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Twenty-four-hour profiles of metabolic and stress hormones in sheep selected for a calm or nervous temperament

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ABSTRACT

Even in the absence of stressors, temperament is associated with changes in the concentration of stress-responsive hormones and, possibly because of such changes, temperament can affect metabolism. We tested whether, in sheep bred for temperament for 14 generations, "nervous" females have greater concentrations of stress-responsive hormones in the absence of stressors than "calm" females, and whether these differences are associated with changes in the concentrations of metabolic hormones. In resting "calm" (n = 8) and "nervous" (n = 8) sheep, concentrations of cortisol, prolactin, leptin, and insulin were measured in blood plasma sampled via jugular catheter every 20 min for 24 h. The animals were individually penned, habituated to their housing and human handling over 7 wk, and fed before sampling began. Diurnal variation was evident for all hormones, but a 24-h cortisol pattern was detected in only 7 individuals. There was no effect of temperament on any aspect of concentrations of cortisol or prolactin, but "calm" animals had greater concentrations of insulin in the early afternoon than "nervous" animals (14.5 ± 1.1 vs 10.0 ± 1.6 μU/mL; $P = 0.038$), and a similar tendency was seen for leptin ($P = 0.092$). We conclude that selection for temperament affects the concentration of metabolic hormones in the absence of stressors, but this effect is independent of stress-responsive hormones.

1. Introduction

Temperament determines, at least in part, how an individual responds to stressful situations and can vary widely even within species and genders [1]. A highly responsive temperament can change the pattern of secretion of the stress-responsive hormones such as cortisol and prolactin in the presence of stressors [1–3] but also in the absence of stressors [1–3]. Furthermore, temperament can also affect metabolism [4–7]; however, the mechanisms behind these relationships are not well understood.

Temperament is reported to be affected by the diurnal pattern of the pulsatile secretion of cortisol in rats and macaques [8,9]. Prolactin secretion is also affected by temperament, but most studies have investigated the prolactin response to stimuli, rather than in the absence of stressors [10–13]. The magnitude of the prolactin response to stimuli is correlated with temperament in humans and cattle [10–13], and, in humans, prolactin responses to stimuli are positively correlated with the cortisol response

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[13–15]. However, to our knowledge, the impact of temperament on the diurnal pattern of prolactin secretion in the absence of stressors, in relationship with the activity of the hypothalamic-pituitary-adrenal (HPA) axis, has not been investigated.

Differences in the diurnal pattern of stress-responsive hormones could help to explain the apparent effects of temperament on metabolism because cortisol directly affects the circulating concentrations of the 2 primary metabolic hormones, insulin and leptin. Glucocorticoids stimulate leptin secretion [16,17], and acute increases in cortisol concentrations suppress insulin secretion in response to nutrient intake [18,19]. The role of prolactin as a regulator of metabolism is still being elucidated but, in rodents and humans, prolactin is reported to stimulate and potentiate leptin secretion [20–25], although a suppressive effect has also been found [25–27]. Therefore, cortisol and prolactin can affect the secretion of metabolic hormones, but it is not known whether an increased responsiveness to stressors can increase the concentrations of the stress-responsive hormones in a nonstressed state, and thereby lead to changes in metabolic hormone balance.

To further understand the relationship between temperament, the resting state of the HPA axis, and metabolic hormones, we measured the concentrations of cortisol, prolactin, insulin, and leptin in sheep that had been selectively bred to be of "calm" or "nervous" temperament on the basis of hyporeactivity or hyperreactivity to isolation and human presence. The diurnal patterns of secretion were described in the absence of any additional stressors (ie, at rest). We hypothesized that, compared with "calm" animals, "nervous" animals will have the following: (1) greater plasma cortisol concentrations, with less pronounced variation over 24 h; (2) greater plasma concentrations of prolactin; (3) greater plasma concentrations of leptin, with leptin values greatest after the peak in the patterns of cortisol and prolactin concentrations; and (4) decreased plasma concentrations of insulin.

2. Methods

2.1. Experimental design

This experiment was carried out in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (7th Edition, 2004) and was approved by the Animal Ethics Committee of The University of Western Australia (RA/3/100/333).

2.2. Animals

The University of Western Australia temperament flock comprises 2 lines of Merino sheep that have been bidirectionally selected for 14 generations for extreme behavioral reactions to the stressors of social isolation and human presence. This phenotypic trait is heritable [28]. Locomotor activity and vocalizations are measured in 2 tests, the isolation box and novel arena tests, as described elsewhere [29,30]. Mobility and vocalization measures are used to calculate a temperament score, and hyporeactive sheep are termed "calm," whereas hyperreactive sheep are termed "nervous" [28,29]. For the present experiment, 2-year-old ewes were selected at random from the calm ($n = 8$) and nervous ($n = 8$) lines of the population. These animals had divergent scores in the arena test (Cross: calm 6.3 ± 2.16 vs nervous 18.4 ± 4.58 crosses; Bleat: calm 5.3 ± 3.36 vs nervous 39.4 ± 13.70) and isolation box text (Box: calm 24.5 ± 10.13 vs nervous 71.8 ± 7.70) conducted at weaning. Furthermore, these animals were subjected to the isolation box test for 1 min, 2 d before commencing habituation to the animal house.

For the duration of the experiment, animals were individually penned indoors, in 1 room, for 7 wk, beginning on November 16 (mid-nonbreeding season), at the University of Western Australia (31′ 58° S). Photoperiod was 12 light:12 dark with lights on at 6 AM. The animals were subjected to minimal human contact and were under constant video surveillance. Pen cleaning and feeding followed a regular schedule, with the animals fed before 7 AM daily. Animals were fed a diet of hammer-milled oaten chaff with 20% lupin seed and 2.5% mineral mixture (Siromin; Narrogin Mineral Stockmix, Narrogin, WA, Australia) providing 9.2 MJ of metabolizable energy and 13% crude protein per kg DM. Each animal was fed enough to maintain body weight. Feed intake was monitored, and all animals finished their daily ration within 90 min.

2.3. Sampling

After 38 d of habituation, the animals were fitted with an indwelling jugular cannula with an extension to the shoulder. The next morning, the animals were fed at 6 AM. We decided to feed the animals before the commencement of sampling because although feeding stimulates secretion of leptin and insulin, missing a feed is a stressor, and not feeding the animals can potentially lead to hypoglycemia, which can trigger cortisol and prolactin responses and disrupt the normal circadian rhythm of these hormones [31,32].

Blood sampling commenced at 7 AM and continued every 20 min for 24 h. Lights were kept on for sampling at night. Samples were centrifuged at 2000 g for 10 min so that plasma could be separated and stored in plastic tubes at −20°C until assay.

2.4. Hormone analysis

2.4.1. Cortisol

Plasma cortisol concentrations were measured in duplicate 100-μL aliquots [33]. The limit of detection was 0.20 ng/mL. Six replicates of 2 control samples were included in the assay to estimate the intra-assay coefficients of variation of 7.9% at 3.8 ng/mL and 13% at 2.5 ng/mL.

2.4.2. Prolactin

Plasma prolactin was measured with a homologous double antibody radioimmunoassay (RIA) [34]. The samples were assayed in duplicate 10-μL aliquots, and the limit of detection was 0.26 ng/mL. The assay included 6 replicates of 3 control samples, which were used to estimate the