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Body temperature changes during simulated bacterial infection in a songbird: fever at night and hypothermia at day

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Abstract

Although fever (a closely regulated increase in body temperature in response to infection) typically is beneficial, it is energetically costly and may induce detrimentally high body temperatures. This can increase the susceptibility to energetic bottlenecks and risks of overheating in some organisms. Accordingly, it could be particularly interesting to study fever in small birds, which have comparatively high metabolic rates and high, variable body temperatures. We therefore investigated two aspects of fever and other sickness behaviours (circadian variation, dose-dependence) in a small songbird, the zebra finch. We injected lipopolysaccharide (LPS) at the beginning of the day and night, respectively, and subsequently monitored body temperature, body mass change and food intake for the duration of the response. We found pronounced circadian variation in body temperature response to LPS-injection, manifested by (dose-dependent) hypothermia at day but fever at night. This resulted in body temperature during the peak response being relatively similar at day and at night. Day-to-night differences might be explained in the context of circadian variation in body temperature: songbirds have a high daytime body temperature that is augmented by substantial heat production peaks during activity. This might require a trade-off between the benefit of fever and the risk of overheating. In contrast, at night when body temperature is typically lower and less variable, fever can be used to mitigate infection. We suggest that the change in body temperature during infection in small songbirds is context-dependent and regulated to promote survival according to individual demands at the time of infection.

Keywords: APR, fever, heterothermy, hypothermia, LPS, sickness behaviour, *Taeniopygia guttata*, zebra finch

Summary statement

This paper provides empirical support for circadian variation in body temperature associated with (mimicked) infection and it highlights how small songbirds may balance fever responses depending on their metabolic status.

Introduction

Fever, a closely regulated increase in the body's set-point temperature in response to infection, is an evolutionary conserved defence mechanism (Kluger et al., 1998; Blatteis 2003) that is widely used across the animal kingdom in organisms ranging from invertebrates (Boorstein and Ewald, 1987; Adamo, 1998) to endotherms (Kurokawa et al., 1996; Escobar et al., 2007; Bingham et al., 2009). Two, not mutually exclusive, hypotheses have been proposed to explain the adaptive value of fever: (I) fever might cause a hostile environment for pathogens, which hampers their growth, proliferation and survival, and/or (II) fever may enhance the efficiency of the host's immune system, thereby facilitating clearance of the infection (Kluger et al., 1998; Blatteis, 2003). However, the role of fever in infections is enigmatic because it is energetically costly (Kluger, 1991; Marais et al., 2011c), its occurrence or absence during infection is equivocal and its benefits are not always obvious (Kluger et al., 1998; Blatteis, 2003).

In vertebrates, fever is an integral part of the acute phase response – the first line of defence against a pathogen – that consists of a suite of physiological and behavioural adjustments (Hart, 1988; Blatteis, 2003). During an acute phase response, animals display typical "sickness behaviours", that (besides fever) include reduced food intake (and even anorexia) and activity (lethargy). These adjustments collectively act to alleviate the effects of infections and facilitate the elimination of the pathogen (Hart, 1988; Kluger et al., 1998). However, because sickness behaviours affect metabolic rate and, hence, an animal's energy budget they may ultimately constrain the amount of energy available for other activities (e.g. Sheldon and Verhulst, 1996). It is perhaps partly for this reason that empirical studies show variation in the strength of the fever response depending on e.g. pathogen load (Maloney and Gray, 1998; Koutsos and Klasing, 2001; Deak et al., 2005; Rudaya et al., 2005), ambient temperature (Rudaya et al., 2005), site of infection (Ashley and Wingfield, 2012), and circadian timing of infection (Nomoto, 1996). In other cases, an organism may respond to infection with hypothermia instead of fever, either as a natural part of the body's defence or as a result of septic shock (Romanovsky et al., 1996; 2005; Martin et al., 2008). Under the former scenario, fever and hypothermia have been suggested to be two alternative strategies to mitigate infection. Hypothermia would be favoured when resources are scarce and the energetic costs of a fever response cannot be supported (Romanovsky and Székely, 1998). This can be the case during very severe infections or in energetically demanding environments (Liu et al., 2012), or in cases where insufficient body insulation and/or small body size precludes any

sustained increase in body temperature (such as in neonates) because the resultant heat loss would be detrimental (Jones et al., 1983; Frafield and Kaplanski, 1998).

Patterns regarding the presence or absence of fever during an acute phase response in birds are equivocal. Even in response to a challenge with similar or identical doses of the same artificial endotoxin, large non-passerine birds such as fowl typically demonstrate fever (Maloney and Gray 1998; Koutsos and Klasing, 2001; Leshchinsky and Klasing, 2001; Marais et al., 2011a), whereas small passerines sometimes respond with fever (Adelman et al., 2010a; 2010b; Coon et al., 2011; Nord et al., 2013) and sometimes with hypothermia (Owen-Ashley et al., 2006; Burness et al., 2010; King and Swanson, 2013), the latter being observed more often during the day (but see Adelman et al., 2010b). The reasons for this variation in the body temperature response to an endotoxin challenge among small passerine birds, and between small and large (e.g. between passerine and non-passerine) birds, are not known. Nor is it currently known if endotoxin-induced hypothermia in birds is adaptive or simply a consequence of improper dosage (Gray et al., 2013). These circumstances make birds interesting study objects when testing hypotheses of the functionality and trade-offs involved in fever responses.

In this study, we used a small bird model (the zebra finch, *Taeniopygia guttata* Vieillot, ca. 14 g) and an endotoxin challenge (*Escherichia coli* lipopolysaccharide) to better understand why the fever response varies among and within birds. Lipopolysaccharide (LPS) is a pyrogenic component of the cell walls of gram-negative bacteria that triggers the host's immune system to react largely as it would do when infected by a live, replicating bacteria (Ashley and Wingfield, 2012). LPS-injection is frequently used to induce an acute phase response and stimulate fever in animals (Nomoto, 1996; Harden et al., 2006; Owen-Ashley et al., 2006; Marais et al., 2011c). Specifically, we assessed the extent to which fever (i.e. a regulated rise in deep body temperature; Blatteis, 2003) and other physiological and behavioural responses that might affect energy expenditure and/or thermoregulation (food intake, body mass changes) (*i*) showed circadian variation, and (*ii*) were dose-dependent during the day. The latter would provide insight into how the body responds to variation in the strength of an endotoxin challenge, which is important to better understand the presence or absence of endotoxin-induced hypothermia. We first challenged birds with different doses of LPS in the morning, and subsequently measured body temperature during the day of the challenge, as well as body mass changes and food intake during the next two days. We predicted that low

doses of LPS would trigger a (dose-dependent) fever response as has previously been found for larger birds (e.g. Maloney and Gray, 1998), whereas higher doses may result in hypothermia (cf. Owen-Ashley et al., 2006; Burness et al., 2012; King and Swanson, 2013). This would be compatible with the idea that fever is not a viable option during severe infection in small birds due to its high energy costs, or that hypothermia is a sign of sepsis caused by severe infection. We further expected body temperature changes to be mirrored by changes in food intake, body mass gain and overnight body mass loss (the latter being attenuated for doses that resulted in use of hypothermia where energy costs of the immune response should be lower). Two months after the first experiment, we administered a single, moderately strong, dose of LPS (that has previously been used to trigger nocturnal fever in passerines; Nord et al., 2013) in the evening and measured the body temperature response and body mass loss during the night, in order to study any variation in these responses that could be related to the circadian timing of the challenge. We predicted that the nocturnal fever response and associated body mass loss should be similar to that observed during the day, which would be compatible with the notion that endotoxin-induced hypothermia develops only during severe infection or sepsis. The results of our study offer important new insights into circadian and functional variation in fever and sickness behaviours, with important implications for our general understanding of costs and benefits of body temperature regulation during infection in homeotherms.

Results

Test statistics for all final models are reported in the Electronic Supplements (Supplementary Table 1).

Responses to a LPS-challenge during the day

LPS-injected birds decreased their body temperature in a dose-dependent manner starting already 20 min after injection (Fig. 1, Table 1), although the *mean* body temperature during this initial period of the acute phase response was not significantly affected by LPS dose (P = 0.11; Fig. 2A). Birds in all treatment categories reached the lowest body temperatures after 3.0 ± 0.33 hrs (Fig. 1), during which time there was a significant negative relationship between *mean* body temperature and LPS dose (P = 0.039; Fig. 2B). After the maximum body temperature response, birds in all four experimental groups steadily increased their body

temperature, with most LPS-dose groups having converged to the body temperatures of the control birds 5 hrs after injection (Fig. 1). However, birds injected with the second to highest LPS-dose (100 µg LPS kg^{-1}) maintained a lower body temperature for an additional 2 hrs (i.e. until 7 hrs after injection). There was no relationship between LPS dose and *mean* body temperature during the last period of the day (12 hrs after injection; $P = 0.26$; Fig. 2C).

Daytime food consumption and body mass gain decreased with increasing LPS dose during the two days after injection (Tables 1, 2; Fig. 3). On the day of injection (day 1), we found a linear dose-dependent reduction in both seed consumption ($P < 0.001$; Fig. 3A) and body mass gain ($P = 0.045$; Fig. 3C). However, body mass loss during the night did not differ between experimental treatments ($P = 0.68$; Table 1). On the day after injection (day 2), only the birds injected with the highest LPS-dose (1000 µg LPS kg^{-1}) showed suppressed body mass gain and food consumption, resulting in a curvilinear relationship between LPS dose and both seed consumption ($P = 0.0035$; Fig. 3B) and body mass gain ($P = 0.013$; Fig. 3D).

Responses to a LPS-challenge during the night

The direction of the body temperature response to a LPS-challenge at night was opposite to that observed during the day (Table 1; Fig. 4). Both LPS-challenged birds and control birds reduced body temperature in a similar way during the first hour after injection. However, from 1 to 3 hrs after injection body temperature continued to decrease in control birds, but increased slightly in LPS-challenged birds. This body temperature increase peaked after 3 hrs, when LPS-challenged birds maintained their body temperature 1.2 °C above that of controls (Fig. 4; Table 1). Body temperature in the LPS-challenged birds subsequently decreased, but was nevertheless 0.7 °C higher than in control birds throughout the night. There was no difference in body temperature between LPS-injected and control birds by the time of the last measurement (at 7 AM, when lights were switched on10 hrs after injection). Birds injected with LPS in the evening did not lose more body mass overnight than did control birds ($P = 0.97$; Table 1).

Comparison of body temperature during the peak response at day and at night

LPS-challenged birds (100 µg LPS kg^{-1}) maintained a relatively similar body temperature at the peak response (3 hrs after injection) regardless of the timing of the challenge (0.4 °C lower at night; $P = 0.058$; Table 1; Fig. 5). In contrast, the body temperature in control birds 3 hrs after injection was 2.1 °C lower at night than at day ($P < 0.001$; Table 1; Fig. 5).

Discussion

By simulating a bacterial infection in male zebra finches we have shown that the body temperature changes during an acute phase response are subject to both pronounced circadian variation (Fig. 5) and dose-dependence during the day (Figs. 1, 2). Specifically, birds challenged with LPS reduced body temperature relative to control birds during the day when challenged in the morning (hypothermia; Fig. 1), but increased body temperature relative to controls during the night when challenged in the evening (fever; Fig. 4). Furthermore, the body temperature response during night lasted longer and was of a larger magnitude than the response during the day. The duration and magnitude of the LPS-induced hypothermia increased with increasing LPS dose during the day (Fig. 1), and was accompanied by a dose-dependent reduction in both seed consumption and body mass gain (Fig. 3). These results imply that the timeframe and strength of physiological and behavioural changes during an acute phase response may depend on both the circadian timing (i.e., day or night) and the magnitude (i.e., LPS-dose) of an endotoxin challenge. We do not think this conclusion would have been different had we continued to monitor birds that were challenged during the day through the night and vice versa. We base this statement on data showing that body temperature in the experimental groups converged at the end of the sampling period during both daytime and night-time sessions, which suggests that there were no further differences between groups from this point onwards (Figs. 1, 4).

A trade-off between foraging and immune function?

We found a dose-dependent reduction in seed consumption and body mass gain during the day following injection with LPS in the morning (Fig. 3). Birds challenged with the highest LPS-dose were still affected on the day after the challenge (i.e., 24-36 h after injection) when this group still consumed less seeds and gained less body mass (Fig. 3). Decreased food intake during infection has been suggested to be beneficial, because by decreasing energy intake the host may restrict access to micronutrients necessary for pathogen proliferation and hence limit the infection (Murray and Murray, 1977; Hart, 1988). In addition, both energy requirements and predation risk (if activity-related; cf. Martin et al., 2000) for the host should decrease if foraging activity is reduced (Hart, 1988). Thus, the decreased food intake (and concomitant reduction in body mass gain) in our study was probably not an undesired consequence of the LPS-challenge, but rather a behavioural adaptation to reduce the negative

effects induced by a real pathogen. The dose-dependence of this response indicates a potential trade-off between foraging and immune function, such that hosts attempt to minimize any negative effects pertaining from reduced food intake by regulating the expression of sickness behaviours to the strength of the infection.

Proximate explanations for the occurrence and lack of fever

Our results do not support the hypothesis that changes in body temperature during an acute phase response occur primarily to create a hostile environment for pathogens (Blatteis, 2003), because LPS-challenged birds did not develop fever during the day, and kept their body temperature within the range of circadian variation in control birds both during the day and at night (Fig. 5). Nor do we think that the primary reasons for diurnal hypothermia after LPS-administration was to create a hostile environment for pathogens, because the maximum decrease in body temperature in LPS-challenged birds never decreased below the minimum nocturnal body temperature in control birds. However, hypothermia may have positive effects on other aspects of pathophysiology independent of the thermal environment for the pathogen. For example, Liu et al. (2012) found that rats that developed a ca. 2 °C hypothermia after inoculation with severe doses of septic or aseptic endotoxins showed supressed leakage of endotoxins into the bloodstream and reduced levels of visceral organ dysfunction, both of which likely contributed to lower mortality in hypothermic subjects. It remains to be seen if the shallow drop in body temperature as observed in this study (≤ 0.5 °C) was large enough to carry any similar anti-pathological benefits.

It is unlikely that hypothermia developed because of energetic constraints on the use of fever (cf. Romanovsky and Székely, 1998), because birds in our study were not constrained by resource availability and should therefore have been able to sustain the potentially increased energy expenditure associated with a fever response as well as the increased heat loss that might accompany febrile body temperatures (cf. Owen-Ashley and Wingfield, 2006; 2007; Burness et al., 2010). It is also unlikely that LPS-induced hypothermia in our study was a consequence of septic shock (Romanovsky et al., 1996; 2005), because a comparatively moderate dose of LPS (100 μg kg^{-1}) induced hypothermia during the day but fever during the night, and shallow daytime hypothermia occurred also in response to injection with very low doses of LPS (Fig. 1). Taken together this suggests that regulated hypothermia might be a more frequent response to infection during the day, whereas fever is the more common

response at night in small birds. However, our data indicate that neither response occur primarily to create an environment that is unsuitable for pathogen replication.

It has been proposed that the immune defence system might work best at a given body temperature or within a range of body temperatures (Nord et al., 2013). The occurrence of daytime hypothermia in our study could then be explained if this optimal body temperature, or body temperature range, is lower than the normal daytime body temperature. Our data do not support the hypothesis of a single optimal temperature for immune function, in which case we would have expected all LPS-challenged birds, irrespective of dose, to maintain this body temperature during the acute phase response (cf. discussion in Nord et al., 2013) However, treatment-wise differences in body temperature were relatively small when integrated over the duration of the response. This might be compatible with the idea that optimum immune function can be realized within a range of body temperatures that are lower than the normal resting body temperature. Further support for this notion is provided by the larger experimental effect at night (Fig. 4) that resulted in a relatively similar body temperature in the immune-challenged birds at the time of the maximum response (3 hrs post injection) regardless of the circadian timing of the immune challenge (Fig. 5). Alternatively, the dose-dependence of the body temperature response might represent a trade-off between optimal immune function and optimal physiological performance, whereby the change in body temperature is determined by production costs of the response on the one hand and the required time-frame for pathogen clearance on the other (Maloney and Gray, 1998). Proper assessment of the dose response to LPS in zebra finches also during the night would provide further insights into the possible existence of such a trade-off.

It is possible that daytime hypothermia was not primarily the result of a thermoregulatory response to LPS. For instance, reduced seed consumption (Fig. 3A, B) might have caused a dose-dependent reduction in the metabolic heat production from digestion (‘heat increment of feeding’; Chappell et al., 1997). This could have been further exacerbated by reduced exercise thermogenesis (Paladino and King, 1984; Prinzinger et al., 1991) if activity was suppressed following LPS-injection (Burness et al., 2010; Sköld-Chiriac et al., 2014). In line with this, decreased locomotor activity has previously been put forward as a possible explanation for LPS-induced hypothermia in the California mouse (*Peromyscus californicus*) (Martin et al., 2008). However, body temperature does not necessarily track changes in such processes, because metabolic heat production can partly or completely substitute for shivering

thermogenesis at temperatures below thermal neutrality (Paladino and King, 1984; Chappell et al., 1997). If so, any reduction in heat supplied by digestion or activity in our study (that was performed some 8°C below thermal neutrality; Calder, 1964) should have been compensated for by increased shivering to maintain a stable body temperature. This remains speculative in the absence of metabolic data. Thus, it is currently unclear if dose-dependent daytime hypothermia was merely a consequence of a reduction in metabolic heat production from digestion and activity, or if it was the result of a direct effect of LPS on birds' thermoregulatory set point.

Based on literature data (Kluger, 1991; Marais et al., 2011c), the increase in body temperature at the maximum response following LPS-injection in the evening (Fig. 4) was estimated to increase resting metabolic rate by 12-28 %. This is somewhat higher than the 10 % increase reported by Burness et al. (2010) for zebra finches injected with a 10 times higher LPS-dose than used at night in our study. It is not known if this 10 % increase in resting metabolic rate was associated with fever, because Burness et al. (2010) did not record body temperature during measurements of metabolic rate. While we did not assess the dose response to LPS at night, the magnitude of fever is proportional to LPS dose in fowl (e.g. Jones et al., 1983; Maloney and Gray, 1998), and great tits (*Parus major*) challenged with LPS in winter maintained similar febrile body temperatures regardless of variation in ambient temperature (Nord et al., 2013). This supports the view that fever in birds might represent a trade-off between optimal immune function and the energy costs of the immune response, and that proper immune function can only be realized within a certain range of body temperatures (see discussion on optimal body temperatures for the immune system, above). Future work should seek to determine if this is true also for nocturnal fever in zebra finches.

Between-species variation in fever responses among birds

We found pronounced circadian variation in the direction of the change in body temperature during an acute phase response in zebra finches (Figs. 1, 3). A review of the avian literature suggests that such circadian variation in the body temperature response is more common in small passerine birds (mean body mass: 22 ± 3 g; range: 14-32 g), whereas larger, non-passerine, birds (mean body mass: 1188 ± 539 g; range: 55-2900 g) develop fever regardless of the circadian timing of the immune challenge (Table 2; although it should be noted that these comparisons suffer from relatively few consistent measurements from both circadian phases in the same species). To the best of our knowledge, diurnal fever in response to LPS

has only been observed once in the Passeriformes, in the 32 g song sparrow (*Melospiza melodia*) (Adelman et al., 2010b). It is possible that the slightly higher active-phase body temperature in the Passeriformes (41.6 °C) compared to other bird orders in Table 2 (41.2 °C) (Prinzinger et al., 1991) may preclude any further rise in body temperature associated with fever during the day (Mackowiak and Boulant, 1996; Gray et al., 2013). However, the consistency of the body temperature change for the larger species in Table 2 (Galliformes, Anseriformes, Columbiformes) suggests that body size may be a more important determinant of the response to LPS than phylogenetic relatedness. For example, exercise hyperthermia scales negatively with body size, such that the activity-induced rise in body temperature is larger in small birds independent of phylogeny (Prinzinger et al., 1991). Moreover, small birds have a comparatively high metabolic intensity (Hulbert et al., 2007) and a limited capacity for fasting (Hohtola, 2012), such that some foraging must occur during the day even during an acute phase response (e.g., Fig. 2). Adding fever to flight-induced peaks in body temperatures associated with foraging during the day may increase the risk of overheating, which comes at high somatic costs (Speakman and Król, 2010). By comparison, larger birds are tolerant to prolonged fasting periods (Sartori et al., 1995; Criscuolo et al., 2000) and might be able to minimize any work-related increase in diurnal body temperature by avoiding excessive activity during the acute phase response. In many (small) bird species, body temperature during the nocturnal roosting period is typically less variable and regulated to a lower set point than during the day (McKechnie and Lovegrove, 2002). Hence, at night even small birds may be able to use fever to clear an infection without a concomitant increase in the risk of overheating. Further studies on circadian variation in the body temperature response to endotoxin in large passerines and small non-passerines would shed light on the relative importance of phylogeny and body size, respectively, in explaining interspecific variation in fever expression.

Conclusions

We found distinct circadian variation in the body temperature response to LPS-injection manifested by the (dose-dependent) use of hypothermia during the day and fever during the night. Thus, the occurrence of diurnal hypothermia in response to an endotoxin challenge does not seem to be sign of sepsis as has previously been suggested for small mammals (above), but may instead be a normal part of the birds' response to infection. We suggest that this might promote survival by optimizing the body's response according to individual demands at the time of infection: the use of hypothermia might be beneficial to minimize the

risk of overheating, or to avoid excessive metabolic costs, during the day when body temperature is high and variable, whereas the use of fever might be more beneficial to counteract infection at night when body temperature is lower and less variable. To fully appreciate this (for us unexpected) circadian rhythmicity in the body temperature response to infection, we need to better understand the proximate mechanisms of fever (Gray et al., 2013) and the extent to which these might vary between distant and related species across a range of body sizes. Based on results from this study in conjunction with those from others (see above), we propose that changes in body temperature regulation in small birds during infection occur as either: *i)* an active response to either maintain body temperature within an optimal range or exceed a body temperature threshold that is required for optimal immune function, or *ii)* a passive change in body temperature resulting from a combination of an increased metabolic heat production during immune system activation (manifested primarily during nights) and a decreased metabolic heat production because of physiological and/or behavioural adjustments during the immune response (manifested primarily during days). Alternatively, it is possible that both these mechanisms together shape the body temperature response seen in our study of zebra finches, because a passive change in body temperature might explain the day-time response to LPS-injection in the morning, and a body temperature threshold for proper immune function might explain the night-time response to LPS-injection in the evening.

Materials and Methods

Four weeks before the experiment started, we implanted a temperature sensitive PIT tag (11.5 × 2.1 mm; 0.06 g; LifeChip BioThermo, Destron Fearing, South St Paul, MN) subcutaneously in the neck of 50 adult male zebra finches. This route of implantation is minimally invasive, and temperatures measured in the neck can be used to accurately predict variation in deep body temperature (Nord et al., 2013). Subsequent to implantation, we measured body mass (to the nearest 0.1 g) and randomly divided birds into four batches (N = 12 – 13 per batch), which contained 2 – 3 individuals from each LPS-treatment (see below). One day before the start of the experiment, we transferred all birds from their regular communal cages to individual experimental cages (32 × 48 × 32 cm), with *ad libitum* access to commercial seed mixture and water. Cages were placed so that birds were able to hear and see each other, but were visually separated from the investigators. Birds were kept under constant artificial light (14 L: 10 D; lights on between 7 AM and 9 PM) and ambient temperature conditions (22 ± 2 °C)

throughout the experiment. There was no difference in body mass ($F_{4, 44}$ = 0.95; P = 0.44) or subcutaneous body temperature ($F_{4, 45}$ = 0.14; P = 0.97) between treatments in any of the four groups of birds at the start of the experiment.

We assessed the relationship between subcutaneous temperature (as measured by the PIT tags) and deep body temperature (as measured by a factory calibrated thermocouple thermometer) on the morning of experimental manipulation ($T_{\text{b-deep}} = 0.94T_{\text{b-subcut}} + 3.23$, R^2 = 0.63, $\Delta T_b = 0.49 + 0.04$°C; see the Electronic Supplements for details). We then measured the birds' body mass and injected them in the pectoral muscle with 50 µl phosphate buffered saline (PBS) or a dose of either 1, 10, 100 or 1000 µg LPS kg^{-1} (based on the mean body mass at the time of PIT tag implantation) derived from *Escherichia coli* (Sigma, cat. no. L2880) diluted in 50 µl PBS. Sample size for each treatment group was N = 10. Implant calibration and injection were commenced between 8.30 and 8.45 AM and were completed within 20 min. Birds were then immediately transferred back to their experimental cages, and were provided with water *ad libitum* and 25.0 g of seeds (equivalent to about 10 times the amount the birds consumed during a day). Starting 20 min after the mean time of injection, we measured subcutaneous body temperature every 20 min for 12.5 h using a handheld racket antenna (Ø 17.5 cm; Destron Fearing) connected to an FS2001F ISO reader (Destron Fearing) through the cage floor. Both the antenna and the observer were outside the birds' field of view at all times. When lights were switched off in the evening (i.e. at 9.00 PM, 12.5 h after injection), we measured the birds' body mass and their seed consumption (by weighing the remaining seeds in the food cup together with any food spill in the cages), after which birds were left undisturbed during the night. Food was not provided again until birds had been weighed in the subsequent morning (below). At 7.30 AM in the following morning (i.e. 30 min after the lights were switched on, and 22.5 h after injection), we weighed all birds and transferred them to larger individual cages (60 × 33 × 57 cm), with *ad libitum* access to water and 25.0 g of seeds. As soon as lights were switched off in the evening of the second day (i.e. 36 h after injection), birds and remaining seeds (including food spill) were weighed, after which the birds were transferred back to their regular cages.

Two months after the daytime trials we measured the body temperature response to LPS-injection in the evening using a subsample (N = 24) of the birds from the first part of the experiment. Previous exposure to LPS does not affect the body temperature response during a second LPS-injection when injections are more than two weeks apart (at least in Pekin ducks;

Marais et al., 2011b). This was true also in our study (previous LPS-dose: $P = 0.61$). One day prior to the evening injection, birds were placed individually in the experimental cages described above, with *ad libitum* access to commercial seed mixture and water. On the evening of experimental manipulation, birds were randomly assigned into experimental treatments, weighed and subsequently injected in the pectoral muscle with either 50 µl PBS (control treatment; $N = 12$) or 100 µg LPS kg^{-1} diluted in 50 µl PBS ($N = 12$). We chose to use a single LPS-dose only because this dose gave the highest response in the daytime tests. Injections started at 8.45 PM and were completed within 20 min. Starting 20 min after the mean time of injection, we then measured subcutaneous body temperature every 20 min for 10 h (i.e., until the lights were switched on in the morning). All body temperature measurements were performed in the darkness without handling the birds (as detailed above). We weighed birds again after the last temperature measurement in the morning and then transferred them back to their regular cages. It is important to note that birds did not eat during the night (when cage rooms were completely dark), so body mass loss should reflect the energy consumption during the night.

Data analysis

All statistical tests were performed using SAS for Windows. We analysed the daytime body temperature response to different LPS-doses using a linear mixed model (PROC MIXED) with a first order autoregressive covariance structure (AR1), with body temperature as the dependent variable, LPS-dose (log-transformed in all analyses) and day of injection (i.e. 'batch') as factors, time and $(time)^2$ (to account for potential non-linearity in the body temperature response) as covariates, and a random intercept for bird identity as random factor. The original model also contained the two-way interactions LPS-dose × time and LPS-dose × $(time)^2$. We then performed separate regressions of body temperature as a function of LPS-dose and $(LPS\text{-}dose)^2$: *i)* in the beginning of the day ("initial" = the mean of the first three body temperature measurements of each individual immediately after the injection; time: 0.67 ± 0.33 hrs post injection), *ii)* at the maximum response ("maximum" = the mean of the three body temperature measurements of each individual at the maximum response; time: 3.00 ± 0.33 hrs post injection) and *iii)* at the end of the day ("late" = the mean of the last three body temperature measurements of each individual during the day; time: 12.00 ± 0.33 hrs post injection). Daytime food consumption following morning injection was tested separately for each day, using seed consumption as the dependent variable, batch as a factor and initial body

mass, LPS-dose and (LPS-dose)^2 as explanatory variables. Body mass gain during the two days after the injection and body mass loss during the night following morning injection were tested in a similar way, *viz.* each day and night was tested separately using body mass gain during the day – or body mass loss during the night (when the birds were not feeding) – as the dependent variable, batch as factor, and body mass at the beginning of the trial, LPS- dose and (LPS-dose)^2 as explanatory variables. We analysed the body temperature response to the LPS-challenge at night in a linear mixed model (PROC MIXED with AR1 covariance structure) with body temperature as the dependent variable, treatment and the previous LPS-dose (from the daytime trial) as factors, time and (time)^2 as covariates, and bird identity as a random intercept. The original model also contained the two-way interactions treatment × time and treatment × (time)^2. Differences in body mass loss during the night following evening injection was compared in a linear model with treatment as a factor and body mass at the beginning of the night as a covariate. Finally, to assess if the body temperature attained during the peak response (defined above) in LPS-challenged birds was different during days and nights, we compared within-treatment differences in body temperature using independent t-tests (control birds and birds injected with 100 µg LPS kg^{-1} only). In multivariate tests, final models were derived using stepwise backward elimination of non-significant variables (P > 0.05) until only significant variables remained. All values are presented as means with standard errors (mean ± SE) and all significances are two-tailed.

Ethical note

The experimental design follows Swedish legislation and was approved by the Malmö/Lund Animal Ethics Committee before the start of the experiment.

Author competing interests

The authors declare no competing or financial interests.

Author contributions

S.S.C. and A. N. conceived and designed the study with input from D.H. and J.-Å.N. The practical work was performed by S.S.C., A.N. and M.T., and S.S.C. and A.N. analyzed the data. All authors participated in writing and preparation of the final manuscript.

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Reference list

Adamo, S.A. (1998). The specificity of behavioral fever in the cricket *Acheta domesticus*. *J. Parasitol.* **84**, 529-533.

Adelman, J.S., Bentley, G.E., Wingfield, J.C., Martin, L.B. and Hau, M. (2010a) Population differences in fever and sickness behaviors in a wild passerine: a role for cytokines. *J. Exp. Biol.* **213**, 4099-4109.

Adelman, J.S., Córdoba-Córdoba, S., Spoelstra, K., Wikelski, M. and Hau, M. (2010b). Radiotelemetry reveals variation in fever and sickness behaviours with latitude in a free-living passerine. *Funct. Ecol.* **24**, 813-823.

Ashley, N.T. and Wingfield, J.C. (2012). Sickness behavior in vertebrates. *Ecoimmunology* (ed. G.E. Demas and R.J. Nelson), pp 45-91. Oxford University Press, New York.

Bingham, J., Green, D.J., Lowther, S., Klippel, J., Burggraaf, S., Anderson, D.E., Wibawa, H., Hoa, D.M., Long, N.T., Vu, P.P., et al. (2009). Infection studies with two highly pathogenic avian influenza strains (Vietnamese and Indonesian) in Pekin ducks (*Anas platyrhynchos*), with particular reference to clinical disease, tissue tropism and viral shedding. *Avian Pathol.* **38**, 267-278.

Blatteis, C.M. (2003). Fever: pathological or physiological, injurious or beneficial? *J. Therm. Biol.*, **28,** 1-13.

Boorstein, S.M. and Ewald, P.W. (1987). Costs and benefits of behavioral fever *in Melanoplus sanguinipes* infected by *Nosema acridophagus*. *Physiol. Zool.* **60**, 586-595.

Burness, G., Armstrong, C., Fee, T. and Tilman-Schindel, E. (2010). Is there an energetic-based trade-off between thermoregulation and the acute phase response in zebra finches? *J. Exp. Biol.* **213**, 1386-1394.

Calder, W.A. (1964). Gaseous metabolism and water relations of the zebra finch, *Taeniopygia castanotis*. *Physiol. Zool.* **37,** 400-413.

Chappell, M.A., Bachman, G.C. and Hammond, K.A. (1997). The heat increment of feeding in house wren chicks: magnitude, duration, and substitution for thermostatic costs. *J. Comp. Physiol. B.* **167**, 313-318.

Coon, C.A.C., Warne, R.W. and Martin, L.B. (2011). Acute-phase responses vary with pathogen identity in house sparrows (*Passer domesticus*). *Am. J. Physiol.* **300**, R1418-R1425.

Criscuolo, F., Gauthier-Clerc, M., Gabrielsen, G.W. and Le Maho, Y. (2000). Recess behaviour of the incubating Common Eider *Somateria mollissima*. *Polar Biol.*, **23**, 571-574.

Deak, T., Bellamy, C. and Bordner, K.A. (2005). Protracted increases in core body temperature and interleukin-1 following acute administration of lipopolysaccharide: Implications for the stress response. *Physiol. Behav.* **85**, 296-307.

Escobar, J., Van Alstine, W.G., Baker, D.H. and Johnson, R.W. (2007). Behaviour of pigs with viral and bacterial pneumonia. *Appl. Anim. Behav. Sci.* **105**, 42-50.

Frafield, V. and Kaplanski, J. (1998). Brain eicosanoids and LPS fever: species and age differences. *Prog. Brain Res.* **115**, 141–146.

Gray, D.A., Marais, M. and Maloney, S.K. (2013). A review of the physiology of fever in birds. *J. Comp. Physiol. B.* **183**, 297-312.

Harden, L.M., du Plessis, I., Poole, S. and Laburn, H.P. (2006). Interleukin-6 and leptin mediate lipopolysaccharide-induced fever and sickness behavior. *Physiol. Behav.* **89**, 146-155.

Hart, B.L. (1988). Biological basis of the behavior of sick animals *Neurosci. Biobehav. Rev.* **12**, 123-138.

Hasselquist, D. and Nilsson, J.-Å. (2012). Physiological mechanisms mediating costs of immune responses: what can we learn from studies of birds? *Anim. Behav.* **83**, 1303-1312.

Hawley, D.M., DuRant, S.E., Wilson, A.F., Adelman, J.S. and Hopkins, W.A. (2012). Additive metabolic costs of thermoregulation and pathogen infection. *Funct. Ecol.* **26**, 701-710.

Hohtola, E. (2012). Thermoregulatory adaptations to starvation in birds. In: McCue, M.D. *Comparative physiology of fasting, starvation, and food limitation*, pp. 155-170. Springer, New York.

Hulbert, A.J., Pamplona, R., Buffenstein, R. and Buttemer, W.A. (2007). Life and death: metabolic rate, membrane composition, and life span of animals. *Physiol. Rev.* **87**, 1175-1213.

Jones C.A., Edens F.W. and Denbow D.M. (1983). Influence of age on the temperature response of chickens to *Escherichia coli* and *Salmonella typhimurium* endotoxins. *Poultry Sci.* **62**, 1553–1558.

King, M.O. and Swanson, D.L. (2013). Activation of the immune system incurs energetic costs but has no effect on the thermogenic performance of house sparrows during acute cold challenge. *J. Exp. Biol.* **216**, 2097-2102.

Kluger, M.J. (1986). Is fever benefical? *Yale J. Biol. Med.* **59**, 89-95.

Kluger, M.J. (1991). Fever: role of pyrogens and cryogens. *Physiol. Rev.* **71**, 93-127.

Kluger, M.J., Kozak, W., Conn, C.A., Leon, L.R. and Soszynski, D. (1998). Role of fever in disease. *Ann. N. Y. Acad. Sci.* **856**, 224-233.

Koutsos, E.A. and Klasing, K.C. (2001). The acute phase response in Japanese quail (*Coturnix coturnix japonica*). *Comp. Biochem. Physiol. C.* **128**, 255-263.

Kurokawa, M., Imakita, M., Kumeda, C.A. and Shiraki, K. (1996). Cascade of fever production in mice infected with influenza virus. *J. Med. Vir.* **50**, 152-158.

Leshchinsky, T.V. and Klasing, K.C. (2001). Divergence of the inflammatory response in two types of chickens. *Develop. Comp. Immunol.* **25**, 629-638.

Liu, E., Lewis, K., Al-Saffar, H., Krall, C.M., Singh, A., Kulchitsky, V.A., Corrigan, J.J., Simons, C.T., Petersen, S.R., Musteata, et al. (2012). Naturally occurring hypothermia is more advantageous than fever in severe forms of lipopolysaccharide- and *Escherichia coli*-induced systemic inflammation. *Am. J. Physiol.* **302**, R1372-R1383.

Mackowiak, P.A. and Boulant, J.A. (1996). Fever's glass ceiling. *Clin. Infect. Dis.* **22**, 525-536.

Maloney, S.K. and Gray, D.A. (1998). Characteristics of the febrile response in Pekin ducks. *J. Comp. Physiol. B* **168**, 177-182.

Marais, M., Maloney, S.K. and Gray, D.A. (2011a). Ambient temperature modulates the magnitude of LPS-induced fevers in Pekin ducks. *J. Therm. Biol.* **36**, 121-127.

Marais, M., Maloney, S.K. and Gray, D.A. (2011b). The development of endotoxin tolerance, and the role of hypothalamo-pituitary-adrenal function and glucocorticoids in Pekin ducks. *J. Exp. Biol.* **214**, 3378-3385.

Marais, M., Maloney, S.K. and Gray, D.A. (2011c). The metabolic cost of fever in Pekin ducks. *J. Therm. Biol.* **36**, 116-120.

Martin, L.B., Weil, Z.M. and Nelson, R.J. (2008). Fever and sickness behaviour vary among congeneric rodents. *Funct. Ecol.* **22**, 68-77.

Martin, T.E., Scott, J. and Menge, C. (2000). Nest predation increases with parental activity: separating nest site and parental activity effects. *Proc. R. Soc. Lond. B.* **267**, 2287-2293.

McKechnie, A.E. and Lovegrove, B.G. (2002). Avian facultative hypothermic responses: a review. *Condor* **104**, 705-724.

Murray, J. and Murray, A. (1977). Suppression of infection by famine and its activation by refeeding--a paradox? *Persp. Biol. Med.* **20**, 471-483.

Nomoto, S. (1996). Diurnal variations in fever induced by intravenous LPS injection in pigeons. *Pfluegers Arch.* **431**, 987-989.

Nord, A., Chiriac, S., Hasselquist, D. and Nilsson, J.-Å. (2013). Endotoxin injection attenuates rest-phase hypothermia in wintering great tits through the onset of fever. *Funct. Ecol.* **27**, 236-244.

Owen-Ashley, N.T., Turner, M., Hahn, T.P. and Wingfield, J.C. (2006). Hormonal, behavioral, and thermoregulatory responses to bacterial lipopolysaccharide in captive and free-living white-crowned sparrows (*Zonotrichia leucophrys gambelii*). *Horm. Behav.* **49**, 15-29.

Owen-Ashley, N.T and Wingfield, J.C. (2007). Acute phase responses of passerine birds: characterization and seasonal variation. *J. Ornithol.* **148**, S583-S591.

Paladino, F.V. and King, J.R. (1984). Thermoregulation and oxygen consumption during terrestrial locomotion by white-crowned sparrows *Zonotrichia leucophrys gambelii*. *Physiol. Zool.* **57,** 226-236.

Prinzinger, R., Pressmar, A. and Schleucher, E. (1991). Body-temperature in birds. *Comp. Biochem. Physiol. A* **99**, 499-506.

Romanovsky, A.A., Almeida, M.C., Aronoff, D.M., Ivanov, A.I., Konsman, J.P., Steiner, A.A. and Turek, V.F. (2005). Fever and hypothermia in systemic inflammation: recent discoveries and revisions. *Front. Biosci.* **10**, 2193-2216.

Romanovsky, A.A., Shido, O., Sakurada, S., Sugimoto, N. and Nagasaka, T. (1996). Endotoxin shock: thermoregulatory mechanisms. *Am. J. Physiol.* **270**, R693-R703.

Romanovsky, A.A. and Székely, M. (1998). Fever and hypothermia: two adaptive thermoregulatory responses to systemic inflammation. *Med. Hyp.* **50**, 219-226.

Rudaya, A.Y., Steiner, A.A., Robbins, J.R., Dragic, A.S. and Romanovsky, A.A. (2005). Thermoregulatory responses to lipopolysaccharide in the mouse: dependence on the dose and ambient temperature. *Am. J. Physiol.* **289**, R1244-R1252.

Råberg, L., Nilsson, J.-Å., Ilmonen, P., Stjernman, M. and Hasselquist, D. (2000). The cost of an immune response: vaccination reduces parental effort. *Ecol. Lett.* **3**, 382-386.

Sartori, D.R.S., Migliorini, R.H., Veiga, J.A.S., Moura, J.L., Kettelhut, I.C. and Linder, C. (1995). Metabolic adaptations induced by long-term fasting in quails. *Comp. Biochem. Physiol. A* **111**, 487-493.

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

Sköld-Chiriac, S., Nord, A., Nilsson, J.-Å. and Hasselquist, D. (2014). Physiological and behavioral responses to an acute-phase response in zebra finches: immediate and short-term effects. *Physiol Biochem. Zool.* **87**, 288-298.

Speakman, J.R. and Król, E. (2010). Maximal heat dissipation capacity and hyperthermia risk: neglected key factors in the ecology of endotherms. *J. Anim. Ecol.* **79**, 726-746.

Figures

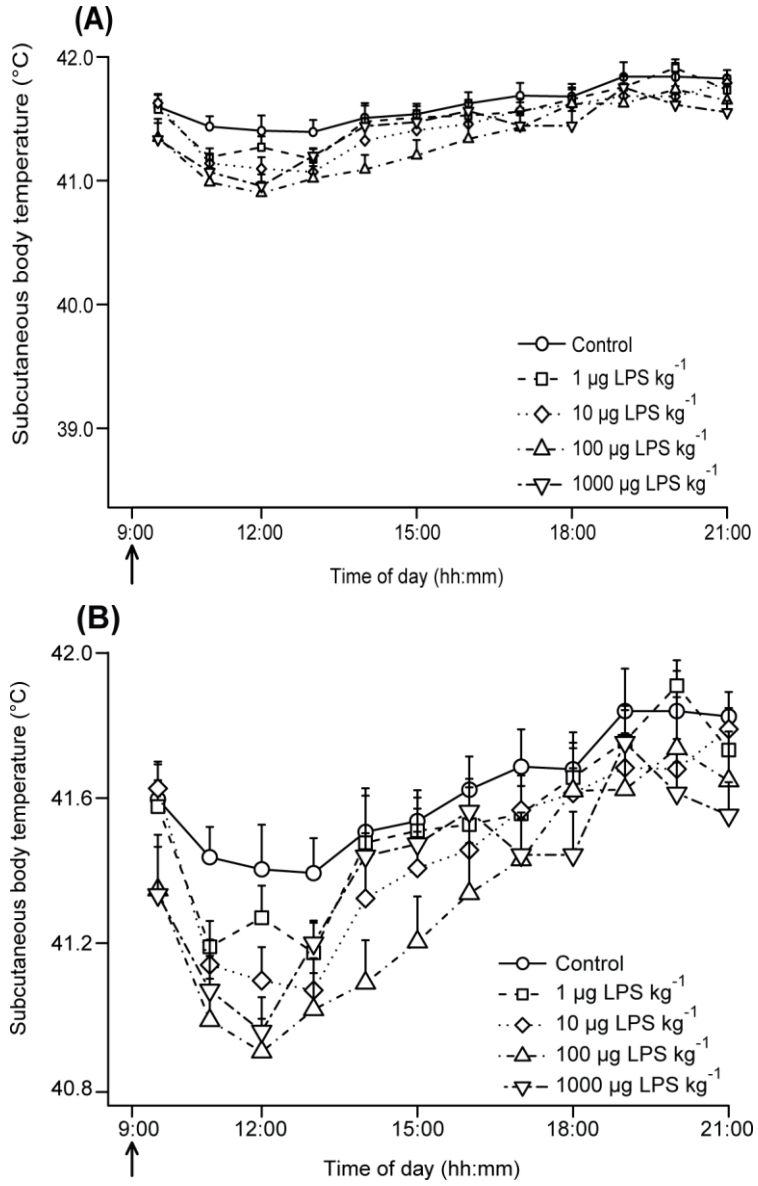

Fig. 1. (A) Mean (+SE) body temperature in male zebra finches as a function of time of day subsequent to injection with saline (Control) or an immune challenge with different doses of the bacterial endotoxin lipopolysaccharide (LPS; 1, 10, 100 or 1000 µg kg^{-1}, respectively). Each data point represents the mean of three consecutive measurements, which were obtained 20 min apart. Panel (B) shows the same data plotted on a finer scale to more clearly illustrate the dose response to LPS. The arrow indicates the time of injection. N = 10 for all treatment groups.

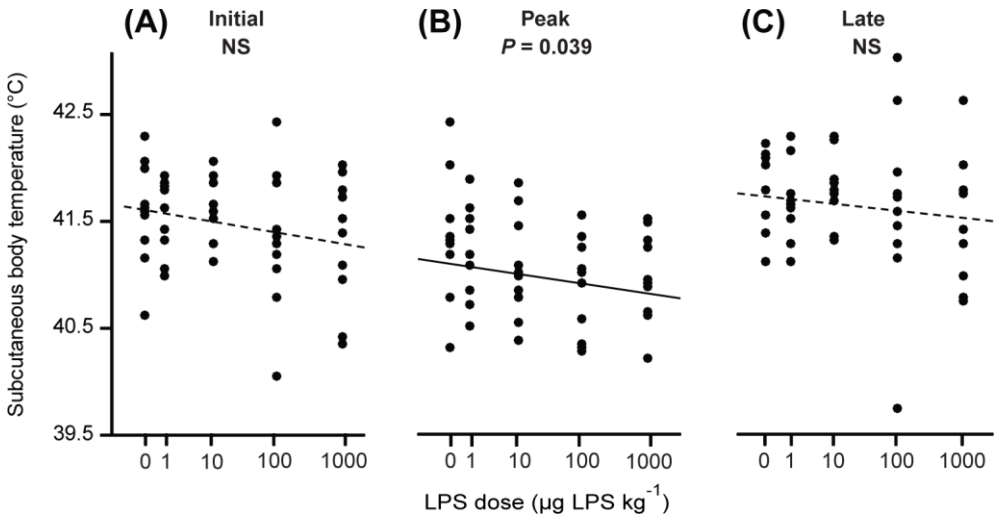

Fig. 2. Body temperature in male zebra finches after injection with either saline (Control) or different doses of LPS (1, 10, 100 or 1000 µg kg^{-1}, respectively), relative to the time elapsed since injection and the strength of the LPS-challenge. Fig. (A) shows body temperature at the time of the initial body temperature response in LPS-challenged birds (time of measurements: 0.7 ± 0.33 h post injection; $P = 0.11$). Fig. (B) shows body temperature during the maximum response to LPS (time of measurements: 3.0 ± 0.33 h post injection; $Y = 41.27 – 0.13x$; $P = 0.039$). Fig. (C) shows body temperature during the late part of the response at the end of the day (time of measurements: 12.0 ± 0.33 h post injection; $P = 0.26$). The controls are illustrated as '0'. Note that the x-axis labels are plotted on a logarithmic scale. $N = 10$ for all treatment groups.

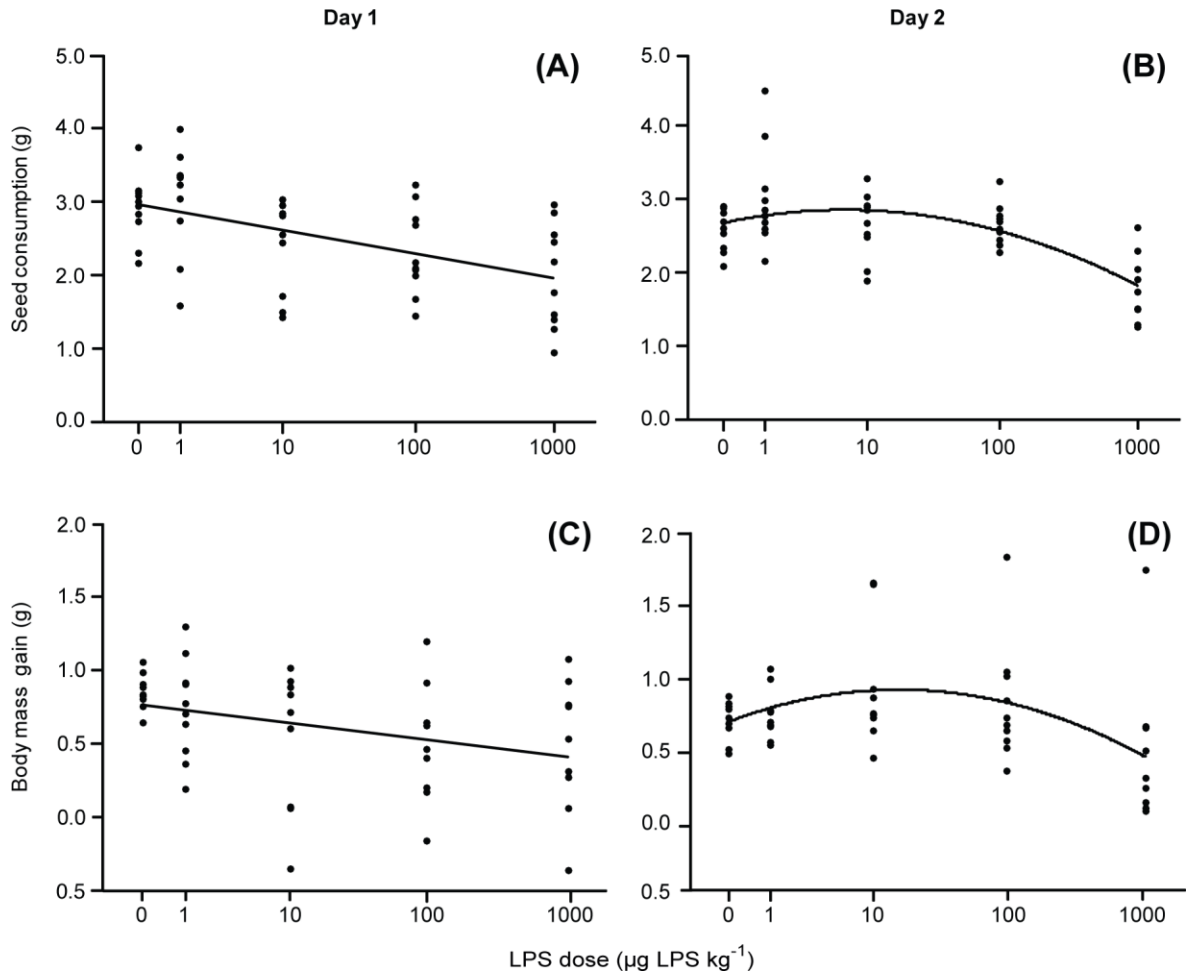

Fig. 3. Seed consumption and body mass gain in male zebra finches during two days after injection with either saline (Control) or different doses of the bacterial endotoxin LPS (1, 10, 100, or 1000 µg kg^{-1}), in relation to the strength of the LPS-challenge. Fig. (A) illustrates seed consumption during the day of the experimental injections, when birds demonstrated a linear dose-dependent decrease in seed consumption ($Y = 2.96 + 0.33x$; $P < 0.0001$). Fig. (B) shows seed consumption during the day after the LPS-challenge, when seed consumption was curvilinearly related to the strength of the LPS-challenge ($Y = 2.69 + 0.41x + 0.23x^2$; $P = 0.0035$). Fig. (C) illustrates body mass gain during the day of the experimental injections, when birds showed a linear dose-dependent decrease in body mass gain ($Y = 0.75 + 0.12x$; $P = 0.045$). Fig. (D) shows body mass gain during the day after injection, when body mass gain showed a curvilinear dose-dependence ($Y = 0.68 + 0.37x + 0.15x^2$; $P = 0.013$). The controls are illustrated as '0'. Note that the x-axis labels are plotted on a logarithmic scale. ' $N = 10$ for all treatment groups

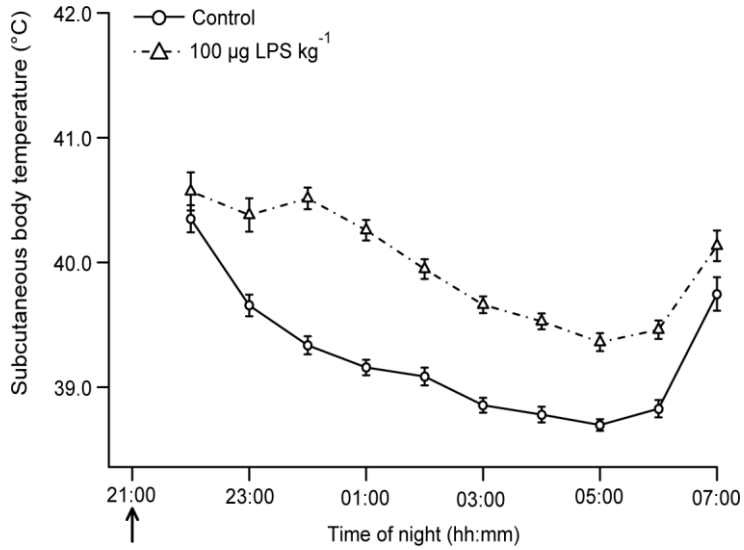

Fig. 4. Mean (± SE) body temperature as a function of time of night in male zebra finches that were injected with either saline (Control; N = 12) or 100 µg kg^{-1} of the bacterial endotoxin LPS (N = 12) in the beginning of the evening. Each data point represents the mean of three consecutive measurements, which were obtained 20 min apart. The arrow indicates the time of injection.

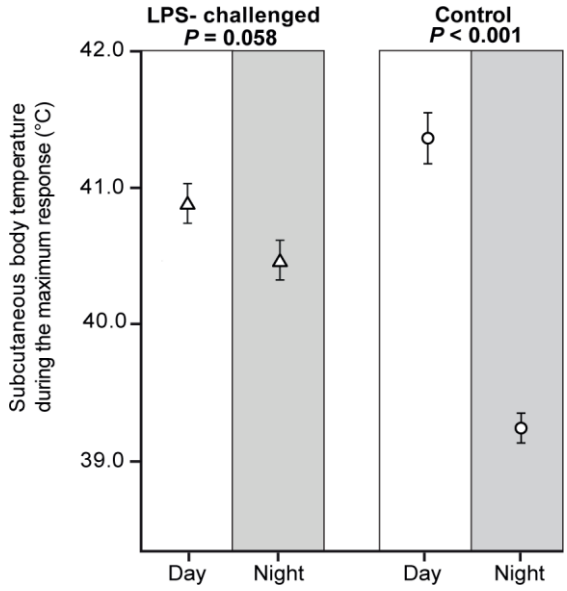

Fig. 5. Mean (± SE) body temperature in male zebra finches that were injected with either saline (Control; N = 12) or the bacterial endotoxin LPS (100 µg kg^{-1}; N = 12), at the time of the maximum body temperature response to the LPS-challenge (3.0 ± 0.33 h post injection). The figure illustrates the maximum response as measured both during the day (following morning injection) and during the night (following evening injection).

Tables

Table 1. Mean (± SE) daytime body temperature, seed consumption and body mass gain (± SE) in male zebra finches that were injected with saline (control) or challenged with different doses of the bacterial endotoxin LPS (1, 10, 100, or 1000 µg kg^{-1} respectively).

	Control	**LPS-challenged**			
		1 µg kg^{-1}	10 µg kg^{-1}	100 µg kg^{-1}	1000 µg kg^{-1}
Mean body temperature at maximum response during the day (3 ± 0.33 hrs; °C)	41.4 ± 0.2	41.2 ± 0.1	41.1 ± 0.2	40.9 ± 0.2	41.0 ± 0.1
Mean body temperature at maximum response during the night (3 ± 0.33 hrs; °C)	39.3 ± 0.1	-	-	40.5 ± 0.1	-
Day 1: Seed consumption (g)	2.9 ± 0.1	3.0 ± 0.2	2.4 ± 0.2	2.3 ± 0.2	2.0 ± 0.2
Day 1: Body mass gain (g)	0.9 ± 0.0	0.7 ± 0.1	0.5 ± 0.2	0.5 ± 0.1	0.5 ± 0.2
Day 2: Seed consumption (g)	2.6 ± 0.1	3.0 ± 0.2	2.7 ± 0.1	2.7 ± 0.1	1.8 ± 0.2
Day 2: Body mass gain (g)	0.7 ± 0.0	0.7 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.4 ± 0.2
Overnight mass loss after morning injection (night 1; g)	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.6 ± 0.1
Overnight mass loss after evening injection (night 1; g)	0.8 ± 0.0	-	-	0.8 ± 0.1	-

Table 2. Overview of studies that first challenged the immune system of birds with the non-pathogenic bacterial endotoxin LPS and subsequently measured the change in the subjects' body temperature during the acute phase response. *Circadian phase:* denotes whether birds were challenged and measured during the photophase (Day) or during the scotophase (Night). *Dose:* refers to the amount of LPS injected (μg kg^{-1}). *Direction of change:* denotes whether body temperature increased (+) or decreased (-) after the LPS-challenge.

Species	**Body mass (g)**	**Circadian phase**	**Dose (μg kg^{-1})**	**Direction of change**	**Reference**
Pekin duck (*Anas platyrhynchos domestica*)	2900	Day	1 to 100	+	Maloney and Gray, 1998
Pekin duck	2800	Day	100	+	Marais et al., 2011a, c
Domestic chicken (*Gallus g. domesticus*)	760	Day	100 to 5000	+	Leshchinsky and Klasing 2001
Pigeon (*Columba livia*)	578	Day	10	+	Nomoto, 1996
Pigeon	578	Night	10	+	Nomoto, 1996
Japanese quail (*Coturnix japonica*)	55	Day	500 to 2500	+	Koutsos and Klasing, 2001
Song sparrow (*Melospiza melodia morphna*)	32	Night	2100	+	Adelman et al., 2010a; b
Song sparrow	32	Day	2100	+	Adelman et al., 2010b
House sparrow (*Passer domesticus*)	28	Day	1000, 5000	-	King and Swanson, 2013
House sparrow	28	Night	1000	+	Coon et al., 2011
House sparrow	28	Day	1000	-	Coon et al., 2011
Gambel's white-crowned sparrow (*Zonotrichia leucophrys gambelli*)	26	Day	1000	-	Owen-Ashley and Wingfield 2006
Great tit (*Parus major*)	19	Night	100	+	Nord et al., 2013
Zebra finch (*Taeniopygia guttata*)	14	Day	1000	-	Burness et al., 2010
Zebra finch	14	Day	100	-	This study
Zebra finch	14	Night	100	+	This study

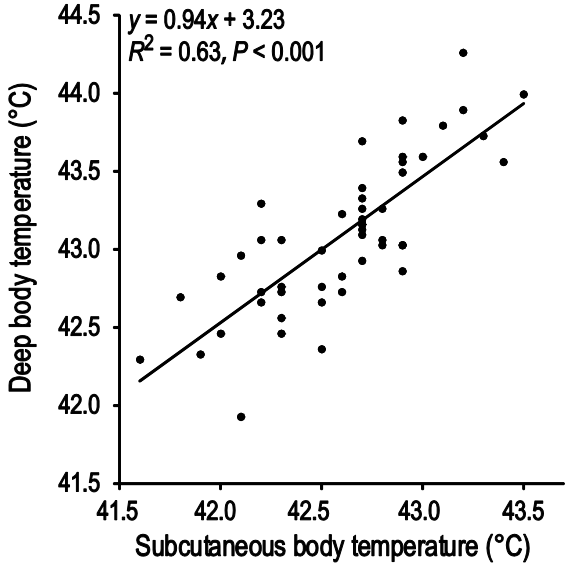

Fig. 1. The relationship between deep body temperature and subcutaneous body temperature in male zebra finches, measured simultaneously prior to experimental manipulation. We measured deep and subcutaneous body temperature simultaneously on each bird from a group in the morning before experimental manipulation in order to validate that the implants accurately reflected variation in deep body temperature. Deep body temperature was measured with a Testo 925 digital thermometer (Testo AG, Lenzkirch, Germany) with a standard Kapton® insulated type K (chromel--alumel) thermocouple (Ø 0.9 mm; ELFA AB, Järfälla, Sweden) inserted 12 mm through the cloaca (further insertion did not alter the temperature reading). Measurements of subcutaneous body temperature were obtained from the implanted PIT tags using a handheld racket antenna (Ø 17.5 cm; Destron Fearing) connected to an FS2001F ISO reader (Destron Fearing). The calibration revealed a strong positive linear relationship between deep body temperature and subcutaneous body temperature.

Table S1. Test statistics, degrees of freedom and resultant P-values for final models. Models in which variation in the dependent variable could not be predicted from any of the explanatory variables are not presented. The table also includes dose-specific model outputs of the body temperature response to a lipopolysaccharide (LPS) challenge during the day, and treatment-specific model outputs of the body temperature response to a LPS-challenge during the night.

Model / Parameter	*F*	d.f.	*P*
Responses to a LPS-challenge during the day:			
Body temperature response:			
LPS-dose	1.64	4, 45	0.18
Time	0.19	1, 1789	0.66
Time^2	17.13	1, 1789	< 0.001
LPS-dose × Time	3.15	4, 1789	0.014
LPS-dose × Time^2	3.70	4, 1789	0.0053
Control:			
Time	0.072	1, 357	0.79
Time^2	3.90	1, 357	0.049
1 µg LPS kg^{-1}:			
Time	54.86	1, 359	< 0.001
10 µg LPS kg^{-1}:			
Time	3.44	1, 358	0.065
Time^2	17.13	1, 358	< 0.001
100 µg LPS kg^{-1}:			
Time	0.065	1, 358	0.80
Time^2	8.34	1, 358	0.0041
1000 µg LPS kg^{-1}:			
Time	55.00	1, 359	< 0.001
Temperature regression – maximum:			
LPS-dose	4.53	1, 48	0.039
Seed consumption day 1:			
LPS-dose	19.81	1, 45	< 0.001
Group	3.41	3, 45	0.025
Seed consumption day 2:			
LPS-dose	3.21	1, 46	0.080
LPS-dose ^{2}	9.50	1, 46	0.0035
Body mass gain day 1:			
LPS-dose	4.26	1, 45	0.045
Initial body mass	7.08	1, 45	0.011
Body mass loss night:			
Group	11.50	3, 43	< 0.001
Body mass gain day 2:			
LPS-dose	4.33	1, 45	0.043
LPS-dose ^{2}	6.75	1, 45	0.013
Responses to a LPS-challenge during the night:			
Body temperature response:			
Treatment	4.49	1, 22	0.046
Time	477.10	1, 692	< 0.001
Time^2	327.76	1, 692	< 0.001
Treatment × Time	37.80	1, 692	< 0.001
Treatment × Time^2	44.86	1, 692	< 0.001
Control:			
Time	534.71	1, 346	< 0.001
Time^2	419.80	1, 346	< 0.001
100 µg LPS kg^{-1}:			
Time	97.54	1, 347.9	< 0.001
Time^2	51.56	1, 348.6	< 0.001