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RESEARCH ARTICLE

Endurance and sprint training affect immune function differently in green anole lizards (*Anolis carolinensis*)

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ABSTRACT

Limited resources must be partitioned among traits that enhance fitness. Although survival-related traits often trade off with reproduction, survival-related traits themselves may trade off with each other under energy limitations. Whole-organism performance and the immune system both enhance survival, yet are costly, but it is unclear how the two might trade off with each other under energy-limited conditions. Resources can be allocated to very different types of performance (e.g. aerobic endurance versus anaerobic sprinting), just as they can be allocated to different components of the immune system (e.g. innate versus acquired) to maximize survival. We forced allocation to different performance traits in green anole lizards (*Anolis carolinensis*) using specialized exercise training, to determine how different components of the immune system would be impacted by shifts in energy use. We measured immunocompetence in endurance-trained, sprint-trained and untrained control lizards by evaluating swelling response to phytohemagglutinin (cell-mediated immunity), antibody response to sheep red blood cells (acquired humoral immunity) and wound healing (integrated immunity). Endurance-trained lizards had reduced cell-mediated immunity, whereas sprint-trained lizards had reduced rates of wound healing. The acquired immune response was not affected by either type of training. Because each immune measure responded differently to the different types of training, our results do not support the hypothesis that simple energy limitation determines overall investment in immunity. Instead, different components of the immune system appear to be affected in ways specific to how energy is invested in performance.

KEY WORDS: Ecoimmunology, Life history, Performance, Trade-offs

INTRODUCTION

Life-history theory predicts that organisms should allocate finite resources to traits in a manner that maximizes fitness (Stearns, 1989, 1992; Roff, 1992). Although many studies have focused on the survival–fecundity trade-off, there are also likely trade-offs within each of these components of Darwinian fitness. Experimental manipulations of reproductive traits, for example, have revealed common patterns of reproduction trade-off resolution (Harshman and Zera, 2007; Roff and Fairbairn, 2007), but fewer studies have focused on how survival-related trade-offs manifest, especially in an experimental context (Lochmiller and Deerenberg, 2000; Zera and Harshman, 2001; Smith and French, 2017). The survival benefits of immunocompetence and whole-organism performance capacity (ability of an organism to accomplish an ecologically relevant task using dynamic movement) are both intuitive and empirically supported (Medzhitov and Janeway, 2000; Zuk and Stoehr, 2002; Klasing, 2004; Irschick et al., 2008; Zimmerman et al., 2010; Husak, 2015), yet how the two interact within the integrated phenotype is underexplored. Both immunity and performance are energetically costly (e.g. Crnokrak and Roff, 2002; Lochmiller and Deerenberg, 2000; Lee, 2006; Hasselquist and Nilsson, 2012; Husak and Lailvaux, 2017), and both are complex manifestations of lower-level biological traits, making them susceptible to trade-offs (Sheldon and Verhulst, 1996; Svensson et al., 2002; Ahtiainen et al., 2019; French et al., 2007; Lailvaux and Husak, 2014; Husak et al., 2016).

Under energetically limiting conditions, the simultaneous maintenance of all immune mechanisms may be too costly, such that energy is diverted away from some components in favor of others (Lee, 2006; Ardia et al., 2011; Demas et al., 2012; Neuman-Lee and French, 2014; Smith et al., 2017; Adamo, 2020). The immune system and its responses comprise multiple functional components that are categorized as innate, acquired and integrated (Janeway et al., 1999). Innate immunity is a general response that acts as the first line of defense against pathogens and includes components such as complement proteins, as well as cellular responses by leukocytes. Acquired immunity is a more specific response that requires initial activation through pathogen exposure, but thereafter allows a fast and more specific response during subsequent exposures to the pathogen, making subsequent costs of use relatively low (Lee, 2006; Muehlenbein, 2010). The general expectation is that when individuals are under energetic stress or food limitation, antibody-based immunity will be increased over systemic inflammatory responses, as well as non-specific and cell-mediated immunity (Lee, 2006).

Integrated immune responses involve complex interactions among innate and acquired aspects of the immune system, as well as other bodily systems. For example, a wound requires activation of the immune system, as well as the circulatory system and the regrowth of tissue (Demas et al., 2011). Given these differences, under limited-resource conditions, any allocation of nutrients, especially protein, toward traits other than the immune system can result in resources being shifted away from some components of the immune system but not others (Venesky et al., 2012; Neuman-Lee and French, 2014; van Dijk and Matson, 2016; Lind et al., 2020). When predicting allocation of limited resources, wound healing, and other integrated responses, should have the highest energetic costs, and may be most susceptible to trade-offs, because additional resources are necessary for tissue growth and remodeling beyond the initial inflammatory and multi-component immune response (Lee, 2006; French et al., 2007). Unless wound healing is prioritized over other aspects of immunity for an adaptive reason, wound healing should trade off when energy, and especially protein, is limited.

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In addition to limited resources, high activity rates are an important and ubiquitous cause of resource re-allocation (Speakman and Selman, 2003; Husak and Lailvaux, 2017). Although, it is debatable whether free-living animals 'exercise' (Killen et al., 2017; Yap et al., 2017), there is certainly high variation in activity among individuals in natural populations, which is affected by such factors as predation and territoriality, among others (Husak et al., 2015; van Dijk and Matson, 2016). Because increased locomotion is associated with greater energetic costs (Dolezal et al., 2000; Speakman and Selman, 2003; Husak and Lailvaux, 2017), increased locomotor activity should create a need to prioritize expenditure of remaining energy to the traits that will maximize fitness, necessitating trade-offs with other traits, including the immune system (Zamora-Camacho et al., 2015; Husak et al., 2016; 2017). Further, extended periods of high locomotor activity result in morphological and physiological changes (i.e. the 'exercise response') that can serve as additional maintenance costs of high activity (Husak and Lailvaux, 2017). For example, mice selectively bred for high maximal metabolic rate showed innate, but not adaptive, immune function, suggesting that high aerobic scope from high activity might trade off with immunocompetence (Downs et al., 2013).

We forced differential energy allocation to locomotor performance via specialized exercise training in green anole lizards (*Anolis carolinensis*) to examine how allocation to components of the immune system would change with increases in different types of activity. Exercise has long been known to affect immunocompetence in humans, although not always in simple or intuitive ways (Pedersen and Hoffman-Goetz, 2000; Muehlenbein et al., 2010). Additionally, the transcriptomic, physiological and systemic responses to different types of strenuous locomotor activity (e.g. resistance versus endurance) may lead to significantly different re-allocation of resources that, in turn, affect allocation to the immune system. Sprint performance is largely anaerobic and depends on muscle size for power output (Atherton and Smith, 2012), whereas endurance performance is aerobic and depends on efficient oxygen delivery to tissues (Wilson, 2013). Both types of locomotion have been shown to enhance survival in a variety of animal taxa (Irschick et al., 2008; Husak, 2015), but enhancing one or the other may result in differential resource allocation and thus differently affect the immune system.

In green anole lizards, calorie restriction and endurance training results in reduced innate and cell-mediated immunocompetence (Husak et al., 2016, 2017). However, it remains unknown whether this is a general response to energy limitation or an effect specific to endurance training. The goal of this study was to test for prioritization of energy within the immune system when energy is allocated to locomotor performance traits that have different types of costs. We experimentally manipulated the allocation of energetic resources in green anole lizards through specialized exercise training (endurance, sprinting or sedentary controls) to detect trade-offs with ecologically relevant aspects of immune function: changes in swelling response to phytohemagglutinin (PHA; cell-mediated immunity), antibody response to sheep red blood cells (SRBCs; acquired humoral immunity) and wound healing (integrated immunity). We hypothesized that different components of the immune system would be prioritized over others when under energetic limitations and tested three alternative hypotheses. First, if trade-offs between performance and immunity are caused by simple energy limitation, then we predicted that all measures of immune function would follow the same pattern of treatment effects regardless of the immune measure (e.g. control>sprint trained>endurance trained). Alternatively, if some (or all) measures are limited by specific macronutrients like protein, then we predicted the pattern control>endurance trained>sprint trained. Finally, there could be prioritization because of training-specific responses that reflect overlap of genetic networks involved with different components of the immune system and performance capacities. For example, both endurance (Smith, 2003) and resistance (Elenkov and Chrousos, 1999) exercise are known to increase humoral over cell-mediated immunity (but via different mechanisms), meaning that trained groups might not differ in humoral immune function. Reactive oxygen species (ROS) that result from endurance training (He et al., 2016), however, can further reduce cell-mediated immunity. The differential effect of training types on the immune system would thus predict different patterns of effects (control versus sprint trained versus endurance trained) for the three immune measures. Because adaptive immunity is predicted to be favored over innate immunity in 'slow-paced' compared with 'fast-paced' species (and vice versa; Lee, 2006), we made the prediction that the endurance training would prioritize humoral over cell-mediated immunity compared with sprint-trained individuals and controls.

MATERIALS AND METHODS

General lizard husbandry

We obtained wild-caught, adult male (N=45) and female (N=45) green anoles (*Anolis carolinensis*, Voigt 1832) from a commercial vendor (Candy's Quality Reptiles, LaPlace, LA, USA). They were housed in male–female pairs in 12-l cages (medium Kritter Keepers; Lee's Aquarium and Pet Products, San Marcos, CA, USA) at 28–31°C on a 12 h:12 h light:dark cycle for 3 weeks of acclimation before the beginning of the experiment (Husak et al., 2015). During acclimation, lizards were fed *ad libitum* with commercially obtained crickets (Fluker Farm, Port Allen, LA, USA) three times a week, with calcium and vitamin D supplemented once a week. All work was conducted with approval from the University of St Thomas Animal Care and Use Committee, and experiments complied with all relevant institutional and national animal welfare laws, guidelines and policies. Once the experiment began, we fed each lizard four crickets during each of the three feeding days per week. This makes resources limited, but does not induce a state of fasting (Lailvaux et al., 2012; Husak et al., 2016). Cages were sprayed with water twice a day, and humidifiers maintained humidity above 40%. Prior to training, we measured the mass (to the nearest 0.1 g with a digital balance) and snout–vent length (SVL; to the nearest 0.01 mm with digital calipers) of each lizard. We then randomly assigned each cage of lizards (male–female pair) to one of three treatment groups described above (control, endurance trained and sprint trained). The treatment groups did not differ from each other in initial SVL (two-way ANOVA treatment effect, P=0.27) or mass (two-way ANOVA treatment effect, P=0.18). Males had longer SVL (two-way ANOVA sex effect, P<0.0001) and higher mass (two-way ANOVA sex effect, P<0.001) than females, and there was no sex×treatment interaction for either SVL (P=0.57) or mass (P=0.88).

Exercise training

Although whether animals exercise in nature is unknown (Yap et al., 2017), our training regime represented a realistic simulation of high-end performance by green anoles. Each 30-min training session (described below) equaled 90 m of slow traveling by each lizard. This is relevant to green anoles in nature (Irschick, 2000), where ~25% of their time budget is spent traveling through their

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territories, with much of this time spent 'creeping' at lower speeds (Jenssen et al., 1995). Green anoles use slow, sustained locomotion for territory patrolling and foraging, in addition to sprinting for predator escape, and there is intra-population variation in the use of locomotion (Jenssen et al., 1995; Irschick and Losos, 1998; Irschick, 2000). Nevertheless, green anoles are sit-and-wait foragers that also rely on sprinting to capture prey, as well as to avoid predators, contexts in which they use near-maximal capacities (Irschick and Losos, 1998; Irschick, 2000). Such sprints can approach a meter in length per run in green anoles (Irschick, 2000), but sprinting distances have not been systematically studied in this species. Our training was performance at the high end of distance traveled in a day and speeds used in nature, but that was precisely our objective – to determine how high levels of performance use (and investment) might lead to trade-offs with immunocompetence.

Endurance-trained lizards were trained 3 days a week for 9 weeks (following Husak et al., 2015, 2016) on a motorized treadmill (PetRun model PR700, modified for lower speeds; GoPet, Ephrata, PA, USA) at 0.18 km h^{-1}. Lizards ran for 30 min in each training session. Training increased in intensity twice for a total of three 3-week 'phases' of training (Husak et al., 2015, 2016). Intensity increased by increasing the treadmill angle from flat (0 deg) to 9 deg and then 13 deg.

Sprint-trained lizards were trained 3 days a week for 9 weeks (Husak et al., 2015), with each lizard being run three times in one day (trials separated by 1 h). 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, BC, Canada) at 0.25 m intervals so that a running lizard broke the beams sequentially and the elapsed time (ms, then converted to m s^{-1}) for each interval was recorded by a computer. The track was placed at 45 deg to simulate natural conditions (Cox et al., 2009). Intensity was increased every 3 weeks by attaching weights to the lizards. Weights consisted of pipette tips affixed with string that was loosely tied around the waist of the lizards. Mass of the weights was increased by adding putty to the pipette tip. The string and pipette combination did not visibly impact the range of motion of the lizards. Lizards started with no weights attached, then progressed to ∼25% of the average body mass by sex (0.3 g for females, 1 g for males) and ended with 50% of the average body mass (0.6 g for females, 2 g for males).

Immune function

We measured cell-mediated immunity by swelling responses after injection with PHA (Martin et al., 2006; Huyghe et al., 2009; Demas et al., 2011). Injection of PHA induces a series of cellular responses, resulting in swelling at the injection site (Martin et al., 2006). We measured the thickness of each lizard's left and right hind foot to the nearest 0.01 mm and then injected 0.05 mg PHA (PHA-P, L8754; Sigma-Aldrich, St Louis, MO, USA) dissolved in 0.01 ml sterile phosphate-buffered saline (PBS) into the right foot. The left foot was injected with the same volume of sterile PBS but without PHA (Huyghe et al., 2009; Husak et al., 2016, 2017). We measured feet at 24 h post-injection and calculated swelling as the change in foot thickness between pre- and post-injection measurements (subtracting swelling from PBS injections). The PHA swelling response assay was performed the week after post-treatment performance measures were collected (see below).

Acquired humoral immunity was measured using a standard SRBC antigenic challenge (Demas et al., 2011) given during the seventh week of training. Lizards of both sexes were injected with 40 µl of a 2% solution of SRBCs diluted in sterile PBS (following Meylan et al., 2010). Although the time frame of antibody production in green anoles specifically is unknown, antibodies are detectable after 10 days in the common lizard, *Zootoca vivipara* (Meylan et al., 2013). Thus, after 10 days, blood samples were taken from the orbit using sterile microhaematocrit tubes and plasma was extracted post centrifugation. Plasma was serially diluted by half (dilution range 1 to 1/256 plasma:PBS) with sterile PBS in 96-well plates before being incubated for 30 min with a 0.5% SRBC dilution. Samples were examined for visible agglutination as a measure of antibody action, and the lowest plasma dilution to show agglutination for each individual was recorded as their response.

Wound healing was performed as an integrated measure of immune function (Demas et al., 2011). We used a 2 mm biopsy punch to create a skin wound on the dorsal surface of each lizard anterior to the base of the tail, between the legs, during the last week of training. Skin wounds were photographed at the time of wound formation and 10 days later, and TpsDig2w32 (http://life.bio.sunysb.edu/morph/) was used to calculate wound area at each time point. We compared the initial wound to that 10 days later to calculate the percentage area of the wound healed.

Post-treatment measurements

At the end of the experiment, the week after training ended, we measured endurance, sprint speed, mass and SVL. Endurance was measured on the same motorized treadmills used for training, but rotating at a higher speed of 0.3 km h^{-1}. Endurance was recorded as the time to exhaustion, when lizards lost their righting response (Cox et al., 2009; Husak et al., 2015). Sprint speed was measured on the same track used for sprint training. Lizards were run three times on each of two consecutive days, with 1 h rest between runs, taking the maximum 0.25-m speed as maximal performance (Husak et al., 2015). Lizards were then removed from their cages and euthanized in <4 min, with blood and organs harvested for a separate study.

Analysis

We first tested whether training increased performance. We conducted two-way analysis of covariance (ANCOVA) with SVL (for $\log_{10}$X+1-transformed sprint speed) and mass (for $\log_{10}$-transformed endurance) used as the covariate and sex and treatment as factors. We used two-way ANOVA, with sex and treatment as factors, to test for differences in PHA swelling response, proportion of wound healed after 10 days (logit transformed) and antibody response to SRBCs (agglutination concentration). None of the immune measures scaled to body size (SVL or mass, $P>0.2$ for all), so we did not include size as a covariate in the analyses.

RESULTS

Training increased performance as expected. Endurance differed among treatments ($F_{2,78}=13.1$, $P<0.0001$; means±s.e.m.: control, 188.0±9.12 s; sprint trained, 241.5±17.1 s; endurance trained, 303.9±20.5 s), with endurance-trained lizards having greater endurance than control [Tukey honest significant difference (HSD) $P<0.01$] or sprint-trained (Tukey HSD $P=0.03$) lizards. Sprint speed also differed among treatments ($F_{2,78}=5.22$, $P=0.007$; means±s.e.m.: control, 0.68.0±0.07 m s^{-1}; endurance trained, 0.55±0.05 m s^{-1}; sprint trained, 0.79±0.07 m s^{-1}), with sprint-trained lizards having higher speeds than endurance-trained (Tukey HSD $P=0.005$), but not control, lizards. Males had greater endurance ($F_{1,78}=4.38$, $P=0.04$) and were faster ($F_{1,78}=6.71$, $P=0.01$) than females, but there were no significant treatment×sex interactions ($P>0.14$ for all). The lack of a difference in speed is not unexpected, as the acclimation to handling during training decreases

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motivation in the sprint-trained group and thus reduces apparent performance (Husak et al., 2015). We note that the difference between the endurance-trained and sprint-trained lizards is a more meaningful comparison, because the endurance-trained lizards are also acclimated to running away from the investigators.

Results indicated a significant difference in some, but not all, immune function measures among treatment groups, as well as differences in the components of the immune system affected by the different treatments. There were no significant treatment×sex interactions for any of the immune response variables (P>0.15 for all). The swelling response to PHA differed across treatments ($F_{2,79}$=24.2, P<0.0001), with controls having the highest response, followed by sprint-trained, then endurance-trained, lizards (all differed from each other, Tukey HSD P<0.01 for all; Fig. 1A). PHA response did not differ between the sexes ($F_{1,79}$=1.62, P=0.21). Proportion of wound healed after 10 days differed across treatments ($F_{2,71}$=3.16, P=0.04; Fig. 1B) but not between sexes ($F_{1,71}$=0.12, P=0.74). Sprint-trained lizards had reduced healing compared with controls (Tukey HSD P=0.04) but not endurance-trained lizards (Tukey HSD P=0.19). Endurance-trained lizards did not differ in wound healing compared with controls (Tukey HSD P=0.80). Antibody production in response to SRBCs did not differ among treatment groups ($F_{2,79}$=0.50, P=0.61; Fig. 1C) or between the sexes ($F_{1,79}$=0.12, P=0.73).

DISCUSSION

Our results highlight the complex nature of how the immune system fits into phenotypic trade-offs of survival-related traits. Cell-mediated immunity (PHA swelling response) was depressed the most by endurance training, and wound healing was depressed most by sprint training. Our measure of acquired immunity (antibody response to SRBCs) was not impacted by either form of training compared with controls. Research into human exercise has suggested that strenuous exercise has a depressive effect on various components of the immune system (Gleeson, 2007), although these studies examined individuals that had already been exercising prior to the investigations rather than experimentally manipulating exercise. Our hypothesis of sprint-trained lizards (or even endurance-trained lizards) having the lowest overall immunocompetence was not supported, suggesting that energy limitation alone does not explain prioritization of immune system components. Instead, our results emphasize how even subtle differences in resource re-allocation to other aspects of the phenotype can have markedly different effects on immune function.

The response to SRBCs was not affected by any form of exercise training. One possible reason behind this is the less resource-intensive nature of an antibody response. Previous studies using an avian model have suggested that the cost of antibody production is low and might not reach a sufficient magnitude to force an energetic trade-off (Eraud et al., 2005; Svensson et al., 2002). It is possible that this humoral response was not greatly impacted because of such a low energy cost. For example, homing pigeons that flew in races had altered leukocyte profiles, but not natural antibody levels, compared with birds that did not fly (Matson et al., 2012). Alternatively, the molecular and cellular responses to exercise could actually enhance humoral immunity over cell-mediated immunity by altering the ratio of types of T helper (T_H) lymphocytes produced (Morel and Oriss, 1998; Romagnani, 2000). Over-strenuous exercise, such as marathon running in humans, increases T_H2 over T_H1 lymphocytes (Smith, 2003), which results in increased humoral immunity at the expense of cell-mediated immunity (Oberholzer et al., 2000). This could be advantageous in energy-limited situations so that resources can be diverted away from the potentially higher costs of cell-mediated immunity and toward other processes or traits (Lee, 2006). Resistance exercise, by comparison, increases IL-6 production (Roberts et al., 2018; Ahtiainen, 2019), which also favors humoral over cell-mediated immunity (Elenkov and Chrousos, 1999). Thus, overall, it is not

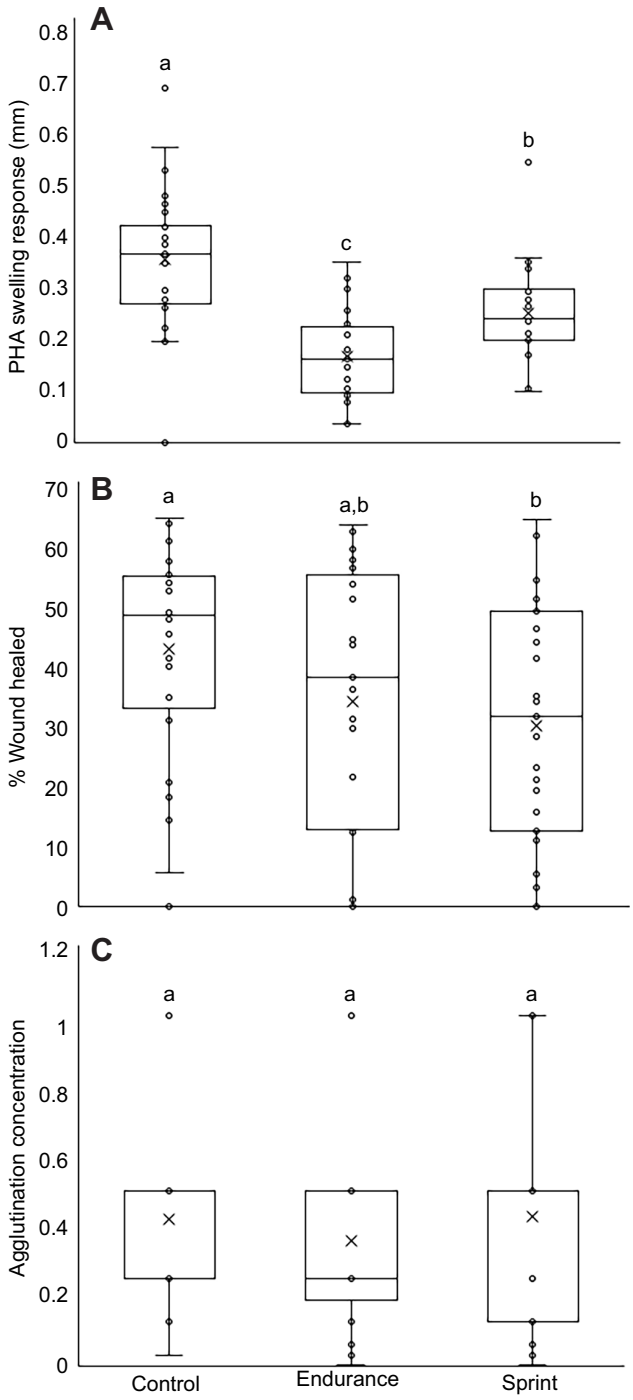

Fig. 1. Specialized exercise training suppresses immune function in *Anolis carolinensis* (green anole lizards). (A–C) Swelling response to phytohemagglutinin (PHA) (A), percentage of wound healed (B) and antibody response to sheep red blood cells (agglutination concentration) (C) by male and female green anoles (combined) that were not trained (controls), endurance trained or sprint trained. Treatments within a panel with different letters above them were significantly different. N=15 per sex per treatment.

surprising that neither form of exercise training suppressed the response to SRBCs.

Endurance training significantly decreased the PHA swelling response to a greater magnitude than sprint training, which makes sense in light of the shifted production of T_H lymphocytes discussed above. However, sprint-trained lizards did not have a significantly suppressed PHA response compared with controls, suggesting that the cost of training to cell-mediated immunity was greatest for endurance-trained lizards. One possible reason behind this difference could lie in how the two types of exercise favor humoral over cell-mediated immunity. Perhaps the shift in T_H lymphocytes caused by endurance training causes a greater decrease in cell-mediated immunity compared with the shift caused primarily by IL-6 during sprint training. Alternatively, the difference could result from increased ROS generation from endurance training, which could lead to oxidative damage to muscle fibers (He et al., 2016). Although regular training enhances the generation of antioxidant enzymes to counteract this deleterious effect, overtraining or instances of energetic limitation could lead to an inability to compensate for ROS generation with only antioxidant production. This would lead to mounting oxidative damage within the organism, possibly leading to further strain on energetic resources (Vider et al., 2001). *In vivo* cell-mediated immunity has been shown to be depressed with strenuous exercise (Bruunsgaard et al., 1997; Nieman, 2000), and the high intensity of our endurance training might have contributed to the significantly lower response. Aerobic exercise in humans transiently reduces the swelling response to PHA owing to a reduction in $CD3^+$ and $CD19^+$ lymphocytes (Shinkai et al., 1992; Vider et al., 2001), and chronic over-exercise, which is likely what our lizards experienced, causes multiple transient changes to become additive in chronic suppression of cell-mediated immunity (Smith, 2003).

Wound healing, an integrated immune response, should have been the costliest of the immune measures we used (Lee, 2006). Accordingly, it was lowest in the sprint-trained group, which should have resulted in the highest protein limitation (Husak et al., 2015). Our previous work on trained green anoles showed that endurance exercise decreases standard metabolic rate, and that green anoles have faster metabolic recovery after running to exhaustion, thus decreasing energy requirements for maintenance (Lailvaux et al., 2018). This more efficient energy usage might at least partially explain why endurance-trained lizards did not have significantly impaired wound healing, because more resources may have been left to be used in wound healing compared with sprint-trained lizards, which invest in larger muscles (Husak et al., 2015). The spared resources in endurance-trained lizards would then become available for other purposes such as faster tissue regrowth. Endurance exercise has been shown to accelerate wound healing in other organisms (Keylock et al., 2008), mostly through the stimulation of type 2 T-cell production, but we did not find heightened wound healing in endurance-trained lizards compared with our controls. Perhaps the high production costs of the training itself prevented the enhancement of wound healing.

Our results reveal a complex set of trade-offs and priorities among whole-organism performance traits and components of the immune system. It appears that producing antibodies is either not very costly for green anoles or that all groups equally invest in antibodies regardless of energy limitation, as predicted by life-history theory (Lochmiller and Deerenberg, 2000; Lee, 2006). Wound healing, by contrast, does appear costly, and high protein use may specifically prevent timely healing when sprinting frequency is high. Although pathogens are ubiquitous in nature, the ecological relevance of a PHA swelling response is less clear (Kennedy and Nager, 2006; Martin et al., 2006), although such plant-produced mitogens may be commonly encountered (Lochmiller and Deerenberg, 2000). Wounds are an important part of the lives of wild green anoles. Sarcophagid fly larvae that parasitize green anoles leave large wounds when they exit the body (Irschick et al., 2006), and fighting males bite each other, at times resulting in large wounds (Lailvaux et al., 2004; McMillan and Irschick, 2010). Further, males bite females on the neck during copulation, sometimes resulting in wounds (Evans, 1935). Thus, green anoles must balance avoiding predators (i.e. using locomotion) and fighting off pathogens (i.e. using the immune system). Based on the mechanisms described above, it is clear that trade-offs that result from increased activity are not caused by energy limitations alone. Instead, increasing the use of different types of locomotor activity 'prioritize' different aspects of the immune system. The evolutionary reasons for the trade-off we discovered are unclear, because few studies have attempted to link performance with immunity in non-human animals (van Dijk and Matson, 2016; Adamo, 2020). Short-lived, 'fast-paced' species such as green anoles are predicted to have greater non-specific immunity over acquired immunity compared with longer-lived species (Lee, 2006). However, when looking within a species, energy limitation should decrease non-specific inflammatory responses and innate immunity to favor stronger antibody responses (Lee, 2006). Previous results in green anoles showed that bacterial killing by plasma, a measure of innate immunity, was reduced by training and diet restriction (Husak et al., 2016), and investment in endurance after training was negatively related to bacteria-killing ability (Husak and Lailvaux, 2017). Our study results partially support the hypothesis that energy limitation should decrease non-specific immunity and favor acquired immunity, which could be why we found little effect of training on antibody production. Alternatively, the activity-specific effects of training on immune function might simply be a byproduct of shared molecular pathways turned on by training but not adaptively altering the immune system. Future studies in non-human animals on the evolution of performance capacities and the immune system should consider both aspects for a fuller picture of how the integrated phenotype evolves.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: A.Z.W., J.F.H.; Methodology: A.Z.W., J.F.H.; Investigation: A.Z.W., J.F.H.; Data curation: A.Z.W.; Writing - original draft: A.Z.W., J.F.H.; Writing - review & editing: A.Z.W., J.F.H.; Project administration: A.Z.W.; Funding acquisition: A.Z.W.

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