snakes' body mass. Four snakes served as control; i.e. were not fed. Continuous open flow respirometry (0.5 s sampling interval) began 3 h before feeding, was briefly interrupted for feeding, and then continued for 43 h after feeding (briefly interrupted for ultrasonography; data not shown). To later match $\dot{V}_{O_2}$ with the changing isotopic profile, we calculated mean (± s.d.) $\dot{V}_{O_2}$ for each 30-min interval before and after feeding. The effect of feeding on the oxygen consumption was first analyzed by a two-way ANOVA with mass-specific $\dot{V}_{O_2}$ as dependent variable, and 'feeding group' and 'time after feeding' as factors. When the general model was significant, we tested within each group for the effects of 'time after feeding' using Tukey's multiple range test. If other tests have been used, it is mentioned in the text. SigmaStat for Windows version 3.5 (Systat Software, Inc., Point Richmond, CA, USA) was used for all statistical analyses.

RESULTS

Oxygen consumption

Standard metabolic rate measured over 24 h during the pre-experimental period averaged 0.03±0.01 ml O_2 h^{-1} g^{-1} (N=11). After feeding, $\dot{V}_{O_2}$ increased rapidly and peaked 20.5 h after feeding at 0.11±0.03 ml O_2 h^{-1} g^{-1} (N=7). During the following 23 h of the experiment, the oxygen consumption was constant at this level (Fig. 1). A two-way ANOVA with mass-specific $\dot{V}_{O_2}$ as dependent variable and 'time after feeding' and 'feeding group' as factors was significant (time after feeding, d.f.=89, F=2.473, P<0.001; feeding group, d.f.=1, F=1212.6, P<0.001). When the effect of time after feeding was tested within each of the groups separately, fasting animals did not show any increase in oxygen consumption rate (one-way ANOVA, d.f.=49, F=1.238, P=0.168). $\dot{V}_{O_2}$ of these animals was, on average, 0.03±0.01 ml O_2 h^{-1} g^{-1} (N=4) and did not differ from SMR (one-sided t-test; d.f.=89, T=1.078, P=0.284). In the group of animals that had been eating, time after feeding had a significant effect on mass-specific $\dot{V}_{O_2}$ (one-way ANOVA, d.f.=89, F=7.273, P<0.001). Tukey's *post-hoc* pairwise comparisons showed that, beginning 8 h after feeding, $\dot{V}_{O_2}$ differed significantly from before feeding (Fig. 1).

Stable isotope ratio

δ^{13}C of fasting animals was, on average, –28.26±0.55‰ (N=10). After feeding seven snakes with isotopically enriched mice, δ^{13}C did not change for 3.5 h. However, beginning 4 h after feeding, we measured continuously increasing δ^{13}C-values (to less-negative values) (Fig. 2). At the end of the measurement period (41.5 h), δ^{13}C reached –23.72±1.54‰ (N=7). This value was slightly more positive then δ^{13}C (–24.27±0.87‰, N=7) measured in the exhaled breath of the mice. The fasting control snakes did not show any change in δ^{13}C over time (δ^{13}C=–27.97±0.44‰). A two-way ANOVA with the δ^{13}C values of the snakes as variable and 'feeding group' and 'time after feeding' as factors was significant (feeding group, d.f.=1, F=256.3, P<0.001; time after feeding, d.f.=57, F=5.68, P<0.001). When the effect of time after feeding was tested within each of the groups separately, δ^{13}C of fasting snakes did not show any change associated with time. By contrast, animals that had been feeding showed a significant change in δ^{13}C (one-way ANOVA, main effect 'time after feeding', d.f.=57, F=17.63, P<0.001). Tukey's *post-hoc* pairwise comparisons showed that, beginning at 12 h after feeding, δ^{13}C differed significantly from before feeding (Fig. 2).

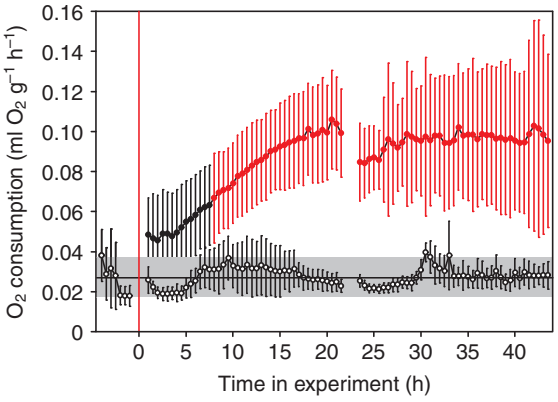

Fig. 1. Oxygen consumption of digesting snakes (filled symbols) and fasting snakes (open symbols). Values are means ± s.d. of seven digesting and four fasting snakes. The moment of feeding is indicated by the red vertical line at time=0. Red symbols are significantly different from before feeding (Tukey's pairwise comparisons, P=0.001). Gray shading indicates 95% confidence interval for SMR.

Mixing model

When the δ^{13}C data were fed into a linear mixing model, we found an exponential increase from zero before and immediately after feeding to ~80% at 30 h after feeding (Fig. 3). A non-linear regression showed that the relationship can best be described by an exponential equation in the form of:

$$p = p_0 + a*(1^{-(-b*time)}), \quad (2)$$

where p is the portion of ^{13}C originating from the mouse, p_0 is p at time zero, and a and b are constant terms (d.f.=2, F=843.92, P<0.001, R^2=0.97). In Fig. 3, the non-linear regression line with upper and lower 95% prediction limits (Eqn 2) is plotted together with the values of p derived from the linear mixing model (Eqn 1). The graph

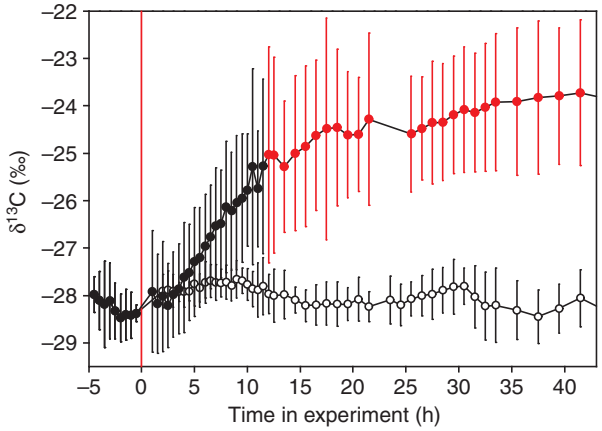

Fig. 2. Changes of isotopic composition of exhaled breath of digesting (filled symbols) and fasting snakes (open symbols) Values are means ± s.d. of seven digesting and three fasting snakes. The moment of feeding is indicated by the red vertical line at time=0. Red symbols are significantly different from before feeding (Tukey's pairwise comparisons, P=0.001).

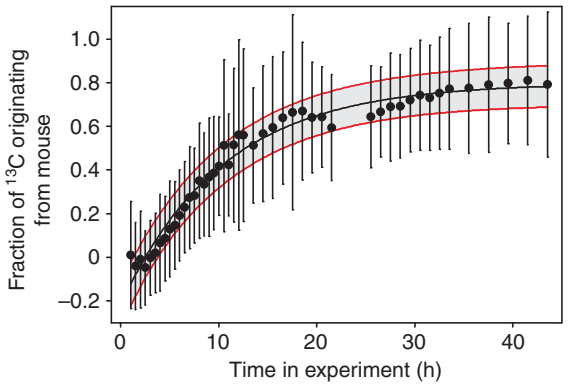

Fig. 3. Fraction of $\delta^{13}C$ originating from the prey as calculated by the linear mixing model in Eqn 2 (see text for details). Gray shading indicates 95% confidence intervals.

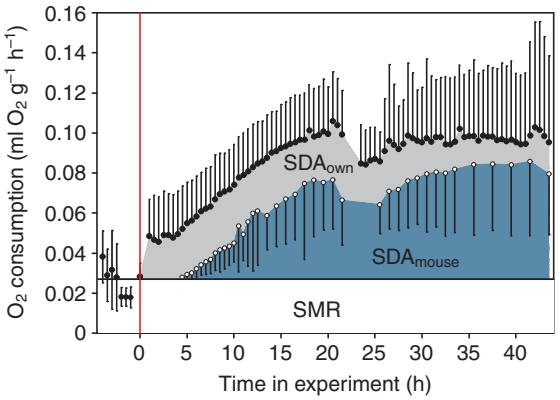

Fig. 4. Partitioning of oxygen consumption during the first 43 h of SDA. Mean SMR is given as a horizontal black line. Total $\dot{V}_{O_2}$ during SDA is given by filled symbols; values are means with positive s.d. The total area underneath this curve (gray and blue area together) is the oxygen consumed during 43 h of SDA. The curve indicated by open symbols, with negative s.d., is the oxygen consumption attributed to oxidising exogenous energy sources; the blue area under this curve is the portion of SDA fuelled by oxidising exogenous energy sources. The gray area between the two curves is the portion of SDA fuelled from the snakes' endogenous energy sources and can roughly be equalled to preabsorptive costs of SDA.

shows clearly that all empirical data range within the 95% prediction limits of the nonlinear regression model.

Energy partitioning

To obtain a robust estimate of energy partitioning during the first two days of SDA, we used $\dot{V}_{O_2}$ curves from all seven snakes. From each individual curve, we calculated the area between the curve describing $\dot{V}_{O_2}$ and the line defining SMR. On average, snakes consumed 2.43±0.91 ml O_2 g^{-1} during 43 h after feeding (Fig. 4), or 2151±1192 ml O_2 per snake.

We then used the values of p derived from the mixing model to determine the portion of $\dot{V}_{O_2}$ that was based on metabolizing the prey and the portion that was based on oxidising the snake's endogenous energy sources. Each of the curves calculated for an individual snake was integrated over time to obtain a measure of the amount of oxygen attributed to the metabolization of the prey. On average, snakes allocated 1.46±0.79 ml O_2 g^{-1} (or 1410.0 ml O_2 per snake) to the oxidation of ^{13}C-enriched mice. The mean difference between SDA and oxygen consumption used from metabolizing mice is 1.04±0.74 ml O_2 g^{-1} (or 943.3±741 ml O_2 per snake). This value equals the amount of oxygen consumed during SDA for metabolizing fuel from the snake's endogenous energy sources (Fig. 4).

DISCUSSION

We measured SDA only during the first 43 h of the postprandial response. Therefore, quantitative comparisons with complete SDA measured in other studies is not directly possible. However, the peak of SDA is usually reached within 24–48 h after feeding and this equals about 50% of the energy spent during SDA. With respect to this first segment of the postprandial response, the snakes in our study showed a typical postprandial response for an average meal size of 8.2% of their body mass at 30°C environmental temperature. Of course, complete documentation of SDA, i.e. until MR returns to the SMR level, may reach much higher values (McCue, 2006; McCue, 2007a; McCue, 2007b; McCue et al., 2005; Overgaard et al., 1999; Secor and Diamond, 1997; Wang et al., 2001; Zaidan and Beaupre, 2003).

The first goal of the present study was to identify the sources of energy during SDA. Using a ^{13}C-label for the prey, we could clearly show and confirm earlier reports (Starck et al., 2004) that energy consumption during SDA is first fuelled from the snakes endogenous energy source and then from exogenous energy sources. Such energy partitioning is not surprising because all active processes during digestion absorption, and assimilation, must initially be fuelled by energy from the animals' endogenous sources. Our previous study (Starck et al., 2004) seems to be the only work on snakes that provides experimental evidence that snakes metabolize their prey shortly after feeding. The second goal of the present study was to determine the time when a snake switches from endogenous to exogenous energy sources. There is no doubt that any ^{13}C-signal picked up from the snake's exhaled breath that exceeds the snake's baseline values must originate from the prey. Under the conditions applied in this study, oxidation of the prey begins 4 h after swallowing the meal and continuously increases quantitatively until it reaches a plateau at 20 h after feeding. Dead mice measured in a metabolic chamber (N=7) at 30°C did not release any noticeable amount of CO_2 until 32 h after death (J.M.S., unpublished data). Thus, our measurements are not affected by microbial CO_2 production. Also, we assume that any CO_2 produced in the stomach of the snake would immediately be bound by the aqueous environment in the stomach. Because we cannot envisage any other source of $^{13}CO_2$ than from complete oxidation of the ^{13}C-enriched tissues of the prey, we conclude that the metabolization of the prey begins shortly after swallowing the meal. The fast turnover of the prey supports our idea that the amount of energy invested in SDA from the animal's endogenous sources might be minimized to extend the safety margins for fasting periods. No comparative data are available for other sit-and-wait foraging snakes.

We can use the change in isotopic signature as a marker to indicate on a time scale the switch in metabolic fuelling. The total oxygen consumption minus $\dot{V}_{O_2}$ due to SMR from the moment after swallowing a meal to the initial change in isotopic signature at 4 h post-feeding indicates the energy requirement necessary to initiate digestion, absorption and assimilation of the meal. Any increase in $\dot{V}_{O_2}$ during those 4 h after feeding counts as preabsorptive energy investment and is exclusively fuelled from the snake's endogenous energy sources. Based on the values presented here, we calculated a mass-specific value of

$0.113\pm0.038\,ml\,O_2\,g^{-1}$ for the preabsorptive energy investment ($=2.25\pm0.75\,J\,g^{-1}$), which is equivalent to 0.35% of the energy contained in an average meal (570±240 kJ). For the time after 4 h, we cannot differentiate between preabsorptive, absorptive and biochemical (post-absorptive) contribution to SDA. A straightforward interpretation in terms of ecological physiology is that such early fuel switching reduces the necessary energy reserves for initiating digesting processes of a large prey.

The third goal of this study was to present a fine time resolution of how total SDA is partitioned into a component that is fuelled from the animal's endogenous energy sources and a component that originates from exogenous energy sources, i.e. the meal. Our linear mixing model provides a simple description with an excellent fit to the empirical data. The interesting point here is that, during the 43 h of the experiment, the fraction of ^{13}C in the snake's exhaled breath that originated from the prey asymptotically reached a plateau of 75% at ~20 h after swallowing the prey. Thus, during SDA, snakes always mixed endogenous and exogenous energy sources, with the exogenous sources representing the major fuel source during SDA. We expected that sit-and-wait foraging snakes would tend to invest a smaller amount of energy allocated into SDA from their endogenous sources than from exogenous sources.

If, for the experimental period of 43 h after feeding, values of $\dot{V}_{O_2}$ allocated to SDA were converted into units of energy [assuming a conversion factor of $19.8\,J\,ml^{-1}\,O_2$ (Secor, 2001)], we calculated a mean mass-specific energy allocation of $48.1\pm18.0\,J\,g^{-1}$ for SDA. Partitioning SDA into the components from the two different fuel sources results in $28.8\pm15.6\,J\,g^{-1}$ from exogenous energy sources and $20.6\pm14.7\,J\,g^{-1}$ from endogenous energy sources, i.e. ~40% of SDA is fuelled from endogenous energy sources (containing the preabsorptive costs equal to 4.7% of SDA) and ~60% from the exogenous sources. The SDA coefficient (amount of energy 64 kJ invested in total SDA as portion of the energy contained in the food; 570 kJ) in our study was 11%. Thus, the amount of energy invested during SDA from the snake's own resources was approximately 4.5% of the energy contained in the meal. This includes the initial start-up costs for digestion and absorption. The remaining SDA energy investment during SDA is fuelled from exogenous sources. Based on resource partitioning of SDA, we estimate that these values partition SDA during the first 43 h after feeding. However, elevated levels of oxygen consumption can usually be observed over a period of 6–10 days, but 50% of energy expenditure occurs during the first 48 h after feeding. Therefore, partitioning fuel use during the entire period of SDA will render values of approximately 50% of what has been presented here.

To our knowledge, these are the first empirical data on resource partitioning during SDA in a snake. Restricting our measurements to a period of 43 h may be somewhat limiting in terms of absolute values, but the goal of this study was to focus on fuel switching and energy partitioning during the early period of SDA. Extending the measurements to the entire period of postprandial elevation of oxygen consumption will not alter the principal findings presented in this study. Using a different approach and asking different questions, a previous study by Secor estimated the costs of gastrointestinal up-regulation to be 4.9% of total SDA (Secor, 2003). We would not place too much emphasis on the similarity of these values to the value calculated here but think that the result of two different approaches shows convincingly that the costs for up-regulation of the gastrointestinal tract are rather low. The values for the preabsorptive costs presented here are certainly conservative, i.e. they rather underestimate the true value, because we used only the 4 h immediately after feeding, when $\dot{V}_{O_2}$ increased but no change in the isotope ratio was observed, to calculate the energy investment.

Our study of only one species does not allow testing evolutionary hypotheses, but we feel that the results presented here are in broad accordance with the expectations and may be considered as a first step towards an evolutionary comparison of fuel partitioning during digestion. As a first model species, ball pythons switch after feeding to fuelling SDA from the prey, the initial energy investment from their own energy depots is very small, and throughout SDA the dominant fuel source is the prey. Thus, carefully extrapolating these findings to a more evolutionary perspective, we suggest the interpretation that ball pythons (as a model species) may expand their safety margins for fasting tolerance and can capture, digest, absorb and assimilate prey with (potentially) largely depleted energy reserves.

ACKNOWLEDGEMENTS

We thank Julia Faltermeier for technical help in the laboratory. We gratefully acknowledge Nina Buchmann for granting access to the stable isotope facility in Zürich. We thank two anonymous reviewers for their insightful and helpful comments on an earlier version of this manuscript.

REFERENCES

Benedict, F. G. (1932). *The Physiology of Large Reptiles.* Washington DC: Carnegie Institute.
Dorcas, M. E., Peterson, C. R. and Flint, M. E. T. (1997). The thermal biology of digestion in rubber Boas (*Charina bottae*): physiology, behavior and environmental constraint. *Physiol. Zool.* **70**, 292-300.
Gerth, N., Redman, P., Speakman, J. R. and Starck, J. M. (2009). Energy metabolism of Inuit sled dogs. *J. Comp. Physiol. B.* doi:10.1007/s00360-009-0432-7.
Großmann, J. and Starck, J. M. (2006). Postprandial responses to feeding in the African rhombig egg-eater (*Dasypeltis scabra*). *Zoology* **109**, 310-317.
Martinez del Rio, C. and Wolf, B. O. (2005). Mass-balance models for animal isotopic ecology. In *Physiological and Ecological Adaptations to Feeding in Vertebrates* (ed. J. M. Starck and T. Wang), pp. 141-174. Enfield: Science Publ., Inc.
McConnachie, S. and Alexander, G. J. (2004). The effect of temperature on digestive and assimilation efficiency, gut passage time and appetite in an ambush foraging lizard, *Cordylus melanotus melanotus. J. Comp. Physiol. B* **174**, 99-105.
McCue, M. D. (2006). Specific dynamic action: a century of investigation. *Comp. Biochem. Physiol.* **144A**, 381-394.
McCue, M. D. (2007a). Snakes survive starvation by employing supply- and demand-side economic strategies. *Zoology* **110**, 318-327.
McCue, M. D. (2007b). Western diamondback rattlesnakes demonstrate physiological and biochemical strategies for tolerating prolonged starvation. *Physiol. Biochem. Zool.* **80**, 25-34.
McCue, M. D., Bennett, A. F. and Hicks, J. W. (2005). The effect of meal composition on specific dynamic action in Burmese pythons (*Python molurus*). *Physiol. Biochem. Zool.* **78**, 182-192.
Ott, B. D. and Secor, S. M. (2007). Adaptive regulation of digestive performance in the genus *Python. J. Exp. Biol.* **210**, 340-356.
Overgaard, J., Busk, M., Hicks, J. W., Jensen, F. B. and Wang, T. (1999). Respiratory consequences of feeding in the snake *Python molurus. Comp. Biochem. Physiol.* **124A**, 359-365.
Overgaard, J., Andersen, J. B. and Wang, T. (2002). The effects of fasting duration on the metabolic response in Python: an evaluation of the energetic costs associated with gastrointestinal growth and up-regulation. *Physiol. Biochem. Zool.* **75**, 360-368.
Secor, S. M. (2001). Regulation of digestive performance: a proposed adaptive response. *Comp. Biochem. Physiol.*. **128A**, 565-577.
Secor, S. M. (2003). Gastric function and its contribution to the postprandial metabolic response of the Burmese python *Python molurus. J. Exp. Biol.* **206**, 1621-1630.
Secor, S. M. and Diamond, J. (1995). Adaptive responses to feeding in Burmese pythons: pay before pumping. *J. Exp. Biol.* **198**, 1313-1325.
Secor, S. M. and Diamond, J. (1997). Determinants of the postfeeding metabolic response of Burmese pythons (*Python molurus*). *Physiol. Biochem. Zool.* **70**, 202-212.
Secor, S. M. and Diamond, J. (1998). A vertebrate model of extreme physiological regulation. *Nature* **395**, 659-662.
Secor, S. M. and Diamond, J. (2000). Evolution of regulatory responses to feeding in snakes. *Physiol. Biochem. Zool.* **73**, 123-141.
Secor, S. M. and Nagy, T. (2000). Postprandial response of plasma lipids and the hormone leptin in pythons. *Am. Zool.* **40**, 557-571.
Secor, S. M., Stein, E. D. and Diamond, J. (1994). Rapid upregulation of snake intestine in response to feeding: a new model of intestinal adaptation. *Am. J. Physiol.* **266**, G695-G705.
Starck, J. M. (2005). Structural flexibility of the digestive system of tetrapods – patterns and processes on the level of cells and tissues. In *Physiological and*

Ecological Adaptations to Feeding in Vertebrates (ed. J. M. Starck and T. Wang), pp. 175-200. Enfield, NH: Science Publishers.
Starck, J. M. and Beese, K. (2001). Structural flexibility of the intestine of Burmese python in response to feeding. *J. Exp. Biol.* **204**, 325-335.
Starck, J. M. and Wimmer, C. (2005). Patterns of blood flow during the postprandial response in ball pythons, *Python regius*. *J. Exp. Biol.* **208**, 881-889.
Starck, J. M., Moser, P., Werner, R. A. and Linke, P. (2004). Pythons metabolize prey to fuel the response to feeding. *Proc. R Soc. Lond.* **271**, 903-908.
Tattersall, G. J., Milsom, W. K., Abe, A. S., Brito, S. P. and Andrade, D. V. (2004). The thermogenesis of digestion in rattlesnakes. *J. Exp. Biol.* **207**, 579-585.
Toeldo, L. F., Abe, A. S. and Andrade, D. V. (2003). Temperature and meal size effect on the postprandial metabolism and energetics in a boid snake. *Physiol. Biochem. Zool.* **76**, 240-246.
Wang, T., Busk, M. and Overgaard, J. (2001). The respiratory consequences of feeding in amphibians and reptiles. *Comp. Biochem. Physiol. A Physiol.* **128**, 535-549.
Wang, T., Hung, C. C. Y. and Randall, D. J. (2006). The comparative physiology of food deprivation: from feast to famine. *Annu. Rev. Physiol.* **68**, 223-251.
Werner, R. A. and Brand, W. A. (2001). Referencing strategies and techniques in stable isotope ratio analysis. *Rapid Commun. Mass Spectrom.* **15**, 501-519.
Westerterp, K. R. (2001). Energy and water balance at high altitude. *News Physiol. Sci.* **16**, 134-137.
Withers, P. C. (1977). Measurement of VO_2, VCO_2, and evaporative water loss with a flow-through mask. *J. Appl. Physiol.* **42**, 120-123.
Zaidan, F., III and Beaupre, S. J. (2003). Effects of body mass, meal size, fast length and temperature on specific dynamic action in the timber rattlesnake (*Crotalus horridus*). *Physiol. Biochem. Zool.* **76**, 447-458.