Energetic costs of performance in trained and untrained *Anolis carolinensis* lizards

Simon P. Lailvaux[1*]

Andrew Z. Wang[2]

Jerry F. Husak[2]

[1]Department of Biological Sciences, University of New Orleans, New Orleans, LA 70148, USA

[2]Department of Biology, University of St. Thomas, St. Paul, MN 55105, USA

[*]Author for correspondence (slailvaux@gmail.com)

ABSTRACT

The energetic costs of performance constitute a non-trivial component of animals’ daily energetic budgets. However, we currently lack an understanding of how those costs are partitioned among the various stages of performance development, maintenance, and production. We manipulated individual investment in performance by training *Anolis carolinensis* lizards for endurance or sprinting ability. We then measured energetic expenditure both at rest and immediately following exercise to test whether such training alters the maintenance and production costs of performance. Trained lizards had lower resting metabolic rates than controls, suggestive of a maintenance saving associated with enhanced performance as opposed to a cost. Production costs also differed, with sprint-trained lizards incurring the largest energetic performance cost and experiencing the longest recovery times compared to endurance trained and control animals. Although performance training modifies metabolism, production costs are probably the key drivers of trade-offs between performance and other life-history traits in this species.

KEY WORDS: Endurance, Exercise, Metabolic rate, Lizard, Locomotion, Performance

INTRODUCTION

Most fundamental fitness-related processes, from growth rate and immune function to reproductive investment, are affected by organisms' overall energy budgets. Measures of daily energetic intake and expenditure therefore delimit the quantity of energetic resources that an organism can dedicate towards supporting key physiological and behavioural processes, including (but not limited to) the **minimal** metabolic machinery that keeps organisms alive. Because that available resource pool is finite, organisms are forced to dynamically prioritize investment in certain traits at the expense of others, resulting in trade-offs in trait expression (De Jong and Van Noordwijk, 1992; Roff and Fairbairn, 2007; Tomkins et al., 2004). Those trade-offs can have clear effects on fitness, and so understanding the costs involved in driving resource allocation decisions is an important goal of life-history evolution.

Activity level constitutes a significant portion of daily energetic expenditure (DEE) in animals such as predators that rely extensively on locomotor activity for foraging (Garland, 1983; Williams et al., 2014). There is mounting evidence that the expression of locomotor performance (and of whole-organism performance in general) can drive trade-offs with other life-history traits that are linked to fitness (reviewed in Lailvaux and Husak, 2014), and that the relative costs of daily locomotor activity and reproduction in particular may be large enough to impinge upon each other in certain species (Lailvaux and Husak, 2017). However, a proper understanding of such trade-offs requires fine-grained estimates of the costs of expression of each trait. The costs of performance expression range from ecological costs imposed by the external environment to intrinsic, energetic costs associated with use of a given performance trait. With regard to these energetic costs specifically, Husak and Lailvaux (2017) distinguished between *maintenance costs* associated with the support and maintenance of those physiological and morphological pathways that bolster performance, and *production costs* which accrue to animals when they use their performance traits to conduct ecological tasks. Quantifying the relative magnitude of these costs is important because changes in either or both can affect how acquired energetic resources are allocated towards other fitness-enhancing traits, and could do so in different, cost-specific ways. Although a large literature is focused on understanding both energetic expenditure associated with locomotor activity and variation in basal/resting metabolic rates in animals following exercise (e.g. Donovan and Gleeson, 2006; Gleeson and Hancock, 2002), no studies thus far have experimentally distinguished between the maintenance and production costs of performance.

A powerful way to disentangle maintenance and production costs of performance is to experimentally manipulate investment in performance through exercise training. Husak et al. (2015) showed that green anole lizards (*Anolis carolinensis*) can be trained for endurance and sprinting, resulting in significant morphological and physiological changes compared to untrained controls. Endurance training in particular not only enhances endurance capacity relative to untrained controls, but also suppresses immune function and fecundity (Husak et al., 2016; Husak et al., 2017). Thus, manipulations of locomotor performance investment through training hold enormous opportunity for understanding not only the life-history consequences of performance expression, but also the various energetic costs of that expression in these organisms.

Here, we test the following explicit hypotheses and associated predictions: 1) Training affects metabolic rate. Because both endurance and sprint training alter aspects of physiology and muscle morphology in measurable ways, we expect that these training regimes will also influence the costs that organisms pay to maintain those performance levels. Specifically, we predict that both sprint and endurance trained lizards will exhibit higher resting metabolic rates than control animals. We expect sprint-trained lizards to have the highest maintenance costs, since enhanced performance is primarily due to increases in tissue mass (Husak et al., 2015) compared to endurance training where training may cause more efficient metabolism (reviewed in Speakman and Selman, 2003). 2) Training affects the energetic production cost of performance expression. Previous studies of lizard locomotion show that the costs of intense locomotor activity are reflected in metabolic rates measured immediately post-exercise (reviewed in Gleeson and Hancock, 2002). We predict that the physiological changes associated with exercise training will drive higher costs of performance production in trained animals as indicated by larger increases in metabolic rate from resting levels during the post-exercise recovery phase in trained versus untrained animals. Furthermore, we predict that sprint-trained lizards would have the longest recovery times of all due to their investment in anaerobic metabolic pathways.

MATERIALS AND METHODS

General husbandry

We obtained juvenile male (*n* = 18) and female (*n* = 18) green anoles from a commercial vendor (Candy's Quality Reptiles, LaPlace, LA, USA), and housed them in male-female pairs in 12-L cages (medium Kritter Keepers, Lee's Aquarium & Pet Products, San Marcos, CA). Lizards were housed at 30-33° C on a 12:12 hr light:dark cycle for four weeks of acclimation before the beginning of the experiment (Husak et al., 2015, 2016). Training and all measures of performance were conducted within this temperature range. Lizards were fed *ad libitum*, commercially obtained crickets (Fluker Farm, Port Allen, LA, USA) three times a week, with calcium and vitamin D supplemented once a week throughout the experiment. Cages were sprayed with tap water three times a day so that water was available *ad libitum*, and humidifiers were kept on in the room to maintain humidity above 40%.

Pre-treatment measurements

Prior to the onset of training, we took several measurements from each lizard. We measured mass and snout-vent length (SVL) of each lizard, as well as sprint speed and endurance capacity. Sprint speed was measured with each lizard being run three times in one day (trials being separated by two hours). **Lizards were motivated by gently tapping their tails with a paintbrush as they ran.** The racetrack was a 2-m long, 5-cm diameter dowel covered in cork (for traction), and was equipped with vertically paired infrared photocells (Trackmate Racing, Surrey, British Columbia, Canada) at 0.25-m intervals so that a running lizard broke the beams sequentially and the elapsed time (msec, then converted to m/sec) for each interval was recorded by a computer. The track was placed at a 45° angle to simulate natural conditions (Cox *et al.* 2009). The fastest 0.25-m speed was considered maximal. Endurance was measured on a motorized treadmill (PetRun model PR700 modified for lower speeds) by recording the time to exhaustion, determined by when lizards lost their righting response while running on a treadmill rotating at 0.3 km/h (Cox et al., 2009; Husak et al., 2016; Husak et al., 2015; Perry et al., 2004). Treadmills were cleaned with ethanol between lizards during measurements and training bouts described below.

After pre-treatment measures, lizards were allowed to rest for 3 days after which we randomly assigned each to one of three treatment groups: sprint trained (*n* = 6 males, 6 females), endurance trained (*n* = 6 males, 6 females), or untrained (control; *n* = 6 males, 6 females). The three treatment groups did not differ from each other in initial SVL (two-way ANOVA with sex and training treatment as factors: *P* > 0.3 for both main effects and interaction), mass (two-way ANOVA with sex and training as factors: *P* > 0.7 for both main effects and interaction), sprint speed (two-way ANCOVA with SVL as a covariate and sex and training as factors: *P* > 0.4 for both main effects and interaction), or endurance (two-way ANCOVA with SVL as a covariate and sex and training as factors: *P* > 0.8 for both main effects and interaction).

Training

Lizards were trained three days a week (Monday, Wednesday, and Friday) for 9 weeks, following previous protocols (Husak et al., 2016; Husak et al., 2015; Husak et al., 2017). Endurance training was conducted on the same treadmill on which we measured endurance capacity, but at a slower speed (0.18 km/h instead of 0.3 km/h). Lizards were run each training session for 30 min, eventually increasing intensity by increasing incline (following a modified procedure in Husak et al., 2016). We began with no incline (weeks 1 – 3) before adding an incline of 9° (weeks 4 – 6), and then 13° for the remaining three weeks.

Sprint speed training consisted of lizards being run up the same racetrack used to measure pre-treatment sprint speed, with intensity increased by adding weights to lizards. In the first phase lizards were run in three sessions of three runs per day with two hours of rest between sessions (for a total of 9 runs per day). In the second phase runs were the same, but individuals had one-quarter of the weight of the average for each sex (1 g for males, 0.3 g for females) tied around their waist with thin monofilament. In the third phase weight was increased to one-half of the average weight of each sex (2 g for males, 0.6 g for females) tied around their waist with thin monofilament. There were no apparent detrimental effects of the added weight beyond reduced speeds, and all lizards were able to run their trials successfully.

The control treatment lizards were handled once daily two times per week. This was meant to stimulate any stress response that may have resulted just from handling the trained lizards. Control lizards were captured, removed from their cage, and held for approximately 30 seconds. Any effect on trained individuals of being in an unfamiliar location during training is unlikely, since the treadmills eventually became familiar places, as they were trained on the same treadmill by the same person.

Respirometry

Standard metabolic rates were measured during normal periods of inactivity (between 2100-0200 hrs). Cages were put into black bags and gently moved from the animal housing room to the laboratory. They were removed from their cage in the dark and placed into a flow-through metabolic chamber (60-mL syringe barrel). These were then placed into an incubator set to 33°C (Orrell et al. 2004). They were left in the chamber for 45 min, and we used the lowest 5 min of **stable** CO_2 production as SMR. CO_2 production was measured **(1 sample/second)** with a Qubit S151 h CO_2 analyzer. Air was drawn from an undisturbed area of the laboratory **(a large, well-ventilated room)** and dried with Drierite **before entering the metabolic chamber**. **Each metabolic rate of interest was determined by calculating an average CO_2 level over the timeframe stated, subtracting stable background levels determined before the trial began, and multiplying by the flow rate (0.4 L/min).**

Resting RMR before running to exhaustion was measured similarly to SMR, but during normal times of activity. Lizards were removed from their cages, placed into a metabolic chamber in an incubator. They acclimated for 10 min before 30 min of CO_2 production was measured, taking the lowest **stable** 5 min as RMR. They were then taken to a treadmill and run to exhaustion at 0.33 km/hr, with the time it took to reach exhaustion being their endurance capacity (Husak et al. 2016, 2107). Immediately after exhaustion (all < 30 sec), lizards were placed in a metabolic chamber. We left lizards in the metabolic chamber until CO_2 consumption reached 1.5 times the previously measured RMR (Hancock et al., 2001; Hancock and Gleeson, 2002). Peak metabolic rate (V**CO**$_2$) was the highest CO_2 **production** achieved during this period **(averaged across the highest 10 sec)**, and recovery time was the period of time from that peak to 1.5 times RMR **(mean time was 15.7 ± 0.94 sec)**. We also calculated the change in metabolic rate (ΔMR) as the difference between peak MR and **minimal MR (either RMR or SMR, coded as metabolic rate type; see Statistical Analysis below). All respirometry measurements were performed after the conclusion of training and other post-training measurements.**

Statistical analysis

<u>Endurance performance</u>**: We compared final endurance times for control, sprint, and endurance trained individuals using a two-way ANCOVA with sex and treatment as factors, SVL as a covariate, and a sex-by-treatment interaction. We used Tukey's Honest Significant Difference post-hoc tests to determine which treatment means differed from each other.**

<u>Resting metabolic rates</u>: Because we measured RMR and SMR on the same individuals, these metabolic measures are not independent of each other. To test for an effect of training treatment on metabolic rate, we therefore used a general linear mixed-model with metabolic rate as a dependent variable; metabolic rate type (i.e. RMR or SMR) as random slopes (Schielzeth and Forstmeier, 2009); treatment, metabolic rate type, mass, and sex as explanatory

variables; and individual as a random factor as implemented in the R package *nlme* (Pinheiro et al., 2013). We also included an interaction between treatment and metabolic rate type to test whether SMR and RMR responded similarly to training regimes.

Metabolic increment data: We used mixed-models with random intercepts for each individual and random slopes for metabolic rate type as above to test for effects of treatment, metabolic rate type, mass, sex, and the interaction between treatment and metabolic rate type on ΔMR. As a further test of the production costs incurred by performance, we also used tested recovery time following final endurance measurement as a function of treatment, resting metabolic rates, sex, mass, endurance time, peak metabolic rate, and final metabolic rate. Because endurance time following training was measured only once per individual, we analyzed these data using a general linear model.

All statistical analyses were conducted using R v3.4.2 (R core team 2013). In all cases, variables were transformed using log or Tukey transformations as required to meet assumptions of normality and homoscedasticity. We based model simplification on log-likelihood ratio tests using maximum likelihood, and re-fit all final models using REML.

RESULTS

We found a significant effect of training treatment on final endurance performance ($F_{2,29}$ = 11.28, P <0.001), but not of sex ($F_{1,29}$ = 0.28, P > 0.6) following correction for SVL. Tukey's HSD test showed that this treatment effect is driven by significantly greater endurance in sprint trained (P < 0.002) and endurance trained (P < 0.001) lizards compared to controls. Thus, both sprint and endurance training increased endurance capacity in these animals, although endurance-trained lizards exhibited the greatest final endurance capacities (Table S1), consistent with earlier studies (e.g. Husak et al. 2015).

The mixed model that best described metabolic rate yielded significant terms for mass, training treatment, and MR type (AIC = -22.13). Control lizards exhibited higher metabolic rates than either sprint or endurance trained lizards, and RMRs were always higher than SMRs (Fig. 1a). This model did not retain an interaction between metabolic rate type and treatment (model with interaction AIC = -19.28), nor did it contain a sex effect (model with sex effect AIC = -20.78; Table 1a). Thus, both types of metabolic rate responded comparably to training in males and females. **(See Table S2 for mean MR values)**.

The mixed model that best described change in metabolic rate following endurance exercise (i.e. ΔMR) retained effects of mass, metabolic rate type, and treatment, as well as an interaction between treatment and metabolic rate type (Table 1b). This model also had the lowest AIC (AIC = -34.31) compared to the model including a sex effect, which was the next most likely (AIC =-32.32). Lizards trained for sprinting thus increase their metabolic rates more following exercise than do untrained controls **when comparing their SMR with their post-exercise peak MR** (Fig. 1b). Results for mass-specific metabolic rates can be found in Table S**2**, and exhibit comparable patterns to those presented here, with the sole exception of a marginal sex effect on Δ mass-specific MR (presented in Table S**3**b for completeness).

The general linear model that best described variation in recovery time retained effects of mass, treatment, Δ**R**MR, and peak V**C**O_2 (Table 2). Sprint-trained individuals had the longest recovery times, and there was a negative relationship between peak V**C**O_2 and recovery time within the context of the final model. Endurance time was not retained in the final model, and thus the recorded recovery times are not influenced by the length of the final endurance trial.

DISCUSSION

The energetic costs of locomotor performance have received a great deal of attention, but measuring the specific costs associated with the maintenance and expression of certain performance traits has long been a challenge (Husak and Lailvaux, 2017). In this study, we trained lizards for either endurance or sprinting and measured their metabolic rates both at rest and immediately following a bout of endurance exercise, comparing to lizards that were not trained. By doing so, we aimed to identify the specific signatures of energetic expenditure associated with enhanced locomotor performance.

Our first hypothesis was that training would alter resting metabolic rates compared to untrained controls, and we specifically predicted that lizards that had undergone either sprint or endurance training would exhibit higher resting metabolic rates than untrained lizards, reflecting the maintenance costs of performance. We did find an effect of training on metabolic rates, albeit in the opposite direction to our prediction: metabolic rates of trained lizards at rest are lower than those of control individuals, and we find no support for a difference between the metabolic rates of endurance and sprint-trained lizards (Table 1a). We also found an additional effect of metabolic rate type, whereby resting metabolic rates measured during the daytime (RMR) are higher than SMRs measured at night. However, the pattern of trained lizards spending less energy than controls at rest is the same for both types (Fig. 1a).

Reduced costs of resting metabolism associated with training might be explicable on the grounds of increased efficiency, whereby trained animals are able to make more efficient use of oxygen, perhaps through **plasticity in mitochondrial function** (Bouchard and Guderley, 2003). Although evidence from trained humans suggests that exercise can increase metabolic efficiency to decrease RMR (Byrne and Wilmore, 2001; Westerterp et al., 1992), this energetic efficiency scenario is not always supported by studies in other animal species, which generally show an increase in resting metabolic rates associated with long-term exercise (reviewed in Speakman and Selman, 2003). However, this trend is not consistent across all species; trained zebra finches (*Taeniopygia guttata*), for example, exhibit reduced resting metabolic rates that are not accounted for by changes in lean body mass (Nudds and Bryant, 2001). In any case, our data suggest that green anoles do not pay maintenance costs associated with performance enhancement via training, but instead realize energetic *savings* associated with the exercise response.

Our second hypothesis (that training would affect the production costs of performance) was supported, with trained lizards showing larger increments in metabolic rates following a bout of endurance exercise (Table 1b), and sprint-trained lizards showing the longest recovery times, as we predicted (Table 2). Given that the excess post-exercise oxygen consumption (EPOC) observed during the recovery phase immediately following exertion is estimated to account for 80-90% of the total energy expended during activity (Baker and Gleeson, 1998; Baker and Gleeson, 1999; Edwards and Gleeson, 2001) this can be interpreted as evidence that sprint-trained lizards pay the highest production costs of performance. The increased recovery time of sprint-trained lizards is likely due to their lower aerobic capacities relative to those of endurance-trained and control individuals because sprint training forces allocation towards pathways and morphology associated with anaerobic metabolism (Husak et al., 2015).

Increased understanding of the effects of performance training on metabolism also sheds light on the nature of life-history trade-offs previously observed in trained green anole lizards, such as reduced reproductive investment and immune function associated with a greater response to training in females. We found no evidence for a sex effect on any of the metabolic variables considered here, which suggests that the sex differences in trade-offs are not driven by sex-specific asymmetries in energetic expenditure. Furthermore, the unexpected finding that locomotor training is associated with energetic savings with regard to resting metabolism implies that it is the production costs of performance as opposed to the posited maintenance costs that drive trade-offs between performance and other life-history traits in green anoles, although future studies, perhaps on exercise-trained juveniles, might consider the energetic development costs, as posited by Husak and Lailvaux (2017), as well.

Acknowledgments

We thank E. Magnuson, K. Reardon, and C. Rohlf for help with lizard husbandry and training, A. Lewno for logistical support, and R. Brown, L. Deichmann, D. Jafari, and N. Pionke for inspiration. All research was conducted under approval by the UST Animal Care and Use Committee.

Competing interests

The authors declare no competing financial interests.

Author contributions

J. F. H., A. Z. W., and S. P. L. designed and ran the experiment. J. F. H. and S. P. L. wrote the manuscript with input from all the authors.

REFERENCES

Baker, E. J. and Gleeson, T. T. (1998). EPOC and the energetics of brief locomotor activity in *Mus domesticus*. *Journal of Experimental Zoology* **280**, 114-120.

Baker, E. J. and Gleeson, T. T. (1999). The effects of intensity on the energetics of brief locomotor activity. *Journal of Experimental Biology* **202**, 3081-3087.

Bouchard, P. and Guderley, H. (2003). Time course of the response of mitochondria from oxidative muscle during thermal acclimation of the rainbow trout, *Oncorhynchus mykiss*. *Journal of Experimental Biology* **206**, 3455-3465.

Byrne, H. K. and Wilmore, J. H. (2001). The relationship of mode and intensity of training on resting metabolic rate in women. *International Journal of Sport Nutrition and Exercise Metabolism* **11**, 1-14.

Cox, R. M., Stenquist, D. S., Henningsen, J. P. and Calsbeek, R. (2009). Manipulating Testosterone to Assess Links between Behavior, Morphology, and Performance in the Brown Anole Anolis sagrei. *Physiological and Biochemical Zoology* **82**, 686-698.

De Jong, G. and Van Noordwijk, A. J. (1992). Acquisition and allocation of resources - genetic (co)variances, selection, and life histories. *American Naturalist* **139**, 749-770.

Donovan, E. R. and Gleeson, T. T. (2006). Metabolic support of moderate activity differs from patterns seen after extreme behavior in the desert iguana Dipsosaurus dorsalis. *Physiological and Biochemical Zoology* **79**, 370-388.

Edwards, E. B. and Gleeson, T. T. (2001). Can energetic expenditure be minimized by performing activity intermittently? *Journal of Experimental Biology* **204**, 599-605.

Garland, T. (1983). Scaling the ecological cost of transport to body mass in terrestrial mammals. *American Naturalist* **121**, 571-587.

Gleeson, T. T. and Hancock, T. V. (2002). Metabolic implications of a 'run now, pay later' strategy in lizards: an analysis of post-exercise oxygen consumption. *Comparative Biochemistry and Physiology A* **133**.

Hancock, T. V., Adolph, S. C. and Gleeson, T. T. (2001). Effect of activity duration on recovery and metabolic costs in the desert iguana (*Dipsosaurus dorsalis*). *Comparative Biochemistry and Physiology a-Molecular and Integrative Physiology* **130**, 67-79.

Hancock, T. V. and Gleeson, T. T. (2002). Metabolic recovery in the Desert Iguana (*Dipsosaurus dorsalis*) following activities of varied intensity and duration. *Functional Ecology* **16**, 40-48.

Husak, J. F., Ferguson, H. A. and Lovern, M. B. (2016). Trade-offs among locomotor performance, reproduction and immunity in lizards. *Functional Ecology* **30**, 1665-1674.

Husak, J. F., Keith, A. R. and Wittry, B. N. (2015). Making Olympic lizards: the effects of specialised exercise training on performance. *Journal of Experimental Biology* **218**, 899-906.

Husak, J. F. and Lailvaux, S. P. (2017). How do we measure the cost of whole-organism performance traits? *Integrative and Comparative Biology* **57**, 333-343.

Husak, J. F., Roy, J. C. and Lovern, M. B. (2017). Exercise training reveals trade-offs between endurance performance and immune function, but does not influence growth, in juvenile lizards. *Journal of Experimental Biology* **220**, 1497-1502.

Lailvaux, S. P. and Husak, J. F. (2014). The life-history of whole-organism performance. *Quarterly Review of Biology* **89**, 285-318.

Lailvaux, S. P. and Husak, J. F. (2017). Predicting life-history trade-offs with whole-organism performance. *Integrative and Comparative Biology* **57**, 325-332.

Nudds, R. L. and Bryant, D. M. (2001). Exercise training lowers the resting metabolic rate of Zebra Finches, Taeniopygia guttata. *Functional Ecology* **15**, 458-464.

Perry, G., Levering, K., Girard, I. and Garland, T. J. (2004). Locomotor performance and social dominance in male *Anolis cristatellus*. *Animal Behaviour* **67**, 37-47.

Pinheiro, J., Bates, D., DeBroy, S., Sarkar, D. and Team, R. C. (2013). nlme: Linear and nonlinear mixed effects models, pp. R package.

Roff, D. A. and Fairbairn, D. J. (2007). The evolution of trade-offs: where are we? *Journal of Evolutionary Biology* **20**, 433-447.

Schielzeth, H. and Forstmeier, W. (2009). Conclusions beyond support: overconfident estimates in mixed models. *Behavioral Ecology* **20**, 416-420.

Speakman, J. R. and Selman, C. (2003). Physical activity and resting metabolic rate. *Proceedings of the Nutrition Society* **62**, 621-634.

Tomkins, J. L., Radwan, J., Kotiaho, J. S. and Tregenza, T. (2004). Genic capture and resolving the lek paradox. *Trends in Ecology and Evolution* **19**, 323-328.

Westerterp, K. R., Meijer, G. A., Janssen, E. M., Saris, W. H. and Hoor, T. F. (1992). Long-term effect of physical activity on energy balance and body composition. *British Journal of Nutrition* **68**, 21-30.

Williams, T. M., Wolfe, L., Davis, T., Kendall, T., Richter, B., Wang, Y., Bryce, C., Elkaim, G. H. and Wilmers, C. C. (2014). Instantaneous energetics of puma kills reveal advantage of felid sneak attacks. *Science* **346**, 81-85.

Figures

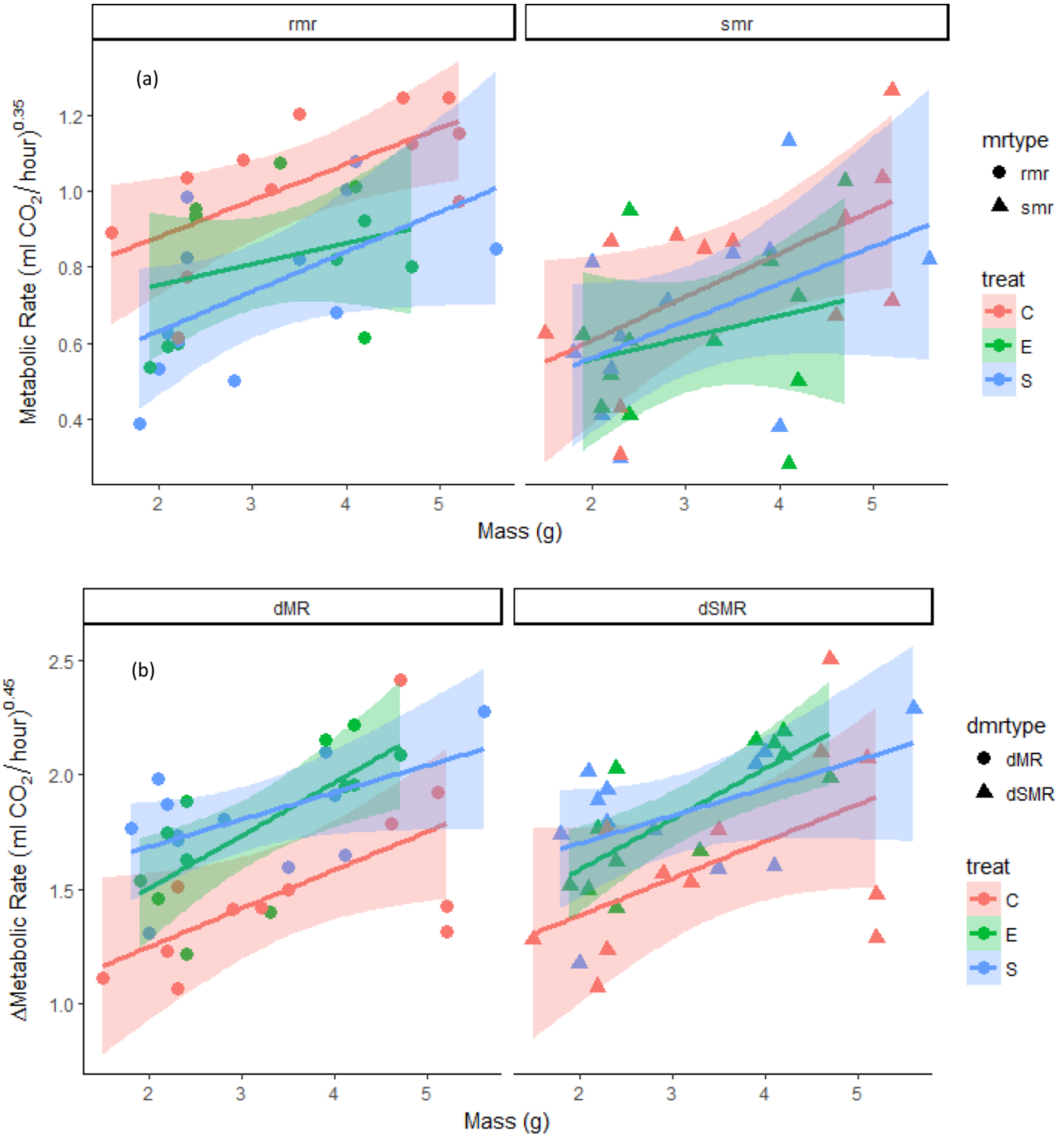

Fig. 1. Influence of mass, MR type, and training treatment on (a) MR$^{0.35}$ and (b) (ΔMR)$^{0.45}$. (a) RMR (left) is higher than SMR (right), and control lizards (red) exhibited higher MRs (of both types) than either sprint- (blue) or endurance-trained (green) lizards. (b) The greatest change in

MR was observed **when comparing SMRs to post-exercise peak MR** (dSMR, right) of sprint-trained lizards (blue), whereas the SMRs of endurance trained lizards (green) were not different from those of controls (red). **Shaded areas represent 95% confidence intervals**; n= 6 for all treatment/sex combinations.

Table 1: Best-fitting models describing the variation in (a) (metabolic rate)$^{0.35}$, and (b) (ΔMR)$^{0.45}$. The baseline category for MR type is RMR, and that for Treat is control (C). Thus, the reported values give estimated change in the dependent variable between the category named in the table and the baseline category. Bold values indicate significant ($P < 0.05$) estimates.

(a) (MR)$^{0.35}$	**Model term**	**Coefficient**	**SE**	**d.f**	**t value**	**P value**
	Intercept	0.667	0.086	35	7.78	**<0.001**
	Mass	0.092	0.02	32	4.517	**<0.001**
	MR type(smr)	-0.17	0.05	35	-3.72	**<0.001**
	Treat(E)	-0.15	0.07	32	-2.63	**0.013**
	Treat(S)	-0.16	0.057	32	-2.77	**0.009**
(b) (ΔMR)$^{0.45}$						
	Intercept	0.921	0.16	33	5.83	**<0.001**
	Mass	0.165	0.039	32	4.28	**<0.001**
	MR type(smr)	0.126	0.034	33	3.67	**<0.001**
	Treat(E)	0.327	0.11	32	2.94	**0.006**
	Treat(S)	0.384	0.112	32	3.44	**0.002**
	MR type(smr):Treat(E)	-0.058	0.049	33	-1.18	0.246
	MR type(smr):Treat(S)	-0.11	0.049	33	-2.27	**0.03**

Table 2: Best-fitting models describing the variation in recovery time following a bout of endurance exercise. The baseline category for Treat is control (C). Degrees of freedom = 30 for all terms. Bold values indicate significant ($P < 0.05$) estimates.

Model term	**Coefficient**	**SE**	**t value**	**P value**
Intercept	9.7	3.03	3.2	**0.003**
Mass	2.17	1.02	2.14	**0.041**
ΔMR	9.83	2.28	4.31	**<0.001**
Treat (E)	1.34	2.35	0.57	0.57
Treat (S)	8.58	2.5	3.44	**0.002**
Peak VCO_2	-9.327	2.27	4.11	**<0.001**

Table S1: Results of final endurance trials in terms of time and distance for control, sprint-trained, and endurance-trained male and female *Anolis carolinensis*. All values are means (± 1 SD).

	Treatment	Time (min)	Distance (m)	Recovery (min)
(a)#Males				
	Control	3.49 (0.97)	176.26 (49.1)	7.46 (4.76)
	Sprint	7.17 (3.12)	360.6 (157.5)	22.38 (7.3)
	Endurance	8.1 (4.4)	409.1 (222.64)	15.35 (3.9)
(b)#Females				
	Control	3.14 (1.24)	158.42 (62.5)	8.15 (4.06)
	Sprint	5.23 (1.49)	264.14 (75.1)	21.6 (8.8)
	Endurance	5.62 (2.38)	284 (120)	12.83 (5.38)

Table S2: Minimal CO_2 production during both active (RMR) and inactive periods (SMR), as well as the difference between peak VCO_2 following exercise and RMR (i.e. ΔRMR) and SMR (ΔSMR) respectively for control, sprint-trained, and endurance-trained (a) male and (b) female *Anolis carolinensis*. All values have units of ml CO_2/hr, and are shown as means (± 1 SD).

(a)#Males	**Treatment**	**RMR**	**SMR**	**ΔRMR**	**ΔSMR**
	Control	1.55 (0.36)	0.87 (0.6)	3.59 (1.96)	4.26 (2.18)
	Sprint	0.73 (0.36)	0.59 (047)	4.14 (1.35)	4.28 (1.41)
	Endurance	0.74 (0.6)	0.41 (0.38)	4.57 (1.33)	4.9 (0.97)
(b)#Females					
	Control	0.8 (0.39)	0.4 (0.31)	1.81 (0.55)	2.22 (0.87)
	Sprint	0.3 (0.33)	0.23 (0.2)	3.5 (0.93)	3.58 (1.17)
	Endurance	5.62 (0.35)	0.24 (0.2)	2.83 (0.88)	3.07 (0.97)

Table S3: Best-fitting models describing the variation in (a) (mass-specific metabolic rate)$^{0.4}$, and (b) (Δ mass-specific MR)$^{0.45}$. The baseline category for MR type is RMR, and that for Treat is control (C). Thus, the reported values give estimated change in the dependent variable between the category named in the table and the baseline category. Bold values indicate significant ($P < 0.05$) estimates.

(a) (MR/mass)$^{0.4}$	**Model term**	**Coefficient**	**SE**	**d.f**	**t value**	**P value**
	Intercept	0.61	0.032	35	19.17	**<0.001**
	MR type(smr)	-0.12	0.032	35	-3.75	**<0.001**
	Treat(E)	-0.11	0.039	33	-2.72	**0.01**
	Treat(S)	-0.12	0.039	33	-2.98	**0.005**
(b) (ΔMR/mass)$^{0.45}$						
	Intercept	0.9	0.066	33	13.81	**<0.001**
	Sex (m)	-0.11	0.064	32	-1.77	0.08
	MR type(smr)	0.09	0.026	33	3.6	**0.001**
	Treat(E)	0.24	0.08	32	2.98	**0.005**
	Treat(S)	0.31	0.08	32	3.88	**<0.001**
	MR type(smr):Treat(E)	-0.039	0.036	33	-1.1	0.278
	MR type(smr):Treat(S)	-0.08	0.036	33	-2.18	**0.036**

Figures

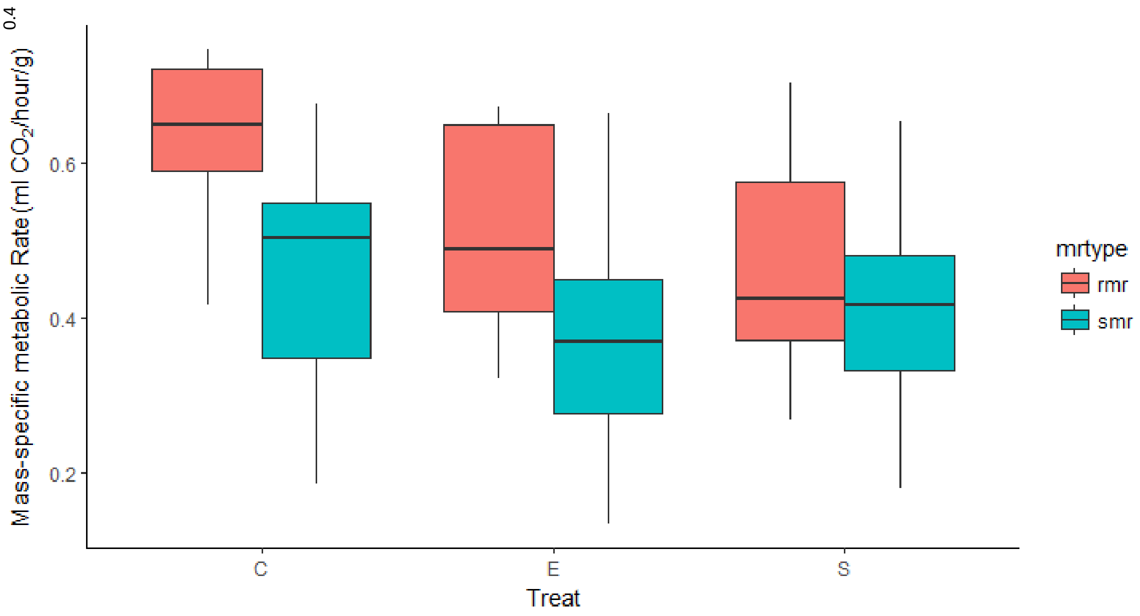

Fig. S1. Influence of MR type and training treatment on mass-specific MR$^{0.4}$. Mass-specific MR is higher in control lizards compared to endurance- or sprint-trained lizards. RMR is higher than SMR overall.

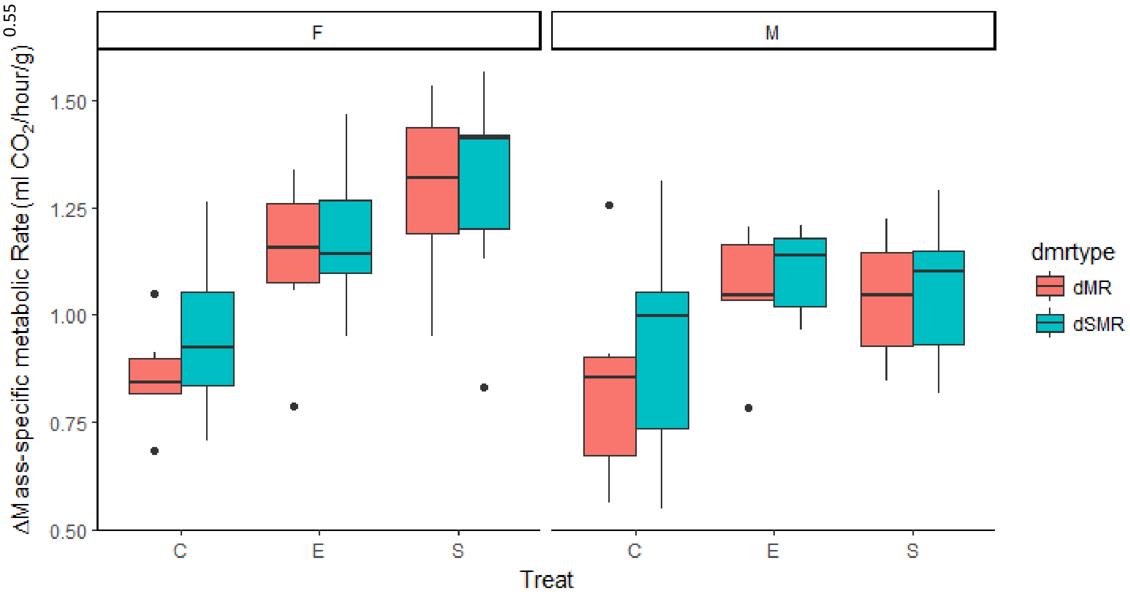

Fig. S2. Influence of MR type, and training treatment on (Δ mass-specific MR)$^{0.55}$. The greatest change in mass-specific MR was observed in sprint-trained lizards when comparing their SMR with their post-exercise peak MR, whereas the change in mass-specific MR of endurance trained lizards was not different from that of controls. The model also retained a sex effect whereby the mass-specific MRs of males changed less than those of females.