The Journal of Experimental Biology 208, 1201-1208
Published by The Company of Biologists 2005
doi:10.1242/jeb.01492



# Physiological and morphological correlates of among-individual variation in standard metabolic rate in the leopard frog *Rana pipiens*

Anthony C. Steyermark*, Alexander G. Miamen, Hesam S. Feghahati and Anthony W. Lewno

*Department of Biology, University of St Thomas, St Paul, MN 55105, USA*

*Author for correspondence (e-mail: acsteyermark@stthomas.edu)

*Accepted 10 January 2005*

## Summary

**Rates of standard metabolism (SMR) are highly variable among individuals within vertebrate populations. Because SMR contributes a substantial proportion of an individual's energy budget, among-individual variation in this trait may affect other energetic processes, and potentially fitness. Here, we examine three potential proximate correlates of variation in SMR: organ mass, serum T4 thyroxine and relative mitochondrial content, using flow cytometry. Body-mass-adjusted kidney mass correlated with SMR, but liver, heart, small intestine and gastrocnemius did not. Thyroxine correlated with SMR, as did mitochondrial content. These results suggest several novel proximate physiological and morphological mechanisms that may contribute to among-individual variation in SMR. Variation in SMR may be maintained by diverse environmental conditions. Some conditions, such as low resource availability, may favor individuals with a low SMR, through small organ size, or low thyroxine or mitochondrial content. Other conditions, such as high resource availability, may favor individuals with a high SMR, through large organ size, or high thyroxine or mitochondrial content.**

Key words: standard metabolic rate, thyroxine, mitochondria, leopard frog, *Rana pipiens*.

## Introduction

Organismal minimal energy costs, referred to as standard metabolic rate (SMR) in ectotherms and basal metabolic rate (BMR) in endotherms (Brody, 1945; McNab, 2002), are a central aspect in understanding animal energetics (Brody, 1945; Kleiber, 1975; McNab, 2002). Standard metabolic rate is an ectotherm's lowest rate of metabolism, measured at a particular temperature in an inactive and postabsorptive organism (McNab, 2002). It differs from BMR in endotherms only by not including the minimal cost of endothermy (McNab, 2002). Both SMR and BMR represent the cost of organismal maintenance (Brody, 1945), and include processes such as maintenance of the mitochondrial $H^+$ gradient, protein turnover, ion transport, hormone production, blood circulation and ventilation (Bennett, 1988; Hochachka and Guppy, 1987; Hulbert, 2000; Hulbert and Else, 1981; Rolfe and Brown, 1997). The cost of these processes can be substantial: in eutherian mammals across a range of body sizes, BMR accounts for about 20–35% of an individual's daily field metabolic rate (Kleiber, 1975; Nagy, 1987); and in turtles, SMR can account for up to 50% of an individual's total daily energy expenditure (Congdon et al., 1982).

Rates of minimal metabolism have been referred to as a fundamental energetic trait (Else and Hulbert, 1985; Konarzewski and Diamond, 1995; Labocha et al., 2004; McNab, 2002; Wikelski et al., 2003), in large part because they represent a fixed cost that all organisms must incur. This fundamental trait, however, appears to be highly variable in vertebrates, both between populations (Wikelski et al., 2003) and among individuals within a population (O'Steen and Janzen, 1999; Steyermark and Spotila, 2000).

Considering the proportion of daily energy expenditure (DEE) accounted for by BMR or SMR among-individual variation in SMR may affect other energetic processes (Steyermark, 2002), and potentially fitness. On the one hand, assuming a fixed energy intake, an individual with a low SMR, as compared to another individual, may have more energy to dedicate to other processes, such as growth (Gadgil and Bossert, 1970). Assuming changing energy intakes, an individual with a low SMR, as compared to another, may have a lower DEE, resulting in less foraging time, and thus less risk of predation. Both scenarios posit that selection should act to keep SMR low because a low SMR results in benefits – energy allocation or a moderated risk – to the individual. On the other hand, selection may act to keep SMR high because a high SMR may be functionally related to increased total daily energy expenditure budgets or a high sustainable metabolic rate (Hammond and Diamond, 1997; Meerlo et al., 1997; Speakman et al., 2003; Tinbergen and Verhulst, 2000; Weiner, 1992), allowing for benefits such as increased lactation, thermoregulatory capacity or aerobic scope. A diversity of environmental conditions may maintain variation in SMR in the population, such that individuals with low SMRs do well

in times of low resource availability, while individuals with high SMRs do well in times of high resources availability (Bateson et al., 2004).

But what is the mechanism underlying variation in BMR and SMR? The mechanism given the most attention has been the positive relationship between body-mass adjusted organ size and SMR (or BMR) in several groups of vertebrates, including mammals (Konarzewski and Diamond, 1995; Selman et al., 2001), birds (Chappell et al., 1999; Daan et al., 1990; Hammond et al., 2000) and lizards (Garland, 1984; Garland and Else, 1987). However, it is not clear whether large, energetically expensive organs cause a high SMR or BMR, or whether in order to support a high SMR or BMR one needs large central organs. Thus, this relationship remains only correlative: which is the cause and which the effect remains unresolved.

Other variables in addition to body-mass-adjusted organ mass may affect SMR, however. Here, we seek to understand potential mechanisms underlying among-individual variation in SMR. In a novel approach, we examine two potential proximate correlates of variation in SMR, in addition to organ mass, in leopard frogs *Rana pipiens*. We measured SMR of the frogs, then measured the masses of energetically expensive organs, serum-free T4 thyroxine, and relative mitochondrial content using flow cytometry (Johnson et al., 1980; Shapiro, 1981). We asked three questions: first, how much among-individual variation exists in the metabolic rate of an amphibian? Second, does organ mass correlate with metabolic rate in frogs, as it does in other vertebrate groups? Third, do sources of among-individual variation in metabolic rate exist other than that correlated to variation in organ mass?

## Materials and methods

### *Animals*

We obtained 29 male leopard frogs *Rana pipiens* Schreber 1782 from Charles D. Sullivan Co. Inc. (Nashville, TN, USA), and kept them at a density of 6–8 individuals per 76 liter aquarium at ≈22°C, 11 h:13 h light:dark photoperiod, filled with ≈20 l $H_2O$. Frogs were housed for 3 weeks and fed mealworms *ad libitum* until 3 days prior to metabolic rate measurements.

### *Determination of standard metabolic rate*

As an index of metabolic rate, we measured carbon dioxide production of postabsorptive leopard frogs at 22°C for 24 h under constant light conditions using an open-flow, push-through respirometry system (Withers, 1977; Sable Systems, Las Vegas, NV, USA). We placed six frogs into individual 237 ml containers at approximately 0800 h. Air was first purged of $H_2O$ and $CO_2$ with an external purge gas generator (Parker Hannifin Corp., Haberhill, MA, USA), and was then pumped into a temperature-controlled chamber (Hotpack, Philadelphia, PA, USA), where the air was further filtered of $CO_2$ and $H_2O$ through a column containing drierite/ascarite/drierite to remove residual $CO_2$. The air then passed through copper tubing to bring the air to 22°C, and then into a respirometer manifold (MF8; Sable Systems) fitted with a bleed valve. The manifold split the airstream into seven paths, six of which were directed into animal chambers, and one was used for baseline measurements. The air was humidified before entering the animal chambers to prevent desiccation, was dried (using drierite) upon exiting the animal chamber, and then entered an air multiplexer (TR-RM8; Sable Systems), which allowed for sampling one individual airstream while venting the rest to the room. The sampled airstream flow was regulated to 100 ml $min^{-1}$ by a thermal mass flow controller (Model 840, Sierra Instruments, Amsterdam, The Netherlands and TR-FC1, Sable Systems). We then subsampled the airstream using a subsampler flow controller (TR-SS1, Sable Systems) at a rate of 75 ml $min^{-1}$, and drew the air through a Licor carbon dioxide analyzer (LI6252, Lincoln, NE, USA).

We recorded baseline measurements with the $CO_2$- and $H_2O$-free air before and after each animal recording. We sampled $CO_2$ and flow rate for each animal chamber at two samples per second for a 10 min duration (one file), after which another set of baselines and the next animal chamber were measured. Chambers were measured in sequence from the first frog to the last, and the sequence was then repeated eight times, for a total of eight 10 min files per frog.

We calculated the minimal $CO_2$ production from the lowest continuous 120 samples (1 min) for each 10 min file, and averaged these values across the final six files of each animal. We disregarded the first two files of each animal because they were significantly higher than the remaining six (most likely caused by animal activity). Standard metabolic rate for each frog was calculated according to the method of Withers (1977). All 29 frogs were measured for $CO_2$ production within 7 days of each other. Body mass measurements taken immediately before and after $CO_2$ measurements indicate that frogs lost less than 5% body mass, suggesting that they remained adequately hydrated throughout the trial.

### *Measurements of organ mass and thyroxine*

Less than 6 h after $CO_2$ measurements ended, we anaesthetized the frogs in a neutral 1:1000 Tricaine methanesulfonate solution (MS-222, Argent Chemical Laboratories, Redmond WA, USA). All frogs were sacrificed between 1200 h and 1700 h, reducing potential effects of diel fluctuations in serum T4 concentrations (Gancedo et al., 1996). We exposed the frog's thoracic cavity, drew ≈1–2 ml blood by cardiac puncture, centrifuged the blood sample (12 min at 894 ***g***), and froze the serum at –20°C for later analysis of T4. Next we removed the liver, blotted it and weighed it whole (±0.00005 g). We then removed approximately one third of its left lobe and weighed it, and then immediately froze the sample for later analysis of mitochondria. Next, we removed and blotted the heart, kidneys, small intestine and right gastrocnemius, trimmed them of fat and connective tissue, and weighed them. We dried the organs at 60°C until constant mass (≈72 h), and then weighed them again to obtain organ dry mass.

We measured total T4 serum thyroxine using a neonatal high

sensitivity microplate enzyme immunoassay (Monobind Inc., Los Angeles, CA, USA). Briefly, enzyme–T4 conjugate and serum sample T4 competed for immobilized T4 antibody sites on the microplate. After a 60 min incubation, unbound T4 was removed by aspiration, and then the bound T4 was measured colorimetrically. Results were compared to a standard curve. We measured each serum and reference sample in triplicate, and read the absorbance of the microplates at 630 nm (ELX800 Microplate Reader, Winooski, VT, USA). We used the mean of the three replicate absorbance readings in the data analyses described below.

### *Measurements of mitochondrial content*

After thawing the liver sample, we washed it several times with Hank's balanced salt solution (HBSS; Sigma-Aldrich Chemical Co., St Louis, MO, USA) until no visible blood remained on the liver. We then transferred the liver into dissociation solution containing 200 U $ml^{-1}$ collagenase IA (Sigma-Aldrich Chemical Co.) and 200 U $ml^{-1}$ hyaluronidase II (Sigma-Aldrich Chemical Co.) together in 10 ml of HBSS, at 37°C, minced it, mixed it by pipetting up and down several times, and then placed it in a 37°C water bath, shaking at 200 r.p.m. for 30 min. Next, we pushed the suspension through a 100 μm sieve (BD Biosciences, Bedford, MA, USA) several times until there was no resistance, and placed it back into a 37°C water bath shaking at 200 r.p.m. for 15 min. Finally, we pushed the suspension through a 40 μm sieve (BD Biosciences) several times.

We collected the disaggregated cells from the suspension by centrifuging the sample at 250 ***g*** for 5 min at room temperature. To lyse red blood cells (RBC), we resuspended the pellet in 5 ml of ice-cold RBC lysis buffer (0.15 mol $l^{-1}$ $NH_4Cl$, 0.1 mmol $l^{-1}$ $KHCO_3$, 0.1 mmol $l^{-1}$ $Na_2EDTA$, pH to 7.2–7.4), and centrifuged as above for a total of three washes. We then resuspended the final hepatocyte pellet in 5 ml of ice-cold HBSS.

We divided the liver cell suspension into two 1 ml samples, and centrifuged them at 250 ***g***, at 4°C for 5 min. We resuspended each cellular pellet in either 1 ml of 100 nmol $l^{-1}$ MitoTracker Deep Red (MDR; Molecular Probes, San Jose, CA, USA) in HBSS (stained cells), or in 1 ml of HBSS alone (unstained cells), incubated the suspensions in a 37°C water bath shaking at 200 r.p.m. for 30 min, and then collected the cells by centrifugation at 250 ***g***, at 4°C for 5 min. Finally, we re-suspended the cells in 1 ml HBSS for fluorescence analysis. We acquired fluorescence emission intensity of 20 000 total counts (a count is any particle, such as a cell, that the flow cytometer detects) at 663 nm with a FACSCaliber flow cytometer (BD Bioscience, San Jose, CA; Johnson et al., 1980; Shapiro, 1981).

Before analyzing frog liver samples, we conducted a series of control experiments. First, we examined the effects of using frozen liver samples by comparing fresh versus frozen samples from the same individual, and found no difference in fluorescence intensity. To determine whether the mitochondrial stain was fully permeable through cell membranes, we permeabilized disaggregated cell membranes with 0.1% Triton X-100 (Sigma-Aldrich Chemical Co.), stained them as above, and then compared their fluorescence intensity with non-permeabilized cells. Fluorescence intensities of the two treatments were similar. As a negative control, we lysed disaggregated cells with 10% sodium dodecyl sulfate (SDS; Sigma-Aldrich Chemical Co.) and then stained them as above. As anticipated, at that concentration the SDS lysed both cell and mitochondrial membranes, resulting in no fluorescence. Lastly, we estimated the repeatability of the method by dividing the solution of disaggregated hepatocytes into five equal samples before the staining procedure. We then calculated a coefficient of variation (3.5%), and repeatability (0.98; Lessells and Boag, 1987).

### *Data analysis*

We used body mass of frogs before $CO_2$ measurements began for all data analysis. We first removed three outliers from the total data set (including metabolic rate, organ mass, serum T4 thyroxine concentration and mitochondrial number) following a Jackknife Distance Outlier Analysis. Thus, all following data analyses are performed on 26 frogs. Using ratios as a size-specific index for either metabolic rate or organ mass can introduce bias into statistical analyses (Packard and Boardman, 1999). Therefore, to investigate relationships between organ mass and metabolic rate, we first computed residuals of SMR on body mass, and residuals of organ mass on body mass, then regressed residual SMR against residual organ mass (Konarzewski and Diamond, 1995). To investigate relationships between serum T4 thyroxine and metabolic rate, we regressed whole body SMR against serum T4 thyroxine concentration.

To investigate relationships between mitochondrial number and SMR, we first used Cellquest Pro 4.0 (BD Bioscience) to plot a histogram of fluorescence intensity on a logarithmic scale, from which we calculated the geometric mean fluorescence for each sample. Geometric mean better represents the typical signal intensity than the arithmetic mean because it is less influenced by high outliers for logarithmic data distributions (Sokal and Rohlf, 1995). The calculated geometric mean of the fluorescence intensity is proportional to the amount of mitochondria in 20 000 counts. To obtain a final mitochondrial fluorescence intensity, we subtracted autofluorescence from the fluorescence of the respective stained sample. To normalize number of liver mitochondria to a relative measure of mitochondrial content, we computed residuals of fluorescence intensity on liver mass, and then regressed residual fluorescence intensity against residual SMR.

All data analyses were performed using JMP 5.0 (SAS 2002; SAS Institute, Inc., Cary, NC, USA).

## Results

### *Body mass and resting metabolic rate*

Mean mass of the 26 frogs was 36.63 g (S.E.M.=0.88, min=28.62, max=45.15). Mean whole body SMR was

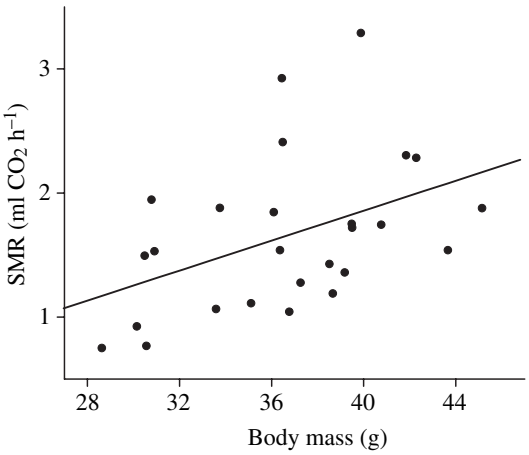


Fig. 1. Standard metabolic rate (SMR) of 26 leopard frogs increased with body mass (SMR=–0.558+0.060×mass; $r^2$=0.19; $P$=0.025).

1.65 ml $CO_2$ $h^{-1}$ (S.E.M.=0.12, min=0.75, max=3.29), and mass-specific MR was 0.045 ml $CO_2$ $h^{-1}$ $g^{-1}$ (S.E.M.=0.003, min=0.025, max=0.082). As expected, whole body metabolic rate increased with body mass ($P$=0.025; $r^2$=0.19; SMR=–0.558+0.060×mass; Fig. 1). Log-scaled body mass and SMR yield the relationship: SMR=–5.13×mass$^{0.19}$.

*Organ mass, T4 concentration and mitochondrial content*

There was considerable variation in organ dry masses between individual frogs, ranging from 18 to 34% CV (Table 1). Dry mass of all organs increased significantly with body mass (Table 1), which accounted for between 17 and 75% of variation in organ mass (Table 1).

Mean T4 thyroxine concentration was 0.810 μg $dl^{-1}$ (S.E.M.=0.006, min=0.753, max=0.873), and did not change with body mass ($P$=0.69). Mean relative mitochondrial content was 2367 (CV=73%), and did not change with body mass ($P$=0.61).

*Relationship between SMR and organ mass, T4 and mitochondrial number*

There was a significant relationship between residuals of SMR and residuals of dry kidney mass ($P$=0.01, $r^2$=0.25; Fig. 2), suggesting that frogs with bigger kidneys had higher SMR values. However, if the circled data point (in Fig. 2) is removed from the analysis, the relationship is no longer significant ($P$=0.4). There were no significant relationships between residuals of SMR and residuals of dry liver, heart, small intestine and gastrocnemius mass (Fig. 2).

There was a significant relationship between serum T4 thyroxine concentration and SMR ($P$=0.04; $r^2$=0.16; SMR=–4.84+8.023[T4]; Fig. 3), suggesting that individuals with higher serum thyroxine T4 levels had higher resting metabolic rates. Residual relative mitochondrial content increased with residual SMR ($P$=0.05; $r^2$=0.15; residual SMR=3.292e$^{-16}$+0.0001238 residual relative mitochondria content; Fig. 4), suggesting that individuals with more mitochondria in the liver have a higher metabolic rate.

Lastly, we examined the relationship between serum T4 concentration and relative mitochondria amount. However, both serum T4 concentration and relative mitochondria amount correlate with SMR. To remove the effects of SMR on both variables of interest, we calculated residuals of serum T4 concentration against SMR, and relative mitochondria amount against SMR. With the effects of SMR on each variable removed, and thereby avoiding colinearity, we plotted residual relative mitochondrial content against residual T4 (Fig. 5). The results indicate a significant relationship ($P$=0.03; $r^2$=0.17; residual mitochondrial content = –6.512×10$^{-13}$+23861× residual T4), suggesting that individuals with high levels of serum T4 also have a high relative mitochondrial content independent of SMR. Alternatively, we performed a multiple regression with relative mitochondrial content as the dependent variable, and SMR and serum T4 concentration as independent variables. Results indicate a marginally significant relationship between relative mitochondrial content and serum T4 concentration ($P$=0.059). Both approaches give qualitatively similar results, both suggesting that high levels of serum T4 correlate with relative mitochondrial content independent of SMR.

## Discussion

*Among-individual variation in SMR*

Resting metabolic rates vary greatly among individuals within populations (Burness et al., 1998; Garland, 1984; Garland and Else, 1987; Johnson et al., 2001; Steyermark and Spotila, 2000). While much attention has been given to

Table 1. *Descriptive statistics of organ masses from 26 leopard frogs*

| | Heart | Liver | Kidney | Small intestine | Gastrocnemius |
|---|---|---|---|---|---|
| Dry mass (g) | 0.0166±0.0006 | 0.316±0.017 | 0.024±0.001 | 0.078±0.005 | 0.156±0.006 |
| Min, Max | 0.012, 0.022 | 0.185, 0.567 | 0.017, 0.039 | 0.047, 0.141 | 0.111, 0.204 |
| CV (%) | 18 | 28 | 22 | 34 | 18 |
| Significance | 0.0001 | 0.0024 | 0.0022 | 0.0384 | 0.0001 |
| $r^2$ | 0.47 | 0.32 | 0.33 | 0.17 | 0.75 |

Dry mass values are means ± S.E.M.
Significance and $r^2$ values describe the relationship between organ mass and body mass.
CV, coefficient of variation.

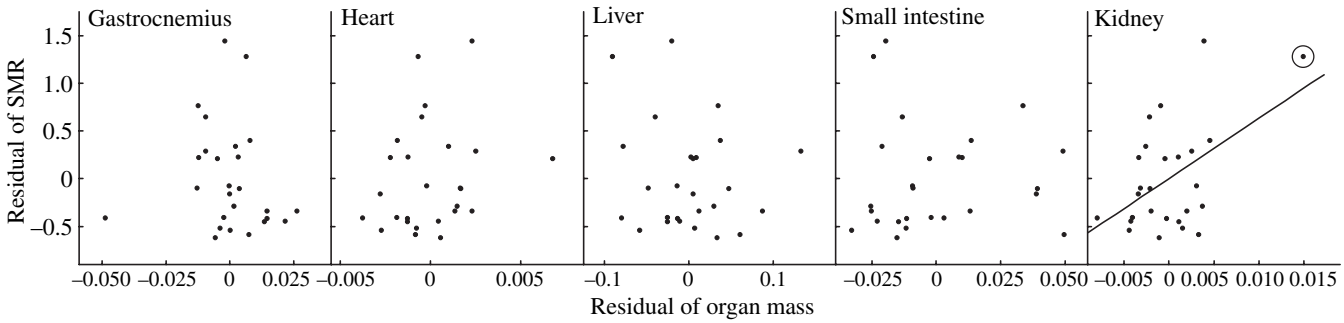


Fig. 2. Residual SMR increased with residual kidney mass ($r^2$=0.25; $P$=0.01), but not with residuals of gastrocnemius, heart, liver or small intestine.

potential consequences of variation in BMR and SMR (Hammond and Diamond, 1997; Meerlo et al., 1997; Speakman et al., 2003; Tinbergen and Verhulst, 2000; Weiner, 1992), little attention has been turned to identifying physiological sources of the variation. This is surprising given the traits' importance to animal energetics in particular, and evolutionary physiology in general (Konarzewski and Diamond, 1995; McNab, 2002). In this study, we report that the high level of among-individual variation in resting metabolic rate (SMR) correlates with among-individual variation in kidney mass, serum T4 thyroxine concentration and relative mitochondrial content.

### *Physiological mechanisms underlying among- individual variation in SMR*

Standard metabolic rate is a sum total of many different body processes, each with several layers of regulatory mechanisms. Therefore, isolating an individual cause of among-individual variation in SMR is illogical. However, previous studies suggest three possible proximate causes of among-individual variation in SMR. First, a recent body of literature suggests that organ mass, when adjusted for body size, may correlate with SMR in both ectotherms (Garland, 1984; Garland and Else, 1987) and endotherms (Burness et al., 1998; Konarzewski and Diamond, 1994; Konarzewski and Diamond, 1995; Meerlo et al., 1997; Speakman and McQueenie, 1996). Though each study found relationships between SMR and different organs, they all focused on a common set of energetically expensive organs, including heart, kidney, small intestine and liver.

Our results indicate a positive correlation between mass-corrected kidney mass and mass-corrected metabolic rate. The frog kidney aids in the regulation of both water and ion re-absorption. Under normal body hydration, water re-absorption in the kidney is typically low, because of the need to excrete water gained through the permeable skin (Vondersaar and Stiffler, 1989). However, absorption of electrolytes is high, and probably energetically expensive (Boutilier et al., 1992). Thus, although kidney mass accounts for a small proportion of total body mass (ca. 0.5%), the kidney's energetic demands may be a partial determinant of whole body SMR. It should be noted

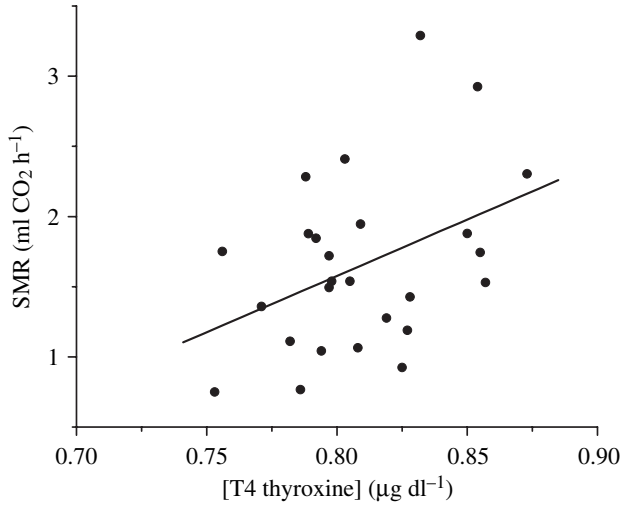


Fig. 3. SMR increased with serum T4 thyroxine concentration (SMR=–4.84+8.023[thyroxine]; $r^2$=0.16; $P$=0.04).

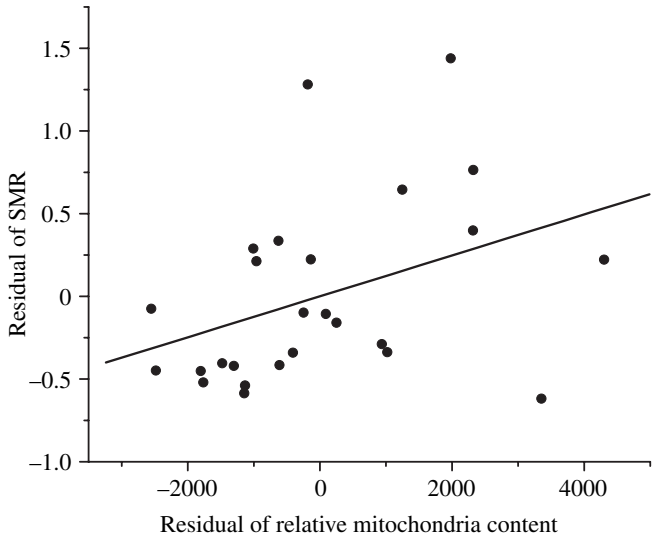


Fig. 4. Residual SMR increased with liver residual relative mitochondrial content ($r^2$=0.15; $P$=0.05). Relative mitochondrial content was determined by incubating disaggregated liver tissue with MitoTracker Deep Red 633 mitochondria stain, and measuring the fluorescence intensity of bound stain by flow cytometry. Fluorescence intensity is dimensionless.

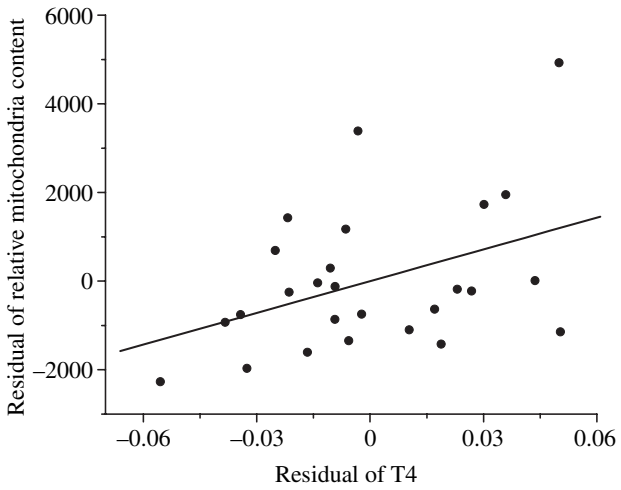


Fig. 5. Residual relative mitochondrial content increased with residual serum T4 ($r^2$=0.17; $P$=0.03).

that the relationship between SMR and kidney mass is significantly affected by a single data point (Fig. 2). However, this data point was not identified as an outlier in the Jackknife Distance Outlier Analysis, and thus should not be considered as an outlier, but rather as a valid data point.

However, any relationships between SMR (or BMR) and organ mass should appropriately be thought of as correlations rather than as cause and effect, because it is not at all clear whether organ size or SMR is the causative factor. One possibility is that a large organ size drives a high metabolic rate, as large organs – whatever their value – have a cost associated with their upkeep and function. In this scenario, large organs may provide benefits such as extra machinery for a high aerobic capacity (Bacigalupe and Bozinovic, 2002; Speakman, 2000). In another scenario, large organs are necessary to provide fuel for a high metabolic rate, which may be driven by some unknown factor (such as high levels of thyroxine, or a high mitochondrial content). This scenario is supported by several studies that have examined the change in organ mass due to increased metabolic demands, such as lactation or thermal stress (Hammond and Diamond, 1992; Hammond et al., 1994; Hammond and Kristan, 2000; Hammond et al., 1996). Because of this organ size plasticity in response to metabolic needs, it may be reasonable to posit that individuals that have high SMR – for whatever reason – may need large organs to provide for the high maintenance energy expenditure.

The second possible cause of among-individual variation in SMR we investigated was T4 thyroxine levels. Thyroxine is well-known as a potential effector on metabolic rate in vertebrates (Hulbert, 2000), including ectotherms (John-Alder, 1983). It is thought to act on metabolic rates through a variety of ways, including changes in mitochondrial membrane composition (Hulbert, 2000), $Na^+$,$K^+$-ATPase pump activity (Ismail-Beigi, 1993) and maintenance of mitochondrial $H^+$ gradients (Gong et al., 1997). Thyroxine supplementation (both T3 and T4) certainly stimulates ectotherm standard metabolic rates (Gupta and Thapliyal, 1991; Joos and John-Alder, 1990). More importantly, however, metabolic rate appears to correlate with endogenous variation of thyroxine levels in both endotherms (Astrup et al., 1996; Stenlof et al., 1993; Toubro et al., 1996) and ectotherms (O'Steen and Janzen, 1999). In the latter study, thyroxine levels of neonatal snapping turtles from different egg incubation temperatures correlated with mean SMR. Their results suggest an ultimate causation of among-individual variation in SMR: egg incubation temperature, as mediated through T4 thyroxine. Results from the present study also show a positive relationship between T4 and SMR, confirming at least a part of the results of O'Steen and Janzen (1999).

In addition to correlations between SMR and organ mass and thyroxine, we also report a correlation between SMR and mitochondrial content. Several mechanisms might explain this correlation. The first is a simple among-individual variation in mitochondrial content, which drives variation in SMR. Though we have found no studies that have detailed among-individual variation in mitochondrial content, variation in correlates to mitochondrial content, and their relationship to performance, have been well studied (e.g. citrate synthase activity; Zimmitti, 1999). Therefore, considering the variation seen in other anatomic traits, such as organ mass (see above), it seems probable that similar variation may exist in mitochondrial content in other organisms.

Two subsequent mechanisms that may explain the mitochondrial content and SMR correlation involve potential effects of thyroxine on mitochondria. First, thyroxine may stimulate the production of mitochondria. We present here a previously unreported relationship between T4 thyroxine and mitochondrial content, suggesting that endogenous high levels of thyroxine may promote an increase in mitochondrial number. Second, thyroxine affects mitochondrial membrane composition (Hulbert, 2000). Thyroxine may affect the amount of inner mitochondrial membrane (Brand et al., 1992), thus resulting in more mitochondrial stain binding. In this scenario, the increase in fluorescence intensity would be due to an increase in inner mitochondria membrane surface area, rather than in mitochondrial content.

Our results suggest that a considerable amount of variation in standard metabolic rate exists in an amphibian, which is comparable with reported variation in SMR in other ectotherms (Steyermark and Spotila, 2000). The finding that variation in SMR correlated only with variation in kidney mass, but not with the mass of other energetically expensive organs, is therefore partially consistent with organ mass–metabolic rate relationships reported for other vertebrate species. Finally, both thyroxine and mitochondrial content correlated with SMR. The relationship between SMR and endogenous thyroxine was not surprising, given the well-known endocrine controls of metabolic rate. However, it is not known what causes the variation in thyroxine levels in the first place. The correlation between mitochondrial content and SMR is a novel result, which again begs the question of the source of that variation. Finally, it is not at all clear how selection may act

on metabolic rate and related life-history traits, though it has been suggested that selection may act on metabolic rate to create diverse life histories that can be adapted for changes in local environment (Wikelski et al., 2003).

We thank S. Chaplin, M. Finkler and T. Watkins for providing valuable feedback on previous drafts of the manuscript, and to J. Manske for use of the flow cytometer. Funding was provided by University of St Thomas Center for Faculty Development to A.C.S. and by the Bush Foundation Program to A.G.M. All protocols were approved by the Institution's IACUC (assurance no. A-3791-01)

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