Life-history traits but not season affect the febrile response to a lipopolysaccharide challenge in highveld mole-rats

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Abstract

Fever is part of an acute phase response that organisms launch to defend themselves against an invasion by microbial pathogens such as bacteria and viruses. The elevation of an individual's body temperature necessary to achieve a fever is considered energetically costly and variation in the expression of the febrile response has been reported with respect to season, sex and the reproductive status of an animal. The effect of these parameters on fever responses are well characterized for laboratory rodents but comparable data from wild rodents are currently lacking. We evaluated the febrile response of wild highveld mole-rats (*Cryptomys hottentotus pretoriae*) to lipopolysaccharide (LPS) during winter and summer. This social rodent retains its breeding potential throughout the year and exhibits a reproductive division of labour. Highveld mole-rats increased their body temperature to a greater degree in response to a dose of 1 mg kg^{-1} LPS than to saline or handling alone. The fever response did not differ between seasons while the stress-induced hyperthermia in response to handling was greater in summer compared winter. In contrast, males and breeders exhibited larger changes in body temperature following LPS administration than females and non-breeders, respectively. These findings are in accordance with those reported for laboratory species and suggest that general principlesgovern the modulation of innate immune responses such as fever among small mammals.

Keywords: Cryptomys, lipopolysacharide, life-history trait, seasonality

Introduction

Fever is part of a suite of physiological and behavioural adaptations labelled acute phase response (APR) that organisms initiate to defend themselves when invaded by bacteria or viruses (Hart 1988, Kluger et al. 1996, Blatteis 2003). Such bacterial infections can be experimentally simulated by the injection of lipopolysaccharide (LPS), a component of the cell wall of gram-negative bacteria resulting in APR's (Danzter 2004). Increases in body temperature (T_b) are associated with substantial energetic costs (a increase of 10% or more in metabolic rate is associated with a 1°C increase in T_b, Kluger 1991, Burness et al. 2010) and these can be further potentiated by the behavioural components of the APR such as anorexia and lethargy that reduce an individual's energy intake (Hart 1988, Adelman & Martin 2009). Consequently, energetic constraints may cause adjustments in the expression of fever and could explain the observed variation in febrile responses within and between species (Martin, Weil & Nelson 2008a).

Both, food availability and thermoregulatory needs, vary seasonally and this in turn may constrain investment in immune defense by an individual (Nelson et al. 2002, Martin, Weil & Nelson 2008b). Indeed, a number of studies have reported seasonal variation in various immune measures (e.g. Lochmiller, Vestey & McMurry 1994, Saino et al. 2000, Nelson et al. 2002). However, in response to the temporal variation in energy balance, animals of many species cease to reproduce during energetic bottlenecks such as winter (Bronson & Heideman 1994, Bronson 2009). The concomitant reduction in energy availability and cessation of reproduction make it often difficult to ascertain whether the seasonal modulation of immune responses is directly linked to changes in gonadal steroids or to other seasonal factors. Laboratory studies have shown that some aspects of the APR such as the change in body mass due to anorexia may not be solely explained by testosterone levels (Prendergast et al. 2008). At the same time some pyrogenic cytokines may not be affected by seasonal factors (e.g. photoperiod) while others are (Prendergast et al. 2007). However,

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irrespective of the source seasonal variations of febrile responses have been reported in a number of studies (e.g Bilbo et al. 2002, Prendergast et al. 2003, Owen-Ashley & Wingfield 2006).

Life-history traits such as an individual's sex and reproductive condition can further modulate the expression of the APR. Sex-differences in febrile responses to endotoxins have been reported for several vertebrate species with males generally expressing stronger increases in T_b than females in a number of rodents (Murakami & Ono 1987, Klein & Nelson 1998, Pittman et al. 1998, Taylor, Tio & Romeo 2005, Yee & Prendergast et al. 2010). The larger febrile responses of males come at a cost and Prendergast et al. (2003) showed that septic shock in response to LPS occurs at lower doses and mortality rates are significantly elevated in reproductive male Siberian hamsters (*Phodopus sungorus*) compared to females.

Another life-history trait that can modulate febrile responses is the reproductive status of an individual. For example, male Siberian hamsters in breeding condition had larger fat reserves and sustained elevated T_b's for longer periods than males in non-breeding condition (Bilbo et al. 2002). Similarly, female Siberian hamsters showed elevated rectal T_b's after LPS administration during long but not short-day priming (Pyter et al. 2005). Like the fever response reproduction requires large energetic investments and concomitant demands from reproductive activity and immune defense can severely impact on an animal's fitness (e.g. Sheldon & Verhulst 1996, Lochmiller & Deerenberg 2000). Accordingly, survival to septic shock induced by the administration of high LPS doses is significantly increased in both male and female Siberian hamsters in breeding condition compared to non-breeding condition (Prendergast et al. 2003).

Trade-offs between APR's such as fever and life-history traits are well established for a number of laboratory rodents while they are comparatively understudied in undomesticated species, particularly in mammals (Muchlinski, Baldwin & Gramajo 2000, Adelman & Martin 2009). The need for studies investigating fever and sickness behaviors in free-living or

undomesticated species has been pointed out recently (Martin et al. 2008b, Adelman & Martin 2009). In the current study we aimed to evaluate the modulatory potential of season and life-history traits on the LPS-induced fever response of wild-caught highveld mole-rats (*Cryptomys hottentotus pretoriae*). This social subterranean rodent exhibits reproductive division of labor with only one female and up to two males per group engaging in procreation (Moolman, Bennett & Schoeman 1998). Although the reproductive potential appears to be maintained year-round pregnancies are restricted to the winter months in this species (van der Walt, Bennett & Schoeman 2001, van Rensburg et al. 2002). There is some indication of a down-regulation of the HPG-axis in non-breeding females with non-breeding females having lower progesterone and estrogen-levels than breeding females and non-breeding females remain unovulatory in the confines of their natal colonies (van Rensburg et al. 2002). In contrast, non-breeding male highveld mole-rats show no signs of reproductive inhibition and testosterone-levels are comparable between breeders and non-breeders (van Rensburg et al. 2002, 2003). Their seasonal breeding pattern and the presence of both breeders and non-breeders of both sexes throughout the year make highveld mole-rats an ideal model to independently study the effects of reproduction and seasonal energetic bottlenecks on fever responses. We firstly aimed to establish that LPS induces fever in highveld mole-rats. Secondly, we hypothesized that males would exhibit greater fever responses than females. Furthermore, if the breeding condition is the main factor affecting febrile responses we expected them to differ between breeders and non-breeders irrespective of the season while the opposite would be true if the seasonally fluctuating energy availability would determine these responses. Alternatively, both of these factors could play a role resulting in significant interaction effects of both of these factors.

Materials and Methods

Capture and housing

Animals were captured from the Tygerpoort area in Tshwane (S25°46'35.45"

E28°21'37.34"), South Africa, between October 2008 to August 2009 using Hickman live-

traps baited with sweet potato. Captured animals were housed in plastic crates (49.5 x 28 cm)

and provided with wood shavings and paper towelling for nesting material. Animals were fed

an *ad libitum* diet of fresh potato and apple on a daily basis. Room temperature was

maintained under constant conditions at $25 \pm 1°C$ and the light cycle of 12:12h LD. Animals

were maintained in the laboratory within their respective colonies for a maximum of two

weeks prior to the onset of the experiment. Mole-rats with a body mass below 50g were

considered juveniles and excluded from the study. Mole-rats were classified into four groups

according to their breeding status namely: breeding female (BF), breeding male (BM), non-

breeding female (NBF) and non-breeding male (NBM). Breeding females were distinguished

by the presence of elongated teats and a perforate vagina, while breeding males were

identified by their large body mass (Moolman et al. 1998).

Experimental design

On the day of the experiment, animals were removed from their colonies, weighed and placed

in separate crates. Experiments commenced around 10:00 am to reduce the possible effects of

circadian variations in body temperature (T_b). All experiments were conducted in a climate

controlled room with the room temperature kept at $25 \pm 1°C$. Rectal T_b's of animals were

measured using a chromel-alumel thermocouple connected to APPA 51 thermometer (APPA

Technology Corporation, Taiwan). The end of the thermocouple was lubricated with

petroleum jelly and carefully inserted one centimeter into the rectum whilst the animals were

hand-restrained. To determine baseline rectal T_b for each animal, measurements were made at

ten minute intervals for a period of one hour. Ten min after the last baseline measurement,

animals received an intraperitoneal administration of either a dose of 1 mg kg^{-1} LPS (from

Escherichia coli serotype 026:B6, Sigma Chemical) dissolved in sterile 0.9% saline or pure

sterile 0.9% saline (0.01ml per gram body mass). Twenty min after the injection the T_b was measured again and this T_b measurement was repeated at intervals of 20 min for a period of 360 min. Measurement intervals were increased from 10 to 20 minute intervals to reduce the handling stress for the animals. After the last measurement the mole-rats were returned to their respective group. The experiment was repeated two days later with individuals that had previously been injected with LPS now receiving saline and *vice versa* such that all animals were subjected to both treatments over the experimental period. To avoid possible order effects, half the subjects were first injected with saline (n=18) and the remainder with LPS (n=19). Room temperature was kept constant throughout experiments at around $25 \pm 1°C$. Animals received *ad libitum* food during the experimental period.

Statistical analysis

We explored the effects of season, sex and breeding status on body mass by employing a general linear model (GLM) using log-transformed body mass data to satisfy the criteria for normality. We classified animals captured from October to February as summer captures (n=19) and those caught between April and August as winter captures (n=18). All two and three-way interactions were included in the model initially.

Temperature data were analyzed using general linear mixed models (GLMM) with individual included as random factor to account for repeated measurements of individuals. Treatment (i.e. saline, LPS), time, season, sex and breeding status (i.e. breeder, non-breeder) as fixed factors. We included body mass as a covariate, however since it did not explain any of the observed variation we report the results excluding body mass here. Initially all two and three-way interactions were included in the model. Models were fitted using the maximum likelihood approach to allow for model comparison (Crawley 2007). Variables were dropped sequentially from the model based on the Akaike information criterion (AIC, Burnham & Anderson 2002) and only the final models are reported here. We firstly compared baseline T_b

measurements, however, since baseline T_b's differed significantly between treatments and increased with time (see Results section) we calculated the difference between the T_b for the last baseline measurement and the T_b at every time-point after treatment (ΔT_b) and used these ΔT_b-values to evaluate the effects of our variables on the febrile response. AIC-values for T_b were 858.71 and 788.69 for the full and final model, respectively. Similarly, AIC was 2456.40 and 2301.11 for the full and final model for ΔT_b, respectively. For significant interaction terms post-hoc paired and unpaired t-tests were carried out. Results are presented as means ± SE.

Results

We measured T_b's for a total of 37 individuals of which 21 were female (7 BF, 14 NBF) and 16 male (6 BM, 10 NBM). Body mass did not differ significantly between seasons ($F_{1,35}=0.696$, p=0.410) or the sexes ($F_{1,35}=1.776$, p=0.192). The interaction between sex and status was significant and BM (123.4±12.3g) were significantly heavier than NBMs (85.1±3.6g, t=-3.341, df= 14, 0.012). In contrast, NBFs and BFs did not differ significantly in body mass (NBF: 96.6±3.1g, BF: 92.4±6.3g, t=,789, df=19, p=0.435). BMs were significantly heavier than BFs (t=-2.383, df=11 , p=0.036) while NBFs were significantly heavier than NBMs (t=2.488, df=22, 0.021).

Baseline measurements

Mean baseline T_b's were significantly lower before the injection of LPS (33.80±0.06°C, n=222) than before the saline treatment (34.03±0.06°C, $F_{1,395}=22.66$, p<0.0001, Figure 1, Table 1). Furthermore, time did significantly affect baseline T_b's ($F_{5,395}=27.54$, p<0.0001) with T_b's increasing from the first to the last measurement (Figure 1). The interaction between treatment and season was significant ($F_{1,395}=21.45$, p<0.0001) with baseline T_b's before injection of LPS being significantly lower than before saline treatment in winter (t=3.584,

df=214, p<0.0001) but not in summer (t=0.127, df=226, p=0.899). Mean T_b's did not differ significantly between the seasons before saline treatment (t=-1.114, df=220, p=0.0263) but were significantly lower during winter compared to summer before LPS injection (t=2.649, df=220, p=0.009). The interaction between time and sex was significant ($F_{5,395}$=2.35, p=0.0400) with male T_b's being lower than female T_b's during the first measurements. None of the other factors had a significant effect on body temperatures measured during the baseline period (Table 1).

Experimental effects

The ΔT_b was significantly higher after LPS compared to saline injection ($F_{1,1238}$=280.2, p<0.0001, Table 2) and changed significantly with time ($F_{17,1238}$=11.9, p<0.0001, Figure 2). In addition, the interaction between treatment and time was significant ($F_{17,1238}$=8.8, p<0.0001) with temperatures significantly higher after the administration of LPS than after saline injection during the period from 100 to 360 min after the treatment (p≤0.030). In response to saline administration a significant increase in ΔT_b was observed from 140 to 160 min (t=-2.248, df=36, p=0.031) but no other consecutive measurements (p≥0.08). In contrast, after LPS administration ΔT_b increased significantly between measurements at 80 and 100 min (t=-2.365, df=36, p=0.024), 120 and 140 min (t=-2.497, df=36, p=0.017), 140 and 160 min (t=-2.499, df=36, p=0.017) as well as 220 to 240 min (t=-3.174, df=36, p=0.003). Furthermore, ΔT_b decreased significantly from 300 to 320 min (t=3.106, df=36, p=0.004) and from 340 to 360 min (t=3.229, df=36, p=0.003).

There was no significant interaction of treatment and season ($F_{1,1238}$=1.2, p=0.2795) but the interaction between treatment and sex was significant ($F_{1,1238}$=10.1, p=0.0016, Figure 3a) with ΔT_b for males being significantly higher than those for females after LPS (males: 0.74±0.05°C, females: 0.51±0.04°C, t=-3.770, df=664, p<0.0001) but not after saline administration (males: 0.15±0.05°C, females: 0.10±0.04°C, t=-0.723, df=664, p=0.470).

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Similarly, the interaction between treatment and breeding status was significant ($F_{1,1238}=20.3$, p<0.0001, Figure 3b) and breeders had a significantly higher ΔT_b after the LPS (breeders: 0.77±0.06°C, non-breeders: 0.52±0.04°C, t=-4.034, df=664, p<0.0001) but not after saline treatment (breeders: 0.10±0.06°C, non-breeders: 0.13±0.04°C, t=-0.379, df=664, p=0.705). The interaction between time and season was significant ($F_{17,1238}=2.3$, p=0.0025, Figure) and ΔT_b was significantly lower in summer for the measurements taken at 40 (summer: -0.14±0.09°C, winter: 0.20±0.11°C, t=-2.344, df=72, p=0.022) and 60 min (summer: -0.13±0.09°C, winter: 0.28±0.11°C, t=-2.878, df=72, p=0.005) compared to the same measurements in winter but none of the other measurements (p≥0.1).

The interaction between treatment, season and breeding status was significant ($F_{2,1238}=4.2$, p=0.0.153, Figure 4). Non-breeders had significantly lower ΔT_b than breeders in response to LPS during both seasons (p≤0.015, Figure 4b). In contrast, after saline administration there was no significant difference in ΔT_b between non-breeders and breeders in summer (t=-1.766, df=340, p=0.079) while non-breeders had significantly higher ΔT_b than breeders in winter (t=2.671, df=322, p=0.008, Figure 4a). After the saline treatment ΔT_b for non-breeders was significantly higher in winter (0.24±0.06°C) than in summer (0.02±0.05°C, t=-2.940, df=430, p=0.003) while this was not the case after LPS (winter: 0.54±0.05°C, summer: 0.49±0.05°C, t=-0.613, p=0.540). In contrast, ΔT_b did not differ significantly between seasons for breeders for either treatment (p≥0.058).

Discussion

We found a significant variation of T_b's between the two treatments during baseline data collection despite the controlled conditions under which our T_b measurements were conducted. These differences were only apparent during winter when small mammals are most likely to exhibit reductions in T_b due to the energetic limitations experienced (Geisser 2004). Indeed the large intra- and inter-individual variation in T_b's measured suggests a

potential for heterothermy in the highveld mole-rat. This hypothesis is supported by the repeated encounter of 'lethargic' individuals of our study species under laboratory conditions (H. Lutermann, personal observation) and similar findings have been reported for other mole-rat species (Marhold & Nagel 1995). The observed increase of T_b from the first to the last baseline measurement suggests that the repetitive handling of the study animals was sufficient to cause significant increases in T_b of highveld mole-rats. Such stress-induced hyperthermia (SIH) is a widespread phenomenon among vertebrates including humans (Bouwknecht, Oliveiri & Paylor 2007 and references therein) and suggests that highveld mole-rats respond to handling in a similar fashion as other vertebrates. The lower initial T_b's for males may be mediated by testosterone and a similar mechanism has previously been suggested for other vertebrates (Feuerbacher & Prinziger 1981, Wikelski et al. 1999). Alternatively, it has been suggested that males lower their body temperature in comparison to females to prevent sperm damage (Setchell 1998).

Despite the augmenting effect of handling on T_b further substantial increases in T_b were observed after the administration of LPS but not saline indicating that the typical febrile response was triggered by this pyrogen. Significant increases in ΔT_b were observed from 100 min after LPS treatment and reached a plateau by 240 min before ΔT_b decreased again after 300 min. At the same time ΔT_b post-LPS was significantly larger than those for saline between 140 and 360 min. Although LPS responses may differ substantially even between closely related species (Martin et al. 2008b) the overall pattern observed in the current study is remarkably similar to that reported in a study on naked mole-rats (Urison, Goelst & Buffenstein 1993). Urison et al. (1993) reported significant differences in ΔT between the two treatments already as early as 50 min after the treatment and they lasted only up to 190 min post-injection. However, these differences in time frame may be attributed to employing an LPS derived from *Staphylococcus aureus* instead of *E. coli* in our study. Treatment affected the time it took for significant increases in rectal temperatures to occur with significantly

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increases in ΔT_b's occurring earlier and more often after LPS treatment compared to saline treatment. This is an indication that LPS produced larger Tb increases than SIH alone.

The initial ΔT_b measurements were elevated in winter compared to summer and this is probably linked to the lower initial T_b's observed during the baseline measurements. In contrast, we did not find an effect of season on treatment suggesting that the APR was not attenuated by season. This suggests that seasonal differences in the febrile response are largely linked to gonadal hormones rather than to other seasonal factors such as fluctuations in energy availability (Prendergast et al. 2007, 2008). The lack of variation in seasonal body mass may indicate that highveld mole-rats do indeed not experience seasonal fluctuations in energy availability. A similar lack of body mass fluctuations has been reported for the closely related Natal mole-rat (*Cryptomys hottentotus natalensis*) (Lutermann & Bennett 2008). However, despite the lack of body mass fluctuation Natal mole-rats exhibit significant reductions in their energy stores suggesting that this may also be the case for highveld mole-rats. In contrast, the *ad libitum* food conditions during the laboratory period may have been sufficient to compensate for such seasonal effects. Alternatively, behavioral mechanisms such as reductions in locomotory activity and digestive activity that were not quantified in the current study may have yielded sufficient energy savings to compensate for the energetic demands of generating and maintaining a fever response (Hart 1988, Adelman & Martin 2009).

In contrast to the febrile response, our data suggests a seasonal modulation of the stress response in non-breeders as evidenced by the significant interaction between treatment, season and breeding status. Seasonal changes in stress responses are well documented in a number of species and animals display more elevated stress responses during winter (Nelson & Martin 2007). These seasonal differences in SIH may be correlated with food availability as has been shown for mice where food restriction increases the ΔT_b's in response to saline but not LPS administration (Conn et al. 1995). This is further supported by the negative

correlation between fat stores and postcapture glucocorticoids found in birds (Nelson & Martin 2007). That this effect was only observed in non-breeders may be due to larger energetic constraints experienced by non-breeders due to a less favorable volume-to-surface ratio as a result of their smaller body masses (Schmidt-Nielsen 1997). At the same time breeders do not participate to the same degree as non-breeders in energetically costly foraging activities and can accumulate larger energy stores (Lutermann & Bennett 2008) which may make them more resistant to the effects of the stressor. The lack of an effect after LPS administration lends support to the accumulating evidence that SIH and febrile response to LPS are functionally different physiological mechanisms that are both associated with elevations of T_b (Bouwknecht et al. 2007, Vinkers et al. 2009).

Male highveld mole-rats exhibited significantly higher ΔT_b's in response to LPS administration than females in our study. This finding is in accordance with other studies in small mammals (Murakami & Ono 1987, Klein & Nelson 1998, Pittman et al. 1998, Taylor et al. 2005, Yee & Prendergast 2010). In mice this sex-difference is mediated by a higher expression of the inflammatory cytokines and endotoxin-induced inflammatory mediators while at the same time the production of anti-inflammatory prostaglandins is reduced in males compared to females (Marriott, Bost & Huet-Hudson 2006). It is likely that a similar mechanism is also responsible for the observed differences in ΔT_b in highveld mole-rats. Our findings suggest that sex-biases in febrile responses to LPS are widespread among rodents.

We recorded higher ΔT_b's for breeders than non-breeders after LPS treatment. In contrast, larger febrile responses in breeding compared to non-breeding individuals could not be found for male Siberian hamsters (*Phodophorus sungorus*, Bilbo et al. 2002). However, the duration of this response was markedly shorter in non-breeding males suggesting that breeding status affected the febrile response in this species. In addition, Bilbo et al. (2002) observed larger reductions in food intake and cytokine concentrations for animals in breeding vs. non-breeding condition. Similarly, in white crowned sparrows (*Zonotrichia leucophrys*

gambelii) sickness behaviours in response to LPS administration were observed for longer durations for males in breeding than non-breeding condition (Owen-Ashley et al. 2006). In both studies, these differences were correlated with higher fat masses of study animals in breeding condition vs. those in non-breeding condition indicating that energy limitations may limit the expression of a febrile response and other sickness behaviours in non-breeders. Fat mass was not measured for the animals in the current study but data from other mole-rat species (Scantlebury et al. 2006, Lutermann & Bennett 2008) suggest that the greater increases of ΔT_b observed for breeders in the current study may be sustained by the larger energy stores of breeders (Adelman & Martin 2009).

In conclusion, wild-caught highveld mole-rats responded to both handling and LPS administration with increases in T_b. However, while the SIH varied seasonally in non-breeders, no seasonal effects were observed on the febrile response. At the same time, both sex and breeding status had significant effects on the febrile response with males and breeders exhibiting larger increases in ΔT_b than females and non-breeders. These effects of sex and breeding status on the febrile response may be linked to sex-specific differences in susceptibility to bacterial infection and larger energy resources as reported for other vertebrate species.

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Figure legends

Figure 1. Mean (± SE) rectal body temperature measured prior to and after treatment with saline (open circles) and LPS (filled circles), respectively. The time of injection is indicated by an arrow.

Figure 2. Mean (± SE) change in body temperature (ΔT_b) in response to intraperitoneal injection of saline (open circles) and LPS (filled circles).

Figure 3. The effect of sex (a) and breeding status (b) on ΔT_b after saline and LPS treatment. Displayed are means ± SE.

Figure 4. The effects of season and breeding status on changes in mean ΔT_b (± SE) after saline (a) and LPS (b) administration.
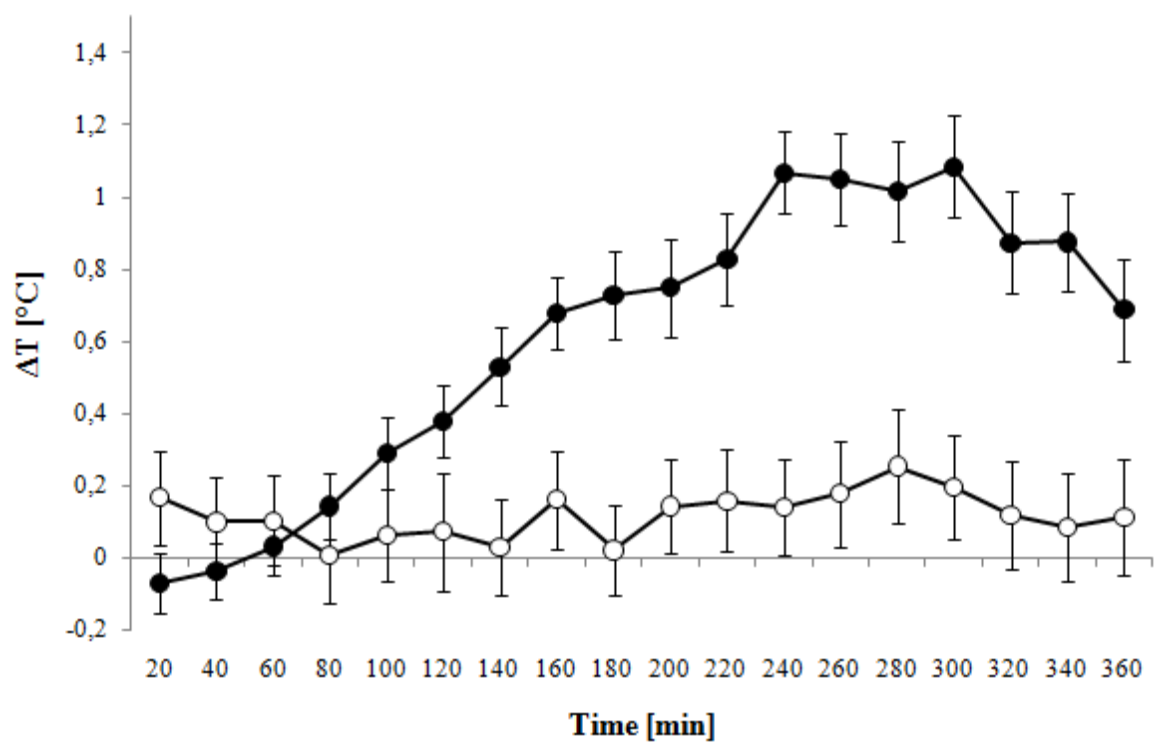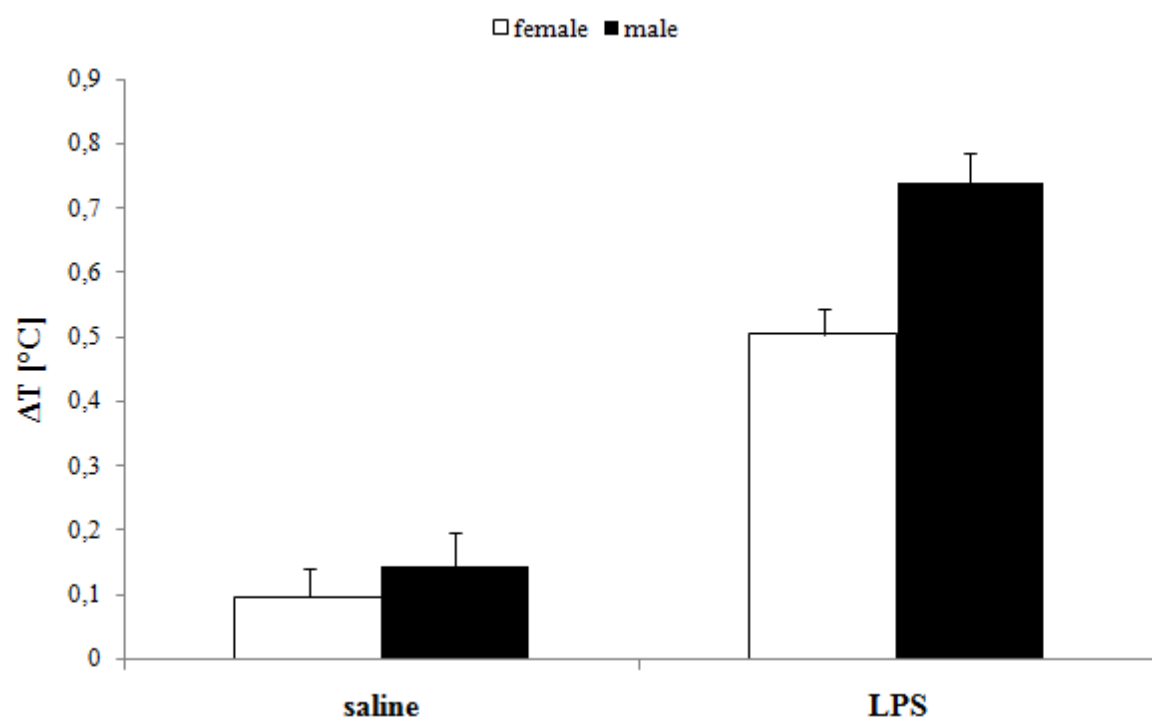
Figure 1
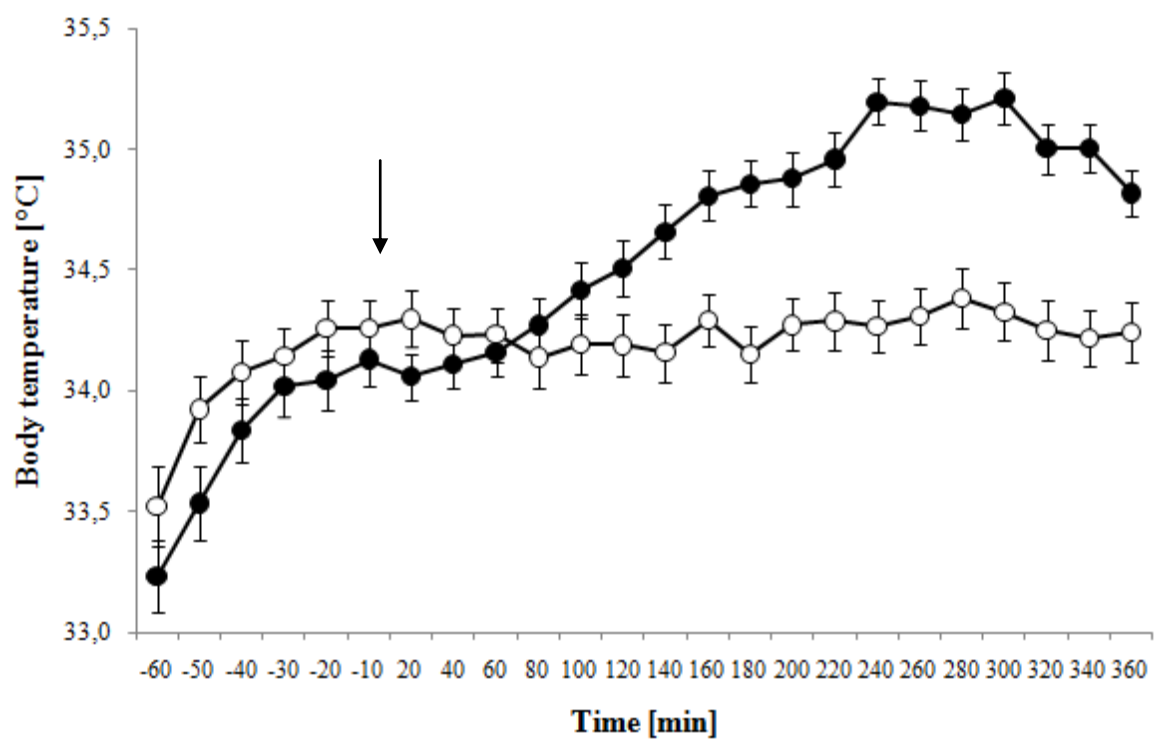
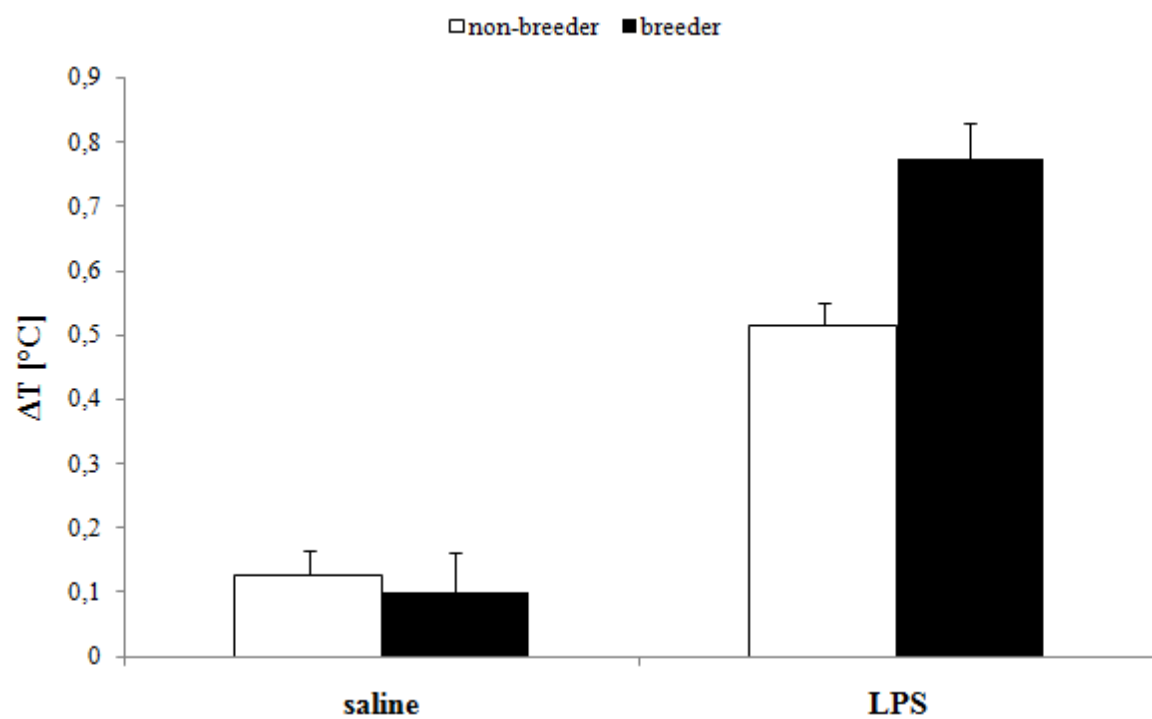**Figure 2**

Figure 3a

Figure 3b

Figure 4a

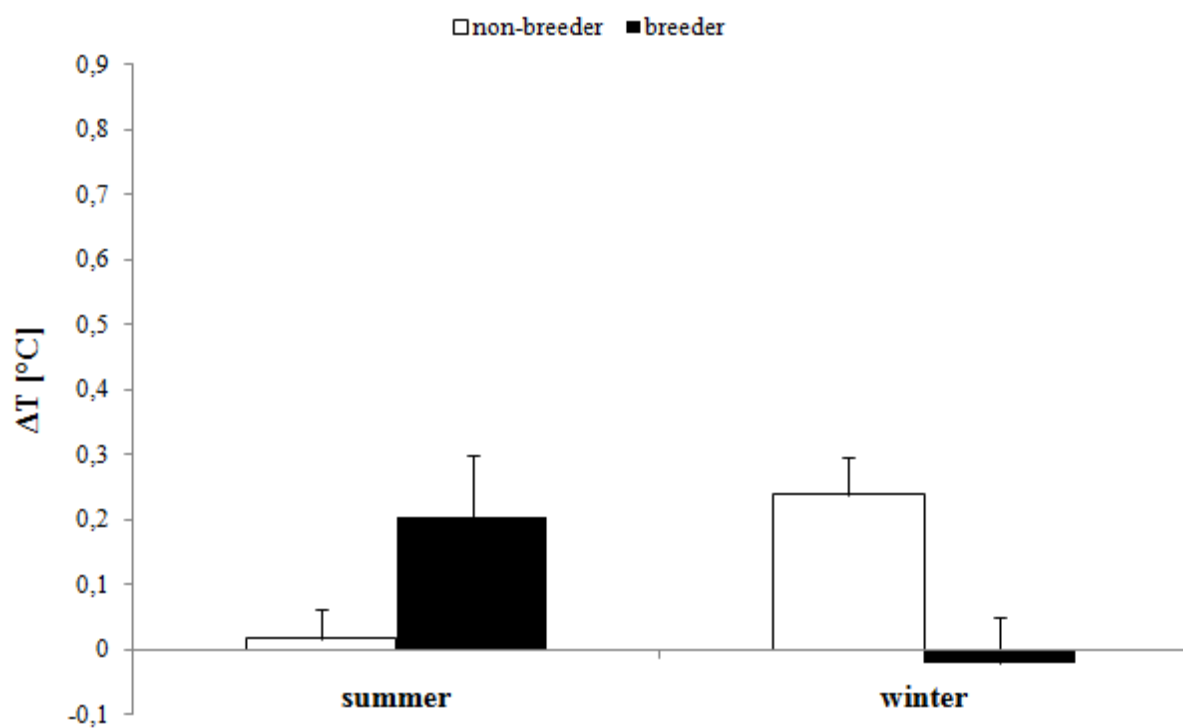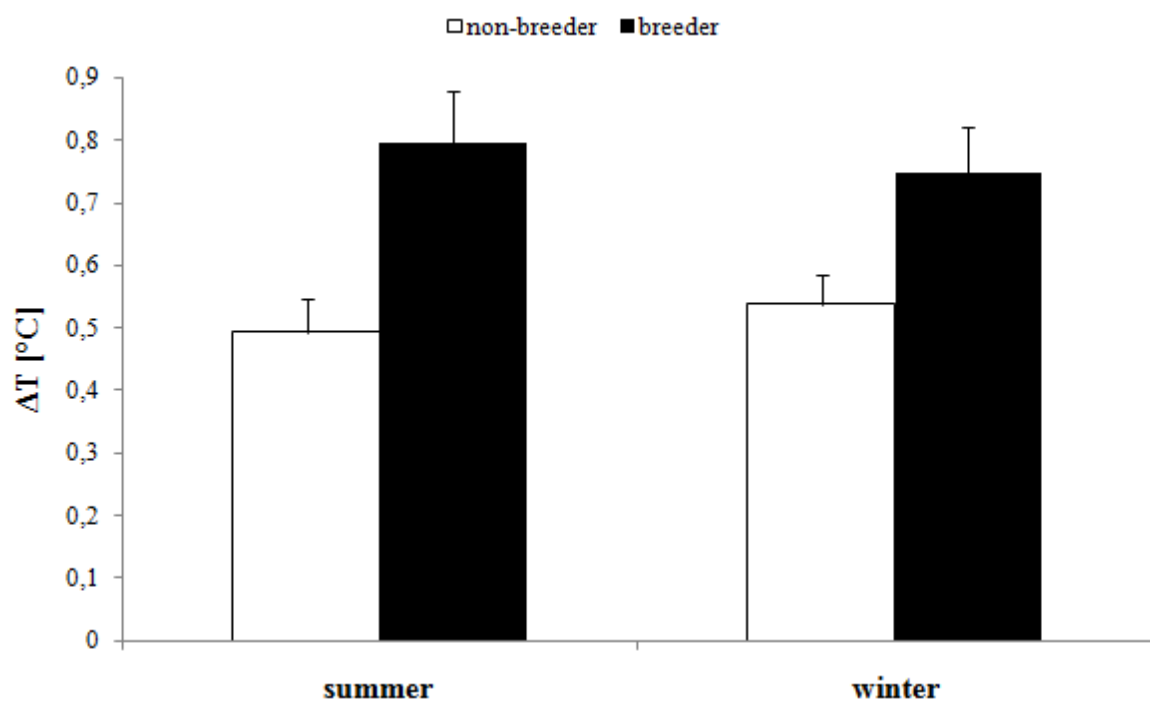**Figure 4b**

Table 1. Results of the final linear mixed model for body temperature during baseline measurements.

Variable	F-value	p
Treatment	22.66	<0.0001*
Time	27.54	<0.0001*
Season	0.25	0.6185
Sex	3.76	0.0609
Treatment*season	21.45	<0.0001*
Time*sex	2.35	0.0400*

* denotes significance at the level of $p < 0.05$.

Table 2. Results of the final general liner mixed model describing the contributions of the independent variables in explaining post-treatment changes in body temperature.

Variable	F-value	p
Treatment	280.2	<0.0001*
Time	11.86	<0.0001*
Season	0.04	0.8528
Sex	0.55	0.4638
Status	0.32	0.5751
Treatment*time	8.80	<0.0001*
Treatment*season	1.17	0.2795
Treatment*sex	10.06	0.0016*
Treatment*status	20.26	<0.0001*
Time*season	2.26	0.0025*
Treatment*season*status	4.19	0.0153*

* denotes significance at the level of $p < 0.05$.