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# Lines of mice with chronically elevated baseline corticosterone levels are more susceptible to a parasitic nematode infection

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## Abstract

Chronically elevated circulating plasma glucocorticoid concentrations can have suppressive effects on immune function in mammals. House mice (*Mus domesticus*) that have been selectively bred for high voluntary wheel running exhibit chronically elevated (two-fold, on average) plasma corticosterone (CORT) levels and hence are an interesting model to study possible glucocorticoid-induced immune suppression. As an initial test of their immunocompetence, we compared the four replicate high runner (HR) lines with their four non-selected control (C) lines by subjecting them to infection by a parasitic nematode, *Nippostrongylus brasiliensis*. At generation 36 of the selection experiment, 10 adult males from each of the eight lines were inoculated subcutaneously with approximately 600 third-stage larval *N. brasiliensis*, and then sacrificed 12 days after injection. Neither spleen mass nor number of adult nematodes in the small intestine differed significantly between HR and C lines. However, the eight lines differed significantly in nematode counts, and the line means for nematode infestation were significantly positively related to baseline circulating CORT concentration measured in males from generations 34 and 39. Therefore, although selective breeding for high locomotor activity may not have resulted in a generally compromised immune response, results of this study are consistent with the hypothesis that glucocorticoids can have immunosuppressive effects.
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***Keywords:*** Experimental evolution; Immune response; Immune suppression; Locomotor activity; Physiological trade-off

## Introduction

The Darwinian fitness of an organism hinges on appropriate allocation of resources to energetic needs, including maintenance metabolism, growth, reproduction, and immunocompetence. When internal resources are limited, increased allocation to one energetic need may come at a cost to another, thus resulting in a "trade-off" (Stearns, 1992). Interest in the physiological mechanisms that underlie trade-offs has increased in recent years, and moved beyond simple energetic considerations (e.g. Zera and Harshman, 2001; Tieleman et al., 2005; Burger et al., 2008; Garland and Rose, 2009). In particular, hormones have been viewed as good candidates for mediators of physiological trade-offs, because a single hormone often affects multiple tissues, organs or physiological processes (Stearns, 1989; Ketterson and Nolan, 1999; Sinervo and Calsbeek, 2003; Zera et al., 2007).

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As one example, glucocorticoid hormones have multiple physiological and behavioral effects (Tharp, 1975; Dallman et al., 2002; Sapolsky, 2002). In vertebrates, glucocorticoids are released throughout the day by the adrenal glands, typically in a strongly circadian pattern, and peak near the onset of the active period (at the time of lights out in nocturnal animals, e.g. Malisch et al., 2008). At basal levels, glucocorticoids are important for sustaining routine intermediary metabolism (Dallman et al., 1993, 2002; Pecoraro et al., 2005, 2006). Glucocorticoid release is elevated in response to various psychological and physical stressors, and this is crucial for rapid energy mobilization. Additionally, glucocorticoids have complex behavioral effects via the central nervous system, including effects on locomotor behavior and potentially on motivation (Lin et al., 1988, 1989; Lin and Singer, 1990; Breuner and Wingfield, 2000; Pecoraro et al., 2005, 2006).

Receptors for glucocorticoids can be found in almost all organs and tissues, including the brain, liver, muscle, spleen, and gonads (McEwen et al., 1997). Therefore, a change in circulating glucocorticoid levels has the potential to alter multiple aspects of behavior and physiology, including locomotion, metabolism, and immune function. Increased plasma corticosterone (CORT, the main glucocorticoid in rodents) increases energy availability by promoting proteolysis, lipolysis, and gluconeogenesis (Tharp, 1975; Dallman et al., 1993, 2002). Because most stressors are associated with increased energetic needs, it is generally presumed that increased release of CORT in response to stress is adaptive and has evolved to help meet these energetic needs while simultaneously curtailing processes that are not immediately necessary, such as immune function, growth, and reproduction (Sapolsky et al., 2000; Sapolsky, 2002). Although increasing the concentration of circulating CORT would seem to be of obvious survival value as a response to acute, short-term stressors, chronically elevated CORT levels have been associated with immune, reproductive, and growth suppression in birds and mammals (Wingfield et al., 1998; Sapolsky, 2002; Romero et al., 2005; Johnson et al., 2006).

Here, we report an initial investigation of immune function in mice selectively bred for high levels of voluntary locomotor activity. As compared with four non-selected control (C) lines, the four replicate high runner (HR) lines exhibit an increase of almost 200% in daily running distance, and a similar increase in home-cage activity when deprived of wheels (Swallow et al., 1998, 1999; Garland, 2003; Rhodes et al., 2005; Malisch et al., 2008). Several types of behavioral and neurobiological evidence indicate that the motivation and/or reward systems of the brains of HR mice have been altered (Bronikowski et al., 2004; Rhodes et al., 2005; Belke and Garland, 2007). In addition, a number of traits have evolved in a way that seem to represent adaptations to permit high levels of sustained, aerobically supported exercise, including elevated maximal oxygen consumption, increased hindlimb symmetry, and larger femoral heads (Garland, 2003; Garland and Freeman, 2005; Rezende et al., 2006; Middleton et al., 2008; references therein).

Mice from the HR lines also exhibit a two-fold increase in baseline plasma CORT levels (Malisch et al., 2007). The increased plasma CORT level has been interpreted as an adaptation to promote or support high locomotor activity (e.g. see Malisch et al., 2007, 2008). However, the elevated CORT levels are associated with reduced growth rates and lower body size in the HR lines (Girard and Garland, 2002; Malisch et al., 2007), which may represent a cost.

The goal of the present study was to test the hypothesis that the elevated CORT levels in the HR lines are associated with a cost in terms of immune function. Among other effects on immune function, elevated glucocorticoids may interfere with clearance of a parasitic infection by inhibiting cytokine release, reducing cytokine receptor levels, blocking maturation of T lymphocytes, and increasing lysis of T lymphocytes (McEwen et al., 1997; Sapolsky et al., 2000). Therefore, we hypothesized that HR lines would have a reduced ability to clear a parasitic infection. In addition, the replicate HR and control lines show significant differences in circulating CORT levels, wheel running, body mass, and other traits (e.g. Swallow et al., 1998, 1999; Rezende et al., 2006; Malisch et al., 2007), so we also tested for a positive relation between line means of circulating CORT levels (obtained from Malisch et al., 2007; Malisch et al., 2008) and the number of nematodes remaining in the small intestine 12 days after inoculation (see below).

## Materials and methods

### Study animals

The replicated selective breeding experiment began from a base population of outbred Hsd:ICR mice. The selection criterion was total revolutions run on days 5+6 of a 6-day exposure to a Wahman-type activity wheel (1.12 m circumference) when mice were ~7–9 weeks of age (Swallow et al., 1998; Garland, 2003; Rhodes et al., 2005). For the present study, 10 adult males from each of the four replicate HR lines and four non-selected C lines were studied, sampled from generation 36. As in the routine selection protocol (Swallow et al., 1998), all animals used in this experiment were weaned at 21 days of age, toe-clipped for identification, and housed in same-sex groups of

four. Following wheel testing, each male was paired with a female for breeding (~18 days), and then housed individually until initiation of nematode inoculations. The immune challenge protocol began approximately 30 days after breeding pairs were separated, so the mice averaged ~13 weeks of age. At all times, mice were maintained on a 12L:12D cycle with lights on at 0700 h and provided with food (Harlan Teklad Rodent Diet 8604) and water *ad libitum*. Animals were housed and maintained in accordance with National Institutes of Health guidelines, and all procedures were approved by the Institutional Animal Care and Use Committee of the University of California Riverside, which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care.

## Immune challenge

The intestinal parasite, *Nippostrongylus brasiliensis*, was used as the immune system challenge. These nematodes infect a range of murid hosts and are frequently used in immune research, because they are potent activators of both systemic and mucosal Th2 immune responses (see Kassai, 1982 for review; see also Katona et al., 1988; Smith et al., 2001; Camberis et al., 2003; Houdijk et al., 2003). Stage-3 infective larvae penetrate the skin, then travel through the circulatory system to the lungs, where they are coughed up the trachea and then swallowed (Kassai, 1982; Camberis et al., 2003). Adult *N. brasiliensis* attach to the wall of the proximal half of the small intestine, where they begin laying eggs that are passed in the feces. Eggs in the external environment mature into stage-3 larvae and penetrate the skin of a murid host (this parasite does not have an intermediate host). Murid hosts attempt to rid themselves of the infection with a generalized inflammatory response (Kassai, 1982).

*N. brasiliensis* from a stock maintained at the University of California, Riverside were acquired from rat fecal cultures using the Baermann procedure (Kassai, 1982; Leventhal and Cheadle, 1989; Voge, 1970). Larvae were suspended in saline and the concentration was adjusted to 6,000 larvae per ml. Each mouse was weighed, so that variation in body mass could be accounted for during statistical analyses, and then inoculated with 0.1 ml of the nematode saline solution (approximately 600 infective worms) subcutaneously in the dorsal neck region under isoflurane anesthesia. Based on preliminary data, immunocompetent mice can clear the infection in approximately 12 days (Platzer, unpublished data); therefore, 12 days after inoculation, mice were sacrificed and weighed, and spleen and small intestine were excised and weighed. To facilitate counting, the small intestine was divided into four equal pieces and frozen at −80 °C. Nematode infestation was quantified by observers blind to experimental groups.

Based on previous studies (Kassai, 1982), we expected most nematodes to be present in the first and second (proximal) quarters of the small intestine. Therefore, these two quarters were individually thawed in a Liquinox soap solution (SPI Supplies, West Chester, PA, USA) and counted under a dissecting microscope. On occasion, the number of nematodes that comprised clumps of worms had to be approximated by the observer because the clumps could not be separated without the risk of breaking worms and thus artificially inflating the apparent number. Following the first counting, the complete contents of the intestine quarters were saved and preserved in 10% formalin. To determine accuracy and repeatability of the first count, a second observer, who was blind to the first count, recounted all nematodes in the first and second quarter. For the third and fourth quarters, where relatively few nematodes were found (see *Results*), only a second count was taken (i.e., after formalin preservation) because results showed them to yield higher numbers (see below).

## Statistical analysis

As an index of the repeatability of nematode counts, we used the Pearson product-moment correlation, which is not sensitive to differences in the mean between counts. A paired *t*-test was used to test for significant differences in mean values of the two counts.

Comparison of line types (HR vs. C) for spleen mass and nematode counts employed a one-way mixed-model nested analysis of covariance (ANCOVA) using SAS PROC MIXED (SAS Institute, Cary, NC, USA). The primary grouping factor was line type (HR vs. C), a fixed effect. Replicate lines were a random effect nested within line type. Degrees of freedom for testing the line type effect were always 1 and 6. Mice from the HR lines are smaller in body size as compared with C lines (Swallow et al., 1999), so body mass at the time of inoculation (for nematode counts) or at sacrifice (spleen mass) was used as a covariate. Dependent variables were transformed as needed to improve normality of the residuals from the ANCOVA models.

We tested for differences among the eight individual lines in two ways. First, we compared the differences in ln-restricted maximum likelihoods for models (see previous paragraph) with and without line in the model: the difference in ln likelihoods asymptotically follows a $\chi^2$ distribution with one degree of freedom. To compute adjusted line means and standard errors for nematode number, we also used SAS PROC MIXED, but excluded line type from the model and treated line as a fixed effect (retaining body mass as a covariate).

The $P$ value for the line effect from this analysis is also reported because the comparison of ln likelihoods described above relies on an asymptotic assumption that may not be warranted.

Finally, line means for nematode count were regressed on line means for baseline plasma CORT concentrations as determined for male mice from studies of two other generations. For generation 34, Malisch et al. (2007) sampled mice between 1600 and 1800 h (12:12 photoperiod with lights on at 0700). For generation 39, Malisch et al. (2008) sampled mice at both 1400 and 1800, and both of these time points were used to compute adjusted line means with SAS PROC MIXED, treating line as a fixed effect. Malisch et al. (2008) also assayed corticosteroid-binding globulin (CBG) capacity, and calculated free corticosterone levels (corticosterone not bound to corticosteroid-binding globulin and potentially biologically active), so we examined both total plasma CORT levels and the calculated free levels. Significance of the regressions of nematode counts on CORT levels was tested with 1-tailed tests because we had an a priori hypothesis about the direction (positive). Baseline plasma CORT concentrations from generation 34 were square-root transformed to improve normality of residuals, whereas CORT and free CORT values for generation 39 were $\log_{10}$ transformed.

Statistical significance was defined at $P<0.05$. We present 2-tailed $P$ values unless otherwise indicated.

## Results

### Repeatability of nematode counts

As might be expected during the course of such an infection, considering all mice, body mass decreased from a mean of 36.5 g at the time of injection to 35.6 g at sacrifice for dissection (paired $t$-test: $t = 4.179$, 2-tailed $P \ll 0.001$). Comparison of the selected and control lines indicated no significant difference in the amount of mass lost (2-tailed $P = 0.1030$).

As can be seen in Fig. 1, mice showed large variation in the number of nematodes remaining 12 days after inoculation. First and second nematode counts were strongly correlated for both the first and second quarters ($R>0.95$, $P \ll 0.001$; Fig. 1). However, values for the second counts were consistently higher (paired $t$-test, $P \ll 0.001$; Fig. 1). We attribute this difference to the process of storing the intestine segment and nematodes in formalin prior to the second count. Observations indicated that most large clumps of worms that had to be approximated during the first round of counting had dissociated from each other by the time of the second count, and fewer worms were still attached to the intestine wall. Although the two counts are strongly correlated, we chose to do all analyses with second counts because we believe they are more accurate.

The number of nematodes counted in the small intestine quarters decreased from the proximal to the distal quarter, and the distribution was strongly right-skewed in all quarters. Ranges (and medians) were 0–505 (100), 0–371 (92), 0–94 (17), and 0–81 (7), respectively. Given the disparity in counts along the length of the small intestine, we subsequently analyzed both the sum of the counts for the first two quarters (Fig. 1) and the sum of the counts for all four quarters.

### Variation in body mass, spleen mass, and nematode counts

Consistent with the results of many previous studies of these mice (e.g. Swallow et al., 1999), on average HR lines were smaller in body mass as compared with C lines, and the replicate lines differed significantly (Table 1). After adjusting for variation in body mass, spleen mass did not significantly differ between HR and C lines or among the replicate lines (Table 1).

As shown in Table 1, nematode counts for the first two quarters or for all four quarters did not significantly differ between HR and C line types ($P\sim0.4$); however, lines differed significantly.

### Line mean correlation of nematode counts and CORT

Line means for nematode count in the first two quarters of the small intestine (Table 2) were significantly positively related to baseline CORT levels for males from generation 34 ($R^2 = 0.510$, 1-tailed $P = 0.023$; Fig. 2). Results were similar for analyses of nematode counts in all four quarters ($R^2 = 0.442$, adjusted $R^2 = 0.349$, 1-tailed $P = 0.036$).

Considering nematodes in the first two quarters, a dummy variable for line type added to the model was not significant (2-tailed $P = 0.485$) and the adjusted $R^2$ for the overall model was decreased to 0.384. Adding both the dummy variable (2-tailed $P = 0.701$) and the interaction between line type and CORT level (2-tailed $P = 0.783$) further reduced the adjusted $R^2$ to 0.246. Again, results (not shown) were similar for total nematode counts. Therefore, the relation between nematode counts and circulating CORT at the level of the eight line means is best represented by a single regression line, as depicted in Fig. 2.

Regressions of line means for nematodes in the first two quarters on both total and free CORT levels from generation 39 (Table 2) yielded similar results ($R^2 = 0.463$, 1-tailed $P = 0.032$ and $R^2 = 0.547$, 1-tailed $P = 0.018$, respectively).

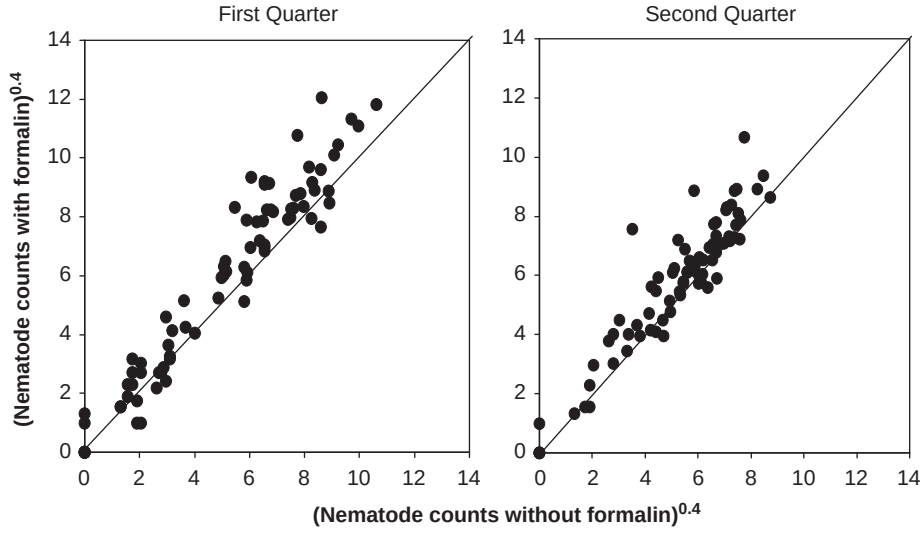


**Fig. 1.** Number of *Nippostrongylus brasiliensis* counted before and after preservation in 10% formalin in the first (left graph) and second (right graph) quarters of the small intestine of male house mice (*Mus domesticus*). Counts were highly correlated (both $R > 0.95$, $P \ll 0.001$). The black line depicts a one-to-one relation and demonstrates that counts after preservation in formalin were consistently higher than first counts (paired *t*-test, $P \ll 0.001$). As explained in the text, only the second counts were used in subsequent analyses. Counts were transformed by raising to the 0.4 power to improve bivariate normality.

## Discussion

Hormones have great potential to mediate physiological trade-offs (Zera et al., 2007). For example, glucocorticoid receptors can be found in almost all body parts. Therefore, a change in circulating CORT has the potential to alter motivational state, reproductive behavior and physiology, aspects of exercise physiology, and immune function. Results of the present study provide evidence that suggests a CORT-mediated trade-off between locomotor activity and immune function. Specifically, among eight lines of mice that vary in locomotor activity, we found a statistically significant positive relation between baseline circulating CORT levels and the inability to clear a parasitic nematode infestation (Fig. 2).

The effects of glucocorticoids on immune function are quite complex, and can be either positive or negative depending on dose and time course (McEwen et al., 1997; Sapolsky et al., 2000). Immune suppression from pharmacological doses of glucocorticoids is exploited for the treatment of some human immunological disorders, including asthma and rheumatoid arthritis (McEwen et al., 1997). In animals other than human beings, several studies have reported decreased immune function following chronic CORT elevation within a normal physiological range (Dhabhar et al., 1994; Dhabhar and McEwen, 1997; McEwen et al., 1997;

**Table 1.** Significance levels from one-way nested ANCOVA models analyzing the effect of line type and line, and from separate one-way ANCOVA models comparing the eight lines.

| Trait | Transformation | *N* | *P* for line type | *P* for line from nested ANCOVA $\chi^2$ | *P* for body mass | *P* for line from one-way ANCOVA | *P* for body mass |
|---|---|---|---|---|---|---|---|
| Body mass at sacrifice | None | 79 | 0.0173 | 0.0005 | 0.5663[a] | <0.0001 | 0.6235[a] |
| Spleen mass | None | 78 | 0.7210[b] | 0.8522 | 0.3241 | 0.4538 | 0.7392 |
| Total nematodes in first two quarters | ^0.65 | 79 | 0.3972[c] | 0.0771 | 0.0912 | 0.0337 | 0.2211 |
| Total nematodes in all four quarters | ^0.8 | 79 | 0.4197[d] | 0.0371 | 0.1171 | 0.0170 | 0.2300 |

Starting *N* was 80, but one mouse died post-injection. For spleen mass, one mouse had an extraordinarily large spleen (0.46 g) and was excluded as a statistical outlier based on the magnitude of its standardized residual. Mean age at the time of inoculation was 114 days with a range of 101–116 days.

[a]For analyses of body mass, age was the covariate.

[b]Removing line 3 from the analysis yielded line type $P = 0.7029$.

[c]Removing line 3 from the analysis yielded line type $P = 0.2047$.

[d]Removing line 3 from the analysis yielded line type $P = 0.2498$.

**Table 2.** Least-square means ± standard errors for spleen mass, number of *Nippostrongylus brasiliensis* in the small intestine, and baseline circulating corticosterone (CORT) levels for males from control (C) and high runner (HR) lines of mice.

| Line number (lab designation) | Line type | Body mass at sacrifice (g) | Spleen mass (g) | First two quarters nematodes$^{0.65}$ | All four quarters nematodes$^{0.80}$ | Square root baseline CORT from generation 35 (ng/ml) | $\log_{10}$ baseline CORT from generation 39 (ng/ml) | $\log_{10}$ baseline free CORT from generation 39 (ng/ml) |
|---|---|---|---|---|---|---|---|---|
| 1 | C | 40.86 ± 0.926 | 0.195 ± 0.016 | 33.04 ± 5.880 | 91.80 ± 16.500 | 7.71 ± 1.086 | 1.795 ± 0.1356 | –0.631 ± 0.2210 |
| 2 | C | 34.33 ± 0.969 | 0.173 ± 0.015 | 33.77 ± 5.485 | 86.42 ± 15.392 | 8.67 ± 1.134 | 1.391 ± 0.1299 | –1.153 ± 0.2115 |
| 4 | C | 40.46 ± 0.917 | 0.210 ± 0.016 | 17.77 ± 5.910 | 43.52 ± 16.583 | 7.79 ± 1.134 | 1.437 ± 0.1318 | –1.173 ± 0.2142 |
| 5 | C | 37.28 ± 0.931 | 0.214 ± 0.014 | 26.66 ± 5.273 | 67.85 ± 14.800 | 6.64 ± 1.126 | 1.481 ± 0.1273 | –0.972 ± 0.2077 |
| 3 | HR | 33.17 ± 0.915 | 0.188 ± 0.015 | 24.73 ± 5.265 | 65.14 ± 14.773 | 8.61 ± 1.246 | 1.703 ± 0.1250 | –0.757 ± 0.2046 |
| 6 | HR | 32.70 ± 0.935 | 0.191 ± 0.015 | 47.86 ± 5.595 | 132.16 ± 15.699 | 12.29 ± 1.171 | 1.940 ± 0.1244 | –0.361 ± 0.2024 |
| 7 | HR | 33.25 ± 0.926 | 0.203 ± 0.014 | 32.88 ± 5.473 | 81.23 ± 15.358 | 11.83 ± 1.154 | 1.720 ± 0.1347 | –0.627 ± 0.2188 |
| 8 | HR | 33.86 ± 0.916 | 0.176 ± 0.014 | 34.53 ± 5.369 | 89.23 ± 15.065 | 10.24 ± 1.076 | 1.795 ± 0.1245 | –0.637 ± 0.2023 |

Age was a covariate for analysis of body mass. Body mass was a covariate for analyses of spleen mass and nematode counts. For analysis of baseline CORT levels from generation 35, covariates were age, time of day, (*z*-transformed time of day)$^2$, and the amount of time from initial disturbance until termination of blood sampling (see Malisch et al., 2007). For analysis of baseline CORT levels from generation 39, covariates were age, hematocrit, the amount of time from initial disturbance until termination of blood sampling, and a 0–1 dummy variable coding for whether the mouse was resting or alert. In addition, the date on which animals were sampled and assay batch were included as random cofactors in the model (see Malisch et al., 2008).




**Fig. 2.** Significant positive regression ($R^2 = 0.510$, 1-tailed $P = 0.023$) of total *Nippostrongylus brasiliensis* counted in the first two quarters of the small intestine (raised to the 0.65 power; see text) on square-root transformed baseline plasma CORT concentrations for each of the eight lines of mice (corresponding to least-squares [adjusted] means presented in Table 2; from Malisch et al., 2007). Open circles represent the four replicate control (non-selected) lines and closed circles are the high runner lines.

Sapolsky, 2002; Berger et al., 2005). For example, temperate house sparrows (*Passer domesticus*) show suppressed cutaneous immune function following CORT implantation (Martin et al., 2005). Furthermore, Martin and coauthors found that baseline and stress-induced CORT levels are higher in temperate house sparrows, where parasitism rates are lower, as compared to tropical house sparrows, where exposure to parasitism is higher. They hypothesized that lower baseline and stress-induced CORT levels may be a benefit to species living in areas where parasitism is high and, therefore, immune function is of greater importance for survival (Martin et al., 2005).

Immune function has been shown to trade-off with other physiological traits, including a negative relation between growth and immunocompetence in *Hirundo rustica* (barn swallow) nestlings and in other bird species (Saino et al., 1998; Szep and Moller, 1999; Soler et al., 2003; Brommer, 2004). Pregnant or lactating rats have a reduced ability to clear parasitic nematode infections (Houdijk et al., 2003; Carlberg and Lang, 2004), and in humans, continuous heavy exercise, such as marathon running, has been linked to immune suppression (see Gleeson, 2007 for review). Interestingly, many of these trade-offs occur during periods of elevated CORT levels (particularly during pregnancy, lactation, and heavy exercise) and as such may represent additional CORT-mediated physiological trade-offs.

As a group, the HR lines have significantly higher baseline CORT levels as compared with the four C lines (Malisch et al., 2007, 2008). However, inspection of

**Table 2.** Least-square means ± standard errors for spleen mass, number of *Nippostrongylus brasiliensis* in the small intestine, and baseline circulating corticosterone (CORT) levels for males from control (C) and high runner (HR) lines of mice.

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Fig. 2 and Table 2 indicates that the HR and C lines can exhibit some overlap in baseline CORT concentrations. In particular, one of the four HR lines (lab-designated #3) has baseline CORT levels that are often more similar to the C lines. Line 3 mice also had nematode counts that were more similar to the C lines than the other HR lines (see Fig. 2 and Table 2). This heterogeneity among the four HR lines renders the overall comparison between HR and C lines not statistically significant for nematode counts (and omitting line 3 from the analysis does not change the result; see footnotes to Table 2). The most notable feature of the line 3 mice is that they have become fixed for an autosomal recessive gene of major effect that causes an approximately 50% reduction in hindlimb muscle mass (Garland et al., 2002; Syme et al., 2005). The so-called "mighty mini-muscle" gene (linkage mapped to a 2.6335-Mb interval on MMU11, but not yet identified; Hartmann et al., 2008) has numerous pleiotropic effects, including a doubling of mass-specific aerobic capacity in mixed hindlimb muscle (Houle-Leroy et al., 2003), altered mitochondrial density and myosin heavy chain composition (Guderley et al., 2006), altered muscle contractile performance (Syme et al., 2005), elevated HSP72 expression in triceps surae muscle (Belter et al., 2004), and an increase in the body mass-adjusted size of their heart, liver, spleen, and lungs (Garland et al., 2002; Swallow et al., 2005; Garland, unpublished observation). Many of these pleiotropic effects would appear conducive to support of sustained aerobic exercise (Garland, 2003; Guderley et al., 2006; Rezende et al., 2006). If and how the mini-muscle gene affects circulating CORT levels and any consequences that may follow are not yet known, but clearly warrant further investigation.

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