Check for updates

Research Article

Innate Immunity

Innate Immunity
17(4) 355–364
© The Author(s) 2010
Reprints and permissions:
sagepub.co.uk/journalsPermissions.nav
DOI: 10.1177/1753425910379144
ini.sagepub.com
SAGE

Temperament influences endotoxin-induced changes in rectal temperature, sickness behavior, and plasma epinephrine concentrations in bulls

Nicole C Burdick[1,2], Jeffery A Carroll[2], Lindsey E Hulbert[2], Jeffery W Dailey[2], Michael A Ballou[3], Ronald D Randel[4], Scott T Willard[5], Rhonda C Vann[6] and Thomas H Welsh Jr[1]

Abstract

This study was designed to determine the influence of temperament on endotoxin-induced changes in body temperature, sickness behavior, and stress hormone concentrations in cattle. Brahman bulls were selected based on temperament score measured 28 d prior to weaning. In dwelling recording devices were used to monitor rectal temperature, and jugular catheters were used to collect blood samples to determine cortisol and epinephrine concentrations before and after LPS administration (0.5 μg/kg body weight). Temperamental bulls had the lowest peak rectal temperature and sickness behavior scores relative to the Calm and Intermediate bulls. Prior to the administration of LPS, Temperamental bulls had greater cortisol and epinephrine concentrations than Calm or Intermediate bulls. Cortisol concentrations increased following LPS administration but were not affected by temperament. Epinephrine concentrations peaked 1 h after LPS administration in Calm bulls. Temperamental bulls did not exhibit an epinephrine response to LPS challenge. These data demonstrate that the temperament of calves can modulate the physiological, behavioral, and endocrine responses of pre-pubertal Brahman bulls to endotoxin challenge. Specifically, temperament differentially affected the rectal temperature, sickness behavior and epinephrine, but not cortisol, responses to LPS challenge.

Keywords

bulls, cortisol, epinephrine, lipopolysaccharide, temperament

Date received: 26 April 2010; revised: 28 May 2010; 24 June 2010; accepted: 28 June 2010

Introduction

Reducing the effects of disease is a major goal in the cattle industry as morbidity is more costly to producers than mortality due to the: (i) expense of treating disease, and (ii) negative impact of disease on performance.[1] Stress is one of the main factors influencing immune function. Chronic stress can negatively impact growth, reproductive, and immune functions.[2–7] In contrast, acute stress is not necessarily detrimental to the health of an animal, and may even elicit beneficial immune responses.[8–11] As data related to the dynamics of stress hormone modulation of immune function are accumulated, the immuno-endocrine concept continues to evolve.

The innate immune system can be activated by molecules or chemicals derived from pathogens that cause acute inflammation and infection.[12,13] Exposure to endotoxin (LPS; a component of the cell wall of Gram-negative bacteria) initiates an inflammatory cascade and increases stress hormone concentrations in

[1]Texas AgriLife Research and Department of Animal Science, Texas A&M University System, College Station, Texas, USA.
[2]USDA ARS Livestock Issues Research Unit, Lubbock, Texas, USA.
[3]Texas Tech University, Department of Animal and Food Sciences, Lubbock, Texas, USA.
[4]Texas AgriLife Research Center, Overton, Texas, USA.
[5]Mississippi Agricultural and Forestry Experiment Station, Mississippi State University, Mississippi, USA.
[6]Brown Loam Branch Experiment Station, Mississippi State University, Raymond, Mississippi, USA.

Corresponding author:
Jeffery A Carroll, USDA ARS Livestock Issues Research Unit, Route 3 Box 215, Lubbock, TX 79403, USA.
Email: jeff.carroll@ars.usda.gov

mammals.[14–17] For example, Williams *et al.*[17] demonstrated that cortisol, epinephrine and norepinephrine increased in young pigs in response to an i.v. LPS challenge.

Often unappreciated is the influence of temperament and stress responsiveness on livestock health and productivity. Temperament is defined as the fear response of cattle to humans or novel or unfamiliar environments.[18] More stress responsive, temperamental cattle characteristically have greater basal concentrations of the stress hormones cortisol and epinephrine,[19–21] depressed immune functions,[22] a slower growth rate,[23,24] and reduced carcass value.[21] Furthermore, the excitable nature of Temperamental cattle make them more difficult to manage and increases the risk of injury to themselves and personnel, and increases damage to facilities.[24] Due to the greater basal concentrations of stress hormones exhibited by Temperamental cattle, it is hypothesized that temperament may affect the stress hormone response to LPS. Thus, this study was designed to determine whether the febrile response and the production of cortisol and epinephrine induced by an acute endotoxin challenge are different due to the temperament of young, growing Brahman bulls.

Materials and methods

Experimental design

All experimental procedures were in compliance with the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* and approved by the Institutional Animal Care and Use Committees of Texas A&M University and the USDA. Bulls (10 months of age) from the Texas AgriLife Research Center's Brahman herd in Overton, Texas were selected for use in this study based on their temperament score measured 28 d prior to weaning (133 ± 3 d of age). Temperament score[21] was an average of exit velocity (EV) and pen score (PS). Exit velocity, the rate of speed of a calf traversing a distance of 1.83 m after its exit from a working chute, was determined using two infrared sensors (FarmTek Inc., North Wylie, TX, USA) and was done by calculating velocity [velocity = distance (m)/time (s)].[19,25] Pen score[26] is a subjective measurement obtained by separating cattle into small groups of three to five and scoring their reactivity to a human observer on a scale of 1 (calm, docile, approachable) to 5 (aggressive, volatile, crazy). Based on temperament score, the 8 most Calm, 8 Intermediate, and 8 most Temperamental were selected from a pool of 60 bulls. However, during the experiment, one calm bull died (1-h post-LPS), researchers intervened to prevent death of another calm bull (3.5-h post-LPS), and one temperamental bull's catheter became dislodged (0-h post-LPS). Therefore, only the data from the 6 most Calm (1.01 ± 0.16 EV and 1.00 ± 0.00 PS), 7 most Temperamental (3.51 ± 0.25 EV and 5 ± 0.00 PS), and the 8 Intermediate bulls (1.59 ± 0.12 EV and 2.25 ± 0.16 PS) are presented. Two days prior to the initiation of the endotoxin challenge part of the study, bulls were fitted with rectal temperature recording devices (A HOBO Pro v2 Temp data logger probe; Part #U23-004, Onset Corp., Pocasset, MA, USA) that measured rectal temperature continuously at 1-min intervals in the absence of a human operator. During these procedures, cattle were restrained in a working chute for approximately 5 min. The factory-calibrated rectal temperature recording devices were tested for accuracy upon receipt from the manufacturer. Rectal temperature recorders became displaced from some animals during the study; temperature data presented include only those bulls that yielded a complete data set ($n = 5$, 6, and 7 for Calm, Intermediate and Temperamental bulls, respectively). On the day prior to the study, bulls were fitted with jugular catheters. During these procedures, cattle were restrained in a working chute for approximately 5 to 10 min. Following these procedures, bulls were moved to the facility that contained individual stalls (2.13-m long × 0.76-m wide) that housed the bulls through the duration of the study. Bulls were randomly placed in their stalls. During the challenge, the bulls had *ad libitum* access to feed and water. The extension tubing of the catheter was extended above the stall to allow researchers to collect blood throughout the study without disturbing the calf, whether the calf was standing or lying down. Blood samples were collected (to harvest serum and plasma) every 30 min beginning 2 h prior to, and continuing 8 h after, administration (0.5 μg/kg body weight LPS; *Escherichia coli* O111:B4; Sigma-Aldrich, St Louis, MO, USA) for determination of cortisol and epinephrine concentrations, respectively.

Sickness behavior

Sickness behavior scores were assigned to animals at 30-min intervals from 0 to 6 h post-LPS challenge. Bulls were scored on a scale of 1 (active or agitated) to 5 (lying on side with labored breathing; Table 1).

Assays for cortisol and epinephrine

Serum concentrations of cortisol were determined using a single antibody radioimmunoassay (DSL-2100; Diagnostic Systems Labs, Webster, TX, USA) utilizing rabbit anti-cortisol antiserum coated tubes according to the manufacturer's directions.[27] The minimum detectable cortisol concentration was 1.2 ng/ml and the intra- and interassay coefficients of variance were 4.3% and 2.4%, respectively. Serum concentrations of cortisol were determined by comparison to a standard curve generated with known concentrations of cortisol and presented as the concentration in nanogram per millilitre.

Plasma concentrations of epinephrine were determined by enzyme immunoassay according to the manufacturer's directions (17-BCTHU-E02; Alpco Diagnostics, Boston, MA, USA) by comparison of unknowns to standard curves generated with known concentrations of epinephrine.[27] Data are presented as picogram per millilitre. The minimum detectable epinephrine concentration was 11 pg/ml and the intra- and interassay coefficients of variation were 3.7% and 7.4%, respectively.

Statistical analysis

Prior to analysis, rectal temperature data were averaged into 30-min intervals. Rectal temperature, cortisol, and epinephrine data were analyzed using the MIXED procedure of SAS (SAS, Inc., Cary, NC, USA) specific for repeated measures with temperament, time, and time*temperament interaction included as fixed effects. Specific pre-planned comparisons were made using Fisher's Protected LSD with $P < 0.05$ considered significant. Data are presented as least squares means (LSM) $\pm$ SEM.

Table 1. Sickness behavior score descriptions

Score	Behavior Description
1	Active or agitated
2	Appeared normal
3	Calm with head distended
4	Clinical signs of sickness, increased respiration
5	Lying on side with labored breathing

Results

Rectal temperature

Prior to the administration of LPS, rectal temperature was greater in Temperamental bulls than Calm and Intermediate bulls ($P < 0.001$), with Intermediate bulls having greater rectal temperature than Calm bulls ($P = 0.05$). Rectal temperatures prior to the administration of LPS were not affected by time ($P > 0.05$; Figure 1). Rectal temperature increased in all bulls following administration of LPS, peaking around 210 min, with Temperamental bulls having the smallest increase in rectal temperature (relative to baseline values) compared to Calm and Intermediate bulls ($P < 0.001$). Twenty-four h (1440 min) after the administration of LPS, rectal temperature of Calm and Intermediate bulls had returned to baseline values; however, Temperamental bulls had lower rectal temperatures than Calm and Intermediate bulls ($P < 0.05$).

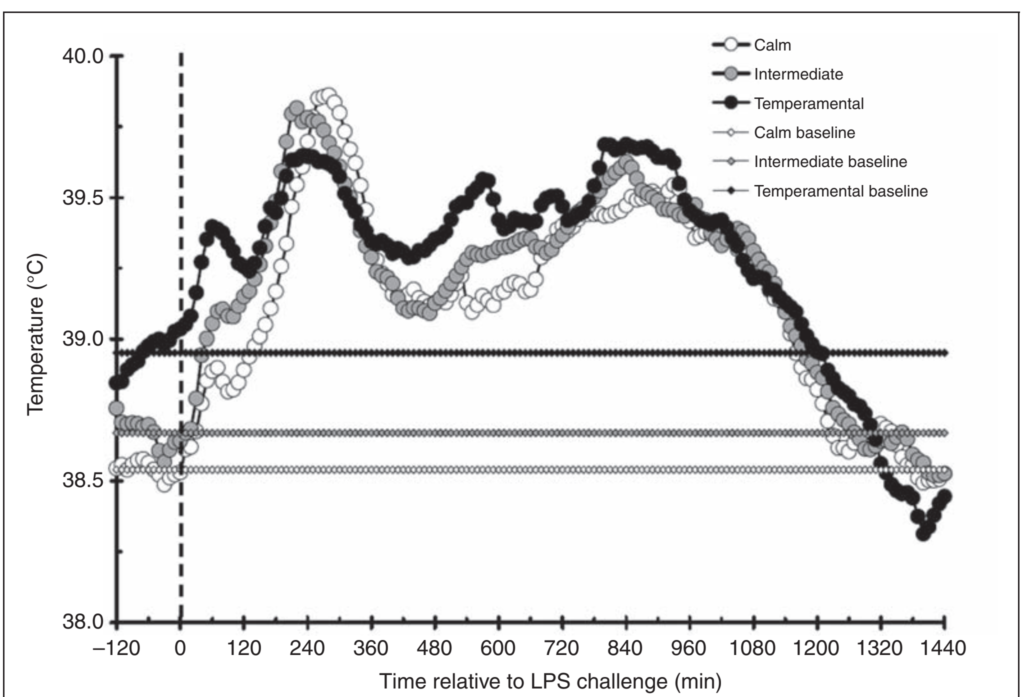

Figure 1. Rectal temperature response to an endotoxin (lipopolysaccharide, LPS; 0.5 µg/kg body weight) challenge (Calm, $n = 5$, Intermediate, $n = 6$, and Temperamental, $n = 7$). Rectal temperature data are presented as LSM (SEM $\pm$ 0.20 for Calm, $\pm$0.16 for Intermediate, and $\pm$0.15 for Temperamental bulls). Baseline rectal temperature data presented as average of LSM for pre-LPS rectal temperatures (–120 to 0 min; SEM $\pm$ 0.17 for Calm, $\pm$0.17 for Intermediate, and $\pm$0.16 for Temperamental bulls).

Sickness behavior

Prior to the administration of LPS, sickness behavior scores were similar amongst temperament groups ($P > 0.05$; Figure 2). Following administration of LPS at time 0, sickness behavior scores increased in all bulls ($P < 0.001$). Calm calves had greater sickness behavior scores when compared to Intermediate ($P = 0.005$) and Temperamental bulls ($P < 0.001$). Additionally, Intermediate bulls had greater sickness behavior scores than Temperamental bulls ($P < 0.001$). There was a tendency for a time*temperament interaction ($P = 0.06$). Specifically, peak sickness behavior scores occurred at 0.5 h post-LPS administration for Calm bulls, and at 1 h post-LPS administration in Intermediate and Temperamental bulls. Peak sickness behavior scores were greater in Calm and Intermediate bulls compared to Temperamental bulls ($P < 0.001$ and $P = 0.007$ for Calm and Intermediate bulls, respectively).

Cortisol and epinephrine

Prior to the administration of LPS, Temperamental bulls had greater concentrations of cortisol than Calm and Intermediate bulls ($P < 0.001$) as depicted in Figure 3. Cortisol concentrations tended to decrease from -2 h to time 0 ($P = 0.07$). Following administration of LPS at time 0, cortisol concentrations increased ($P < 0.001$) through 2 h before declining, and were similar among temperament groups ($P = 0.80$).

Prior to the administration of LPS, Temperamental bulls had greater concentrations of epinephrine than Calm and Intermediate bulls ($P < 0.001$) as depicted in Figure 4. There was a significant time effect ($P < 0.001$) and time*temperament interaction ($P = 0.014$) prior to the administration of LPS with epinephrine concentrations increasing from –2 h to –1 h in Calm ($P = 0.05$) and Temperamental ($P < 0.001$) bulls, and increasing from –2 h to −0.5 h in Intermediate bulls ($P = 0.02$). Relative to time 0, epinephrine concentrations did not change in response to the LPS challenge in Intermediate bulls ($P > 0.05$). Epinephrine concentrations tended to peak 1 h after the administration of LPS in Calm bulls ($P = 0.06$). In contrast, epinephrine concentrations in Temperamental bulls decreased from time 0 h to 0.5 h after the administration of LPS ($P = 0.01$). Epinephrine concentrations then increased from 0.5 h post-LPS, with significantly higher concentrations of epinephrine at 1, 2, 3, 3.5, 4, 4.5, 5, and 5.5 h post-LPS compared to values at 0.5 h ($P < 0.05$). Post-LPS administration, Temperamental bulls maintained greater epinephrine concentrations than either the Calm or the Intermediate bulls ($P < 0.05$).

Discussion

These data demonstrate that temperament modifies the endocrine, behavioral, and physiological responses of pre-pubertal Brahman bulls to endotoxin challenge. Rectal temperature, sickness behavior, cortisol, and

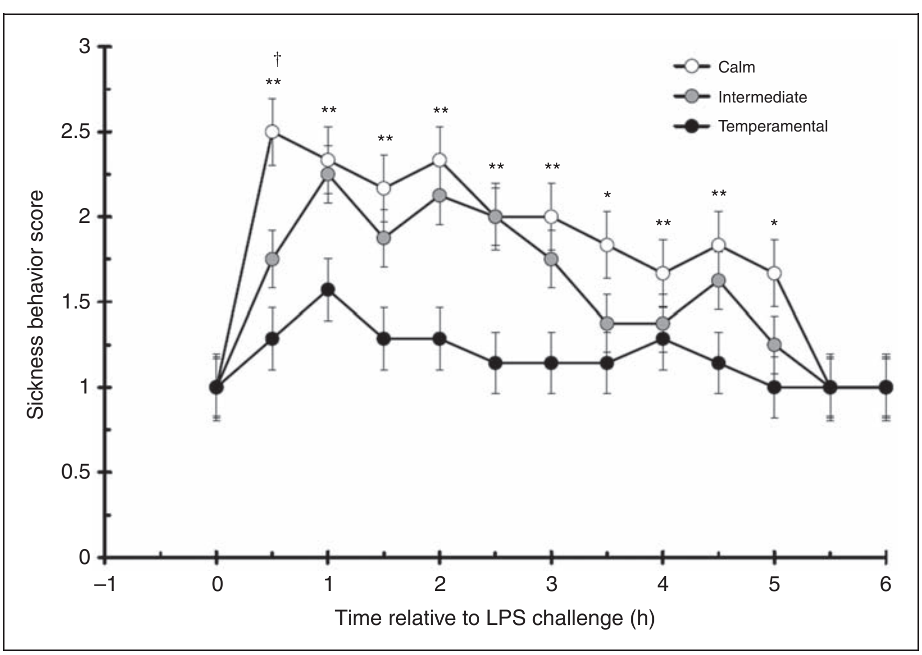

Figure 2. Sickness behavior response to an endotoxin (LPS; 0.5 μg/kg body weight) challenge (Calm, $n = 6$; Intermediate, $n = 8$; and Temperamental, $n = 7$ bulls). Data presented as LSM ± SEM. †Intermediate bulls differ compared to Calm bulls ($P < 0.05$). *Temperamental bulls differ compared to Calm bulls ($P < 0.05$). **Temperamental bulls differ compared to Intermediate and Calm bulls ($P < 0.05$).

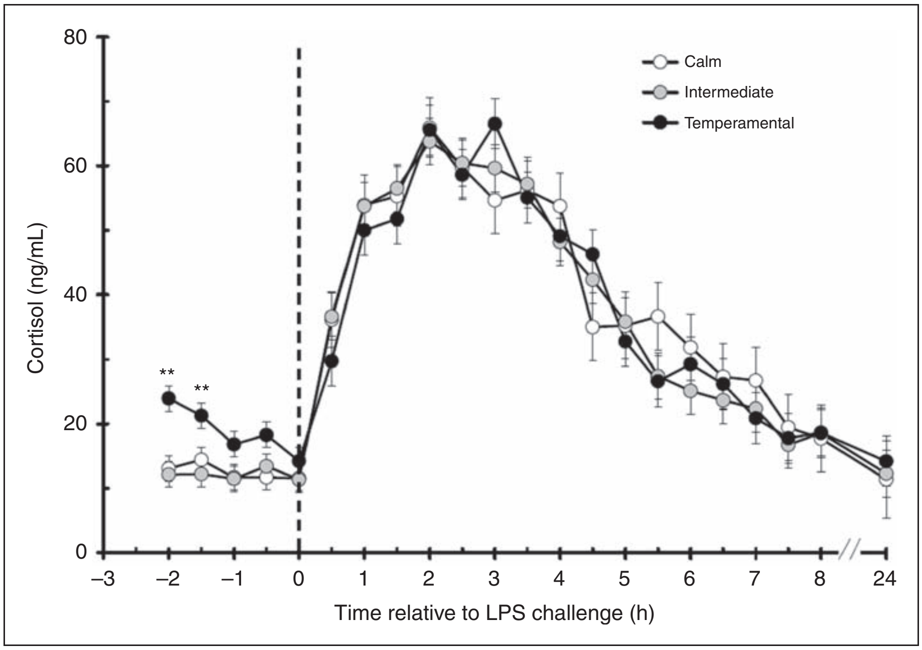

Figure 3. Serum cortisol response to an endotoxin (LPS; 0.5 μg/kg body weight) challenge (Calm, $n = 6$; Intermediate, $n = 8$; and Temperamental, $n = 7$ bulls). Data presented as LSM ± SEM. **Temperamental bulls differ compared to Intermediate and Calm bulls ($P < 0.05$).

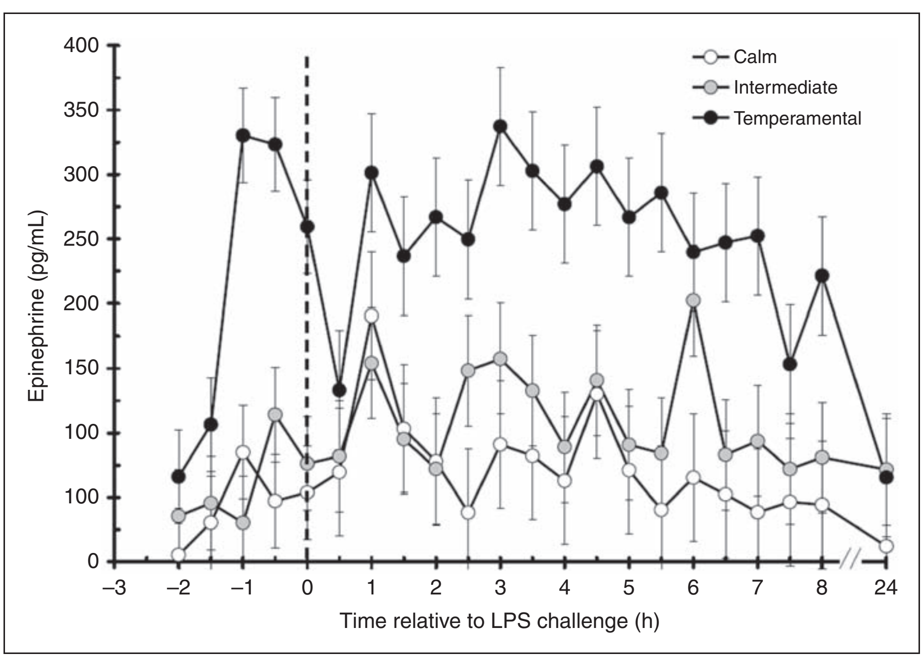

Figure 4. Plasma epinephrine response to an endotoxin (LPS; 0.5 μg/kg body weight) challenge (Calm, $n = 6$; Intermediate, $n = 8$; and Temperamental, $n = 7$ bulls). Data presented as LSM ± SEM.

epinephrine increased in response to administration of LPS. Specifically, temperament differentially affected the rectal temperature, sickness behavior, and epinephrine, but not cortisol, responses to LPS challenge.

No differences in baseline body temperature were found in rats selectively bred to differ in behavior (high-anxiety and low-anxiety behavior lines).[28] However, documentation regarding the potential for temperament to influence thermoregulation is limited in cattle. In the present study, there was an effect of temperament on pre-LPS rectal temperatures in bulls, with Temperamental bulls having greater rectal temperatures than Intermediate and Calm bulls. This is similar to our prior study which demonstrated that rectal temperature was greater in Temperamental bulls than Calm and Intermediate bulls immediately after loading into a trailer.[29]

An increase in body temperature has been utilized as a characteristic response of cattle to LPS challenge, and as a sign of inflammation.[30–37] An increase in body temperature in response to LPS is stimulated by pro-inflammatory cytokines, mainly TNF-α, IL-1β and IL-6.[38–40] In response to LPS, peak rectal temperatures in the pre-pubertal Brahman bulls used in this study were attained within 3.5 h, which was earlier than the 6 h required for Holstein cows to attain peak rectal temperatures post-LPS challenge.[32] Also, in the current study, attainment of peak rectal temperature occurred 1 h earlier than previously reported for Angus steers (peak rectal temperature attained at 4.5 h post-LPS).[35] However, the Jacobsen study[32] used a lower dose of LPS (0.1 μg/kg) and was performed in mature dairy cows, in contrast to the current study which utilized a higher LPS dose (0.5 μg/kg) in young, pre-pubertal Brahman beef bulls. The study on Angus steers[35] used a larger dose of LPS (2.5 μg/kg) than the current study. Therefore, although similar febrile responses have been demonstrated in cattle studies, the dose administered and the breed of cattle can vary the time to reach peak values and the duration in which rectal temperatures are elevated.

Twenty-four hours after the administration of LPS, the rectal temperature of Calm and Intermediate bulls had returned to baseline. However, at this time, Temperamental bulls had significantly lower rectal temperature compared to their baseline value. The mechanism for LPS to reduce rectal temperature below baseline values in Temperamental cattle is unclear. Several rodent models have been employed to study LPS-induced hypothermia.[41,42] However, in many rodent models, hypothermia can precede fever, or is the only temperature response. The manifestation of this hypo- or hyperthermic response in rodents depends on the dose of LPS administered.[41,42] This sequence of lower followed by higher temperature in rodents following LPS administration is not what was observed in the current study that utilized cattle. However, it is difficult to compare the response of rodents to cattle due to the relative refractoriness of rodents to endotoxin.

Although changes in body temperature following LPS administration have been studied in cattle, the authors restricted data collection to the initial 12 h post-LPS which prevents assessment of further temporal changes.[34,37,43] Nevertheless, a study by Borderas *et al.*[33] using dairy calves did not find a decrease in rectal temperature below baseline values when measurements were collected 24-h post-LPS challenge. Application of the rodent models enabled detection of interactions between cytokines, prostaglandins, and lipid mediators, each of which have a role in the regulation of hypothermia.[41,42,44] It has been suggested that the capability to minimize or lessen the increase in body temperature in response to an endotoxin (such as LPS) increases survival.[45] While not the only reason for survival, the change in rectal temperature may be a factor in this study, as all of the Temperamental and Intermediate bulls survived, yet one of the Calm bulls died, and the authors intervened in order to prevent the death of another Calm bull. However, this can be argued as increases in body temperature are typically considered to inhibit the growth of prokaryotic organisms, thus serving as a host survival mechanism. Additionally, many other factors regulate survival (cardiovascular output, electrolyte concentrations) and are not necessarily dependent on changes in body temperature. Future studies are needed to determine the mechanisms resulting in LPS-induced changes in body temperature, particularly in cattle. The elevated stress hormones in more Temperamental cattle may serve as a protective mechanism when challenges with endotoxin occur.

Thermoregulation in response to LPS may involve catecholamines within the central nervous system.[46,47] Tolchard *et al.*[47] demonstrated that agonists for the α_2-adrenergic receptor, the receptor that inhibits responses to the catecholamines epinephrine and norepinephrine, inhibited LPS-induced hypothermia, suggesting a role for catecholamines. This is interesting as epinephrine concentrations were greater in Temperamental bulls prior to, and following, the administration of LPS, as discussed in more detail below. Thermoregulation induced by substances such as LPS is regulated by thermosensitive central nervous system neurons that innervate the hypothalamus.[46] In mice, Jüttler *et al.*[46] demonstrated that the neurons responsible for mediating actions of pro-inflammatory cytokines within this region contain the active transcription factor NF-κB, which is responsible for downstream actions of LPS, including an increase in inflammatory response. It will be necessary to determine if the cytokine response to LPS is affected by temperament, thereby explaining the differences in rectal temperature observed in Temperamental bulls.[48]

Animals respond behaviorally to illness in various ways, simultaneously with physiological changes, in order to help the animal cope with the illness.[33] These behaviors include weakness, malaise, inability to concentrate, depression, lethargy, and decreases in eating and drinking.[49,50] Sickness behavior is induced by pro-inflammatory cytokines including IL-1, IL-6, TNF-α and interferons.[49] The systemic administration of these cytokines results in the onset of sickness behavior in both humans and in animals, with these responses mimicked by the administration of LPS.[49] Whereas there are several studies utilizing rodent and human models, there are limited studies describing the effect of LPS on sickness behavior in cattle.

A study in young dairy calves found an increase in the amount of time calves were lying inactive, and found a decrease in the time eating hay and ruminating 2 h before and after the peak in rectal temperature in response to low doses (0.025 and 0.05 μg/kg body weight) of LPS.[33] In the current study, calves of Calm and Intermediate temperament spent more time lying, as indicated by their sickness behavior scores. The bull calves lying on their side may be a mechanism for which an animal increases heat loss through transfer of heat from the body to the ground; however, this is debatable as cattle most often use evaporative cooling through the production of sweat, which would be inhibited by cattle lying down. Additionally, calves lying down may be a way for the calves to conserve energy, as it takes less energy to lay down then it does for an animal to remain standing. Our observations that Temperamental bulls had a lower sickness behavior score than did either Intermediate or Calm bulls suggest that temperament can influence sickness behavior in cattle. Several research articles have put forth the concept that sickness behavior is not a manifestation of innate behavioral or psychological weakness but is an expression of a motivational state.[49–51] Specifically, Dantzer[49] stated that motivation to exhibit fear behavior competes with sickness behavior, with fear taking priority over sickness-related behavior. Therefore, it may be that Temperamental bulls did not appear as sick as Intermediate and Calm bulls due to an increase in fear behavior to their environment and the human workers. Further research is needed in order to elucidate the mechanisms by which cytokines (both peripherally and in the brain) regulate sickness behavior, and the mechanisms by which temperament influences this response.

Peripheral blood concentrations of cortisol and epinephrine prior to the administration of LPS were greater in Temperamental bulls than in Intermediate and Calm bulls. This is in agreement with our previous studies[19–21] and studies conducted by others,[52] in which basal concentrations of cortisol and epinephrine were greater in Temperamental bulls. The elevation in plasma epinephrine concentrations prior to the LPS challenge is most likely in response to the presence of human researchers, as humans were not present in the facility prior to the collection of the –2-h sample. The increase in baseline epinephrine concentrations in response to the presence of humans is not surprising, as temperament is defined as the reactivity to humans. Therefore, Temperamental bulls reacted to the presence of humans with a greater epinephrine response compared to Calm and Intermediate bulls prior to the LPS challenge. It is unclear as to the exact causes for the difference in cortisol concentrations in Calm versus Temperamental cattle. Grandin[53] suggests that temperament is a genetic factor due to its heritability. However, temperament can be reduced with repeated handling,[19,52,54] which suggests a complex interaction between genetics and environment resulting in changes in physiology. In humans, morning plasma cortisol concentrations had a heritability of 0.45, with unbound (free or active) cortisol having a higher heritability of 0.51, which suggests a genetic basis for cortisol concentrations.[55] Studies in both humans and livestock have indicated a relationship between cortisol output, including individual variability in cortisol secretion, with disease risk.[56,57] Although the cortisol response to LPS challenge was not affected by temperament, the difference in cortisol concentrations due to temperament prior to the LPS challenge may have influenced the overall response to LPS challenge.

Cortisol is a potent anti-inflammatory hormone that is secreted in response to endotoxin challenge. Temperament differentially affected the response of epinephrine, but not cortisol, to endotoxin challenge. Previous studies in cattle have established that LPS induces an increase in cortisol concentrations.[35–37,58] The cortisol response to LPS in this study was similar to that described by Carroll *et al.*[35] In contrast, Kahl *et al.*,[58] utilizing pubertal beef heifers, found that LPS stimulated an increase in cortisol concentrations 2 h after administration, and concentrations remained elevated through 7 h post-challenge. It should be noted that Kahl *et al.*[58] used a 5-fold larger dose (2.5 μg/kg body weight) of LPS than was used in the current study.

Whether *Bos taurus* and *Bos indicus* vary in sensitivity to endotoxin merits investigation. *Bos indicus* cattle, such as those used in this study, appear to be more sensitive than *B. taurus* cattle to endotoxin. Other studies suggested a breed difference in response to immunological stimuli.[43,59] For example, Blecha *et al.*[59] reported a greater response to a phytohemagglutinin skin test by Angus steers than by Brahman × Angus cross steers. Compared to the current study, Carroll *et al.*[35] delivered a 5-fold greater dose of LPS to pure-bred Angus steers (of undefined temperament), yet produced a cortisol response similar in duration and magnitude to that which we observed for pure-bred Brahman bulls. This suggests that the *B. taurus* genotype may convey added protection or resistance to the detrimental actions of LPS. Similarly, it has been demonstrated that *B. indicus* influenced cattle are more sensitive to stimulation by gonadotropins (luteinizing hormone and follicle stimulating hormone) than *B. taurus* influenced cattle.[60,61] Therefore, breed of cattle, as well as temperament of the cattle, should be considered during the design and the comparison of results of experiments in which LPS is utilized to stimulate the innate immune system as well as an inflammatory response.

While there was no difference among the temperament groups with respect to the cortisol response to LPS challenge, there were clear differences due to temperament in the epinephrine response to LPS challenge. Specifically, epinephrine concentrations in Temperamental bulls did not increase in response to LPS compared to time 0 (prior to the administration of LPS). However, as discussed earlier, epinephrine concentrations increased in Temperamental bulls prior to the administration of LPS; therefore, time 0 epinephrine values may not be a true representation of baseline values. A longer collection period prior to the administration of LPS is required in order to characterize baseline epinephrine concentrations more fully. Epinephrine concentrations decreased from time 0 to 0.5 h post-challenge before increasing at 1 h post-challenge. Therefore, in response to an endotoxin challenge, temperament may differentially affect the response of the adrenal medulla, not the adrenal cortex. This is supported by the report that more aggressive mice have greater concentrations of phenylethanolamine *N*-methyltransferase (PNMT), the enzyme that converts norepinephrine to epinephrine, in the adrenal medulla, with no differences in cortisol production.[62] However, there is no prior documentation on the effect of temperament on the stress hormone response to LPS challenge. Based on our survey of the relevant literature, this appears to be the first or at least among the earliest demonstration of the temporal epinephrine response of Brahman cattle to an endotoxin challenge. The peak epinephrine response of the Calm bulls is similar in magnitude to the response in young pigs, but occurred 45 min later.[17] Studies have suggested that epinephrine tolerance protects mice and dogs against endotoxin-induced shock.[63,64] Taking into consideration that the Temperamental bulls had greater basal concentrations of cortisol when compared to bulls of Calm and Intermediate temperament, the greater basal concentrations of cortisol may protect Temperamental bulls. A recent study by Frank *et al.*[65] found that pre-treatment with glucocorticoids prior to stimulation with LPS increased pro-inflammatory cytokines in the hippocampus, which may have priming actions of the nervous system and mediate the manner in which the mice respond to the inflammatory stimuli. However, this does not explain why epinephrine concentrations did not increase in response to the challenge with LPS. The response may indicate that Temperamental bulls did not require an increase in epinephrine in order to stabilize and maintain the cardiovascular system, as concentrations of epinephrine were already elevated. Thus, the epinephrine response exhibited by Calm bulls represents a greater response to stabilize the cardiovascular system in response to LPS challenge, a response that was not necessary for Temperamental bulls. The endocrine response of Temperamental bulls to LPS is unique and requires more attention to elucidate the mechanism in which temperament differentially affects the production of stress hormones by the adrenal gland.

Conclusions

Lipopolysaccharide induced the secretion of the stress hormones cortisol and epinephrine, and increased rectal temperature and sickness behavior. While rectal temperature, sickness behavior, and epinephrine differentially responded based on temperament, temperament did not affect the response of the adrenal cortex to endotoxin challenge. It is not yet clear whether this differential innate response to LPS (*i.e.* diminished febrile response, sickness behavior, and failure to produce an epinephrine response) is beneficial or potentially detrimental to either the near- or long-term health of Temperamental bulls. These data suggest that temperament of cattle should be accounted for in the design of experiments, as it may be a significant source of variation. Future studies should determine if temperament affects properties of both the innate and adaptive immune response to LPS challenge. A clearer understanding of the inter-relationship between stress hormones and the inflammatory process may lead to methods of early intervention to minimize the debilitating impacts of illness on growth and productivity.

Acknowledgements

Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. The authors acknowledge the technical support of D.A. Neuendorff, A.W. Lewis, L.C. Caldwell, A.N. Loyd, and J. Lyons. N.C. Burdick is a Tom Slick Senior Graduate Fellow at Texas A&M University, College Station, Texas.

Funding

This study was supported in part by Texas A&M AgriLife Research, USDA-NRI-CSREES Grant 2005-01671 and USDA SG 2006-34564-1700.

References

1. Smith RA. Impact of disease on feedlot performance: a review. *J Anim Sci* 1998; 76: 272–274.
2. Moberg GP. A model for assessing the impact of behavioral stress on domestic animals. *J Anim Sci* 1987; 65: 1228–1235.
3. Dobson H, Tebble JE, Smith RF and Ward WR. Is stress really all that important? *Theriogenology* 2001; 55: 65–73.

4. Shi Y, Devadas S, Greeneltch KM, Yin D, Allan Mufson R and Zhou JN. Stressed to death: implication of lymphocyte apoptosis for psychoneuroimmunology. *Brain Behav Immun* 2003; 17: S18–S26.
5. Siberman DM, Wald MR and Genaro AM. Acute and chronic stress exert opposing effects on antibody responses associated with changes in stress hormone regulation of T-lymphocyte reactivity. *J Neuroimmunol* 2003; 144: 53–60.
6. Compas BE, Connor-Smith J and Jaser SS. Temperament, stress reactivity, and coping: implications for depression in childhood and adolescence. *J Clin Child Adolesc Psychol* 2004; 33: 21–31.
7. Zhao J, Kim KD, Yang X, Auh S, Fu YX and Tang H. Hyper immune responses in neonates lead to increased morbidity and mortality after infection. *Proc Natl Acad Sci USA* 2008; 105: 7528–7533.
8. Galyean ML, Perino LJ and Duff GC. Interaction of cattle health/immunity and nutrition. *J Anim Sci* 1999; 77: 1120–1134.
9. Dhabhar FS. Stress-induced augmentation of immune function—the role of stress hormones, leukocyte trafficking, and cytokines. *Brain Behav Immun* 2002; 16: 785–798.
10. Duff GC and Galyean ML. Board-invited review: Recent advances in management of highly stressed, newly received feedlot cattle. *J Anim Sci* 2007; 85: 823–840.
11. Sorrells SF and Sapolsky RM. An inflammatory review of glucocorticoid actions in the CNS. *Brain Behav Immun* 2007; 21: 259–272.
12. Andreasen AS, Krabbe KS, Krogh-Madsen R, Taudorf S, Pedersen BK and Møller K. Human endotoxemia as a model of systemic inflammation. *Curr Med Chem* 2008; 15: 1697–1705.
13. Männel DN. Advances in sepsis research derived from animal models. *Int J Med Microbiol* 2007; 297: 393–400.
14. Webel DM, Finck BN, Baker DH and Johnson RW. Time course of increased plasma cytokines, cortisol, and urea nitrogen in pigs following intraperitoneal injection of lipopolysaccharide. *J Anim Sci* 1997; 75: 1514–1520.
15. Iwasaki Y, Taguchi T, Nishiyama M, et al. Lipopolysaccharide stimulates proopiomelanocortin gene expression in AtT20 corticotroph cells. *Endoc J* 2008; 55: 285–290.
16. Borghetti P, Saleri R, Mocchegiani E, Corradi A and Martelli P. Infection, immunity and the neuroendocrine response. *Vet Immunol Immunopathol* 2009; 130: 141–162.
17. Williams PN, Collier CT, Carroll JA, Welsh Jr TH and Laurenz JC. Temporal pattern and the effect of sex on lipopolysaccharide-induced stress hormone and cytokine response in pigs. *Domes Anim Endo* 2009; 37: 139–147.
18. Fordyce G, Dodt RM and Wythes JR. Cattle temperaments in extensive beef herds in northern Queensland. 1. Factors affecting temperament. *Aust J Exp Agric* 1988; 28: 683–687.
19. Curley Jr KO, Paschal JC, Welsh Jr TH and Randel RD. Exit velocity as a measurement of cattle temperament is repeatable and associated with serum concentration of cortisol in Brahman bulls. *J Anim Sci* 2006; 84: 3100–3103.
20. Curley Jr KO, Neuendorff DA, Lewis AW, Cleere JJ, Welsh Jr TH and Randel RD. Functional characteristics of the bovine hypothalamic-pituitary-adrenal axis vary with temperament. *Horm Behav* 2008; 53: 20–27.
21. King DA, Schuehle Pfeiffer CE, Randel RD, et al. Influence of animal temperament and stress responsiveness on the carcass quality and beef tenderness of feedlot cattle. *Meat Sci* 2006; 74: 546–556.
22. Oliphint R, Burdick N, Laurenz J, Curley K, Vann R and Welsh T. Relationship of temperament with immunization response of lymphocyte proliferation in Brahman bulls. *J Anim Sci* 2006; 84 (Suppl 2): 32 (Abstract 98).
23. Petherick JC, Doogan VJ, Venus BK, Holroyd RG and Olsson P. Quality of handling and holding yard environment, and beef cattle temperament: 2. Consequences for stress and productivity. *Appl Anim Behav Sci* 2009; 120: 28–38.
24. Voisinet BD, Grandin T, Tatum JD, O'Connor SF and Struthers JJ. Feedlot cattle with calm temperaments have higher average daily gains than cattle with excitable temperaments. *J Anim Sci* 1997; 75: 892–896.
25. Burrow HM, Seifert GW and Corbet NJ. A new technique for measuring temperament in cattle. *Proc Aust Anim Prod* 1988; 17: 154–157.
26. Hammond AC, Olson TA, Chase Jr CC, et al. Heat tolerance in two tropically adapted Bos taurus breeds, Senepol and Romosinuano, compared with Brahman, Angus, and Hereford cattle in Florida. *J Anim Sci* 1996; 74: 295–303.
27. Burdick NC, Banta JP, Neuendorff DA, et al. Interrelationships among growth, endocrine, immune, and temperament parameters in neonatal Brahman calves. *J Anim Sci* 2009; 87: 3202–3210.
28. Liebsch G, Linthorst ACE, Neumann ID, Reul JMHM, Holsboer F and Landgraf R. Behavioral, physiological, and neuroendocrine stress responses and differential sensitivity to diazepam in two Wistar rat lines selectively bred for high- and low-anxiety-related behavior. *Neuropschopharmacology* 1998; 19: 381–396.
29. Burdick NC, Carroll JA, Hulbert LE, et al. Relationships between temperament and transportation with rectal temperature and serum concentrations of cortisol and epinephrine in bulls. *Live Sci* 2010; 129: 166–172.
30. Elsasser TH, Richards M, Collier R and Hartnell GF. Physiological responses to repeated endotoxin challenge are selectively affected by recombinant bovine somatotropin administration to calves. *Domes Anim Endo* 1996; 13: 91–103.
31. Bieniek K, Szuster-Ciesielska A, Kaminska T, Kondracki M, Witek M and Kandeter-Szerszen M. Tumor necrosis factor and interferon activity in circulation of calves after repeated injection of low doses of lipopolysaccharide. *Vet Immunol Immunopathol* 1998; 62: 297–307.
32. Jacobsen S, Toelboell T and Andersen PH. Dose dependency and individual variability in selected clinical, haematological and blood biochemical responses after system lipopolysaccharide challenge in cattle. *Vet Res* 2005; 36: 167–178.
33. Borderas TF, de Passillé AM and Rushen J. Behavior of dairy calves after low dose of bacterial endotoxin. *J Anim Sci* 2008; 86: 2920–2927.
34. Reuter RR, Carroll JA, Dailey JW, Cook BJ and Galyean ML. Effects of dietary energy source and level and injection of tilmicosin phosphate on immune function in lipopolysaccharide-challenged beef steers. *J Anim Sci* 2008; 86: 1963–1976.
35. Carroll JA, Reuter RR, Chase Jr CC, et al. Profile of the bovine acute-phase response following an intravenous bolus-dose lipopolysaccharide challenge. *Innate Immun* 2009; 15: 81–89.
36. Waggoner JW, Löest CA, Mathis CP, Hallford DM and Petersen MK. Effects of rumen-protected methionine supplementation and bacterial lipopolysaccharide infusion on nitrogen metabolism and hormonal responses of growing beef steers. *J Anim Sci* 2009; 87: 681–692.
37. Waggoner JW, Löest CA, Turner JL, Mathis CP and Hallford DM. Effects of dietary protein and bacterial lipopolysaccharide infusion on nitrogen metabolism and hormonal responses of growing beef steers. *J Anim Sci* 2009; 87: 3656–3668.
38. Dinarello CA. Endogenous pyrogens: The role of cytokines in the pathogenesis of fever. In: Mackowiak PA (ed.) *Fever, Basic Mechanisms and Management*. New York: Raven, 1996, pp.22–48.
39. Steiger M, Senn M, Altreuther G, et al. Effect of a prolonged low-dose lipopolysaccharide infusion on feed intake and metabolism in heifers. *J Anim Sci* 1999; 77: 2523–2532.
40. Black PH. Stress and the inflammatory response: a review of neurogenic inflammation. *Brain Behav Immun* 2002; 16: 622–653.
41. Ochalski SJ, Hartman DA, Belfast MT, Walter TL, Glaser KB and Carlson RP. Inhibition of endotoxin-induced hypothermia

and serum TNF-α levels in CD-1 mice by various pharmacological agents. *Inflamm Res* 1993; 39 (Suppl 1): C52–C54.
42. Roth J, Rummel C, Barth SW, Gerstberger R and Hübschle T. Molecular aspects of fever and hyperthermia. *Immunol Allergy Clin North Am* 2009; 29: 229–245.
43. Carroll JA, Chase Jr CC, Coleman SW, Riley DG, Spiers DE and Reuter RR. Angus and Romosinuano steers exhibit differential acute phase response following an endotoxin challenge. *J Anim Sci* 2007; 85(Suppl 2): 26 (Abstract #72).
44. Steiner AA, Hunter JC, Phipps SM, et al. Cyclooxygenase-1 or -2 - which mediates lipopolysaccharide-induced hypothermia? *Am J Physiol* 2009; 297: R485–R494.
45. Romanovskey AA and Székely M. Fever and hypothermia: two adaptive thermoregulatory responses to systemic inflammation. *Med Hypoth* 1998; 50: 219–226.
46. Jüttler E, Inta I, Eigler V, et al. Neuronal NF-κB influences thermoregulation and survival in a sepsis model. *J Neuroimmunol* 2007; 189: 41–49.
47. Tolchard S, Burns PA, Nutt DJ and Fitzhjohn SM. Hypothermic responses to infection are inhibited by α-adrenergic agonists with possible clinical implications. *Br J Anaesth* 2009; 103: 554–560.
48. Kovalovsky D, Refojo D, Holsboer F and Arzt E. Molecular mechanisms and the Th1/Th2 pathways in corticosteroid regulation of cytokine production. *J Neuroimmunol* 2000; 109: 23–29.
49. Dantzer R. Cytokine-induced sickness behavior: where do we stand? *Brain Behav Immun* 2001; 15: 7–24.
50. Dantzer R. Cytokine-induced sickness behaviour: a neuroimmune response to activation of innate immunity. *Eur J Pharmacol* 2004; 500: 399–411.
51. Dantzer R and Kelly KW. Twenty years of research cytokine-induced sickness behavior. *Brain Behav Immun* 2007; 21: 153–160.
52. Fell LR, Colditz IG, Walker KH and Watson DL. Associations between temperament performance and immune function in cattle entering a commercial feedlot. *Aust J Exp Agric* 1999; 39: 795–802.
53. Grandin T. Assessment of stress during handling and transport. *J Anim Sci* 1997; 75: 249–257.
54. Burrow HM and Dillon RD. Relationships between temperament and growth in a feedlot and commercial carcass traits of. *Bos indicus* crossbreds. *Aust J Exp Agric* 1997; 37: 407–411.
55. Meikle AW, Stringham JD, Woodward MG and Bishop TD. Heritability of variation of plasma cortisol levels. *Metabolism* 1988; 37: 514–517.
56. Federenko IS, Nagamine M, Hellhammer DH, Wadhwa PD and Wüst S. The heritability of hypothalamus pituitary adrenal axis responses to psychological stress is context dependent. *J Clin Endocrinol Metab* 2004; 89: 6244–6250.
57. Mormède P. Genetic variation of HPA axis activity and function in farm animals. *Encyclopedia of Stress*, 2nd edn, 2007, pp.150–152.
58. Kahl S, Elsasser TH and Li CJ. Variability in tumor necrosis factor-alpha, nitric oxide, and xanthine oxidase responses to endotoxin challenge in heifers: effect of estrous cycle stage. *Domes Anim Endo* 2009; 36: 82–88.
59. Blecha F, Boyles SL and Riley JG. Shipping suppresses lymphocyte blastogenic responses in Angus and Brahman X Angus feeder calves. *J Anim Sci* 1984; 59: 576–583.
60. Rhodes RC, Randel RD and Long CR. Corpus luteum function in the bovine: in vivo and in vitro evidence for both a seasonal and breedtype effect. *J Anim Sci* 1982; 55: 159–167.
61. Massey JM and Odgen AJ. No seasonal effect on embryo donor performance in the southwest region of the USA. *Theriogenology* 1983; 21: 196–217.
62. Sorensen DB, Johnsen PF, Bibby BM, et al. PNMT transgenic mice have an aggressive phenotype. *Horm Metab Res* 2005; 37: 159–163.
63. Motsay GJ, Alho A, Jaeger T, Schultz LS, Dietzman RH and Lillehei RC. Effects of methylprednisolone, phenoxybenzamine, and epinephrine tolerance in canine endotoxin shock: study of isogravimetric capillary pressures in forelimb and intestine. *Surgery* 1971; 70: 271–279.
64. Baykal A, Kaynaroglu V, Hascelik G, Sayek I and Sanac Y. Epinephrine and endotoxin tolerance differentially modulate serum cytokine levels to high-dose lipopolysaccharide challenge in murine model. *Surgery* 1999; 125: 403–410.
65. Frank MG, Miguel ZD, Watkins LR and Maier SF. Prior exposure to glucocorticoids sensitizes the neuroinflammatory and peripheral inflammatory responses to *E. coli* lipopolysaccharide. *Brain Behav Immun* 2009; 24: 19–30.