Endotoxin exposure during late pregnancy alters ovine offspring febrile and hypothalamic–pituitary–adrenal axis responsiveness later in life

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Abstract
A growing number of studies indicate that maternal infection during pregnancy is associated with adverse fetal development and neonatal health. In this study, late gestating sheep (day 135) were challenged systemically with saline (0.9%) or Escherichia coli lipopolysaccharide endotoxin (400 ng/kg × 3 consecutive days, or 1.2 µg/kg × 1 day) in order to assess the impact of maternal endotoxemia on the developing fetal neuroendocrine–immune system. During adulthood, cortisol secretion and febrile responses of female offspring and the cortisol response of the male offspring to endotoxin (400 ng/kg), as well as the female cortisol response to adrenocorticotropic hormone (ACTH) challenge, were measured to assess neuroendocrine–immune function. These studies revealed that maternal endotoxin treatment during late gestation altered the female febrile and male and female cortisol response to endotoxin exposure later in life; however, the response was dependent on the endotoxin treatment regime that the pregnant sheep received. The follow-up ACTH challenge suggests that programing of the adrenal gland may be altered in the female fetus during maternal endotoxemia. The long-term health implications of these changes warrant further investigation.

Keywords: Endotoxin, fetal development, fever, hypothalamic–pituitary–adrenal axis, inflammatory stress, sheep

Introduction
The risk of certain diseases is increased during adulthood by prenatal exposure to maternal stressors such as undernutrition, psychological stress, metabolic disease, and bacterial infection (Drake et al. 2007; Hodyl et al. 2007a; Le Clair et al. 2009; Simeoni and Barker 2009). These conditions appear to disrupt the normal uterine environment and this can alter the programing of genes during critical windows of development that permanently affect the function of fetal organs or systems (Seckl and Holmes 2007).

Bacterial endotoxin lipopolysaccharide (LPS), a major component of the cell membrane of Gram-negative bacteria, has been used extensively to study neuroendocrine–immune interaction during bacterial infection because endotoxin elicits the release of pro-inflammatory cytokines that activate the febrile response as part of the host innate response (Roth 2006), and the autonomic nervous system and hypothalamic–pituitary–adrenal axis (HPAA), two important regulators of the host innate and acquired immune response (Karrow 2006). This model has also been used in studies designed to assess the impact of inflammatory stress associated with bacterial infection on the developing prenatal and neonatal rodent neuroendocrine–immune system (Shanks et al. 2000; Hodgson et al. 2001; Boissé et al. 2004; Ellis et al. 2005; Owen et al. 2005; Spencer et al. 2006; Walker et al. 2006; Hodyl et al. 2007a,b, 2008). These studies have revealed that the HPAA is susceptible to programing by endotoxin exposure during late gestation and neonatal development, and that this can affect regulation of the immune system (Shanks et al. 2000; Boissé et al. 2004; Ellis et al. 2005; Spencer et al. 2006; Walker et al. 2006; Hodyl et al. 2007a, 2008).
In the ovine species, there is evidence that the fetal HPAA is highly susceptible during late gestation to changes in the fetal environment because it is more responsive to increasing levels of circulating glucocorticoids (GCs) around parturition (Sloboda et al. 2008). Given this, we hypothesize, similarly, that the ovine HPAA will be sensitive to endotoxin-induced inflammatory stress during late gestation, and that this will alter HPAA responsiveness and the febrile response in the offspring later in life. Sheep are widely used as a model for immunological (Hein and Griebel 2003) and fetal HPAA programing studies (Chadio et al. 2007), and are susceptible to a variety of different Gram-negative bacterial infections during pregnancy and lactation (Sammin et al. 2006; Mavrogianni and Fthenakis 2007).

Materials and methods

Endotoxin challenge studies

Eighteen pregnant crossbred Rideau-Arcott sheep were used for the endotoxin challenge studies. These ewes were subjected to either a 2 ml i.v. bolus injection of saline or 400 ng/kg of *Escherichia coli* 055:B5 LPS endotoxin (Sigma-Aldrich, St Louis, MO, USA) between 9 and 11 h on days 135, 136, and 137 of pregnancy (400 ng/kg × 3 consecutive days) to simulate a more typical prolonged bacterial infection, or 1.2 μg/kg of LPS endotoxin on day 135 of pregnancy to model an acute infection (Figure 1); the average gestation cycle of this breed of sheep is 145 days. The timing of endotoxin exposure corresponded with the onset of fetal adrenal function, which is thought to occur beyond day 130 of gestation (Kabaroff et al. 2006a,b) and those studies were based on previous studies performed in our laboratory (Kabaroff et al. 2006a,b) and those previously reported in the literature (Grigsby et al. 2003). The offspring were weighed at birth and weekly until 5 months of age.

At 5 months of age, the offspring (eight females + four males from the saline treatment, six females + five males from the 1.2 μg/kg endotoxin treatment, and nine females + seven males from the 400 ng/kg endotoxin treatment × 3 consecutive days) were challenged with a 2 ml i.v. bolus injection of *E. coli* 055:B5 LPS endotoxin (400 ng/kg) to assess the effects of maternal endotoxin exposure on the juvenile HPAA (females and males) and febrile response (females) (Figure 1).

All animals were born and housed at the University of Guelph Ponsonby sheep research station in covered group pens. During the endotoxin challenge, animals were separately penned adjacent to each other, and given *ad libitum* access to water and feed. The University of Guelph Animal Care Committee approved the experimental protocol in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Measurement of the febrile response during endotoxin challenge

The Flir ThermaCAM SC 2000 infrared camera (Flir Systems, Burlington, Ont., Canada) was used to capture infrared images of the female offspring ear and eye immediately before, and 1, 2, 3, 4, 5, and 6 h post endotoxin challenge. The instrument was calibrated to the ambient temperature at a distance of 0.1 m and an emissivity of 0.98. Ear and eye temperatures were determined from the ThermaCAM images using the ThermaCAM Researcher 2000 software. Since insufficient numbers of male offspring were included in the infrared camera analysis to obtain statistical validity, their data have not been included in the manuscript.

Serum collection during endotoxin challenge and cortisol analysis

Blood samples were collected from the pregnant ewes and juvenile offspring at 5 months of age into vacutainers by jugular venous-puncture before, and at 2-h intervals post endotoxin challenge. The blood was allowed to clot at room temperature for 30 min, and the serum was obtained following centrifugation at 1122g for 15 min. Serum was stored at −80°C for cortisol analysis.

Cortisol concentrations were measured using a cortisol luminescent immune-assay kit (Pheonix Biotech Corp., Oakville, Ont., Canada). Briefly, 96-well NUNC brand microtiter plates that were coated with rabbit anti-cortisol antibody were incubated with 20 μl of serum in triplicate in conjunction with 100 μl horseradish peroxidase enzyme conjugate for 3 h. The plates were washed four times with 250 μl/well of the supplied 10% phosphate-buffered saline–Tween solution. The provided peroxide and luminal substrate solution was then added at 50 μl per well and incubated for 30 min at room temperature. Luminescence was measured using a Wallac Victor 3 plate reader (Perkin-Elmer, Woodbridge, Ont., Canada). The interassay coefficient of variation for the ewes and offspring was 15 and 9%, respectively, and the limit of detection for this assay was 0.414 nmol/l.

Figure 1. Experimental design for the ewe endotoxin challenge and subsequent offspring endotoxin challenge.
Adrenocorticotropic hormone challenge

An adrenocorticotropic hormone (ACTH) challenge was performed on individually penned female offspring from the saline control and 1.2 μg/kg endotoxin treatment groups at 18 months of age to assess responsiveness of the adult female adrenal glands to this stress hormone (Figure 1). Females were selected based on their cortisol responsiveness to the previous endotoxin trial and only the female offspring exhibiting high cortisol responses were chosen. Therefore, the female offspring born to mothers treated with 1.2 μg/kg endotoxin were retained while the low cortisol responding females born to mothers treated with 400 ng/kg endotoxin × 3 days were removed from the flock. Male offspring were removed from the trial and therefore were not under an ACTH challenge because the research facility could not accommodate housing for these individuals. Animals were challenged with a 2 ml bolus of 0.25 μg/kg of ACTH (Sigma-Aldrich), and serum was obtained 0, 0.25, 0.5, 1, 2, and 4 h post challenge for cortisol analysis as described above. The interassay coefficient of variation for the offspring was 7%.

Statistical analysis

The experimental design for the ewe response was a completely randomized design, with three treatments (saline treatment and 400 ng/kg endotoxin treatment on days 135, 136, and 137; 1.2 μg/kg endotoxin treatment on day 135). Mixed model ANOVAs were used to analyze the repeated measurements on cortisol over time for each ewe, according to the method given by Wang and Goonewardene (2004), and the best-fitting covariance structure over time was determined according to the Akaike criterion (Kuehl 1999; Wang and Goonewardene 2004). Linear and quadratic orthogonal polynomial contrasts were assessed over time, and treatment differences in time trends were assessed from interactions among the treatment and time contrasts.

The experimental design for the offspring response was also based on this completely randomized design with the three treatments applied to the ewes (saline control, 1.2 μg/kg endotoxin treatment, and 400 ng/kg treatment for 3 consecutive days), but included the offspring of each ewe as a sub-sampling effect, and offspring gender (for cortisol data) in a factorial arrangement with treatments. The cortisol and febrile responses to the endotoxin challenge for the offspring were assessed in the same way as for the ewes, and mixed models were used to analyze repeated temperature and cortisol measurements over time for each offspring per ewe, as described above. Linear and quadratic orthogonal polynomial contrasts were used to assess cortisol and febrile responses over time, and interactions of these with treatments and gender (for cortisol data) were used to compare the changes in cortisol and febrile responses over time among treatments and gender.

The model used for the analysis of offspring cortisol response to ACTH was similar to that described for offspring cortisol response to the endotoxin challenge, except that only two treatment groups of ewes were used, saline control and 1.2 μg/kg endotoxin treatment, and gender was not included in the model since only female offspring were measured.

All statistical analyses were performed using the SAS® software system. The MIXED procedure was used for all analyses. Residual plots were examined for all analyses, and showed no evidence of variance heterogeneity. Results were considered to be significantly different at \( P < 0.05 \), and trends were reported at \( P < 0.10 \).

Results

HPAA response of ewes challenged with endotoxin

Repeated measures over time demonstrated that serum cortisol concentrations increased significantly over time on day 1 \((P < 0.01, F = 29.60, df = 2)\) for the endotoxin-challenged ewes. Peak cortisol concentrations were measured around 2 h post challenge for the ewes in the 1.2 μg/kg endotoxin treatment group, and around 4 h post challenge for the ewes that received 400 ng/kg endotoxin (Figure 2(A)). The ewe cortisol response to the 400 ng/kg endotoxin treatment on days 2 and 3 was also significant \((P < 0.02, F = 7.48, df = 1)\) but attenuated and shorter in duration when compared to the first day of challenge (Figure 2(B),(C)).

Offspring survival and body weights

All the offspring survived the studies, and none were born premature as a result of the maternal endotoxin challenge. Birth weights and body weight gain throughout the trial were similar among the three treatment groups (data not shown).

Female offspring febrile response to endotoxin challenge at 5 months of age

Repeated measures mixed model ANOVAs demonstrated that all female offspring responded to the endotoxin challenge with a change in body temperature. Orthogonal polynomial contrasts revealed significant linear \((\text{eye} = P < 0.01, F = 11.46, df = 1)\) and quadratic \((\text{ear} = P < 0.01, F = 15.36, df = 1)\) contrasts for endotoxin-challenged ewes. The eye temperature response increased following endotoxin challenge and remained elevated at 6 h post challenge. In contrast to the eye temperature measurements, there was a significant decrease in the ear temperature from \(31.01^\circ C \pm 1.03\) to \(21.83^\circ C\)
The ear temperature then returned to, and eventually exceeded the basal ear temperature (36.94 ± 0.37 °C) between 4 and 6 h.

A significant treatment interaction was detected for the mean eye temperature response ($P < 0.01$, $F = 11.81$, df = 2; Figure 4), but not the mean ear temperature response (statistics not shown). Females in the 1.2 μg/kg endotoxin treatment group, for example, exhibited a greater mean eye temperature than females from the group given 400 ng/kg endotoxin for 3 consecutive days ($P < 0.01$, $F = 22.75$, df = 1).

**Juvenile offspring cortisol response during the endotoxin challenge at 5 months of age**

Repeated measures mixed model ANOVA demonstrated that quadratic trends over time revealed that all offspring responded to the endotoxin challenge with an increase in serum cortisol concentration ($P < 0.01$, $F = 135.92$, df = 1; Figure 5(A)). For the females in all treatments and the control males, this increase peaked approximately 2 h post challenge. In contrast, the peak responses of the males that had been subjected to maternal endotoxin challenge occurred later, and they were not significantly different from each other (Figure 6(A)). Linear trends over time were significantly different between the average of the endotoxin treatments and the control group ($P < 0.01$, $F = 7.85$, $P < 0.01$, $F = 11.81$, df = 2; Figure 4).

**Figure 2.** Ewe serum cortisol response (A–C) during challenge with 400 ng/kg of endotoxin for three consecutive days (mENDO-400) ($n = 7$), 1.2 μg/kg of endotoxin (mENDO-1.2) ($n = 5$), or saline (mSALINE) ($n = 6$) beginning on day 135 of gestation. Data are presented as least square mean ± SE. Day 1 treatment differences were reported at $P \leq 0.01$; days 2 and 3 treatment differences were reported at $P \leq 0.02$.

**Figure 3.** Combined ear and eye temperature response over time of juvenile offspring ($n = 39$) from all three treatment groups challenged i.v. with endotoxin (400 ng/kg). Data are presented as least square mean ± SE. Significant differences were seen in both quadratic and linear responses over time at $P \leq 0.01$.

**Figure 4.** Temperature responses of juvenile female offspring to an acute endotoxin challenge: prenatal treatment interactions for eye (A) and ear (B) temperature responses. Mothers were challenged with 1.2 μg/kg of endotoxin (nENDO-1.2) ($n = 3$ females for eye and ear temperature), or 400 ng/kg of endotoxin (nENDO-400) ($n = 4$ females for eye and ear temperature) or saline (nSALINE) ($n = 3$ females for eye and ear temperature) for three consecutive days beginning day 135 of gestation. Data are presented as least square mean ± SE.
df = 1; Figure 5(A)), and between the two endotoxin treatment groups (P < 0.05, F = 3.86, df = 1; Figure 5(B)). Sex by treatment interactions demonstrated a higher trend in the cortisol response of female offspring from mothers treated with 1.2 μg/kg endotoxin during gestation compared to female offspring from mothers treated with 400 ng/kg endotoxin for three consecutive days (P < 0.08, F = 3.24, df = 1; Figure 5(B)).

Cortisol response of adult female offspring from the saline control and 1.2 μg/kg endotoxin treatment groups to ACTH challenge at 18 months of age

Linear and quadratic trends over time revealed that all female offspring responded to the ACTH challenge with an increase in serum cortisol concentration over time (P < 0.01, $F_{\text{linear}} = 20.39$, $F_{\text{quadratic}} = 97.99$, df = 1; Figure 6). Serum cortisol concentrations peaked 15 min post challenge for both treatment groups, and returned to basal levels within 2 h. The offspring peak mean ± SEM cortisol concentrations were mean ± SEM, 220 ± 20 nmol/l and 161 ± 17 nmol/l for the 1.2 μg/kg endotoxin treatment and saline control, respectively. Repeated measures mixed model ANOVAs demonstrated quadratic trends over time for the treatment by time interaction revealing an increased trend in the cortisol response of female offspring from the 1.2 μg/kg endotoxin treatment compared to the saline control offspring (P = 0.06, F = 4.43, df = 1).

Discussion

The purpose of this study was to investigate the effects of maternal endotoxin-induced inflammatory stress on programing of the fetal ovine HPAA and febrile response, as well as assess potential target tissues susceptible to fetal programing. The maternal exposure period used in this study was selected on the basis of major developmental changes that occur within the HPAA during this period (Liggins and Thorburn 1994). Results of the present study demonstrated that maternal endotoxin exposure induces temporal changes in the female offspring febrile response to the bacterial endotoxin challenge later in life, and these changes were dependent on the endotoxin treatment regime. As expected, eye temperatures were increased in the female offspring challenged with endotoxin. However, an unexpected simultaneous decrease and then increase in ear temperature was also observed in these animals. This change in ear...
temperature was likely attributed to vasoconstriction associated with fever (Blatteis 2006). Lowe et al. (2005) also demonstrated that ear temperature was reduced in sheep following exercise-induced and isolation stress; they attributed this to input from the sympathetic nervous system (SNS), since activation of the SNS during stress has been shown to induce vasoconstriction in the body’s extremities and skin surface causing a redistribution of blood from the peripheral capillary vessels to more vital central regions (Hilton 1982; Bell et al. 1983; Reichlin 1993; Blatteis 2006).

Programing of the offspring febrile response appeared to be treatment specific, with female offspring from 1.2 µg/kg endotoxin-treated mothers having a significantly greater eye temperature response to endotoxin challenge than female offspring from mothers treated with 400 ng/kg endotoxin for three consecutive days. This suggests that the dose, duration of endotoxin exposure, or the combination of both, influence programing of the febrile response in female sheep.

Results of the present study also demonstrated that maternal endotoxin exposure induces temporal and gender-specific changes in the offspring HPAA response to endotoxin challenge later in life; these changes were also dependent on the endotoxin treatment regime. The overall HPAA response of males subjected to maternal endotoxin challenge was greater regardless of the endotoxin treatment. Prolonged activation of the HPAA in both endotoxin treatments suggests that mechanisms regulating cortisol concentration in the circulation may have been affected in the male offspring. Further studies are underway to investigate if this hypercortisol responsiveness is due to changes in the expression of GC receptors in the brain, and/or differences in the concentration and activity of enzymes and binding proteins that regulate cortisol bioavailability.

The HPAA response of female offspring was also affected by maternal endotoxin exposure; however, the response was different for each endotoxin treatment. Female offspring from mothers subjected to 1.2 µg/kg endotoxin, for example, showed cortisol hyperresponsiveness when subjected to endotoxin later in life, whereas females from mothers subjected to 400 ng/kg × 3 consecutive days of endotoxin were hyporesponsive. The hyperresponsive cortisol status of the female offspring from the 1.2 µg/kg maternal endotoxin treatment was later confirmed during adulthood by ACTH challenge. These results suggest that fetal programing was disrupted at the level of, or immediately downstream from the adrenal cortex in these female offspring. A study from Chadio et al. (2007) provides support to the current results. In this study, a CRF challenge (0.5 mg/kg) was used to assess the responsiveness of the HPA axis in lambs born to undernourished mothers. They found that, at 5.5 months of age, offspring from the undernourished group had a significantly higher cortisol response compared to those of the control group (Chadio et al. 2007). Weaver et al. (2000) also found increased cortisol binding globulin (CBG), decreased cortisol concentrations, and increased plasma ACTH concentrations in boars at the time of slaughter after they had been handled during critical postnatal periods. They reported that these results might be related to changes in the adrenal response to ACTH.

Previous studies have shown that GCs play an important role in regulating the febrile response. Adrenalectomized rats, for example, exhibited an augmented febrile response to IL-1β and this was controlled by dexamethasone treatment (Watanabe et al. 1995). Likewise, guinea pigs implanted with cortisol-releasing pellets for 10 days exhibited an attenuated febrile response to endotoxin (Roth 2006).

Numerous studies have investigated the effects of maternal or neonatal stress on programing of the febrile and HPAA responses. However, the different stressors and stress challenge levels, the duration and timing of stress, and the species used in these studies have made it difficult to formulate hypotheses on the mechanisms involved in developmental programing of the febrile and HPAA responses. Galic et al. (2009) recently reviewed potential mechanisms related to neonatal programing. In the context of prenatal programing, a study with guinea pigs showed that maternal endotoxin treatment (50 µg/kg) on gestation days 46, 48, 50, and 52 was sufficient to attenuate the cortisol response to endotoxin challenge in the adult female offspring, but not in male offspring (Hodyl et al. 2007a). The authors also showed a unique drop in body temperature 3 h post challenge in offspring from mothers that had received the endotoxin during pregnancy. Other guinea pig studies have demonstrated that strobe light stress during gestational days 50–52 (PS50) and days 60–62 (PS60) was sufficient to attenuate stress-induced cortisol concentrations in adult females, but this was only detected during the estrus phase of their reproductive cycle (Kapoor and Matthews 2008); in contrast, male PS50 offspring exhibited increased basal plasma cortisol concentrations and male PS60 offspring were hypercortisol responsive to HPAA activation (Kapoor et al. 2008).

In an earlier study of effects of exposing pregnant rats to endotoxin (30 µg/kg) or human red blood cell (HRBC), adult male offspring from both treatments exhibited increased basal corticosterone concentrations, whereas only male offspring from the HRBC treatment exhibited increased ACTH and corticosterone concentrations after being subjected to novelty stress (Reul et al. 1994). In pigs, 8-week-old female offspring born to sows that had been treated with hydrocortisone acetate during early and late pregnancy exhibited a heightened febrile
response to endotoxin (de Groot et al. 2007). In contrast, the cortisol response to endotoxin of the offspring was not significantly impacted by the maternal cortisol treatment, but Kranendonk et al. (2006) demonstrated that female offspring had an exacerbated cortisol response to ACTH challenge at 6 weeks of age.

In the present study, we demonstrated that the female offspring from the 400 ng/kg for three consecutive days treatment exhibited attenuated eye temperature and HPAA responses to acute endotoxin when compared to the other prenatal treatment groups used in this study. Since both of these responses are triggered by prostaglandin-E2, acting through G-protein-coupled receptors (Furuyashiki and Narumiya 2009), and GCs contributing to the regulation of the febrile response (Roth 2006), it seems reasonable that an attenuated febrile response would be accompanied by an attenuated cortisol response. In contrast, however, female offspring from the 1.2 µg/kg endotoxin treatment appeared to be cortisol hyperresponsive, yet no significant attenuation of eye temperature was observed. We can only speculate at this time why this occurred. If the febrile response were down-regulated by threshold levels of circulating cortisol, it might be possible for the febrile response to be normally down-regulated even in the presence of excessive or sustained circulating cortisol concentrations. A study by Kanashiro et al. (2009), however, does not support this hypothesis, since dose-dependent anti-pyretic activity for dexamethasone was demonstrated using a zymozan-induced arthritis rat model. Another possibility is that the concentration of CBG, which controls the bioavailability of cortisol, was affected by the endotoxin treatments (Hodyl et al. 2007a). If this were the case, then measurements of total cortisol in this study may not accurately reflect concentrations that are bioavailable for regulating the febrile response. Lastly, it is possible that other mechanisms are involved in regulating the febrile response independent of cortisol concentration; these may include neural input from the vagus and splenic nerves (Rosas-Ballina et al. 2008), circulating prostaglandin-E2, and pro- and anti-inflammatory cytokines (Blatteis 2006).

Some of the observed gender differences in this study are likely attributed to sex-related differences in sensitivity to fetal stress. Zagron and Weinstock (2006), for example, demonstrated that mild restraint stress during pregnancy exacerbated female rat offspring anxiogenic behavior, while in males, spatial learning was impaired. Adrenal steroids were responsible for changes in these hippocampus-associated behaviors, since maternal adrenallectomy completely abolished the anxiety and learning defects (Zagron and Weinstock 2006). In contrast, another recent rat study demonstrated that a more severe restraint stress protocol during pregnancy led to increased male offspring anxiety-like behavior and decreased a number of neurobiological parameters (Zuena et al. 2008). However, female offspring from this study had reduced anxiety-like behavior and increased spatial learning. While the results from these two studies appear conflicting, they demonstrate that variation in the severity and timing of stress application during gestation can lead to dramatic changes in fetal programing.

Aside from the work of Hodyl et al. (2007a,b) and Reul et al. (1994), we are unaware of any other studies that have investigated the effects of maternal endotoxin exposure on programing of the offspring HPAA response, and none have been carried out using sheep. The classic studies carried out by Shanks and Meaney (1994) and Shanks et al. (1995) demonstrated that neonatal exposure to endotoxin leads to HPAA hyperactivity in response to restraint stress in adult rats, and that gonadal steroids contribute to gender differences in endotoxin-mediated programing of the HPAA. These authors suggested that the prolonged GC response to restraint stress was attributed to decreased negative-feedback sensitivity to GCs because the response was associated with reduced GC receptor density within various HPAA regulatory regions of the brain (Shanks et al. 1995). It is possible that changes in GC receptor binding and density may account for the endotoxin-induced changes seen in the present ovine study and this should be explored further in future studies, especially in the cortisol hyperresponsive male offspring where mechanisms that regulate circulating concentrations of cortisol may have been affected.

It is unclear from the present study if fetal programing of the HPAA is due to fetal stress, secondary to increased exposure to maternal cortisol during endotoxemia, or a combination of both possibilities. Kabaroff et al. (2006a,b) demonstrated in pregnant sheep that the HPAA response to systemic endotoxin challenge (400 ng/kg) was not attenuated, as previously demonstrated in rodent studies (Neumann 2001), but rather the cortisol response was exaggerated. This raises the possibility that fetal programing of the HPAA may be attributed to exposure to maternal or placental GCs. However, Grigsby et al. (2003) demonstrated that fetal hypoxia occurs in response to maternal endotoxin challenge (2 µg/kg) at 130 days of gestation. This was associated with sustained increases in fetal plasma cortisol and PGE2 concentrations, which are suggestive of fetal stress, and these increases persisted for up to 48 h after maternal endotoxin challenge. In the end, it is likely that both possibilities contribute to fetal programing of the HPAA, and that their contribution varies depending on the endotoxin dose, treatment regime, as well as timing of exposure.

In summary, the present ovine study has demonstrated that maternal exposure to endotoxin
(1.2 μg/kg or 400 ng/kg of endotoxin × 3 days) during late pregnancy induces temporal-specific changes in the offspring febrile and HPAA response to the bacterial endotoxin challenge later in life, and that these changes are dependent on the endotoxin treatment regime. Future studies are needed to investigate if these changes are permanent, how they occur, and if they influence susceptibility to disease during adulthood.

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