The Journal of Experimental Biology 216, 4212-4221

doi:10.1242/jeb.087361

RESEARCH ARTICLE

Immune response to a *Trichinella spiralis* infection in house mice from lines selectively bred for high voluntary wheel running

Elizabeth M. Dlugosz[1,*], Heidi Schutz[1,†], Thomas H. Meek[1,‡], Wendy Acosta[1], Cynthia J. Downs[2,§], Edward G. Platzer[1,3], Mark A. Chappell[1] and Theodore Garland, Jr[1]

[1]Department of Biology and Graduate Program in Evolution, Ecology and Organismal Biology, University of California, Riverside, Riverside, CA 92521, USA, [2]Department of Biology and Program in Ecology, Evolution and Conservation Biology, University of Nevada, Reno, NV 89557, USA and [3]Department of Nematology, University of California, Riverside, Riverside, CA 92521, USA

*Author for correspondence at present address: Mitrani Department of Desert Ecology, The Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, 84990 Midreshet Ben-Gurion, Israel (edlug001@ucr.edu)

†Present address: Department of Biology, Pacific Lutheran University, Tacoma, WA 98447, USA

‡Present address: Diabetes and Obesity Center of Excellence, Department of Medicine, University of Washington, Seattle, WA 98109, USA

§Present address: Department of Natural Resources and Environmental Science, University of Nevada, Reno, NV 89557, USA

SUMMARY

Four lines of mice bred for high voluntary wheel running (HR lines) have high baseline circulating corticosterone levels and increased daily energy expenditure as compared with four non-selected control (C) lines. High corticosterone may suppress immune function and competing energy demands may limit ability to mount an immune response. We hypothesized that HR mice have a reduced immune response and therefore a decreased ability to fight an infection by *Trichinella spiralis*, an ecologically relevant nematode common in mammals. Infections have an acute, intestinal phase while the nematode is migrating, reproducing and traveling throughout the bloodstream, followed by a chronic phase with larvae encysted in muscles. Adult males (generation 55 of the selection experiment) were sham-infected or infected by oral gavage with ~300 J1 *T. spiralis* larvae. During the chronic phase of infection, mice were given wheel access for 6 days, followed by 2 days of maximum aerobic performance trials. Two weeks post-infection, infected HR had significantly lower circulating immunoglobulin E levels compared with infected C mice. However, we found no statistical difference between infected HR and C mice in numbers of encysted larvae within the diaphragm. As expected, both voluntary running and maximum aerobic performance were significantly higher in HR mice and lower in infected mice, with no line type-by-infection interactions. Results complement those of previous studies suggesting decreased locomotor abilities during the chronic phase of *T. spiralis* infection. However, despite reduced antibody production, breeding for high voluntary wheel exercise does not appear to have a substantial negative impact on general humoral function.

Supplementary material available online at http://jeb.biologists.org/cgi/content/full/216/22/4212/DC1

Key words: artificial selection, corticosterone, experimental evolution, immune function, trade-off, trichinosis, voluntary exercise, wheel running.

Received 23 February 2013; Accepted 7 August 2013

INTRODUCTION

A major theme in the field of ecological immunology (Sheldon and Verhulst, 1996; Wikelski and Ricklefs, 2001; Martin et al., 2007) is the potential for physiological trade-offs that are presumed to occur when various functions compete for limited resources and/or influence the behavior of animals under natural conditions (Clobert et al., 2000; Careau and Garland, 2012). A number of studies, mostly in birds, have investigated potential trade-offs between immune function and other energetically expensive traits. Energetically costly activities are particularly likely to suffer if a limited supply of energy must support immune upregulation in addition to other functions (Demas et al., 1997; Lochmiller and Deerenberg, 2000; Derting and Compton, 2003; French et al., 2009). Maintenance and upregulation of immune function are assumed to be expensive for most organisms, though many costs related to immune functions remain difficult to quantify (Lochmiller and Deerenberg, 2000; Ots et al., 2001; Bonneaud et al., 2003; Freitak et al., 2003; Pilorz et al., 2005; Amat et al., 2007; Colditz, 2008). Moreover, it may be less energetically costly to live with a low-level infection rather than investing the energy to completely remove it (Bonneaud et al., 2003; Pilorz et al., 2005; Colditz, 2008).

Parasites are common in wild animals and produce wide-ranging effects on their hosts, including immune responses elicited through a variety of mechanisms. Behavioral changes that occur in the host as a result of infection are well studied, and various parasites (particularly helminths) have substantial impacts on energetically expensive traits (e.g. a host's locomotor behavior or performance abilities), which may directly or indirectly alter the host's ability to survive or reproduce (Anderson and May, 1991; Poirier et al., 1995; Meagher and Dudek, 2002; Careau and Garland, 2012). Assuming limited resources, it is reasonable to expect that parasitized animals may redirect resources to allow for an upregulation of immune function, while compromising other functions.

In this study we investigated the effects of a common nematode parasite, *Trichinella spiralis*, with an infective biology known to directly affect its host (for example, effects on muscle physiology); it also elicits immune responses with potentially indirect effects (e.g.

changes in energy availability *via* alterations in allocation). *Trichinella spiralis* is geographically widely distributed and ecologically relevant in many wild mammal species that consume meat (even occasionally). Its biology has been intensely studied because of is relevance to human health and the husbandry of stock animals (Campbell, 1983; Capó and Despommier, 1996). During *T. spiralis* infections, a host experiences distinct acute and chronic phases (Frenkel, 1989; Meagher and Dudek, 2002). Infection begins when a host animal eats infected meat and larvae emerge in the stomach. During the initial acute phase, larvae mature in the small intestines of the host. Adults mate, females reproduce, and new larvae travel through the host's bloodstream and preferentially enter active skeletal muscle. Upon entering an individual muscle fiber, larvae induce a suite of changes, including de-differentiation of host muscle fibers (making them non-functional in contraction) and considerable upregulation of angiogenesis. The chronic phase of the infection begins when larvae encyst in 'nurse cells' within a muscle, where they can remain for years (Campbell, 1983; Despommier, 1998). Effects of acute trichinosis in rodents include increased weight loss, decreased locomotor activity, decreased run time to exhaustion in a forced-exercise test, decreased home-cage activity and decreased running speed (Von Brand et al., 1954; Bernard, 1959; Goodchild and Frankenberg, 1962; Zohar and Rau, 1984; Zohar and Rau, 1986; Poirier et al., 1995).

The primary goal of the present study was to test for a trade-off between voluntary locomotor activity in wheels and immune function. We also tested whether maximal metabolic rates elicited during forced exercise were impacted by infection. Assuming competing energetic demands and a limited energy supply or ability to process food, we predicted that parasitized animals would reduce voluntary activity levels to allow for an upregulation of immune function. A long-term experiment selectively breeding mice for high voluntary wheel running (Swallow et al., 1998; Rhodes et al., 2005; Garland et al., 2011a; Garland et al., 2011b) is particularly well-suited to studies involving possible trade-offs between locomotion and other metabolically costly functions, including immune responses (Malisch et al., 2009a; Downs et al., 2012). Prior work shows that voluntary running can account for substantial fractions of daily energy expenditure (Rezende et al., 2009), and running in high voluntary wheel running (HR) lines has remained unchanged for >40 generations, despite continued selection, suggesting that it has reached an upper limit (Rezende et al., 2009; Kolb et al., 2010; Careau et al., 2013). In addition, daily running distances of the four replicate HR lines are approximately threefold greater than those of mice from four non-selected control (C) lines, thus providing greater protection against possible 'floor effects' as compared with mice that run at much lower levels. Finally, HR mice have evolved increased maximal oxygen consumption ($\dot{V}_{O2max}$), as measured during forced treadmill exercise (Rezende et al., 2009), and another selection experiment with laboratory house mice that targeted $\dot{V}_{O2max}$ found evidence for altered innate immune function (e.g. decreased cytokine production in response to a lipopolysaccharide injection) in their selected lines (Downs et al., 2013).

Regarding the effects of a *T. spiralis* infection on HR mice, two competing (although not mutually exclusive) hypotheses warrant examination. First, because of possible energetic trade-offs associated with very high activity levels (Rezende et al., 2009), we expect that HR mice may allocate less energy toward resisting infection and would therefore be susceptible to higher infection levels as compared with C mice. Assuming reduced energy allocation toward an immune response in HR mice, we would also expect lower immunoglobulin levels in HR mice as compared with C mice. Here, we measure immunoglobulin E (IgE), which is part of a broader T helper (Th)2 immune response associated with helminth infections. Briefly, Th1 and Th2 cells represent two lymphocyte subpopulations. Both subpopulations are components of adaptive immunity and primarily work through the secretion of cytokines, but Th1 broadly promotes cellular immunity, whereas Th2 promotes humoral immunity (Elenkov, 2004; Viveros-Paredes et al., 2006). Specifically, IgE is thought to directly induce expulsion of *T. spiralis* from the gut (Gurish et al., 2004; Watanabe et al., 2005). We expect higher parasite loads in HR mice *versus* C mice because constraints on IgE levels and/or other immune functions should reduce the host's ability to expel *T. spiralis*. Additionally, if energy allocation is the primary factor driving IgE production and, consequently, the number of larvae eventually encysted in skeletal muscles, then we expect infected HR mice to show reduced muscle function and hence a greater decrease in running performance as compared with infected C mice. That finding would support the idea that HR mice are more energetically challenged by a *T. spiralis* infection.

An alternate hypothesis is focused on elevated circulating corticosterone (CORT) concentrations in HR mice. CORT is a steroid hormone well known to mobilize energy stores, particularly in times of increased stress. Malisch et al. (Malisch et al., 2007) showed that CORT levels changed in response to selection in HR mice, whose CORT levels are approximately double those of C mice (see also Malisch et al., 2008; Malisch et al., 2009a; Downs et al., 2012). Running on wheels further elevates CORT levels (Girard and Garland, 2002; Malisch et al., 2007), as CORT levels generally increase during exercise (Coleman et al., 1998). CORT is thought to be immunosuppressive (Sapolsky et al., 2000); however, it is not clear which aspect of an immune response is most affected by CORT. Malisch et al. showed that, although HR and C mice did not differ statistically in the ability to clear an intestinal nematode infection (*Nippostrongylus brasiliensis*), an analysis of the eight line means revealed a negative relationship between clearance ability and baseline CORT levels, supporting the hypothesis that CORT has immunosuppressive effects (Malisch et al., 2009a). More recently, a study by Downs and colleagues also found no statistical difference between the HR and C lines following an immune challenge with lipopolysaccharide (Downs et al., 2012). Thus, evidence to date indicates that HR and C mice may be capable of similar immune responses to infection. Despite the general prediction that CORT is immunosuppressive, some studies have suggested that CORT affects Th1/Th2 balance and works synergistically with certain cytokines (specifically, interleukin 4) to induce IgE synthesis (Zieg et al., 1994; Wiegers and Reul, 1998; Elenkov, 2004; Viveros-Paredes et al., 2006). Therefore, if IgE levels are increased in HR mice as a result of increased baseline circulating CORT levels, then we may expect these mice to be more resistant to *T. spiralis* infection and with fewer encysted larvae as compared with C mice.

Direct (invasion and deactivation of skeletal muscle cells) and presumed indirect (costly immunological responses) influences of *T. spiralis* on hosts are expected to decrease running performance in both HR and C mice. Thus, an intuitive prediction is a negative relationship between parasite load and voluntary wheel running or maximal performance ability [e.g. as indexed by maximal oxygen consumption (Kolb et al., 2010)]. Assuming immunological and energetic costs associated with infection and potentially anorexigenic effects of parasite infection (Kyriazakis et al., 1998; Adamo et al., 2010), overall body mass and many organ masses are expected to be negatively affected by infection. However, the spleen is often enlarged in organisms with parasitic infections, perhaps indicating

heightened immune response caused by increased production/storage of lymphocytes and immunoglobulins. As the spleen is considered to be an immunologically important organ (Corbin et al., 2008; Cowan et al., 2009; Schulte-Hostedde and Elsasser, 2011), spleen mass is expected to increase with infection. Additionally, hematocrit is expected to be positively correlated with both infection (Meagher, 1998; Downs et al., 2012) and $\dot{V}_{O2max}$ performance.

Finally, we tested for effects of mini-muscle status (Garland et al., 2002; Kelly et al., in press). The primary characteristic of the mini-muscle phenotype is a dramatic reduction in hindlimb muscle mass (~50%) coupled with approximately doubled mass-specific aerobic capacity in hindlimb muscle (Houle-Leroy et al., 2003). This phenotype is caused by a simple Mendelian recessive allele and is associated with alterations in various morphological, physiological and behavioral traits, including organ size, response to a lipopolysaccharide (LPS) immune challenge, and wheel running under some conditions (e.g. Garland et al., 2002; Houle-Leroy et al., 2003; Swallow et al., 2005; Syme et al., 2005; Rezende et al., 2006; Dlugosz et al., 2009; Downs et al., 2012).

MATERIALS AND METHODS

Study animals and experimental design

Mice (*Mus musculus domesticus* Linnaeus) were from generation 55 of an ongoing selection experiment, which includes four lines bred for high voluntary wheel running (HR) and four control (C) lines bred without regard to wheel running (Swallow et al., 1998). Briefly, mice from each generation are given wheel access at ~6–8 weeks of age, within-family selection is used, and a minimum of 10 mating pairs are used to produce litters. In the HR lines, the mice that run the most on days 5 and 6 of the 6-day period of wheel access are bred to produce the next generation. C mice are also given access to wheels for 6 days, but are bred without regard to their amount of wheel running. All eight lines have been reproductively isolated since the beginning of the selection experiment. Mice are housed on a 12 h:12 h light:dark cycle and given *ad libitum* food and water. One hundred and one male mice were used for this experiment (males were used to avoid potential complications from estrous cycles). Mice were weaned at 21 days according to the selection protocol (Swallow et al., 1998) and housed four to a cage.

At approximately 7 weeks of age (Fig. 1), half of the mice (chosen randomly) were infected (*via* a stomach tube) with approximately 300 *T. spiralis* (Beltsville strain) J1-stage larvae in 0.1 ml saline [shown to be an ecologically relevant dose for mice (Despommier, 1983; Meagher and Dudek, 2002)]. Larvae were recovered from artificially digested skeletal muscle of a previously infected laboratory mouse host. The remaining mice received a sham infection (0.1 ml saline). IgE levels were obtained from 75 µl blood samples taken from each mouse 2 weeks following infection using an infraorbital socket protocol (Hoff, 2000). Blood was centrifuged for 12 min at 46,000 ***g*** and 4°C, and plasma was stored at –20°C until IgE assays.

At approximately 4 weeks after the initial infection, larvae were expected to be encysted in host skeletal muscle [the chronic phase of infection (Despommier, 1998)]. At this time, mice were exposed to the running wheels used in the selection experiment (1.12 m circumference) for 6 days. Wheel running was recorded for 23.5 h each day in 1 min intervals, and total revolutions per day, total number of 1 min intervals with at least one revolution, mean speed (total revolutions per number of active intervals) and maximum speed in any 1 min interval were calculated. Wheel freeness (measured by the number of revolutions recorded following acceleration to a standard revolutions per minute) was measured prior to wheel testing and at the conclusion of the study. Wheel data were recorded and downloaded each day.

Immediately following the 6-day wheel running test, we measured $\dot{V}_{O2max}$ during forced exercise using open-flow respirometry in a small enclosed treadmill (Rezende et al., 2006) (details below). Following $\dot{V}_{O2max}$ trials, mice were euthanized by decapitation and dissected. Liver, ventricles, lungs, diaphragm, spleen and the right triceps surae muscle were collected and weighed. Liver, ventricles and lungs were then dried to a constant mass and re-weighed. Diaphragm and spleen were frozen for future analyses. Right triceps surae mass was used to determine mini-muscle status (Garland et al., 2002).

Diaphragm muscle compression for larvae detection

The diaphragm is one of the most common sites of *T. spiralis* larval encystment, so we used counts of larvae encysted in this tissue as an index of infection level (Gottstein et al., 2009). Diaphragms were removed, weighed and stored in a –20°C freezer. We counted total larvae number in the diaphragm as a measure of final parasite load for each mouse. Prior to counting, diaphragms were stained in Giemsa Stain (see Ramirez-Melgar et al., 2007) and compressed between two glass microscope slides. All nurse cell–larvae complexes in the diaphragm were counted (Zeiss microscope, 100× total magnification) four times and averaged. Additionally, diaphragms from sham-infected mice were inspected to ensure they were not infected.

IgE analyses

Plasma samples collected 2 weeks post-infection (i.e. in the acute state of infection) were used to assess the primary immune response to infection. Two weeks post-infection is the predicted time of maximum IgE response (Jarrett and Haig, 1976; Dearman et al., 1998; Salagianni et al., 2007; Gurish et al., 2004). A double antibody enzyme-linked sandwich immunoperoxidase assay (Mouse IgE ELISA Kit E90-E; Immunology Consultants Laboratory, Portland, OR, USA) was used to determine plasma IgE levels. Samples were diluted and measured in duplicate on a pre-coated anti-mouse IgE plastic microtiter plate. Absorbance was determined at 450 nm using a SpectraMax Plus microplate reader (Molecular Devices, Sunnyvale, CA, USA). Values (ng ml^{-1}) were compared with a standard curve generated individually for each plate.

Maximal metabolic rate ($\dot{V}_{O2max}$)

Each mouse was run individually and was given two $\dot{V}_{O2max}$ tests on consecutive days. Mice were given several minutes to acclimate to the motionless treadmill (volume 850 ml). When the test began,

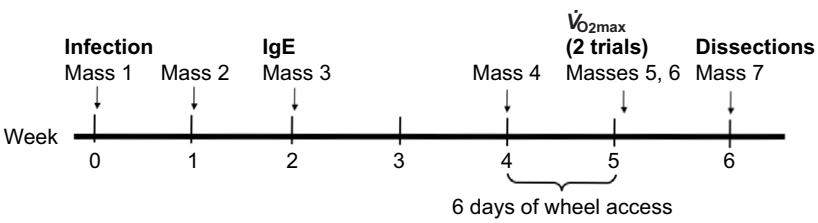

Fig. 1. Timeline of experimental design.

speed was initially low. Mice oriented quickly and ran well. Treadmill speed was matched to the behavior of the animals, and was increased approximately every 30 s until $\dot{V}_{O_2}$ did not increase or until the mouse could not keep pace with the treadmill or failed to continue running. At that point the treadmill was stopped and mice were allowed to recover before being removed. Flow rates (2000 ml min^{-1}) and gas concentrations were recorded using LabHelper software (www.warthog.ucr.edu) every 1 s with references taken at the beginning and end of each trial. Excurrent air was subsampled at 150 ml min^{-1}, scrubbed and dried (soda lime and Drierite), and then passed through an O_2 analyzer (Applied Electrochemistry S-3A, Sunnyvale, CA, USA). Instantaneous corrections (Bartholomew et al., 1981) were used to account for mixing and washout characteristics and accurately record rapid changes in metabolism. The effective volume for instantaneous calculations was 900 ml, as determined from washout curves generated with step changes in incurrent O_2 concentration. Oxygen consumption ($\dot{V}_{O_2}$) was calculated as:

$$\dot{V}_{O_2} = \dot{V}(F_{IO_2} - F_{EO_2}) / (1 - FeO_2), \quad (1)$$

where F_{IO_2} and F_{EO_2} are the fractions of inspired and expired O_2, respectively.

The $\dot{V}_{O_2max}$ was defined as the highest consecutive 1 min of $\dot{V}_{O_2}$ during exercise. The higher of the two $\dot{V}_{O_2max}$ values was used in analyses.

Statistical analyses

Proc Mixed in SAS version 9.2 was used for mixed-model ANOVA analyses (SAS Institute, Cary, NC, USA). The mixed-model ANOVA included infection (referring to differences between infected and uninfected mice), line type (referring to differences between HR and C mice) and the infection-by-line type interaction. These effects were tested relative to the random effect of replicate line nested within line type, with 1 and 6 d.f. Our prediction that HR mice were more affected by infection than C mice was tested *via* the interaction between line type and infection. Mini-muscle [which may affect certain aspects of performance (Houle-Leroy et al., 2003; Syme et al., 2005; Dlugosz et al., 2009; McGillivray et al., 2009)] was coded as a dummy variable and included as a fixed effect, tested relative to the error term with d.f. of 1 and ~76 (or ~38 when only infected mice were analyzed). On a trait-by-trait basis, mice were excluded from analyses in which their standardized residual was greater than |3|.

Body mass was recorded seven times over the course of the experiment (see timeline in Fig. 1). Body mass 1 was analyzed with a one-way mixed-model ANOVA (with line included as a random effect), as mice were not yet infected at that time. All other body mass data were analyzed with the general mixed-model ANOVA described above. Age was included as a covariate in analyses of body mass and body mass was included as a covariate in analyses of organ masses. Spleen, ventricle and lung masses were $\log_{10}$-transformed to achieve approximate normality of residuals.

To test for the effects of infection on wheel running, we used mixed-model ANOVAs (as described above) for distance (revolutions day^{-1}), time (min day^{-1}), mean speed (revolutions min^{-1}) and maximum speed (revolutions min^{-1}). For consistency with the standard selection protocol, only values averaged for days 5 and 6 of the 6-day test were analyzed. Wheel freeness measures were averaged and used as a covariate in wheel-running analyses, as was age. Distance and time were square-root transformed to reduce skewness of residuals.

For $\dot{V}_{O_2max}$, we used the mixed-model ANOVA described above. As is typical for metabolic measurements, $\dot{V}_{O_2max}$ was $\log_{10}$-transformed and $\log_{10}$-tranformed body mass was used as a covariate. Because IgE levels for uninfected mice were, as expected, very low and often did not reliably fall on the standard curve, only infected mice were included in IgE analyses. The number of larvae encysted in the diaphragm was used as our measure of final infection intensity, with body mass as a covariate.

For traits measured close to the time of dissection [body masses 5, 6 and 7 (Fig. 1), organ masses, wheel running and $\dot{V}_{O_2max}$ (ml min^{-1})], we also performed analyses of only the infected mice, using diaphragm larvae count as a covariate and line type and mini-muscle status as factors.

RESULTS

Body mass

Consistent with many previous studies of these lines, uninfected C mice were approximately 8–9% larger than uninfected HR mice at all seven body mass measurements, although none of the differences reached statistical significance (Fig. 1, supplementary material Tables S1, S2) (e.g. Swallow et al., 1999; Malisch et al., 2009a; Meek et al., 2009; Rezende et al., 2009; Kolb et al., 2010). Mini-muscle HR mice were always significantly smaller than normal mice. One week after infection (body mass 2), infection status was not significantly associated with body mass. However, starting 2 weeks after infection and continuing through the end of the experiment, infected mice were significantly smaller than uninfected mice. Overall, from the time of infection (bleed 1 mass) until dissection, uninfected C mice showed a 10.75% increase in body mass and uninfected HR mice increased their body mass by 10.01% compared with initial body mass. However, infected C mice only showed a 0.33% increase in body mass, whereas infected HR mice increased their body mass by 2.87%. There were no significant line type-by-infection interactions for body mass at any point of the experiment (i.e. body masses of both line types were approximately equally affected by infection). Considering only the infected mice, diaphragm larvae counts had a significant negative relationship with body mass (supplementary material Table S1).

Organ masses

Body mass at the time of dissection was a significant positive predictor of all organ masses except wet and dry lung mass (Table 1). HR mice had significantly larger wet and dry ventricle masses compared with C mice. Spleen mass [thought to be a good indicator of immunological activity and parasitic infection in mammals (Cowan et al., 2009; Schulte-Hostedde and Elsasser, 2011)] was significantly larger in infected mice than uninfected mice. Infected mice also had significantly smaller wet and dry lung masses than uninfected mice. Line type-by-infection interactions were not statistically significant for any organ mass. In previous studies, the mini-muscle gene has been shown to have many pleiotropic effects, including increases in body-mass-adjusted organ masses (e.g. Garland et al., 2002; Swallow et al., 2005; Downs et al., 2012). In the present study, mini-muscle mice had significantly larger wet and dry liver masses than normal mice, but mini-muscle was not a significant predictor of any other relative organ masses (Table 1). In infected mice, diaphragm larvae counts had a significant negative relationship with ventricle mass (supplementary material Table S3).

Hematocrit

Hematocrit was never statistically different between HR and C mice, and body mass was never a significant predictor of hematocrit

Table 1. Significance levels from SAS Proc Mixed analyses of organ masses

		Least squares means ± s.e.m.					Two-tailed *P* (d.f.)				
Organ mass (*N*)	Transform	Control uninfected	Control infected	High runner uninfected	High runner infected	Normal muscle	Mini-muscle	Infection	Line type	Infection × line type	Mini-muscle	Body mass
Spleen (94)	Log_{10}	−1.0619± 0.0416	−0.9982± 0.0413	−1.0580± 0.0382	−0.9416± 0.0389	−1.0281± 0.0209	−1.0017± 0.0339	**0.0451 (+)** (1,6)	0.4982 (+) (1,6)	0.4798 (1,6)	0.4132 (+) (1,76)	**<0.0001 (+)** (1,76)
Wet liver (95)	None	1.8861± 0.0725	2.0069± 0.0683	2.0078± 0.0563	2.0211± 0.0600	1.7981± 0.0360	2.1692± 0.0769	0.1750 (+) (1,6)	0.3947 (+) (1,6)	0.2241 (1,6)	**<0.0001 (+)** (1,77)	**<0.0001 (+)** (1,77)
Dry liver (95)	None	0.6068± 0.0259	0.6382± 0.0246	0.6475± 0.0203	0.6496± 0.0215	0.5746± 0.0132	0.6965± 0.0275	0.3031 (+) (1,6)	0.3717 (+) (1,6)	0.3203 (1,6)	**<0.0001 (+)** (1,77)	**<0.0001 (+)** (1,77)
Wet ventricle (94)	Log_{10}	−0.8478± 0.0134	−0.8415± 0.0128	−0.7954± 0.0108	−0.8136± 0.0113	−0.8319± 0.0069	−0.8172± 0.0144	0.4557 (–) (1,6)	**0.0299 (+)** (1,6)	0.1233 (1,6)	0.3329 (+) (1,76)	**<0.0001 (+)** (1,76)
Dry ventricle (93)	Log_{10}	−1.4809± 0.0135	−1.4827± 0.0131	−1.4272± 0.0113	−1.4484± 0.0116	−1.4674± 0.0074	−1.4521± 0.0142	0.1503 (–) (1,6)	**0.0261 (+)** (1,6)	0.1748 (1,6)	0.2948 (+) (1,75)	**<0.0001 (+)** (1,75)
Wet lungs (94)	Log_{10}	−0.5967± 0.0215	−0.6714± 0.0190	−0.6037± 0.0154	−0.6336± 0.0171	−0.6314± 0.0087	−0.6212± 0.0214	**0.0180 (–)** (1,6)	0.4452 (+) (1,6)	0.1843 (1,6)	0.6659 (+) (1,76)	0.6478 (+) (1,76)
Dry lungs (93)	Log_{10}	−1.2599± 0.0215	−1.3241± 0.0189	−1.2632± 0.0157	−1.2922± 0.0170	−1.2888± 0.00833	−1.2809± 0.0215	**0.0308 (–)** (1,6)	0.4660 (+) (1,6)	0.2912 (1,6)	0.7375 (+) (1,75)	0.6749 (+) (1,75)
Hematocrit (bleed 1) (101)	None	47.01± 0.6851		46.94± 0.55		46.42± 0.36	47.53± 0.89	–	0.9415 (–) (1,6)	–	0.2635 (+) (1,91)	0.6871 (–) (1,91)
Hematocrit (bleed 2) (98)	None	45.43± 1.09	48.23± 1.04	44.97± 0.8552	47.08± 0.85	47.18± 0.50	45.67± 1.17	**0.0148 (+)** (1,6)	0.4679 (–) (1,6)	0.6416 (1,6)	0.2409 (–) (1,80)	0.6169 (+) (1,80)
Hematocrit (bleed 3) (96)	None	44.11± 1.15	38.86± 1.08	44.28± 0.88	40.35± 0.92	43.24± 0.48	40.56± 1.12	**0.0028 (–)** (1,6)	0.4293 (+) (1,6)	0.4891 (1,6)	**0.0311 (–)** (1,78)	0.7277 (+) (1,78)

Positive signs following *P*-values indicate infected>uninfected, HR>C, mini-muscle>normal or positive effect of body mass. Negative signs indicate the opposite relationships. *P*-values <0.05 are in **bold**.
Body mass was not transformed for use as a covariate in analyses of organ masses.

(Table 1). One week after infection, infected mice had significantly higher hematocrit than uninfected mice (P=0.0148), but this difference was reversed a week later (P=0.0028). Two weeks post-infection, mini-muscle mice had significantly lower hematocrit compared with normal mice (P=0.0311).

Voluntary wheel running

Four components of voluntary wheel running (means for days 5 and 6 of the 6-day test) were analyzed: distance, time, mean speed and maximum speed. Neither age nor mini-muscle was significantly associated with any measure of wheel running (Table 2). As expected based on many previous studies, HR male mice ran significantly farther, for more minutes per day, and at a higher mean running speed than C mice. As predicted, distance run, time run and maximum speed were significantly lower in infected mice, although the decrease in mean speed did not reach statistical significance. There was no infection-by-line type interaction for any aspect of voluntary wheel running; thus, based on back-transformed least squares means, the proportional reduction in distance run resulting from infection was similar in HR and C mice (−43% *versus* −46%, respectively). However, the absolute reduction in distance run due to infection was approximately fourfold larger in HR as compared with C mice (Fig. 2). In the infected mice, diaphragm larvae counts had a significant negative relationship with all four measures of wheel running while HR mice also ran more than C mice (Table 2).

$\dot{V}_{O2max}$

The two measurements of $\dot{V}_{O2max}$ were significantly correlated between trial days (r=0.3982, P<0.0001), and did not differ on average between trials (paired t-test, P=0.1645). As expected, $\log_{10}$ body mass was a highly significant predictor of $\log_{10}$ $\dot{V}_{O2max}$ (P<0.0001; Table 3). After accounting for $\log_{10}$ body mass, HR mice had significantly higher $\dot{V}_{O2max}$ than C mice (P=0.0170). Infection decreased $\dot{V}_{O2max}$ (P=0.0396), although the magnitude of the decrease was small (2–6%). Similar to wheel running, there was no infection-by-line type interaction (P=0.1286) and mini-muscle mice did not have different $\dot{V}_{O2max}$ from normal mice (P=0.9009). In the infected mice, diaphragm larvae counts were not significantly related to $\dot{V}_{O2max}$ (Table 3).

Immunoglobulin E

In infected animals, plasma IgE levels at 2 weeks post-infection were significantly higher (~67%) in C mice as compared with HR mice (P=0.0077; Table 3). Surprisingly, although all mini-muscle mice are HR mice, mini-muscle mice had significantly higher IgE levels compared with mice without mini-muscle (P=0.0361; Table 3). Although uninfected mice were not included in IgE analyses, preliminary tests suggested that baseline, uninfected IgE levels were similar in HR and C mice (P=0.9682) and IgE levels in uninfected mini-muscle mice were not different from those of normal mice (P=0.4728).

Diaphragm larvae counts

Among infected mice, the number of larvae per diaphragm ranged from 285 to 2140 (mean ± s.e.m.=1261±54.5). $\log_{10}$ body mass was strongly negatively correlated with infection level, as measured by counts of larvae in diaphragms (P<0.0001; Table 3). Contrary to our prediction, infected HR and C mice did not have significantly different numbers of larvae in their diaphragms (least squares means were 1278 *versus* 1132 larvae in C *versus* HR mice, respectively; P=0.2060). Mini-muscle status was not a significant predictor of larvae encysted in the diaphragm (P=0.2605). Results

Table 1. Significance levels from SAS Proc Mixed analyses of organ masses

		Least squares means ± s.e.m.						Two-tailed *P* (d.f.)				
Organ mass (*N*)	Transform	Control uninfected	Control infected	High runner uninfected	High runner infected	Normal muscle	Mini-muscle	Infection	Line type	Infection × line type	Mini-muscle	Body mass
Spleen (94)	Log_{10}	−1.0619± 0.0416	−0.9982± 0.0413	−1.0580± 0.0382	−0.9416± 0.0389	−1.0281± 0.0209	−1.0017± 0.0339	**0.0451 (+)** (1,6)	0.4982 (+) (1,6)	0.4798 (1,6)	0.4132 (+) (1,76)	**<0.0001 (+)** (1,76)
Wet liver (95)	None	1.8861± 0.0725	2.0069± 0.0683	2.0078± 0.0563	2.0211± 0.0600	1.7981± 0.0360	2.1692± 0.0769	0.1750 (+) (1,6)	0.3947 (+) (1,6)	0.2241 (1,6)	**<0.0001 (+)** (1,77)	**<0.0001 (+)** (1,77)
Dry liver (95)	None	0.6068± 0.0259	0.6382± 0.0246	0.6475± 0.0203	0.6496± 0.0215	0.5746± 0.0132	0.6965± 0.0275	0.3031 (+) (1,6)	0.3717 (+) (1,6)	0.3203 (1,6)	**<0.0001 (+)** (1,77)	**<0.0001 (+)** (1,77)
Wet ventricle (94)	Log_{10}	−0.8478± 0.0134	−0.8415± 0.0128	−0.7954± 0.0108	−0.8136± 0.0113	−0.8319± 0.0069	−0.8172± 0.0144	0.4557 (–) (1,6)	**0.0299 (+)** (1,6)	0.1233 (1,6)	0.3329 (+) (1,76)	**<0.0001 (+)** (1,76)
Dry ventricle (93)	Log_{10}	−1.4809± 0.0135	−1.4827± 0.0131	−1.4272± 0.0113	−1.4484± 0.0116	−1.4674± 0.0074	−1.4521± 0.0142	0.1503 (–) (1,6)	**0.0261 (+)** (1,6)	0.1748 (1,6)	0.2948 (+) (1,75)	**<0.0001 (+)** (1,75)
Wet lungs (94)	Log_{10}	−0.5967± 0.0215	−0.6714± 0.0190	−0.6037± 0.0154	−0.6336± 0.0171	−0.6314± 0.0087	−0.6212± 0.0214	**0.0180 (–)** (1,6)	0.4452 (+) (1,6)	0.1843 (1,6)	0.6659 (+) (1,76)	0.6478 (+) (1,76)
Dry lungs (93)	Log_{10}	−1.2599± 0.0215	−1.3241± 0.0189	−1.2632± 0.0157	−1.2922± 0.0170	−1.2888± 0.00833	−1.2809± 0.0215	**0.0308 (–)** (1,6)	0.4660 (+) (1,6)	0.2912 (1,6)	0.7375 (+) (1,75)	0.6749 (+) (1,75)
Hematocrit (bleed 1) (101)	None	47.01± 0.6851		46.94± 0.55		46.42± 0.36	47.53± 0.89	–	0.9415 (–) (1,6)	–	0.2635 (+) (1,91)	0.6871 (–) (1,91)
Hematocrit (bleed 2) (98)	None	45.43± 1.09	48.23± 1.04	44.97± 0.8552	47.08± 0.85	47.18± 0.50	45.67± 1.17	**0.0148 (+)** (1,6)	0.4679 (–) (1,6)	0.6416 (1,6)	0.2409 (–) (1,80)	0.6169 (+) (1,80)
Hematocrit (bleed 3) (96)	None	44.11± 1.15	38.86± 1.08	44.28± 0.88	40.35± 0.92	43.24± 0.48	40.56± 1.12	**0.0028 (–)** (1,6)	0.4293 (+) (1,6)	0.4891 (1,6)	**0.0311 (–)** (1,78)	0.7277 (+) (1,78)

Positive signs following *P*-values indicate infected>uninfected, HR>C, mini-muscle>normal or positive effect of body mass. Negative signs indicate the opposite relationships.

P-values <0.05 are in **bold**.

Body mass was not transformed for use as a covariate in analyses of organ masses.

Table 2. Significance levels from SAS Proc Mixed analyses of wheel running on days 5 and 6

		Least squares means ± s.e.m.					Two-tailed *P* (d.f.)			
Trait (*N*)	Transform	Control uninfected	Control infected	High runner uninfected	High runner infected	Normal muscle	Mini-muscle	Infection	Line type	Infection × line type	Mini-muscle	Diaphragm larvae counts
Mean distance (revolutions) (95)	Square	37.30±	21.12±	66.59±	45.37±	41.15±	44.03±	**0.0125 (–)**	**0.0034 (+)**	0.6510 (1,6)	0.6914 (+)	–
	root	6.78	6.38	5.33	5.28	2.85	6.71	(1,6)	(1,6)		(1,76)	
Mean distance (revolutions) (with diaphragm larvae counts) (49)	Square	–	11.53±	–	42.81±	34.83±	19.52±	–	**0.0073 (+)**	–	0.1068 (+)	**0.0015 (–)**
	root		6.92		5.40	3.96	8.56		(1,6)		(1,37)	(1,37)
Mean time (min) (95)	Square	12.78±	7.29±	16.19±	12.03±	12.36±	11.78±	**0.0023 (–)**	**0.0076 (+)**	0.5102 (1,6)	0.6678 (-)	–
	root	1.24	1.16	0.96	0.95	0.52	1.25	(1,6)	(1,6)		(1,76)	
Mean time (min) (with diaphragm larvae counts) (49)	Square	–	6.09±	–	11.80±	10.24±	7.65±	–	**0.0190 (+)**	–	0.2095 (–)	**0.0003 (–)**
	root		1.56		1.23	0.90	1.89		(1,6)		(1,37)	(1,37)
Mean speed (revolutions min^{-1}) (95)	None	8.25±	6.60±	16.64±	12.93±	10.02±	12.20±	0.1102 (–)	**0.0029 (+)**	0.4491 (1,6)	0.2321 (+)	–
		1.76	1.69	1.43	1.42	0.76	1.70	(1,6)	(1,6)		(1,76)	
Mean speed (revolutions min^{-1}) (with diaphragm larvae counts) (49)	None	–	4.40±	–	12.31±	9.42±	7.29±	–	**0.0054 (+)**	–	0.3412 (+)	**0.0208 (–)**
			1.64		1.28	0.94	2.04		(1,6)		(1,37)	(1,37)
Mean maximum speed (revolutions min^{-1}) (95)	None	16.26±	11.22±	30.35±	22.11±	18.96±	21.01±	**0.0194 (–)**	**0.0014 (+)**	0.4750 (1,6)	0.4491 (+)	–
		2.61	2.49	2.10	2.09	1.12	2.52	(1,6)	(1,6)		(1,76)	
Mean maximum speed (revolutions min^{-1}) (with diaphragm larvae counts) (49)	None	–	8.04±	–	21.37±	16.76±	12.64±	–	**0.0058 (+)**	–	0.2580 (+)	**0.0055 (–)**
			2.77		2.19	1.60	3.35		(1,6)		(1,37)	(1,37)

Positive signs following *P*-values indicate infected>uninfected, HR>C, mini-muscle>normal or positive effect of diaphragm larvae counts. Negative signs indicate the opposite relationships.
Age and a measure of wheel freeness were covariates in all analyses (results not shown).
P-values <0.05 are in **bold**.
Age was never a significant covariate. Wheel freeness was only a significant covariate in analyses of mean distance (with diaphragm larvae counts) and mean time (with diaphragm larvae counts).

Fig. 2. Wheel running averaged on days 5 and 6 of voluntary wheel running test. Untransformed means (±s.e.m.) for control (C) sham (*N*=18), C infected (*N*=23), high voluntary wheel running (HR) sham (*N*=27) and HR infected (*N*=26) groups. Note that statistical analyses were performed on square-root-transformed values (see Table 2).

were similar when body mass was not included as a covariate (Table 3).

DISCUSSION

Trichinella spiralis infections elicit a suite of behavioral and physiological responses in a wide range of hosts (Campbell, 1983; Capó and Despommier, 1996). Many of these are expected to affect energy metabolism, and our HR mice (the products of 55 generations of selective breeding for high voluntary wheel running) seem particularly likely to exhibit a decrease in voluntary wheel running [which is performed at extraordinarily high levels and has apparently reached a selection limit (Careau et al., 2013)] and a decrease in immune response as a result of a *T. spiralis* infection. As expected, we found effects of both line type and infection on behavior and performance. However, our results suggest that despite increased voluntary wheel running and hence energetic demands in HR mice (Rezende et al., 2009), selection for a highly aerobic lifestyle does not appear to have had substantial negative effects on immune function. Moreover, the infection caused similar proportional decreases in wheel running in both line types (Fig. 2).

Consistent with this interpretation, Malisch and colleagues found that HR and C mice did not significantly differ in their ability to clear another parasite, the intestinal nematode *Nippostrongylus brasiliensis* (Malisch et al., 2009a). However, previous findings also indicate that HR mice have increased baseline CORT levels (thought to be immunosuppressive), which were negatively associated with the ability to clear an *N. brasiliensis* infection when analyzed at the level of the eight line means (Girard and Garland, 2002; Malisch et al., 2007; Malisch et al., 2009a). More recently, Downs et al. challenged mice from the same generation as in the present study with LPS to investigate inflammatory responses (Downs et al., 2012). Despite their elevated baseline CORT levels, HR mice did not have a suppressed inflammatory response to a classic LPS immune challenge.

In the present study, infected mice had significantly higher hematocrit values compared with uninfected mice 1 week after infection. Increased hematocrit is typical of inflammatory responses and parasitic infections (Meagher, 1998; Downs et al., 2012). Two

Table 2. Significance levels from SAS Proc Mixed analyses of wheel running on days 5 and 6

		Least squares means ± s.e.m.						Two-tailed *P* (d.f.)				
Trait (*N*)	Transform	Control uninfected	Control infected	High runner uninfected	High runner infected	Normal muscle	Mini-muscle	Infection	Line type	Infection × line type	Mini-muscle	Diaphragm larvae counts
Mean distance (revolutions) (95)	Square root	37.30± 6.78	21.12± 6.38	66.59± 5.33	45.37± 5.28	41.15± 2.85	44.03± 6.71	**0.0125 (–)** (1,6)	**0.0034 (+)** (1,6)	0.6510 (1,6)	0.6914 (+) (1,76)	–
Mean distance (revolutions) (with diaphragm larvae counts) (49)	Square root	–	11.53± 6.92	–	42.81± 5.40	34.83± 3.96	19.52± 8.56	–	**0.0073 (+)** (1,6)	–	0.1068 (+) (1,37)	**0.0015 (–)** (1,37)
Mean time (min) (95)	Square root	12.78± 1.24	7.29± 1.16	16.19± 0.96	12.03± 0.95	12.36± 0.52	11.78± 1.25	**0.0023 (–)** (1,6)	**0.0076 (+)** (1,6)	0.5102 (1,6)	0.6678 (-) (1,76)	–
Mean time (min) (with diaphragm larvae counts) (49)	Square root	–	6.09± 1.56	–	11.80± 1.23	10.24± 0.90	7.65± 1.89	–	**0.0190 (+)** (1,6)	–	0.2095 (–) (1,37)	**0.0003 (–)** (1,37)
Mean speed (revolutions min^{-1}) (95)	None	8.25± 1.76	6.60± 1.69	16.64± 1.43	12.93± 1.42	10.02± 0.76	12.20± 1.70	0.1102 (–) (1,6)	**0.0029 (+)** (1,6)	0.4491 (1,6)	0.2321 (+) (1,76)	–
Mean speed (revolutions min^{-1}) (with diaphragm larvae counts) (49)	None	–	4.40± 1.64	–	12.31± 1.28	9.42± 0.94	7.29± 2.04	–	**0.0054 (+)** (1,6)	–	0.3412 (+) (1,37)	**0.0208 (–)** (1,37)
Mean maximum speed (revolutions min^{-1}) (95)	None	16.26± 2.61	11.22± 2.49	30.35± 2.10	22.11± 2.09	18.96± 1.12	21.01± 2.52	**0.0194 (–)** (1,6)	**0.0014 (+)** (1,6)	0.4750 (1,6)	0.4491 (+) (1,76)	–
Mean maximum speed (revolutions min^{-1}) (with diaphragm larvae counts) (49)	None	–	8.04± 2.77	–	21.37± 2.19	16.76± 1.60	12.64± 3.35	–	**0.0058 (+)** (1.6)	–	0.2580 (+) (1,37)	**0.0055 (–)** (1,37)

Positive signs following *P*-values indicate infected>uninfected, HR>C, mini-muscle>normal or positive effect of diaphragm larvae counts. Negative signs indicate the opposite relationships.

Age and a measure of wheel freeness were covariates in all analyses (results not shown).

P-values <0.05 are in **bold**.

Age was never a significant covariate. Wheel freeness was only a significant covariate in analyses of mean distance (with diaphragm larvae counts) and mean time (with diaphragm larvae counts).

Table 3. Least squares means and significance levels from SAS Proc Mixed analyses of $\dot{V}_{O2max}$, IgE and diaphragm larvae counts

		Least squares means ± s.e.m.					Two-tailed *P* (d.f.)					
Trait (*N*)	Transform	Control uninfected	Control infected	High runner uninfected	High runner infected	Normal muscle	Mini-muscle	Infection	Line type	Infection × line type	Mini-muscle	Log_{10} body mass	Diaphragm larvae
$\dot{V}_{O2max}$ (ml min^{-1}) (95)	Log_{10}	0.8405± 0.0206	0.8286± 0.0198	0.9317± 0.0163	0.8794± 0.0178	0.8715± 0.0106	0.8686± 0.0220	**0.0396 (–)** (1,6)	**0.0170 (+)** (1,6)	0.1286 (1,6)	0.9009 (–) (1,77)	**<0.0001 (+)** (1,77)	–
$\dot{V}_{O2max}$ (ml min^{-1}) (with diaphragm larvae counts) (49)	Log_{10}	–	0.8119± 0.0230	–	0.8671± 0.0201	0.8424± 0.0132	0.8366± 0.0305	–	0.0939 (+) (1,6)	–	0.8618 (–) (1,38)	**0.0019 (+)** (1,38)	0.3569 (–) (1,38)
IgE (ng ml^{-1}) (49)	None	–	340.7± 32.5	–	203.8± 23.5	221.19± 17.3864	323.22± 42.5011	–	**0.0077 (–)** (1,6)	–	**0.0361 (+)** (1,40)	–	–
Diaphragm larvae counts (49)	None	–	1278± 91	–	1132± 77	1289± 50	1120± 131	–	0.2060 (–) (1,6)	–	0.2605 (–) (1,39)	**<0.0001 (–)** (1,39)	–
Diaphragm larvae counts (without body mass) (49)	None	–	1358± 138	–	1343± 104	1224± 78	1477± 170	–	0.9233 (–) (1,6)	–	0.1783 (–) (1,39)	–	–

Positive signs following *P*-values indicate infected>uninfected, HR>C, mini-muscle>normal or positive effect of log_{10} body mass. Negative signs indicate the opposite relationships. *P*-values <0.05 are in **bold**.

weeks after infection, however, infected mice had lower hematocrit than uninfected ones (Table 1). This may be expected if *T. spiralis* individuals are physically damaging the host (for example, burrowing through the gut or into muscle tissue) and causing blood loss or, in severe cases, perhaps even anemia. Similar to previous results (Swallow et al., 2005), we found no significant differences in hematocrit values between male HR and C mice (Table 1). Swallow et al. found significantly lower hematocrit values for mini-muscle mice in females (when body mass was not included as a covariate), but not males (Swallow et al., 2005). We found significantly lower values for mini-muscle male mice only for the third blood sample, 2 weeks after infection.

As expected, spleen mass (adjusted for body mass) was significantly higher in infected mice than in uninfected mice (Table 1) and, although not statistically significant, liver masses also tended to be slightly heavier in infected mice. Generally, increased spleen and liver masses are associated with a classic inflammatory response (Hart, 1988). Many studies have demonstrated that spleen mass is commonly negatively associated with overall body condition and positively correlated with parasite infection (Corbin et al., 2008; Cowan et al., 2009; Schulte-Hostedde and Elsasser, 2011). As seen previously (Swallow et al., 2005; Meek et al., 2009; Downs et al., 2012), spleen and liver masses were not significantly different between HR and C mice, and mini-muscle mice had heavier livers. Additionally, lung mass was significantly lower in infected mice. Decreased lung mass may be one of the causes of the decreased $\dot{V}_{O2max}$ measured in infected mice (Table 3), and in turn this could contribute to decreased wheel running in infected mice (Table 2). HR mice had significantly greater $\dot{V}_{O2max}$ (Table 3) and larger ventricles than C mice (Table 1), both of which would support their higher aerobic locomotor activity (see also Swallow et al., 2005; Rezende et al., 2006; Kolb et al., 2010).

Consistent with previous studies, HR mice had higher $\dot{V}_{O2max}$ and voluntary wheel running than C mice (e.g. Swallow et al., 1998; Rezende et al., 2005; Rezende et al., 2006; Kolb et al., 2010). As expected, our results show that both wheel-running behavior and $\dot{V}_{O2max}$ decreased when mice were infected. Following the acute phase of a *T. spiralis* infection, larvae encyst in active skeletal muscle and dedifferentiate skeletal muscle fibers, thereby decreasing the number of functional muscle fibers available for locomotion. HR mice showed a much larger absolute decrease in daily wheel running as compared with C mice; however, they had similar factorial decreases. The latter observation indicates that HR mice are not proportionally more affected by *T. spiralis* infection compared with C mice.

The proportional reduction in infection-related aerobic performance ($\dot{V}_{O2max}$) was much smaller than decreases in wheel running. By definition, performance tests (such as $\dot{V}_{O2max}$) require that an individual is maximally motivated, thereby providing a measure of ability only (Careau and Garland, 2012). One potential reason for a larger change in voluntary running behavior is the motivational factors underlying wheel running (see also Kolb et al., 2010). It is reasonable to expect that discomfort resulting from skeletal muscle damage due to larval encystment may decrease motivation to maintain normal levels of locomotor activity in infected mice (see also Li et al., 2004). Additionally, for both voluntary wheel running and $\dot{V}_{O2max}$ there were no significant interactions between line type and infection, thus indicating a similar relative response to infection in both HR and C mice.

Our results are consistent with the well-established association between helminth infections and increased IgE production (Gurish et al., 2004; Watanabe et al., 2005; Erb, 2007). As wheel running

Table 3. Least squares means and significance levels from SAS Proc Mixed analyses of $\dot{V}_{O2max}$, IgE and diaphragm larvae counts

		Least squares means ± s.e.m.						Two-tailed *P* (d.f.)					
Trait (*N*)	Transform	Control uninfected	Control infected	High runner uninfected	High runner infected	Normal muscle	Mini-muscle	Infection	Line type	Infection × line type	Mini-muscle	Log_{10} body mass	Diaphragm larvae
$\dot{V}_{O2max}$ (ml min^{-1}) (95)	Log_{10}	0.8405± 0.0206	0.8286± 0.0198	0.9317± 0.0163	0.8794± 0.0178	0.8715± 0.0106	0.8686± 0.0220	**0.0396 (–)** (1,6)	**0.0170 (+)** (1,6)	0.1286 (1,6)	0.9009 (–) (1,77)	**<0.0001 (+)** (1,77)	–
$\dot{V}_{O2max}$ (ml min^{-1}) (with diaphragm larvae counts) (49)	Log_{10}	–	0.8119± 0.0230	–	0.8671± 0.0201	0.8424± 0.0132	0.8366± 0.0305	–	0.0939 (+) (1,6)	–	0.8618 (–) (1,38)	**0.0019 (+)** (1,38)	0.3569 (–) (1,38)
IgE (ng ml^{-1}) (49)	None	–	340.7± 32.5	–	203.8± 23.5	221.19± 17.3864	323.22± 42.5011	–	**0.0077 (–)** (1,6)	–	**0.0361 (+)** (1,40)	–	–
Diaphragm larvae counts (49)	None	–	1278± 91	–	1132± 77	1289± 50	1120± 131	–	0.2060 (–) (1,6)	–	0.2605 (–) (1,39)	**<0.0001 (–)** (1,39)	–
Diaphragm larvae counts (without body mass) (49)	None	–	1358± 138	–	1343± 104	1224± 78	1477± 170	–	0.9233 (–) (1,6)	–	0.1783 (–) (1,39)	–	–

Positive signs following *P*-values indicate infected>uninfected, HR>C, mini-muscle>normal or positive effect of log_{10} body mass. Negative signs indicate the opposite relationships.
P-values <0.05 are in **bold**.

can have a substantial energy cost in mice and HR mice have higher daily energy expenditure than C mice (e.g. Koteja et al., 1999; Rezende et al., 2005; Rezende et al., 2006), they are thought to be more energetically challenged than C mice (although this may not be important under conditions of *ad libitum* food). Accordingly, if there is a trade-off between locomotion and immune function, then we expected HR mice to be less resistant (more susceptible) to infection. As we expected, among infected animals, IgE levels were significantly lower in HR mice than in C mice. Assuming general immunosuppressive effects of CORT (Sapolsky et al., 2000; Galon et al., 2002; Spencer et al., 2011) and our hypothesis that HR mice would allocate less energy toward immune upregulation, a smaller increase in IgE levels among HR mice appears to be a reasonable response to infection. However, the effects of glucocorticoids on the immune system are complex. Despite an enormous number of studies, the extent to which glucocorticoids influence innate *versus* acquired immune function or humoral *versus* cell-mediated responses is not clear (for a review, see Spencer et al., 2011). In addition to being part of a general immune response to parasites, IgE is thought to bind directly to *T. spiralis* and directly aid in expulsion of the parasite from the gut of the host, and there is evidence that IgE may also help kill larval stages of *T. spiralis* (Gurish et al., 2004). Lower IgE levels in HR mice would be expected to result in a smaller immune upregulation, less ability to expel *T. spiralis* adults and, ultimately, increased numbers of larvae encysted in skeletal muscle.

Surprisingly, HR and C mice did not significantly differ in diaphragm loads of *T. spiralis* larvae. Immune responses to a parasite infection are very complex, and IgE is not the only important factor in a primary immune response. We assume that other components of immunity may compensate for differences in IgE in the two line types.

A number of possibilities could explain the apparent contradiction regarding IgE levels and diaphragm larvae counts. First, during the initial 4 weeks of the infection (i.e. the acute phase) mice were individually housed without access to running wheels; under those circumstances, HR mice are expected to be less energetically limited than if wheel running were possible. However, HR mice without wheel access demonstrate increased home cage activity and increased food consumption as compared with C mice, so there are still differences in energy demands between the HR and C mice (Koteja et al., 2001; Swallow et al., 2001; Malisch et al., 2009b). Our results suggest that despite selection for increased voluntary wheel running and the increased energy needs of HR mice, both HR and C mice have adequate energy for upregulation of the immune system when housed with *ad libitum* food. Nonetheless, a direct measure of food consumption (e.g. Koteja et al., 1999; Koteja et al., 2001; Koteja et al., 2003) during the acute infection would be useful in determining the extent to which energetic demands of HR and C mice have the capacity to influence immune function.

Second, particularly in energy-limited wild animals, it is often thought to be less energetically costly and of greater net benefit to live with a low-level parasitic infection than to completely eliminate parasites, which could be quite expensive (Bonneaud et al., 2003; Pilorz et al., 2005; Colditz, 2008). In this sense, our results do not provide evidence of the proportional extent to which HR and C mice allocated energy toward an immune response. In other words, it is not clear whether immune upregulation (IgE production specifically) is maximized in either HR or C mice.

Third, because HR mice are, on average, smaller than C mice and all mice received an approximately equal infection dose (~300 J1 larvae total; ~12–15 larvae per gram body mass), HR mice received a slightly larger dosage relative to their body mass. One prediction that might follow would be higher encysted larvae loads in HR mice, particularly given that line type's higher CORT levels, but this was not observed.

Finally, the time course of IgE production may not be the same in HR and C mice. Previous rodent studies (Jarrett and Haig, 1976; Gurish et al., 2004) have shown that IgE production is maximized approximately 2 weeks after infection. If the time course of an immune response was altered (Wiegers and Reul, 1998) by selective breeding for high voluntary wheel running, then IgE production in HR and C mice may peak at different times. These results underscore the importance of measuring clearance of diseases and parasites – not just markers of immune function (again, IgE is only one feature of a highly complicated Th2 response) – when trying to understand physiological trade-offs that may involve the immune system (Viney et al., 2005).

Within the HR line type, the various phenotypic alterations associated with the mini-muscle morphology are known to include a number of effects on physiology (e.g. Garland et al., 2002; Houle-Leroy et al., 2003; Swallow et al., 2005; Dlugosz et al., 2009; McGillivray et al., 2009; Kolb et al., 2010). Results from Downs et al. (Downs et al., 2012) suggest that mini-muscle mice show greater sickness behaviors than normal mice, although cytokine levels were not significantly different. In the present study, body mass measurements, hematocrit, liver mass and IgE were the only traits affected by mini-muscle status. Taken together, the results of Downs et al. (Downs et al., 2012) and this study suggest that mini-muscle mice differ from normal mice in the activity of molecules associated with an inflammatory response. Additionally, given the smaller body masses and larger mass-corrected organ masses typically associated with the mini-muscle phenotype, sickness behavior or inflammatory response may be further accentuated in mini-muscle mice compared with normal mice.

A more complete picture of both innate and adaptive immunity would be useful in determining the specific mechanisms by which artificial selection for high levels of voluntary exercise has affected immune function HR and C mice. Nevertheless, the results of this study and others (Malisch et al., 2009a; Downs et al., 2012) clearly indicate that selection for increased activity levels, and hence increased levels of daily energy expenditure, has not had a strong negative effect on some aspects of immune function. This is one of various examples in which predictions about trade-offs derived from basic physiological principles are not necessarily met in practice, perhaps because nature has more 'degrees of freedom' than expected (e.g. Garland, 1988; Downs et al., 2013).

ACKNOWLEDGEMENTS

We thank D. Hill (USDA) for the original *T. spiralis* Beltsville strain infection source. We thank L. Holness, S. Hyunh, R. Marsik and K. Vu for their help with data collection, and B. N. Harris for help generating assay standard curves.

AUTHOR CONTRIBUTIONS

E.M.D. conceived the experiment and designed it with the help of E.G.P., M.A.C. and T.G. E.G.P. assisted with isolating the *Trichinella* from the original host, infecting the mice used in this experiment, and manuscript revisions. E.M.D., H.S., T.H.M., W.A. and C.J.D. assisted with experimental design and data collection. E.M.D., M.A.C. and T.G. wrote the paper.

COMPETING INTERESTS

No competing interests declared.

FUNDING

This study was supported by the National Science Foundation (IOB-0543429 and IOS-112127 to T.G.) and UC Riverside Academic Senate Funds (to M.A.C.).

REFERENCES

Adamo, S. A., Bartlett, A., Le, J., Spencer, N. and Sullivan, K. (2010). Illness-induced anorexia may reduce trade-offs between digestion and immune function. *Anim. Behav.* **79**, 3-10.

Amat, J. A., Aguilera, E. and Visser, G. H. (2007). Energetic and developmental costs of mounting an immune response in greenfinches (*Carduelis chloris*). *Ecol. Res.* **22**, 282-287.

Anderson, R. M. and May, R. M. (1991). Infectious diseases of humans: dynamics and control. New York: Oxford University Press.

Bartholomew, G. A., Vleck, D. and Vleck, C. M. (1981). Instantaneous measurements of oxygen consumption during pre-flight warm-up and postflight cooling in sphingid and saturnid moths. *J. Exp. Biol.* **90**, 17-32.

Bernard, G. R. (1959). Experimental trichinosis in the golden hamster. I. Spontaneous muscular activity patterns. *Am. Midl. Nat.* **62**, 396-401.

Bonneaud, C., Mazuc, J., Gonzalez, G., Haussy, C., Chastel, O., Faivre, B. and Sorci, G. (2003). Assessing the cost of mounting an immune response. *Am. Nat.* **161**, 367-379.

Campbell, W. (1983). *Trichinella and Trichinosis*. New York: Plenum Press.

Capó, V. and Despommier, D. D. (1996). Clinical aspects of infection with *Trichinella* spp. *Clin. Microbiol. Rev.* **9**, 47-54.

Careau, V. and Garland, T., Jr (2012). Performance, personality, and energetics: correlation, causation, and mechanism. *Physiol. Biochem. Zool.* **85**, 543-571.

Careau, V., Wolak, M. E., Carter, P. A. and Garland, T., Jr (2013). Limits to behavioral evolution: the quantitative genetics of a complex trait under directional selection. *Evolution* [Epub ahead of print] doi:10.1111/evo.12200.

Clobert, J., Oppliger, A., Sorci, G., Ernande, B., Swallow, J. G. and Garland, T., Jr (2000). Trade-offs in phenotypic traits: endurance at birth, growth, survival, predation, and susceptibility to parasitism in a lizard, *Lacerta vivipara*. *Funct. Ecol.* **14**, 675-684.

Colditz, I. G. (2008). Six costs of immunity to gastrointestinal nematode infections. *Parasite Immunol.* **30**, 63-70.

Coleman, M. A., Garland, T., Jr, Marler, C. A., Newton, S. S., Swallow, J. G. and Carter, P. A. (1998). Glucocorticoid response to forced exercise in laboratory house mice (*Mus domesticus*). *Physiol. Behav.* **63**, 279-285.

Corbin, E., Vicente, J., Martin-Hernanado, M. P., Acevedo, P., Perez-Rodriguez, L. and Gortazar, P. (2008). Spleen mass as a measure of immune strength in mammals. *Mammal Rev.* **38**, 108-115.

Cowan, K. M., Shutler, D., Herman, T. B. and Stewart, D. T. (2009). Splenic mass of masked shrews, *Sorex cinereus*, in relation to body mass, sex, age, day of the year, and bladder nematode, *Liniscus* (=*Capillaria*) *maseri*, infection. *J. Parasitol.* **95**, 228-230.

Dearman, R. J., Basketter, D. A., Blaikie, L., Clark, E. D., Hilton, J., House, R. V., Ladics, G. S., Loveless, S. E., Mattis, C., Sailstad, D. M. et al. (1998). The mouse IgE test: interlaboratory evaluation and comparison of BALB/c and C57BL/6 strain mice. *Toxicol. Mech. Methods* **8**, 69-85.

Demas, G. E., Chefer, V., Talan, M. I. and Nelson, R. J. (1997). Metabolic costs of mounting an antigen-stimulated immune response in adult and aged C57BL/6J mice. *Am. J. Physiol.* **273**, R1631-R1637.

Derting, T. L. and Compton, S. (2003). Immune response, not immune maintenance, is energetically costly in wild white-footed mice (*Peromyscus leucopus*). *Physiol. Biochem. Zool.* **76**, 744-752.

Despommier, D. D. (1983). Biology. In *Trichinella and Trichinosis* (ed. W. C. Campbell), pp. 75-152. New York: Plenum Press.

Despommier, D. D. (1998). How does *Trichinella spiralis* make itself at home? *Parasitol. Today* **14**, 318-323.

Dlugosz, E. M., Chappell, M. A., McGillivray, D. G., Syme, D. A. and Garland, T., Jr (2009). Locomotor trade-offs in mice selectively bred for high voluntary wheel running. *J. Exp. Biol.* **212**, 2612-2618.

Downs, C. J., Schutz, H., Meek, T. H., Dlugosz, E. M., Acosta, W., de Wolski, K. S., Malisch, J. L., Hayes, J. P. and Garland, T., Jr (2012). Within-lifetime trade-offs but evolutionary freedom for hormonal and immunological traits: evidence from mice bred for high voluntary exercise. *J. Exp. Biol.* **215**, 1651-1661.

Downs, C. J., Brown, J. L., Wone, B., Donovan, E. R., Hunter, K. and Hayes, J. P. (2013). Selection for increased mass-independent maximal metabolic rate suppresses innate but not adaptive immune function. *Proc. Biol. Sci.* **280**, 2012-2636.

Elenkov, I. J. (2004). Glucocorticoids and the Th1/Th2 balance. *Ann. New York Acad. Sci.* **1024**, 138-146.

Erb, K. J. (2007). Helminths, allergic disorders and IgE-mediated immune responses: where do we stand? *Eur. J. Immunol.* **37**, 1170-1173.

Freitak, D., Ots, I., Vanatoa, A. and Hõrak, P. (2003). Immune response is energetically costly in white cabbage butterfly pupae. *Proc. Biol. Sci.* **270 Suppl. 2**, S220-S222.

French, S. S., Moore, M. C. and Demas, G. E. (2009). Ecological immunology: the organism in context. *Integr. Comp. Biol.* **49**, 246-253.

Frenkel, J. K. (1989). Tissue-dwelling intracellular parasites: infection and immune responses in the mammalian host to *Toxoplasma*, *Sarcocystis* and *Trichinella*. *Am. Zool.* **29**, 455-467.

Galon, J., Franchimont, D., Hiroi, N., Frey, G., Boettner, A., Ehrhart-Bornstein, M., O'Shea, J. J., Chrousos, G. P. and Bornstein, S. R. (2002). Gene profiling reveals unknown enhancing and supressive actions of glucocorticoids on immune cells. *FASEB J.* **16**, 61-71.

Garland, T., Jr (1988). Genetic basis of activity metabolism. I. Inheritance of speed, stamina, and antipredator displays in the garter snake *Thamnophis sirtalis*. *Evolution* **42**, 335-350.

Garland, T., Jr, Morgan, M. T., Swallow, J. G., Rhodes, J. S., Girard, I., Belter, J. G. and Carter, P. A. (2002). Evolution of a small-muscle polymorphism in lines of house mice selected for high activity levels. *Evolution* **56**, 1267-1275.

Garland, T., Jr, Kelly, S. A., Malisch, J. L., Kolb, E. M., Hannon, R. M., Keeney, B. K., Van Cleave, S. L. and Middleton, K. M. (2011a). How to run far: multiple solutions and sex-specific responses to selective breeding for high voluntary activity levels. *Proc. Biol. Sci.* **278**, 574-581.

Garland, T., Jr, Schutz, H., Chappell, M. A., Keeney, B. K., Meek, T. H., Copes, L. E., Acosta, W., Drenowatz, C., Maciel, R. C., van Dijk, G. et al. (2011b). The biological control of voluntary exercise, spontaneous physical activity and daily energy expenditure in relation to obesity: human and rodent perspectives. *J. Exp. Biol.* **214**, 206-229.

Girard, I. and Garland, T., Jr (2002). Plasma corticosterone response to acute and chronic voluntary exercise in female house mice. *J. Appl. Physiol.* **92**, 1553-1561.

Goodchild, C. and Frankenberg, D. (1962). Voluntary running in the golden hamster, *Mesocricetus auratus* (Waterhouse, 1839), infected with *Trichinella spiralis* (Owen, 1835). *Trans. Am. Microsc. Soc.* **81**, 292-298.

Gottstein, B., Pozio, E. and Nöckler, K. (2009). Epidemiology, diagnosis, treatment, and control of trichinellosis. *Clin. Microbiol. Rev.* **22**, 127-145.

Gurish, M. F., Bryce, P. J., Tao, H., Kisselgof, A. B., Thornton, E. M., Miller, H. R., Friend, D. S. and Oettgen, H. C. (2004). IgE enhances parasite clearance and regulates mast cell responses in mice infected with *Trichinella spiralis*. *J. Immunol.* **172**, 1139-1145.

Hart, B. L. (1988). Biological basis of the behavior of sick animals. *Neurosci. Biobehav. Rev.* **12**, 123-137.

Hoff, J. (2000). Methods of blood collection in the mouse. *Lab. Anim.* **29**, 47-53.

Houle-Leroy, P., Guderley, H., Swallow, J. G. and Garland, T., Jr (2003). Artificial selection for high activity favors mighty mini-muscles in house mice. *Am. J. Physiol.* **284**, R433-R443.

Jarrett, E. E. E. and Haig, D. M. (1976). Time course studies on rat IgE production in *N. brasiliensis* infection. *Clin. Exp. Immunol.* **24**, 346-351.

Kelly, S. A., Bell, T. A., Selitsky, S. R., Buus, R. J., Hua, K., Weinstock, G. M., Garland ,T., Jr, Pardo-Manuel de Villena, F. and Pomp, D. (in press). Myosin heavy polypeptide 4 gene is responsible for the Mini-Muscle phenotype characterized by major reduction in hindlimb muscle mass in mice. *Genetics*.

Kolb, E. M., Kelly, S. A., Middleton, K. M., Sermsakdi, L. S., Chappell, M. A. and Garland, T., Jr (2010). Erythropoietin elevates $V_{O_2,max}$ but not voluntary wheel running in mice. *J. Exp. Biol.* **213**, 510-519.

Koteja, P., Swallow, J. G., Carter, P. A. and Garland, T., Jr (1999). Energy cost of wheel running in house mice: implications for coadaptation of locomotion and energy budgets. *Physiol. Biochem. Zool.* **72**, 238-249.

Koteja, P., Swallow, J. G., Carter, P. A. and Garland, T., Jr (2001). Maximum cold-induced food consumption in mice selected for high locomotor activity: implications for the evolution of endotherm energy budgets. *J. Exp. Biol.* **204**, 1177-1190.

Koteja, P., Carter, P. A., Swallow, J. G. and Garland, T., Jr (2003). Food wasting by house mice: variation among individuals, families, and genetic lines. *Physiol. Behav.* **80**, 375-383.

Kyriazakis, I., Tolkamp, B. J. and Hutchings, M. R. (1998). Towards a functional explanation for the occurrence of anorexia during parasitic infections. *Anim. Behav.* **56**, 265-274.

Li, G., Rhodes, J. S., Girard, I., Gammie, S. C. and Garland, T., Jr (2004). Opioid-mediated pain sensitivity in mice bred for high voluntary wheel running. *Physiol. Behav.* **83**, 515-524.

Lochmiller, R. L. and Deerenberg, C. (2000). Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos* **88**, 87-98.

Malisch, J. L., Saltzman, W., Gomes, F. R., Rezende, E. L., Jeske, D. R. and Garland, T., Jr (2007). Baseline and stress-induced plasma corticosterone concentrations of mice selectively bred for high voluntary wheel running. *Physiol. Biochem. Zool.* **80**, 146-156.

Malisch, J. L., Breuner, C. W., Gomes, F. R., Chappell, M. A. and Garland, T., Jr (2008). Circadian pattern of total and free corticosterone concentrations, corticosteroid-binding globulin, and physical activity in mice selectively bred for high voluntary wheel-running behavior. *Gen. Comp. Endocrinol.* **156**, 210-217.

Malisch, J. L., Kelly, S. A., Bhanvadia, A., Blank, K. M., Marsik, R. L., Platzer, E. G. and Garland, T., Jr (2009a). Lines of mice with chronically elevated baseline corticosterone levels are more susceptible to a parasitic nematode infection. *Zoology* **112**, 316-324.

Malisch, J. L., Breuner, C. W., Kolb, E. M., Wada, H., Hannon, R. M., Chappell, M. A., Middleton, K. M. and Garland, T., Jr (2009b). Behavioral despair and home-cage activity in mice with chronically elevated baseline corticosterone concentrations. *Behav. Genet.* **39**, 192-201.

Martin, L. B., II, Weil, Z. M. and Nelson, R. J. (2007). Immune defense and reproductive pace of life in *Peromyscus* mice. *Ecology* **88**, 2516-2528.

McGillivray, D. G., Garland, T., Jr, Dlugosz, E. M., Chappell, M. A. and Syme, D. A. (2009). Changes in efficiency and myosin expression in the small-muscle phenotype of mice selectively bred for high voluntary running activity. *J. Exp. Biol.* **212**, 977-985.

Meagher, S. (1998). Physiological responses of deer mice (*Peromyscus maniculatus*) to infection with *Capillaria hepatica* (Nematoda). *J. Parasitol.* **84**, 1112-1118.

Meagher, S. and Dudek, S. N. (2002). Effects of Trichinella spiralis on survival, total mass, and organ mass of oldfield mice (*Peromyscus polionotus*). *J. Parasitol.* **88**, 833-838.

Meek, T. H., Lonquich, B. P., Hannon, R. M. and Garland, T., Jr (2009). Endurance capacity of mice selectively bred for high voluntary wheel running. *J. Exp. Biol.* **212**, 2908-2917.

Ots, I., Kerimov, A. B., Ivankina, E. V., Ilyina, T. A. and Hõrak, P. (2001). Immune challenge affects basal metabolic activity in wintering great tits. *Proc. Biol. Sci.* **268**, 1175-1181.

Pilorz, V., Jäckel, M., Knudsen, K. and Trillmich, F. (2005). The cost of a specific immune response in young guinea pigs. *Physiol. Behav.* **85**, 205-211.

Poirier, S. R., Rau, M. E. and Wang, X. (1995). Diel locomotory activity of deer mice (*Peromyscus maniculatus*) infected with *Trichinella nativa* or *Trichinella pseudospiralis*. *Can. J. Zool.* **73**, 1323-1334.

Ramírez-Melgar, C., Gómez-Priego, A. and De-la-Rosa, J.-L. (2007). Application of Giemsa stain for easy detection of *Trichinella spiralis* muscle larvae. *Korean J. Parasitol.* **45**, 65-68.

Rezende, E. L., Chappell, M. A., Gomes, F. R., Malisch, J. L. and Garland, T., Jr (2005). Maximal metabolic rates during voluntary exercise, forced exercise, and cold exposure in house mice selectively bred for high wheel-running. *J. Exp. Biol.* **208**, 2447-2458.

Rezende, E. L., Kelly, S. A., Gomes, F. R., Chappell, M. A. and Garland, T., Jr (2006). Effects of size, sex, and voluntary running speeds on costs of locomotion in lines of laboratory mice selectively bred for high wheel-running activity. *Physiol. Biochem. Zool.* **79**, 83-99.

Rezende, E. L., Gomes, F. R., Chappell, M. A. and Garland, T., Jr (2009). Running behavior and its energy cost in mice selectively bred for high voluntary locomotor activity. *Physiol. Biochem. Zool.* **82**, 662-679.

Rhodes, J. S., Gammie, S. C. and Garland, T., Jr (2005). Neurobiology of mice selected for high voluntary wheel-running activity. *Integr. Comp. Biol.* **45**, 438-455.

Salagianni, M., Wong, K. L., Thomas, M. J., Noble, A. and Kemeny, D. M. (2007). An essential role for IL-18 in CD8 T cell-mediated suppression of IgE responses. *J. Immunol.* **178**, 4771-4778.

Sapolsky, R. M., Romero, L. M. and Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr. Rev.* **21**, 55-89.

Schulte-Hostedde, A. I. and Elsasser, S. C. (2011). Spleen mass, body condition, and parasite load in male American mink (*Neovison vison*). *J. Mammal.* **92**, 221-226.

Sheldon, B. C. and Verhulst, S. (1996). Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends Ecol. Evol.* **11**, 317-321.

Spencer, R. L., Kalman, B. A. and Dhabhar, F. S. (2011). Role of endogenous glucocorticoids in immune system function: regulation and counterregulation. *Compr. Physiol.* **23**, 381-423.

Swallow, J. G., Carter, P. A. and Garland, T., Jr (1998). Artificial selection for increased wheel-running behavior in house mice. *Behav. Genet.* **28**, 227-237.

Swallow, J. G., Koteja, P., Carter, P. A. and Garland, T., Jr (1999). Artificial selection for increased wheel-running activity in house mice results in decreased body mass at maturity. *J. Exp. Biol.* **202**, 2513-2520.

Swallow, J. G., Koteja, P., Carter, P. A. and Garland, T., Jr (2001). Food consumption and body composition in mice selected for high wheel-running activity. *J. Comp. Physiol. B* **171**, 651-659.

Swallow, J. G., Rhodes, J. S. and Garland, T., Jr (2005). Phenotypic and evolutionary plasticity of organ masses in response to voluntary exercise in house mice. *Integr. Comp. Biol.* **45**, 426-437.

Syme, D. A., Evashuk, K., Grintuch, B., Rezende, E. L. and Garland, T., Jr (2005). Contractile abilities of normal and 'mini' triceps surae muscles from mice (*Mus domesticus*) selectively bred for high voluntary wheel running. *J. Appl. Physiol.* **99**, 1308-1316.

Viney, M. E., Riley, E. M. and Buchanan, K. L. (2005). Optimal immune responses: immunocompetence revisited. *Trends Ecol. Evol.* **20**, 665-669.

Viveros-Paredes, J. M., Puebla-Pérez, A. M., Gutiérrez-Coronado, O., Sandoval-Ramírez, L. and Villaseñor-García, M. M. (2006). Dysregulation of the Th1/Th2 cytokine profile is associated with immunosuppression induced by hypothalamic-pituitary-adrenal axis activation in mice. *Int. Immunopharmacol.* **6**, 774-781.

Von Brand, T., Weinstein, P. P. and Wright, W. H. (1954). The working ability of rats infected with *Trichinella spiralis*. *Am. J. Hyg.* **59**, 26-31.

Watanabe, N., Bruschi, F. and Korenaga, M. (2005). IgE: a question of protective immunity in *Trichinella spiralis* infection. *Trends Parasitol.* **21**, 175-178.

Wiegers, G. J. and Reul, J. M. H. M. (1998). Induction of cytokine receptors by glucocorticoids: functional and pathological significance. *Trends Pharmacol. Sci.* **19**, 317-321.

Wikelski, M. and Ricklefs, R. E. (2001). The physiology of life histories. *Trends Ecol. Evol.* **16**, 479-481.

Zieg, G., Lack, G., Harbeck, R. J., Gelfand, E. W. and Leung, D. Y. M. (1994). *In vivo* effects of glucocorticoids on IgE production. *J. Allergy Clin. Immunol.* **94**, 222-230.

Zohar, A. S. and Rau, M. E. (1984). The effect of the intestinal phase of *Trichinella spiralis* on the open-field behavior of mice. *J. Parasitol.* **70**, 927-930.

Zohar, A. S. and Rau, M. E. (1986). The role of muscle larvae of *Trichinella spiralis* in the behavioral alterations of the mouse host. *J. Parasitol.* **72**, 464-466.

Table S1. Significance levels (2-tailed *P* values; degrees of freedom in parentheses) from SAS Procedure Mixed Analyses of seven body mass measurements at different infection time points.

Trait (N)	Transform	Mean Age (days)	Infection	Linetype	Infection*Linetype	Mini-muscle	Age	Diaphragm Larvae Counts
Bleed 1 Mass (at infection) (101)	None	51	--	0.1004 (-) (1,6)	--	**0.0027 (-)** (1,91)	**0.0168 (+)** (1,91)	--
Bleed 2 Mass (100)	None	58	0.0969 (-) (1,6)	0.2069 (-) (1,6)	0.3671 (1,6)	**0.0097 (-)** (1,82)	**0.0224 (+)** (1,82)	--
Bleed 3 Mass (97)	None	65	**0.0055 (-)** (1,6)	0.2322 (-) (1,6)	0.1716 (1,6)	**0.0097 (-)** (1,79)	0.0881 (+) (1,79)	--
Mass On Wheel (96)	None	82	**0.0303 (-)** (1,6)	0.1646 (-) (1,6)	0.6088 (1,6)	**0.0012 (-)** (1,79)	0.7893 (+) (1,79)	--
Mass On Wheel (with Diaphragm Larvae Count) (49)	None	82	--	0.1790 (-) (1,6)	--	**0.0037 (-)** (1,38)	0.5197 (+) (1,38)	**<0.0001 (-)** (1,38)
$\dot{V}O_2$max (1) (Mass Off) (95)	None	88	**0.0180 (-)** (1,6)	0.2052 (-) (1,6)	0.6001 (1,6)	**0.0013 (-)** (1,77)	0.7414 (+) (1,79)	--
$\dot{V}O_2$max (1) (Mass Off) (with Diaphragm Larvae Count) (49)	None	88	--	0.0646 (-) (1,6)	--	**0.0153 (-)** (1,38)	0.7871 (+) (1,38)	**<0.0001 (-)** (1,38)
$\dot{V}O_2$max (2) Mass (95)	None	88	**0.0177 (-)** (1,6)	0.1705 (-) (1,6)	0.7121 (1,6)	**0.0036 (-)** (1,77)	0.5879 (+) (1,77)	--

$\dot{V}O_2$max (2) Mass (with Diaphragm Larvae Count) (49)	None	88	--	**0.0377 (-)** (1,6)	--	**0.0153 (-)** (1,38)	0.6447 (+) (1,38)	**<0.0001 (-)** (1,38)
Dissection Mass (95)	None	97	**0.0140 (-)** (1,6)	0.2126 (-) (1,6)	0.4813 (1,6)	**0.0081 (-)** (1,77)	0.3477 (+) (1,77)	--
Dissection Mass (with Diaphragm Larvae Count) (49)	None	97	--	0.1206 (-) (1,6)	--	**0.0269 (-)** (1,38)	0.6962 (+) (1,38)	**<0.0001 (-)** (1,38)

Positive signs following *P*-values indicate infected>uninfected, HR>C, mini-muscle>normal or positive effect of age.
P-values < 0.05 are in **bold**.

Table S2. Least squares means from SAS Procedure Mixed Analyses of seven body mass measurements, as shown in Table S1.

	Least Squares Means ± Standard Error					
Trait	Control Uninfected	Control Infected	High Runner Uninfected	High Runner Infected	Normal Muscle	Mini-muscle
Bleed 1 Mass (at infection)	31.196 ± 1.134		28.489 ± 0.966		31.668 ± 0.698	28.017 ± 1.209
Bleed 2 Mass	31.126 ± 1.282	29.063 ± 1.266	28.404 ± 1.113	27.709 ± 1.099	30.670 ± 0.720	27.481 ± 1.234
Bleed 3 Mass	32.875 ± 1.390	28.427 ± 1.353	29.678 ± 1.166	27.614 ± 1.153	31.469 ± 0.755	27.828 ± 1.368
Mass On Wheel	34.796 ± 1.531	31.690 ± 1.500	31.656 ± 1.318	29.549 ± 1.315	34.318 ± 0.834	29.527 ± 1.449
Mass On Wheel (with diaphragm larvae count)	--	32.027 ± 1.145	--	29.919 ± 0.951	32.993 ± 0.693	28.953 ± 1.277
$\dot{V}O_2max$ (1) (Mass Off)	33.971 ± 1.507	31.263 1.479	31.093 ± 1.312	29.178 ± 1.302	33.701± 0.873	29.052 ± 1.449
$\dot{V}O_2max$ (1) (Mass Off) (with diaphragm larvae count)	--	32.153 ± 0.978	--	29.613 ± 0.768	32.495 ± 0.564	29.271 ± 1.184
$\dot{V}O_2max$ (2) (Mass)	34.688 ± 1.540	31.864 ± 1.509	31.655 ± 1.333	29.434 ± 1.322	34.069 ± 0.877	29.751± 1.486
$\dot{V}O_2max$ (2) (Mass) (with diaphragm larvae count)	--	32.666 ± 0.930	--	29.891 ± 0.715	32.895 ± 0.524	29.662 ± 1.169
Dissection Mass	34.954 ± 1.650	31.300 ± 1.620	31.672 ± 1.443	29.332 ± 1.433	33.844 ± 0.941	29.786 ± 1.560
Dissection Mass (with diaphragm larvae count)	--	31.926 ± 1.074	--	29.691 ± 0.845	32.419 ± 0.620	29.208 ± 1.298

Table S3. Least squares means and significance levels (2-tailed *P* values; degrees of freedom in parentheses) from SAS Procedure Mixed Analyses of organ masses where diaphragm larvae counts are used as a covariate. Body mass was not transformed for analyses of organ masses.

		Least Squares Means ± S.E.M.				2-tailed P values (d.f.)			
Organ Mass (N)	Transform	Control Infected	High Runner Infected	Normal Muscle	Mini-muscle	Linetype	Mini-muscle	Body Mass	Diaphragm Larvae Count
Spleen (with diaphragm larvae count) (49)	Log_{10}	-1.0206 ± 0.0368	-0.9506 ± 0.0328	-0.9923 ± 0.0230	-0.9789 ± 0.0398	0.1844 (+) (1,6)	0.7341 (+) (1,38)	**0.0010 (+)** (1,38)	0.2962 (-) (1,38)
Wet Liver (with diaphragm larvae count) (49)	None	1.9247 ± 0.0918	1.8994 ± 0.0785	1.7384 ± 0.0540	2.0857 ± 0.1130	0.8270 (+) (1,6)	**0.0064 (+)** (1,38)	**<0.0001 (+)** (1,38)	0.4228 (-) (1,38)
Dry Liver (with diaphragm larvae count) (49)	None	0.6149 ± 0.0329	0.6101 ± 0.0282	0.5554 ± 0.0195	0.6695 ± 0.0402	0.9081 (+) (1,6)	**0.0110 (+)** (1,38)	**0.0001 (+)** (1,38)	0.3524 (-) (1,38)
Wet Ventricle (with diaphragm larvae count) (49)	Log_{10}	-0.8618 ± 0.0110	-0.8216 ± 0.0093	-0.8439 ± 0.0061	-0.8395 ± 0.0150	**0.0205 (+)** (1,6)	0.7909 (+) (1,38)	**<0.0001 (+)** (1,38)	**0.0219 (-)** (1,38)
Dry Ventricle (with diaphragm larvae count) (49)	Log_{10}	-1.5007 ± 0.0100	-1.4589 ± 0.0085	-1.4824 ± 0.0056	-1.4773 ± 0.0134	**0.0119 (+)** (1,6)	0.7293 (+) (1,38)	**<0.0001 (+)** (1,38)	**0.0373 (-)** (1,38)

Wet Lungs (with diaphragm larvae count) (48)	Log_{10}	-0.6981 ± 0.0298	-0.6462 ± 0.0253	-0.6531 ± 0.0174	-0.6912 ± 0.0373	0.1974 (+) (1,6)	0.3468 (+) (1,37)	0.8286 (+) (1,37)	0.4936 (+) (1,37)
Dry Lungs (with diaphragm larvae count) (48)	Log_{10}	-1.3595 ± 0.0299	-1.3093 ± 0.0254	-1.3048 ± 0.0175	-1.3639 ± 0.0369	0.2121 (+) (1,6)	0.1429 (+) (1,37)	0.6851 (+) (1,37)	0.2972 (+) (1,37)

Positive signs following *P*-values indicate HR>C, mini-muscle>normal or positive effect of diaphragm larvae count.
P-values < 0.05 are in **bold**.