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RESEARCH ARTICLE

Activation of the immune system incurs energetic costs but has no effect on the thermogenic performance of house sparrows during acute cold challenge

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SUMMARY

Trade-offs between the immune system and other condition-dependent life-history traits (reproduction, predator avoidance and somatic growth) have been well documented in both birds and mammals. However, no studies have examined the impact of immune activation on thermoregulatory performance during acute cold exposure. Because of their high surface-area-to-volume ratios, small birds incur high energetic costs associated with thermoregulation during cold exposure. Consequently, we predicted that the immune system and the thermoregulatory system would compete for energetic resources. To test this, we immunologically challenged adult house sparrows (*Passer domesticus*) with 5 mg kg^{-1} of lipopolysaccharide (LPS) to induce an acute phase response and measured both resting (RMR; minimum metabolic rate) and summit ($\dot{M}_{sum}$; maximal metabolic rate during cold exposure) metabolic rates. We found that birds injected with LPS had significantly higher RMR and $\dot{M}_{sum}$ than birds injected with phosphate-buffered saline, indicating that LPS-treated birds were able to support the cost of both immune activation and thermoregulation under conditions eliciting maximal thermogenic performance. These results suggest that, in the absence of a pathogen, birds that experience short-term activation of the immune system have higher energetic costs during cold exposure, but immune activation does not compromise maximum thermoregulatory performance.

Key words: immune, life history, thermogenic capacity, trade-off, lipopolysaccharide.

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INTRODUCTION

Condition-dependent life-history traits require organisms to invest energetic resources among several potentially competing physiological and behavioral functions. Physiological processes such as immune activation, thermoregulation, reproduction, growth and cellular maintenance all require organismal expenditures of energy (French et al., 2009; Zuk and Stoehr, 2002). However, energetic resources are limited and investment in one process can potentially reduce the investment in others, resulting in decreased functionality (French et al., 2009; Stearns, 1989). Immune activation (Martin et al., 2003) and thermoregulation in endothermic organisms (Swanson, 2010) both incur high energetic costs that are reflected by changes in metabolic output. How metabolic energy is allocated during concurrent activation of these two functions, and between potentially competing physiological functions in general, is poorly understood.

Small passerine birds overwintering in cold climates are faced with high energy expenditures for thermoregulation. This is, in part, due to their high surface-area-to-volume ratios (Schmidt-Nielsen, 1984). Birds can mediate the negative effects of cold exposure through metabolic adjustments that facilitate cold tolerance (McKechnie, 2008; Swanson, 2010). Cold tolerance is defined as the period of time over which a bird can maintain its body temperature by thermogenesis (principally shivering) at a given level of cold exposure (Swanson, 2001). Winter-acclimatized or cold-acclimated birds from cold winter climates generally show seasonal increases in both basal (BMR; minimum metabolic rate required for maintenance) and summit ($\dot{M}_{sum}$; maximal metabolic rate during cold exposure) metabolic rates relative to summer-acclimatized or warm-acclimated birds (Cooper and Swanson, 1994; Swanson, 2010). $\dot{M}_{sum}$ assumes importance not only because it sets the upper limit of thermogenic capacity and cold tolerance for endothermic organisms, but also because it is correlated with increased shivering endurance at submaximal levels of cold exposure (Marsh and Dawson, 1989; Swanson, 2001; Swanson and Liknes, 2006) and increased survival rates (Hayes and O'Connor, 1999; Sears et al., 2006). However, the energetic demands of thermoregulation are likely exacerbated by the activation of the immune system during cold exposure.

Overwintering birds confronted with a pathogen must cope with the dual challenge of activating and maintaining both thermoregulatory and immune functions during cold exposure. Like thermoregulation, activation of the immune system, both innate (Martin et al., 2003; Ots et al., 2001) and adaptive (Svensson et al., 1998), increases metabolism in birds. However, the effects of cold on avian immunity are not consistent. In regards to performance effects, one study reported that cold-challenged birds show increased activity of the adaptive immune system (antibody production) (Hangalapura et al., 2004b), while another study found a depression (Svensson et al., 1998). In contrast, components of the innate immune system (phagocytes, natural antibodies, cytokines, etc.) are enhanced in birds challenged both immunologically and by cold exposure (Dabbert et al., 1997; Hangalapura et al., 2003; Hawley et al., 2012). Metabolically, individuals experiencing the combined effects of moderate cold exposure and immune challenge experience elevated resting metabolic rates (RMR; minimum metabolic rate at rest in immune-challenged birds) compared with individuals immunologically

challenged at thermoneutrality (Burness et al., 2010; Hawley et al., 2012). Based on the aforementioned studies, though, no clear effect of cold exposure on the immune system has emerged in studies that have focused on the effect of cold challenge on immune function. To our knowledge, no studies have examined the alternative scenario, the impact of immune activation on thermoregulatory performance during acute cold exposure.

In the present study we examine the effects of immune activation on the ability of house sparrows [*Passer domesticus* (Linnaeus 1758)] to thermoregulate during extreme cold challenge, when they are challenged to their maximal thermogenic capacity ($\dot{M}_{sum}$). Under these conditions, the cost of reduced thermogenic function or performance is expected to be much higher than documented in previous studies because of the risk of hypothermia. To test this we simulated a bacterial infection by inducing an acute phase response (APR) using lipopolysaccharide (LPS). LPS is an immunogenic component of gram-negative bacteria that stimulates a short-lived inflammatory response, but is non-pathogenic (Kent et al., 1992). The APR has become an important tool in examining the effects of immune activation on the performance and functionality of other condition-dependent life-history traits (Bonneaud et al., 2003; Burness et al., 2010; Lee et al., 2005; Owen-Ashley and Wingfield, 2007). Activation of the APR results in both behavioral and physiological changes (Owen-Ashley et al., 2006; Owen-Ashley and Wingfield, 2007) that may interfere with cold tolerance. It is characterized by heterothermia, the release of endogenous pro-inflammatory cytokines (IL-1, IL-6 and TNF-α) (Kluger et al., 1998), the release of glucocorticoids (Owen-Ashley et al., 2006) and the presentation of sickness behaviors (anorexia, lethargy, adipsia and hypergesia) (Coon et al., 2011; Hart, 1988; Owen-Ashley et al., 2006). We expected sparrows challenged under these conditions to be more sensitive to cold challenge and demonstrate a reduction in thermogenic capacity due to the physiological and behavioral changes induced by the APR.

MATERIALS AND METHODS

Animal capture and care

We collected house sparrows by mist nets near Alcester, Union County, South Dakota (43°1′N, 96°38′W), and Vermillion, Clay County, South Dakota (42°47′N, 96°56′W). The house sparrow is a widely distributed, invasive, non-migratory species that overwinters in both temperate and tropical climates (Anderson, 2006). Upon capture, we measured body mass to the nearest 0.1 g and wing and tarsus length to the nearest 0.01 mm. We captured birds used in this study in October 2011 and June 2012. After capture, we immediately brought birds back to University of South Dakota campus where they were housed indoors under controlled temperature (23±1°C) and light (12 h:12 h light:dark) conditions. We housed birds in flight cages (56×30×38 cm) with food (wild birdseed) and vitamin-enriched water provided *ad libitum*. We allowed birds to acclimate to the captive environment for at least 2 weeks before experimentation began. We used birds captured in October 2011 exclusively for $\dot{M}_{sum}$ measurements and those captured in June 2012 for RMR measurements. An additional four birds were captured in May 2012 for use in an RMR pilot study, but their metabolic measurements were not included in the final analysis because they were measured on the same day as capture. Because we maintained birds for both time periods under identical captive conditions for at least 2 weeks prior to experiments, a time during which body mass stabilized, and limited our comparisons to control *versus* treatment birds within each period, we expect no seasonal effects on our results.

Immune challenge

We randomly assigned birds to receive a single injection of either LPS (L4005, serotype 055:B5; Sigma-Aldrich, St Louis, MO, USA) dissolved in phosphate-buffered saline (PBS) or PBS (control).

We injected birds with a high LPS dose of 5 mg kg^{-1} body mass to maximize the detection of the effects of immune activation on thermogenic performance and acute cold tolerance. We derived this dose from a pilot dose–response study where birds used in the cold challenge study were randomly challenged with either PBS or with varying doses of LPS (1 and 5 mg kg^{-1} body mass). We measured their body temperature (T_b) at the time of injection (0 h) and 3, 6 and 24 h post-injection. We found that the dose of 5 mg kg^{-1} induced a significantly more pronounced and longer lasting bout of hypothermia (3 to 6 h post-injection) than the 1 mg kg^{-1} dose (Fig. 1). The birds used for the pilot experiment were given an additional 2 week rest period prior to $\dot{M}_{sum}$ measurements. In small passerine bird species, hypothermia, as opposed to fever, has previously been reported during LPS challenge (Owen-Ashley et al., 2006; Owen-Ashley and Wingfield, 2007). A 5 mg kg^{-1} dose of LPS has been used in other avian (Cheng et al., 2004) and small mammal studies (Barsig et al., 1995; Qin et al., 2007).

Summit metabolic rate

We injected birds with either LPS (N=6) or PBS (N=6) 3 h prior to cold challenge. We measured $\dot{M}_{sum}$ *via* sliding cold exposure in a helox (79% helium and 21% oxygen) gas mixture (Liknes et al., 2002; Swanson, 2001; Swanson et al., 1996). We initiated the cold exposure at 0 to −6°C for 15 min, then decreased the bath temperature at a rate of −0.3°C min^{-1} until a steady decline in oxygen consumption indicative of hypothermia was induced. We modified the initial temperature for each individual, according to body mass (lower temperatures for larger birds), so that hypothermia did not occur too rapidly (<30 min) or too slowly (>1 h).

We placed individual birds into a 1.91 metabolic chamber designed from a paint can with the inner surface painted flat black to provide emissivities near 1.0. The chamber was equipped with a perch. The mean effective volume of this chamber, calculated according to Bartholomew et al. (Bartholomew et al., 1981), was 1917 ml. We achieved temperature control within the metabolic chamber by immersing it into a bath of water and ethylene glycol

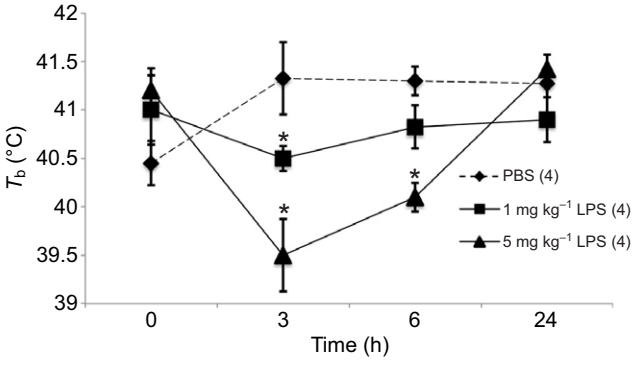

Fig. 1. Cloacal body temperature (T_b; °C) of house sparrows maintained at room temperature that were injected with either phosphate-buffered saline (PBS; control) or with varying doses of lipopolysaccharide (LPS; 1 and 5 mg kg^{-1}) and measured at 0, 3, 6 and 24 h post-injection for a pilot dose–response study. Values are expressed as means ± s.e.m. Asterisks denote statistically significant differences between the body temperatures of PBS- and LPS-challenged birds as determined by Student's *t*-test (P<0.05). Sample sizes are in parentheses beside the treatment dose.

(Forma Scientific model 2095, Marietta, OH, USA), which regulated chamber temperature to ±0.2°C. Prior to immersion, we flushed the chamber for at least 5 min with helox to replace air. We scrubbed incurrent and excurrent gas of water and CO_2 by passing the gas stream through a column of Drierite and Ascarite. We maintained flow rates of dry, CO_2-free helox at 1010–1030 ml min^{-1} over the course of the experiments using a Cole-Parmer Precision Rotameter (model FM082-03ST, Chicago, IL, USA), previously calibrated with a soap bubble meter to ±1% accuracy. We monitored chamber temperature continuously with a Cole-Parmer thermocouple thermometer (model 8500-40) and recorded chamber temperature every 60 s.

We measured oxygen consumption during helox cold exposure by open-circuit respirometry with an Ametek S-3A oxygen analyzer (Pittsburgh, PA, USA). We recorded measurements of dry, CO_2-free efflux gas every 5 s on a computer using Datascan 5.0 (Sable Systems, Henderson, NE, USA) data collection software. We computed oxygen consumption according to the instantaneous equations of Bartholomew et al. (Bartholomew et al., 1981) using Expedata 2.0 software (Sable Systems). For calculations of oxygen consumption, we recorded the oxygen content of incurrent gas before and after each metabolic trial and adjusted baseline oxygen content for drift over the test period using the drift correct function in Expedata 2.0. We then calculated consecutive 5 min means for oxygen consumption rates over the test period and considered the highest 5 min mean, excluding the initial 10 min of measurements, as $\dot{M}_{sum}$ (Dawson and Smith, 1986). We corrected all values for oxygen consumption to STPD. Tests were conducted between 09:00 and 12:00 h. Birds were in the chamber for no more than 1 h and no more than two birds were cold-challenged per day.

At the end of each test, we promptly removed birds from the chamber and recorded their body mass and cloacal temperature. We measured T_b with a Cole-Parmer Model 8500-40 thermocouple thermometer by inserting a lubricated 20 gauge copper–constantan thermocouple into the cloaca (~1 cm). We considered birds with T_b<37°C as hypothermic. For each bird, we noted the temperature at cold limit (T_{CL}; the temperature producing hypothermia during exposure of an individual bird to a declining series of temperatures) (Saarela et al., 1989).

We completed body mass and temperature measurements at the time of injection, 3 h post-injection, immediately after cold challenge and 24 h after injection.

Resting metabolic rate

We measured RMR using procedures similar to those for $\dot{M}_{sum}$ measurements except that air was used as the respiratory gas instead of helox. We randomly selected birds to receive LPS or PBS treatments. We measured RMR in LPS- (N=5) and PBS-injected (controls; N=5) birds from 19:00 to 07:00 h. We injected birds immediately before placing them into the metabolic chamber. We maintained flow rates of dry, CO_2-free air at 290 ml min^{-1} and kept the chamber temperature at 30°C, which is within the thermal neutral zone for house sparrows (Arens and Cooper, 2005; Hudson and Kimzey, 1966), throughout the RMR trials. We kept birds within the metabolic chambers overnight for ~12 h. Because we did not fast birds prior to metabolic measurements, and to allow the APR to develop, we excluded the first 3 h of the metabolic trial from our RMR calculations. We recorded oxygen content in the excurrent gas every 5 s and calculated oxygen consumption according to steady-state equations (Withers, 1977) corrected to STPD. We calculated 10 min running mean values for oxygen consumption over the test period and considered the lowest 10 min running mean as RMR (Bartholomew et al., 1981). Only one individual was measured per night.

We recorded body mass and temperature measurements at the time of injection and 12 h post-injection, when the birds were removed from the metabolic chambers.

Data analysis

We carried out all statistical analyses using JMP 7.0.1 (SAS Institute, Cary, NC, USA) and present data as means ± s.e.m. We accepted statistical significance at $P \leq 0.05$. We analyzed the effects of treatment on RMR and $\dot{M}_{sum}$ *via* ANOVA and analysis of covariance (ANCOVA), with body mass (measured immediately prior to birds being placed in the metabolic chamber) acting as the covariate. We used repeated-measures ANOVA to detect any treatment by time effects on the body mass and T_b of birds sampled at multiple time points, followed by Bonferroni-corrected Student's t-test at each time point. Student's t-test were also used for pairwise comparisons of the magnitude of change in body mass and T_b, and also to detect variation in T_{CL}.

RESULTS

Effects of immune challenge on $\dot{M}_{sum}$

LPS-treated birds had significantly higher $\dot{M}_{sum}$ than PBS-treated birds both with (ANCOVA: $F_{3,8}$=5.14, P=0.029) and without (ANOVA: $F_{1,10}$=8.14, P=0.017) body mass included as a covariate (Fig. 2). For birds having T_b and body mass measured at multiple time points, a significant treatment by time interaction for T_b (repeated-measures ANOVA: $F_{2,20}$=22.67, P<0.0001), but not body mass (repeated-measures ANOVA: $F_{2,20}$=0.4402, P=0.65), was detected. At the time of injection (3 h prior to cold exposure), body mass (t_{10}=2.23, P=0.68) and T_b (t_{10}=2.23, P=0.19) did not differ significantly between LPS- and PBS-treated birds (Table 1). Immediately prior to cold challenge (3 h post-injection), LPS-treated birds had significantly lower T_b compared with PBS-treated birds (t_{10}=2.28, P=0.0002; Fig. 3; Table 1) and the change in T_b between pre-injected and 3 h post-injected birds was also significantly greater in LPS- than in PBS-treated birds (t_{10}=2.23, P=0.0003), with the T_b of the LPS-treated birds decreasing to a greater extent than that of the PBS-treated birds. However, there was no significant difference in body mass between LPS- and PBS-treated birds immediately prior to cold exposure (t_{10}=2.23, P=0.46; Table 1), nor was there a significant change in body mass (t_{10}=2.23, P=0.21) for birds between the time of injection and 3 h post-injection.

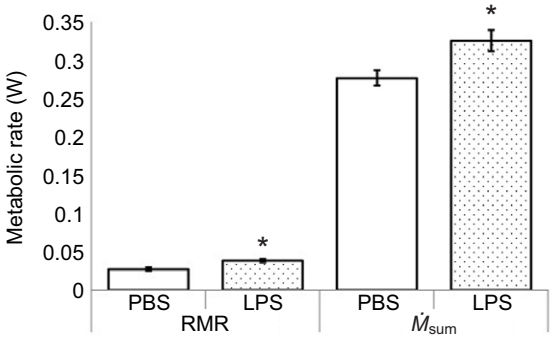

Fig. 2. The effect of immune challenge on summit metabolic rates ($\dot{M}_{sum}$; cold challenge) and resting metabolic rates (RMR; thermoneutrality) of house sparrows challenged with either PBS or LPS. Values are expressed as means ± s.e.m. Asterisks denote statistically significant differences between PBS and LPS-challenged birds (P<0.05) as determined by Student's t-test.

Table 1. Cloacal body temperature (T_b) and body mass of PBS- and LPS-challenged house sparrows undergoing acute cold challenge during the course of the experimental timeline

	Pre-injection		Pre-cold-challenge (3 h post-injection)		24 h post-injection	
Treatment	Body mass (g)	T_b (°C)	Body mass (g)	T_b (°C)	Body mass (g)	T_b (°C)
PBS	28.3±0.67	40.6±0.21	28.1±0.78	41.2±0.20	27.9±0.94	40.9±0.35
LPS	27.9±0.59	41.3±0.17	27.4±0.52	39.5±0.22	27.8±0.64	41.4±0.21
P-value	0.68	0.19	0.46	0.0002*	0.97	0.31

PBS, phosphate-buffered saline; LPS, lipopolysaccharide.
Values are expressed as means ± s.e.m.
Asterisks denote statistically significant differences between the body temperatures of PBS and LPS (P<0.025) as determined by Student's *t*-test.

All of the birds that underwent the helox cold challenge were confirmed to be hypothermic at the termination of the cold exposure treatment. Immediately following cold challenge there was no significant difference in body mass between LPS- and PBS-treated birds (t_{10}=2.23, P=1). However, PBS-treated birds lost significantly more body mass than LPS-treated birds (t_{10}=2.23, P=0.0014) during cold challenge (change in body mass for birds measured immediately before and after cold challenge). At 24 h post-injection there was no significant difference in body mass (t_{10}=2.23, P=0.97) or T_b (t_{10}=2.23, P=0.31; Table 1) between LPS- and PBS-treated birds. T_{CL} was not significantly different between LPS- and PBS-treated birds (t_{10}=2.23, P=0.81; Fig. 4).

Effects of immune challenge on RMR

LPS-treated birds had significantly higher RMR than PBS-treated birds both with (ANCOVA: $F_{3,6}$=11.41, P=0.0068) and without (ANOVA: $F_{1,8}$=11.63, P=0.0092) body mass included as a covariate (Fig. 2). For birds that had T_b and body mass measured at multiple time points, no significant treatment by time interaction was detected for either T_b (repeated-measures ANOVA: $F_{1,8}$=2.55, P=0.1490) or body mass (repeated-measures ANOVA: $F_{1,8}$=3.97, P=0.0816). Prior to injection, birds did not differ significantly in body mass (t_8=2.31, P=0.098) or T_b (t_8=2.31, P=0.83). Upon removal from the metabolic chamber, the change in body mass was significantly greater in LPS-treated birds than in PBS-treated birds (t_{12}=2.31, P=0.013; experimental data combined with pilot data), but treatment groups showed no significant difference in T_b (t_8=2.17, P=0.09).

DISCUSSION

The results of this study suggest that mounting an immune response (APR) incurs a significant energetic cost and that this cost is additive to thermoregulatory costs during severe cold challenge. This is similar to the results of Burness et al. (Burness et al., 2010) and Hawley et al. (Hawley et al., 2012), who documented additive costs and no trade-offs between thermoregulation and immune activation during much milder cold exposure treatments. Despite the additive cost of the APR and $\dot{M}_{sum}$ in this study, the thermogenic performance of cold-challenged birds was not affected by immune activation, suggesting that immune-challenged birds were able to afford the energetic costs of concurrent activation of both thermoregulatory and immune systems, even under conditions eliciting maximal thermoregulatory performance. To our knowledge, this is the first study to examine the effects of immune activation on thermoregulation during acute cold challenge.

Stimulation of the acute phase response, *via* LPS, resulted in birds having significantly higher RMR (40% increase) than those challenged with PBS (Fig. 2). This is in agreement with previous data for avian species, where activation of the immune system resulted in elevated RMR (Marais et al., 2011; Martin et al., 2003). LPS-treated house sparrows also lost significantly more body mass than control birds during the 12 h period that they spent in the metabolic chamber. Birds in the metabolic chamber did not have access to food or water. As such, the loss of body mass is reflective of birds using their stored energetic resources to maintain bodily functions. In a previous study, the loss of body mass was ameliorated by providing *ad libitum* access to food and water during immune challenge, suggesting that the energetic consequences of immune activation can be offset by resource availability at temperatures both within and below thermoneutrality (Burness et al., 2010).

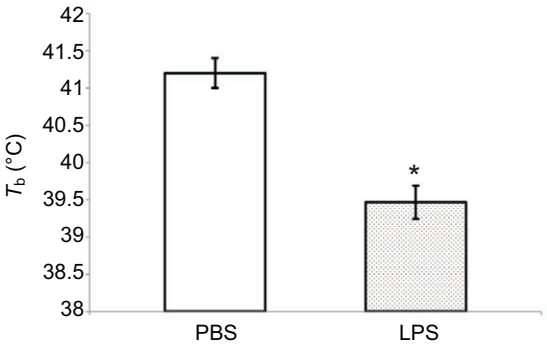

Fig. 3. T_b (°C) of PBS- and LPS-treated house sparrows 3 h post-injection. Values are expressed as means ± s.e.m. Asterisk denotes statistically significant differences between PBS- and LPS-challenged birds (P<0.02) as determined by Student's *t*-test.

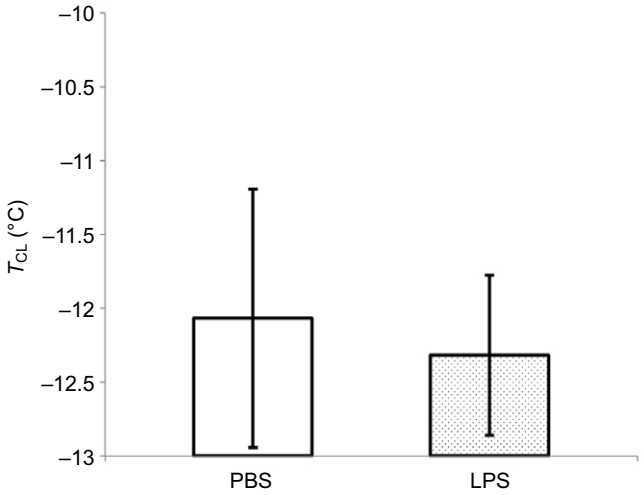

Fig. 4. Average temperature producing hypothermia (T_{CL}) in PBS- and LPS-challenged house sparrows exposed to a declining series of temperatures (°C). Values are expressed as means ± s.e.m.

Simultaneous activation of the thermoregulatory and immune systems significantly elevated the $\dot{M}_{sum}$ of house sparrows by 17% relative to PBS-treated birds, but did not affect the cold tolerance of birds challenged with severe cold temperatures (Figs 2, 4). This was contrary to our original prediction that thermoregulatory performance would be compromised (i.e. lower $\dot{M}_{sum}$ and higher T_{CL}) in birds undergoing an APR. The LPS dose and cold-challenge temperatures used in this experiment were more extreme than those used in other life-history trade-off experiments (Burness et al., 2010; Hangalapura et al., 2003; Hawley et al., 2012; Owen-Ashley et al., 2006). For example, mild cold exposure in other passerine bird thermoregulatory–immune trade-off studies (Burness et al., 2010; Hawley et al., 2012) resulted in metabolic rate elevations above those for thermoneutral temperatures of 1.3- to 2.6-fold, whereas the severe cold exposure in our study resulted in metabolic rate elevations exceeding eightfold. Nevertheless, we found that thermogenic performance was not hindered by immune activation, even though it required elevated energy expenditure.

An identifying characteristic of the acute phase response is the presentation of heterothermia. In this study, LPS-challenged house sparrows experienced a brief bout of hypothermia in lieu of hyperthermia. This was confirmed in 100% of our LPS-treated birds, immediately prior to cold challenge. This contrasts with T_b data from house sparrows following 1 mg kg^{-1} LPS injections (Coon et al., 2011), where sparrows showed a slight nocturnal hyperthermia. Part of the difference between our study and that of Coon et al. (Coon et al., 2011) may be due to the higher LPS dose used in our study, as the thermoregulatory response to LPS is dose-dependent in rodents, with hypothermia occurring at high doses (Blatteis, 2006; Rudaya et al., 2005). However, our pilot studies suggested a similar, albeit less pronounced, response for 1 mg kg^{-1} LPS-treated birds. The LPS-induced hypothermia documented in our study is consistent with previous data from Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*) (Owen-Ashley et al., 2006) and, to a lesser extent, zebra finches (*Taeniopygia guttata*) (Burness et al., 2010). Owen-Ashley and Wingfield (Owen-Ashley and Wingfield, 2007) attribute the hypothermic response during APR to the fact that small passerine species possess high thermal set points and high surface-area-to-volume ratios, making prolonged elevation of T_b metabolically unfeasible. However, variation in T_b, at the onset of cold challenge, had no effect on $\dot{M}_{sum}$ or T_{CL}, which also contradicted our earlier assertion that the APR would negatively impact thermoregulatory performance.

Body mass also did not vary significantly between LPS- and PBS-treated birds during acute cold challenge (Table 1). Although PBS-treated birds lost significantly more mass during the course of cold challenge, we suspect that birds treated with PBS likely consumed more water and food during the 3 h interim between injection and cold challenge, as APR typically reduces feeding in birds (Burness et al., 2010; Owen-Ashley et al., 2006). This would result in higher mass loss from urination and defecation during the course of cold challenge. Although we challenged our birds under more rigorous conditions than those described in other thermo-immune studies, our findings on body mass are still consistent with other avian studies that examined the effects of moderately cold temperatures and immune activation on body condition (Burness et al., 2010; Hawley et al., 2012).

This study demonstrated that while there was a significant energetic cost attached to the concurrent activation of both the thermoregulatory and immune systems, thermogenic performance was not affected by immune activation. Under natural conditions, the thermoregulatory abilities of cold-challenged birds may be compromised by pathogen exposure and resource availability (Gutierrez et al., 2011; Hangalapura et al., 2004a; Hawley et al., 2012). In this study we activated the immune system using LPS, which is non-pathogenic. In the absence of a pathogen, trade-offs between thermogenic and immunologic function may not be readily apparent, as pathogenicity can greatly affect body condition (Pantin-Jackwood et al., 2012) and thus thermoregulatory ability (Verbeek et al., 2012). Although we found no effect of immune activation on thermogenic performance, further investigation into the immediate and long-term effects of thermo-immune interactions is warranted.

Life-history theory assumes an energetic ceiling such that elevated energetic costs in one energetically demanding trait are linked to reduced energetic investment in another (French et al., 2009; Stearns, 1989). Although we did not measure the intensity of the APR during cold exposure in this study, the significant elevation of $\dot{M}_{sum}$ for LPS-treated birds suggests that additive energetic costs, at least for some potentially competing systems, can be accommodated. If this is the case, then the assumption of competition among condition-dependent life-history traits for strictly limited energetic resources needs to be applied cautiously and tested empirically when exploring the evolution of avian life histories. This is consistent with the idea that the energetic cost of an immune response is not necessarily the currency mediating potential trade-offs between immunity and other condition-dependent life-history traits, but that other factors such as competition for other shared resources (e.g. proteins) or degradation of body condition could be involved (Burness et al., 2010). In addition, competition among condition-dependent life-history traits may only occur under conditions approaching maximum levels of organismal energy expenditure. Because $\dot{M}_{sum}$ is typically lower than exercise-induced maximal metabolic rates for birds (Swanson et al., 2012; Wiersma et al., 2007), compromised metabolic performance due to activation of the immune system, or other energetically costly activities, might not be detected for $\dot{M}_{sum}$, but only at energetic expenditures approaching maximum metabolic output. Distinguishing among these possibilities will require additional research.

LIST OF SYMBOLS AND ABBREVIATIONS

APR	acute phase response
BMR	basal metabolic rate
LPS	lipopolysaccharide
$\dot{M}_{sum}$	summit metabolic rate
PBS	phosphate-buffered saline
RMR	resting metabolic rate
T_b	body temperature
T_{CL}	temperature at cold limit

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AUTHOR CONTRIBUTIONS

M.O.K. was responsible for the conception, design and execution of the study. M.O.K and D.L.S. interpreted the results and drafted and revised the article.

COMPETING INTERESTS

No competing interests declared.

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