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Influence of feeding status, time of the day, and season on baseline adrenocorticotropic hormone and the response to thyrotropin releasing hormone-stimulation test in healthy horses

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ABSTRACT

Equine pituitary pars intermedia function can be assessed by the measurement of baseline and thyrotropin releasing hormone (TRH)-induced concentrations of adrenocorticotropic hormone (ACTH); however, these measurements may be affected by the environment. Therefore, a prospective observational study evaluated the influence of feeding, time of the day, and season on baseline and TRH-induced concentrations of ACTH in healthy horses. Baseline ACTH was measured in 50 horses before and 2 h after feeding. Six research horses were subjected to a crossover study in which 6 TRH tests were performed in 2 different seasons, March-April (MA) and July-September (JS), at 2 different times of the day, 8 AM and 8 PM, and, under 2 different conditions relative to feeding status, fasted and 2 h after feeding. Differences between fasted and fed horses were found in baseline ACTH, 17.1 ± 1.8 versus 46.1 ± 7.6 pg/mL ($P = 0.003$) and TRH-stimulated ACTH: 124.1 ± 21.3 versus 192.6 ± 33.1 pg/mL ($P = 0.029$) at 10 min, and 40.1 ± 4.9 versus 73.2 ± 13.4 pg/mL ($P = 0.018$) at 30 min post TRH injection. No differences were found between tests performed at different times of the day. Basal ACTH concentrations were greater in JS than in MA, 17.1 ± 1.8 versus 11.9 ± 0.6 pg/mL ($P = 0.006$). A seasonal influence was also found in stimulated ACTH values, which were much greater in JS 122.7 ± 36.7 versus 31.2 ± 7.4 pg/mL, at 10 min ($P = 0.03$) and 39.0 ± 7.2 versus 19.8 ± 3.1 pg/mL, at 30 min ($P = 0.03$). In addition to season, feeding is a potential confounding factor when measuring baseline or stimulated ACTH in horses. In conclusion, feeding status should be standardized for the diagnosis of equine pituitary pars intermedia dysfunction.

1. Introduction

Diagnosis of equine pituitary pars intermedia dysfunction (PPID), the most common endocrine disorder of aged horses, is generally established on the basis of typical clinical signs and hormonal measurements, mainly quantification of plasma adrenocorticotropic hormone (ACTH) concentration, which is increased in horses with PPID. The use of ACTH measurements for the diagnosis of PPID has been validated in numerous studies [1–3]. However, high variability and wide reference ranges have been reported and the results of ACTH measurements may be confounded by many factors such as stress [4], disease [5], or exercise [6]. In addition, measurement of resting plasma ACTH concentration for the purpose of the diagnosis of PPID lacks sensitivity and specificity in younger horses with earlier stages of the condition. Thus, simple resting plasma ACTH may be inaccurate for horses with subclinical disease [7,8].

The activity of the pars intermedia (PI) is stimulated by the thyrotropin-releasing hormone (TRH), likely via stimulation of TRH R1 receptors located in the PI and pars

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distalis of the pituitary gland [9]. An increase in ACTH concentration after TRH administration has been reported both in clinically normal horses and in horses with PPID [7], but ACTH concentrations were significantly greater in the latter. The ACTH response to TRH has been found to be useful in diagnosis of PPID, particularly in horses with baseline ACTH concentrations within reference ranges [7,8,10].

Interpretation of any PPID diagnostic test is complicated by seasonal variation in the activity of the pituitary-adrenal axis in the horse [11]. Plasma concentrations of the PI hormones, including ACTH, increase as daylight decreases from summer solstice to autumnal equinox [2], an adaptation that has been suggested to prepare the animal for the winter [12]. In the past, seasonal influence was considered a problem for diagnosis of PPID and measuring ACTH in autumn was not recommended. However, several authors have demonstrated that using appropriate cut-off values for this time of the year, autumn is in fact the season when ACTH measurement has a higher sensitivity and specificity for the diagnosis of PPID [3,13,14]. Although seasonal influence on baseline plasma ACTH is well known [2,11,12,14–17], its impact on TRH stimulation tests has not been studied in detail [7,15].

Many other factors that may influence ACTH measurements and TRH stimulation tests have not been thoroughly investigated in horses. Circadian variation of ACTH levels has been studied in 3 recent articles [13,18,19]. However, the influence of time of the day over TRH stimulation tests is unknown. This is important because in humans a circadian effect has been demonstrated in combined stimulation tests with Gonadotropin-Releasing Hormone, TRH, and ACTH [20]. An increase in both ACTH and cortisol after feeding has been reported in rats [21,22] and humans [23,24]. However, to our knowledge, food-related effects on baseline and stimulated plasma ACTH concentrations have not been investigated in horses.

The objective of this study was to evaluate the influence of season, time of the day, and feeding on baseline plasma ACTH concentrations and on plasma ACTH concentrations measured after TRH stimulation in healthy horses.

2. Materials and methods

2.1. Animals

This study was approved by the Ethical Committee for Animal Research of the University of Cordoba. Client-owned horses were included with informed consent. Fifty privately owned horses: 12 mares, 10 geldings, and 28 stallions, aged 9.7 ± 0.7 (mean ± Standard Error) yr, belonging to a stud farm, and 6 research horses: 3 mares, 2 geldings, and 1 stallion, aged 9.2 ± 1.4 (mean ± Standard Error) yr, belonging to the University of Cordoba, were studied. The research horses were all Andalusians and the privately owned horses comprised: 32 Andalusians, 14 Crossbreds, 3 Arabians, and 1 Thoroughbred. The diet of all these horses consisted of alfalfa hay administered twice daily, although some of the stud farm horses were supplemented with concentrate, depending on the horses' reproductive status. Alfalfa hay consumed by research horses through all the study period came from the same batch. Horses from the stud farm were kept in stalls during most of the day with daily turnout for exercise. Research horses lived in a dirt (sand) paddock all the year round. The privately owned horses were used to obtain samples for measurement of nonstimulated ACTH concentrations and the research horses to perform the TRH stimulation tests. Horses were healthy based on clinical examination and periodical blood tests. None of them had clinical or biochemical signs of PPID or other endocrine disease (normal leukogram and normal plasma concentrations of glucose and insulin). Basal ACTH was <20 pg/mL in all research horses and they had a normal TRH response when tested in springtime (Fig. 1).

2.2. Study design

2.2.1. The effect of feeding on baseline ACTH

Two blood samples were obtained from 50 horses by jugular venipuncture. The first sample was drawn after the horses had been fasted for 12 h, at 8 AM. Subsequently, horses were fed 3 kg of alfalfa hay and 2 h later, after the hay had been consumed, a second sample was obtained at 10 AM.

2.2.2. The effect of feeding, time of the day, and season on TRH stimulation tests

The same 6 research horses were used throughout the entire study. A crossover study design in which each horse was tested 6 times was performed: 4 tests were conducted between July and September 2012 (JS) and 2 tests were performed during the months of March and April 2013 (MA).

In the first period (JS), each horse was tested twice in the morning and twice in the evening. The 2 morning tests were carried out at 8 AM, one with the horses fasted for 12 h (FAST) and the other 2 h after being fed 3 kg of alfalfa hay (FOOD). The 2 evening tests were conducted at 8 PM and the horses were also tested after fasting for 12 h (FAST), and 2 h after being fed 3 kg of alfalfa hay (FOOD).

In the second period (MA), TRH stimulation tests were performed only in the mornings (8 AM) and each horse was tested twice, fasted (FAST), and after being fed (FOOD) (Fig. 2).

A minimum of 2-wk washout period was elapsed between TRH stimulation tests. To avoid stress-associated artefacts, before the experiments horses were accustomed to the fasting-feeding protocol and the routine of being handled and confined in a stall during the time required to perform the experiments. Horses had free access to water during the experiments except at the time of being sampled. Daylight hours during each experiment period were calculated based on the data from: www.tutiempo.net/calendario-solar. Maximum, mean, and minimum temperatures of the days when the experiments were conducted were also recorded. A decreasing photoperiod, from 14 h 44 min to 12 h 59 min, and mean temperature of 28°C were recorded in JS; and an increasing photoperiod, from 11 h 55 min to 12 h 58 min, and mean temperature of 14°C in MA.

2.3. TRH stimulation tests

Synthetic TRH (Sigma-Aldrich Co, St Louis, MO, USA) was reconstituted under a biohazard hood with sterile