Growth Hormone and Prolactin Secretion in Horses: Effects of Multiple and Extended Exercise Bouts, β -Hydroxy- β -methyl Butyrate Feeding, and Arginine and Thyrotropin Releasing Hormone Pretreatment

Donald L. Thompson Jr.^{a,*}, Peggy A. Miller-Auwerda^b, Liv Sandberg^{b,**}

^aSchool of Animal Sciences, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, LA

^bDepartment of Animal Science, Iowa State University, Ames, IA 50011

Approved for publication by the Director of the Louisiana Agricultural Experiment Station as manuscript number 2016-230-26093.

*Corresponding author at: Donald L. Thompson, Jr., School of Animal Sciences, Louisiana State University, Baton Rouge, LA 70803-4210.

E-mail address: dthompson@agctr.lsu.edu (D.L. Thompson Jr.).

**Current address: Department of Animal Sciences, University of Wisconsin, Madison, WI.

ABSTRACT

Five experiments were performed to test the overall hypothesis that exercise might be a useful indicator of growth hormone (GH) and prolactin status in horses. In experiment 1, geldings were exercised for 5 minutes four times at hourly intervals. The prolactin response ($P < 0.05$) to the first two exercise bouts was small and increased with successive bouts. There was a consistent GH response ($P < 0.05$) for only the first two bouts. In experiment 2, geldings were exercised for 29 to 39 minutes on a treadmill. After the initial bout, half the geldings were supplemented daily with Ca- β -hydroxy- β -methyl butyrate, and all geldings were conditioned for 12 weeks. Exercise bouts at 7 and 12 weeks indicated no effect ($P > 0.1$) of supplementation. In experiment 3, treatment of geldings with arginine before exercise increased ($P < 0.001$) prolactin concentrations but had no effect ($P > 0.1$) on the GH response to exercise. In experiment 4, the repeatability of the GH response to 5 minutes of exercise was determined by exercising eight stallions on six separate occasions. In addition to a large variation in GH response among stallions, there was large variation within each stallion. In experiment 5, pretreatment with thyrotropin releasing hormone 2 hours before exercise did not normalize the GH response to exercise. In conclusion, factors affecting the GH response to exercise likely preclude its usefulness as an indicator of GH status in horses.

Key Words: Arginine, Exercise, Growth Hormone, Horses, Prolactin, Thyrotropin Releasing Hormone

1. Introduction

Short-term exercise is a potent secretagogue for growth hormone (GH) and prolactin secretion in horses [1-3]. Similar responses to exercise and other stimuli of the sympathetic nervous system have been reported for various species [4-6]. In humans, several physiologic and

pharmacologic secretagogues of GH have been described for the assessment of GH status of individuals, including exercise, sleep, and administration of GH releasing hormone (GHRH), GH releasing peptides, thyrotropin releasing hormone (TRH), insulin, and arginine [7-9]. Arginine administration in some cases does not stimulate GH release directly but potentiates the release of GH in response to other secretagogues [10-12]. In horses, TRH, which stimulates prolactin secretion [13,14], suppresses GH secretion for up to 2 hours and may potentiate the GH response to exercise 2 to 3 hours later [15,16].

The present experiments were performed to test the overall hypothesis that exercise might be a useful indicator of GH and prolactin status in horses. A series of five experiments tested 1) the effects of four sequential, 5-minute exercise bouts performed once per hour for 4 hours, 2) the effects of extended periods (29 to 39 minutes) of varying intensity exercise in horses fed either a control supplement or the same supplement containing Ca- β -hydroxy- β -methyl butyrate (HMB), 3) the effects of pretreatment with arginine, 4) the repeatability of GH responses, and 5) the effects of pretreatment with TRH on the repeatability of the GH responses to exercise.

2. Materials and Methods

Horses used in experiments 1, 3, 4, and 5 were housed at the Louisiana State University Agricultural Center Horse Unit in Baton Rouge, Louisiana. Geldings used in experiment 2 were housed in facilities at the Iowa State University in Ames, Iowa. All procedures in these experiments were approved by the appropriate Institutional Animal Care and Use Committees on the respective campuses.

2.1. Experiment 1

The effects of multiple, 5-minute bouts of exercise on GH and prolactin secretion were

studied. Six mature geldings of light horse breeds, aged 8 to 12 years, with body weights (BW) of 500 to 570 kg and body condition scores (BCS [17]) of 6 to 7, were used in July. These geldings had not been exercised or worked in the previous several months, and had basically roamed their pasture 24 hours a day. On the night before exercise, all geldings were brought in from pasture and were placed in a dry lot with ad libitum access to water but no feed overnight. Feed restriction was used to avoid complications with prolactin concentrations due to eating (well documented by us in previous reports [18-20]). In the morning, each gelding was fitted with a 14-gauge indwelling jugular catheter and subsequently tethered in a stall for a minimum of 1 hour. The geldings were then exercised, one at a time, by lunging at a fast trot, with occasional cantering, in a round pen for 5 minutes. They were again exercised in the same manner at 60, 120, and 180 minutes after the start of the first exercise bout, for a total of four bouts. Between exercise bouts, they were returned to their stall and kept tethered without access to feed or water. Samples of jugular blood (10 mL) were drawn via the catheter at 10-minute intervals starting 10 minutes before the first exercise bout and finishing 60 minutes after the fourth bout; samples were timed such that the sixth sample after exercise immediately preceded the next exercise bout. Blood samples were placed in tubes containing sodium heparin (20 units/mL of blood) and were kept at 5°C until centrifugation at 1600 x g for 15 min at 5°C; plasma was harvested and stored at -15°C.

2.2. Experiment 2

The effects of extended, graded exercise, with and without HMB supplementation, on GH and prolactin secretion were studied. Ten mature, previously unconditioned (not worked or exercised), light horse geldings (3 to 7 years old; 430 to 610 kg BW) were used. Housing and pre-trial preparations were the same as for geldings in experiment 1. These geldings were exercised on a treadmill at 1.6 m/s for 5 minutes (warm-up), at approximately 3.6 m/s for 10

minutes (heart rate [HR] of 160), at approximately 6.0 m/s for 2 minutes (HR of 200), and then again at HR of 160 for 10 minutes and 200 for 2 minutes. Blood samples were drawn before exercise, at the end of each exercise period, and again at 5, 30, and 60 minutes of warm down and recovery (5 minutes at 1.6 m/s and standing thereafter).

After the first test (August 19), geldings were fed either a control supplement (alfalfa based pellet containing ground limestone; $n = 5$) or the same supplement in which HMB (BETA Advantage©, Metabolic Technologies Inc., Ames, IA) was substituted at approximately 22 mg/kg of BW for the limestone ($n = 5$). While on the experimental diets, all geldings were conditioned for 12 weeks, and endurance tests were repeated at 7 weeks and again at the end of conditioning. These latter two tests consisted of the exercise protocol described above plus an additional 10 minutes of exercise at 3.6 m/s (HR of 160) added immediately prior to recovery.

2.3. Experiment 3

The effects of arginine pretreatment on the exercise-induced secretion of GH and prolactin were studied. Ten mature light horse geldings (6 to 17 years old; 440 to 600 kg BW; BCS 7 to 8.5) with no recent work or exercise were used during June. Five of the geldings had been used in experiment 1 the previous year.

On the night before exercise, geldings were brought in from pasture, where they had been kept for several months. They were placed in a drylot with ad libitum access to water but no feed overnight. In the morning, each gelding was fitted with a 14-gauge indwelling jugular catheter and subsequently tethered in a stall for a minimum of 1 hour. After withdrawal of three 10-mL blood samples 15 minutes apart, geldings (5/group) were infused intravenously with either 1) arginine at 0.5 g/kg BW as a 25% solution in water (pH = 7.4), or 2) 0.15 M saline at an equivalent volume (2 mL/kg BW). Infusion typically took about 8 minutes, so the start of infusion was timed so that it

would be completed 15 minutes after the last blood sample. A post-infusion blood sample was drawn immediately (time 0), and the geldings were then taken to a round pen and exercised in two successive bouts in the same manner as described for experiment 1. Blood samples were drawn at 10-minute intervals relative to the start of exercise through 60 minutes, at which time the geldings were again exercised in the same manner and sampled every 10 minutes for the next 60 minutes; all samples were collected and processed as described for experiment 1.

2.4. Experiment 4

The repeatability of the GH response to single exercise bouts was tested. Eight mature light horse stallions (4 to 18 years old; 480 to 580 kg BW; BCS 4 to 6) were used. They were maintained in individual pens approximately 50 x 80 m² in size, and were fed a commercially available complete grain mix (Country Acres Horse Complete, Country Acres Feed Company, Brentwood, MO) at 1.2% of their BW daily. They also had native grass hay available for ad libitum consumption. They were not fed their grain meal on the mornings of exercise until after the exercise bout for that day.

The stallions were exercised once for 5 minutes as described in experiment 1 on six different occasions. The six occasions were randomly selected for each stallion from a 21-day period (November 12 through December 2), and were unique for each stallion, with the exception that there was at least 1 day of no exercise between successive days of exercise. For each bout, samples of blood (10 mL) were collected via jugular catheters at -10, 0, 10, 20, 30, 40, 50, 60, and 90 minutes relative to onset of exercise. All exercise bouts were performed in the morning (between 0700 and 1200).

2.5. Experiment 5

Because previous reports [16,21] indicated that the initial TRH-induced suppression of GH secretion was followed by a rebound in secretion 2 to 3 hours later, the effect of pretreatment with TRH 2 hours before exercise on the repeatability of the GH response to exercise was tested. Six of the eight stallions used in experiment 4 (7 to 15 years old; 495 to 560 kg BW; BCS scores 5 to 6) were exercised on eight separate occasions between March 18 and April 24 (16 months following experiment 4) in the same manner as described for experiment 4. On the first occasion, three stallions were randomly selected to receive TRH (10 µg/kg BW intravenously in saline) exactly 120 minutes before exercise; the remaining three stallions received an injection of saline (0.01 mL/kg BW). Thereafter, TRH or saline treatment was alternated for each subsequent exercise bout. Samples of jugular blood were collected at -120, -105, -90, -75, -60, -45, -30, -15, 0, 10, 20, 30, 45, 60, 90, 120, 150, and 180 minutes relative to onset of exercise.

2.6. Sample Analyses

Plasma concentrations of prolactin were analyzed by radioimmunoassay previously validated for horse samples [22]. Intra- and interassay coefficients of variation and assay sensitivity were 7%, 12%, and 0.2 ng/mL.

Plasma concentrations of GH were measured with double-antibody radioimmunoassay based on an antiserum generated in a rabbit against recombinant equine GH (EquiGen, BresaGen Ltd., Adelaide, Australia; no longer available commercially) and radioiodinated equine GH (AFP-7112B). The antiserum was diluted 1:2,000 in 0.01 M phosphate-buffered 0.155 M saline (PBS) containing 0.05 M EDTA, 1% normal rabbit serum, 50% normal cow serum, and 0.1% Triton-X100; 200 µL of this solution was used per tube. The equine GH was radioiodinated by the chloramine-T method (1 mCi of carrier-free ¹²⁵I plus 1 µg chloramine-T/µg protein for 20 sec at 1°C), which was terminated by addition of 2.5 µg of sodium metabisulfite/µg protein. The

radiolabeled protein was separated from the unincorporated iodine on Sephadex G-15 columns prepared in 5-mL disposable pipettes. Specific binding of the radiolabeled GH averaged 35%; nonspecific binding averaged 3.0%.

For the assay of GH in horse plasma, duplicate aliquots of 200 μ L of plasma in 12 x 75-mm disposable glass tubes were used. Eight serial dilutions of an equine pituitary extract (equivalent to 96 ng/mL equine GH) in horse serum were included, as well as 6 buffer control (zero standard) and six nonspecific binding (0.95% normal rabbit serum) tubes. After addition of the diluted anti-GH serum to all tubes except the nonspecific binding tubes, all tubes were vortexed briefly and incubated at 5°C for 48 hours. A fixed amount of radioiodinated equine GH (approximately 60 nCi) was then added to all tubes in 200 μ L of PBS containing 1.0 g/L gelatin; the tubes were vortexed briefly and incubated for 48 hours at 5°C. Precipitation of the primary antibody complexes was achieved by the addition of 200 μ L of anti-rabbit gamma globulin serum (diluted 1:4.5) generated in a sheep. After the tubes were vortexed, they were incubated for at least 24 hours at 5°C. Final separation of bound from free was achieved by centrifugation of the tubes at 1,200 x g for 30 minutes at 5°C and decanting of the supernatant; the pellets were washed once with 0.8 mL of PBS at 5°C and centrifuged and decanted a second time. Radioactivity in the pellets was assessed by solid scintillation counting for 1 minute. The resulting data were used in a logit-log transformation analysis, and GH concentrations in unknowns were determined from the predictive equation derived from regression analysis of the standard curve data.

Inhibition curves generated by serial dilutions of horse plasma pools (mares, geldings, and stallions), horse pituitary extracts, and the equine GH standard were parallel. Cross-reactivities of various hormones in the GH assay were (%): LH, <0.005; FSH, <0.004; TSH, <0.04; prolactin, <0.0032; IGF-I, <0.16; insulin, <0.016; ACTH, <0.0008; and eCG, <0.16. Recovery of graded amounts of equine GH added to a pool of equine plasma selected for low GH concentration

resulted in quantitative recovery (101%) with high correlation between added and measured (correlation coefficient = 0.99). Assay of six plasma pools that had been previously measured in an assay based on anti-porcine GH serum and equine GH [2] resulted in good agreement between the two assays ($r = 0.99$; $\text{ng/mL measured} = 0.91 \times \text{ng/mL in old assay} - 0.95 \text{ ng/mL}$). Estimates of intra- and interassay coefficients of variation averaged 4 and 10%, respectively. Sensitivity of the assay, based on 200 μL sample size, was 0.4 ng/mL.

2.7. Statistical Analyses

For each experiment, data were analyzed by analysis of variance using the PROC GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Data from experiment 1 were analyzed as a factorial design with horses and sampling times as main effects; the horse x sampling time interaction term was used to test the main effects [23]. Follow-up analyses were performed for each hormone on the net changes from pre-exercise (sample immediately before the start of exercise for each bout, i.e., at 0, 60, 120, and 180 minutes) to maximum post-exercise for each bout (typically the sample at 10 or 20 minutes after start of exercise). These data were calculated for each bout for each gelding, and were then individually analyzed in one-way analyses of variance for each bout that tested the hypothesis that the mean differences differed from zero (indicating a significant response to exercise).

Data from the individual exercise bouts in experiment 2 and the data from experiment 3 were analyzed as a completely randomized design (HMB treatment in experiment 2 or arginine pretreatment in experiment 3) with repeated sampling (split-plot; [24]). The horses within treatment term was used to test the effects of treatment, and the residual error term was used to test the effects of time and its interaction with treatment [23]. In addition, an overall factorial analysis of the data in experiment 2 was performed, ignoring HMB treatment, with horses and time as the

main effects (tested with the horse x time interaction term), and successive exercise bouts as a subplot; bout and its interaction with time was tested with residual error.

Data from experiment 4 were analyzed as a factorial design with horse, exercise bout, and sampling times as main effects; the effects of exercise bout and time were tested with their interactions with horse, and the exercise bout x sampling time interaction was tested with the three-way interaction [23]. Also, the net increase in GH concentrations (highest GH concentration within 60 minutes of exercise minus pre-exercise GH concentration) was calculated for each exercise bout, and these data were analyzed in a factorial arrangement with horse and exercise bout as main effects. Data from experiment 5 were analyzed as a factorial design with repetitive sampling; the effects of horse and TRH treatment were tested with the horse x treatment interaction term, and the effects of time and its interaction with treatment were tested with residual error [23]. In addition, individual means and standard deviations for each stallion were calculated. From these statistics, the resulting coefficients of variations of the stallions were calculated and used for comparison of repeatability of responses.

3. Results

3.1. Experiment 1

There was an effect of time ($P < 0.05$) for both GH (Fig. 1a) and prolactin (Fig. 1b) concentrations in experiment 1. Analysis of the net changes in GH after each bout revealed that there was a significant GH response to exercise for the first two exercise bouts ($P < 0.05$), but GH secretion thereafter was inconsistent. In contrast, the prolactin response to exercise was small for the first two bouts and tended to get larger from the first to the fourth bout; all were significant responses (i.e., were greater than zero; $P < 0.05$).

3.2. Experiment 2

Supplementation with HMB had no effect ($P > 0.1$) on the GH or prolactin responses to the two post-treatment endurance tests, thus the pooled means are presented. During the first (preconditioning and pretreatment) endurance test (Fig. 2), GH concentrations increased ($P < 0.01$) from 1.6 ng/mL before exercise to >30 ng/mL during the four exercise intervals, began to decrease but remained elevated ($P < 0.05$) at 5 minutes into recovery, and then decreased to 9.6 and 2.2 ng/mL at 30 and 60 minutes of recovery, respectively ($P > 0.1$). Prolactin concentrations averaged 3.6 ng/mL before exercise and gradually increased ($P < 0.01$) with each successive exercise interval, peaked at 19.6 ng/mL at 5 minutes of recovery, and remained elevated ($P < 0.05$) at 30 and 60 minutes of recovery (14.0 and 10.4 ng/mL, respectively). During the next two tests, the patterns of GH and prolactin in response to exercise were very similar to those obtained in the first test, except that prolactin concentrations before exercise and the magnitude of the prolactin response were lower ($P < 0.05$) with each successive test.

3.3. Experiment 3

Concentrations of GH increased ($P < 0.001$) in response to the first exercise bout in both groups of geldings (Figure 3a); there was a minor GH response to the second exercise bout in about half the geldings. There was no effect ($P > 0.1$) of pretreatment with arginine on the GH response to either exercise bout.

For prolactin concentrations (Fig. 3b), there was an effect of treatment ($P < 0.01$), time ($P < 0.001$) as well as a treatment x time interaction ($P < 0.001$). Infusion of arginine immediately before exercise increased ($P < 0.01$) prolactin concentrations, which continued to increase after the first exercise bout. Control geldings also had the expected rise in prolactin concentrations in response to exercise, with the responses to the first and second bouts being approximately the

same. Relative to those in control geldings, prolactin concentrations in geldings infused with arginine were higher ($P < 0.05$) in all time periods from 0 to 120 minutes.

3.4. Experiment 4

When eight stallions were exercised on six different occasions, there was an effect of time ($P < 0.01$; Fig. 4a) and stallion ($P < 0.001$; Fig. 4b), but no effect ($P > 0.1$) of exercise bout (1 through 6), in the analysis of variance. The individual GH responses for the six bouts for each stallion are shown in Fig. 4c to illustrate how variable they were, depending on the stallion. Although almost all exercise bouts resulted in a net positive GH response in the first 30 minutes, the variability in responses for any given stallion was large. For example, the mean response for the six stallions ranged from 1.9 to 22.2 ng/mL; the coefficients of variation for these individual means ranged from 88 to 203% across the eight stallions.

3.5. Experiment 5

When stallions were pretreated with TRH 120 minutes before exercise, there was an effect of treatment ($P = 0.058$) and time ($P < 0.001$) in the analysis of variance as well as an interaction between treatment and time ($P = 0.018$). Plasma GH concentrations were lower ($P < 0.1$) from 45 minutes before exercise through 20 minutes after onset of exercise (Fig. 5a) when TRH was administered relative to when saline was administered. Treatment with TRH increased ($P < 0.001$) prolactin concentrations (Fig. 5b) within the first 15 minutes after treatment, and they remained elevated ($P < 0.05$) throughout the sampling period. The net GH responses to exercise for the individual stallions when treated with saline (Fig. 6a) or TRH (Fig. 6b) were not affected ($P > 0.1$) by treatment or exercise bout. The coefficients of variation for net GH response ranged from 82 to 180% when stallions were pretreated with saline and 88 to 193% when stallions were pretreated

with TRH.

4. Discussion

Although concentrations of both GH and prolactin increase in response to exercise, it is apparent that they exhibit differential patterns of response to multiple and extended exercise bouts. In experiment 1, the prolactin response to the first two exercise bouts was small and tended to increase from the first to last bout. In contrast, the GH response was variable or absent after the first two bouts. This difference likely reflects the difference in hypothalamic control of secretion of the two hormones; i.e., prolactin secretion is believed to be primarily regulated by a single, inhibitory substance (dopamine), whereas GH secretion is regulated by two peptide hormones, GHRH and somatostatin [25]. Assuming somatostatin is released in response to a rise in GH secretion in the horse, as has been reported for other species [25], it likely would interfere with subsequent GH stimulation in the later exercise bouts.

Another differential response in the release of GH and prolactin was revealed in experiment 2, in which geldings were exercise for extended periods of time. The concentrations of both hormones increased immediately after onset of exercise, but GH concentrations peaked and began to decrease before the exercise bout was over, whereas prolactin concentrations did not peak until the first blood sample after the cessation of exercise. Again, this difference may indicate that somatostatin secretion was stimulated by the early rise in GH concentrations, and began to suppress GH secretion even though the horses were still exercising. The gradual decrease in prolactin response from the first bout (performed on August 19) to the last (performed on November 18) was likely due to the seasonal influence on prolactin secretion, which is consistently observed in horses of all reproductive states [26,27].

An interesting aspect of the results of experiment 2 was the continued increase in plasma

concentrations of both GH and prolactin with continued physical exertion. Most of the data on exercise or stress in which these two pituitary hormones have been measured have been after brief stimuli (e.g., 5 minutes of trotting, as in experiment 1, or after semen collection [22,28]). In all of the previous studies, brief stimuli have produced immediate but brief responses; basically an initial dumping of hormone into the blood followed by the concentrations decreasing down an expected clearance curve. The 29- to 39-minute bouts reported herein, perhaps due to their general increasing in speed on the treadmill (i.e., intensity of exercise), resulted in rising plasma concentrations through 20 to 30 min for GH, and up to 34 to 44 minutes for prolactin, which was beyond the intense exercise period and into the recovery period. Thus, the question arises whether plasma prolactin concentrations would continue to increase if exercise was continued or further increased in intensity.

Arginine infusion is commonly used to stimulate GH secretion in various species [29-31], and is one of several standard clinical methods of assessing GH status in humans [32,33]. Arginine infusion also has been reported to potentiate the GH response to hexarelin in elderly patients, in which the hexarelin itself has little effect [12]. In horses, arginine infusion stimulates GH secretion to a minor extent but is a much better secretagogue for prolactin [34]; aspartic acid, on the other hand, is a potent secretagogue for GH in horses [34,35]. Pretreatment of geldings in experiment 3 with arginine did not alter the exercise-induced GH response, and in fact did not alter the pattern of GH at any time during the 2-h sampling period. Sticker et al. [34] reported that arginine infusion resulted in a delayed GH response in horses, with concentrations peaking at 30 minutes after onset of infusion, compared with aspartic acid infusion, after which GH concentrations peaked in the first 10 minutes. Sticker et al. [34] suggested that this difference in responses may indicate different underlying mechanisms by which the two amino acids induce GH secretion.

Unlike the two consistent GH responses to the first two exercise bouts in experiment 1, the

second exercise bout in experiment 3 caused little response in GH secretion in either group of geldings. The duration and intensity of exercise was similar in the two experiments, as was the pattern of the GH response. Also, there was no increase in the prolactin response in experiment 3 from the first to the second bout like observed in experiment 1, although the patterns were again similar in the two experiments. Perusal of the GH secretion patterns for individual horses in experiment 1 and 3 indicated considerable variation in magnitude of the hormonal responses. To better characterize this variation, experiment 4 was designed to determine how repeatable the GH responses might be for a given horse. Due to their availability, stallions were used rather than geldings; however, previous results from our laboratory [3] indicated that stallions and geldings were similar in their GH secretory patterns.

The individual GH responses presented in Fig. 4c for the eight stallions over six different exercise bouts in experiment 4 illustrate the extreme variation in responses, not only from stallion to stallion, but also within a given stallion from bout to bout. There was no overall effect of bout (1 vs 2 vs 3, etc.), indicating that no time-related changes occurred (e.g., a depletion of GH from the pituitary or a conditioned response-like effect). Stallions that had the largest responses tended to repeat that magnitude of response at least once, and there were also stallions that consistently had small responses relative to the greatest responders. However, even the stallion with the greatest GH responses (ANT) had three occasions when the responses were only 2, 3, and 13% of his maximal response. In general, about one-half of all responses for any given stallion were low relative to his maximal response(s). Again, due to the episodic nature of GH secretion in horses [2,36], any time chosen for exercise at random will fall before, during, or after an endogenous surge in GH secretion. Assuming the horse is similar to other species in its somatostatin response, this means that exercise could occur at a period of greatest sensitivity to a stimulus (just before a GH surge) or during a period of reduced sensitivity (during or after a surge). This timing of

exercise relative to an endogenous GH surge is suspected to be a major cause of the variation in exercise-induced GH responses in these horses.

Gentry et al. [21] was the first to report that TRH administration seemed to interfere with GH secretion induced by EP51389, a potent GH-releasing tripeptide. Kennedy et al. [15] subsequently reported that TRH administration inhibited the GH response both to EP51389 and to GHRH itself. Most recently, Pruett et al. [16] demonstrated that TRH inhibits the GH response to exercise and aspartic acid infusion and inhibits endogenous GH secretion as well. The inhibitory effect of TRH lasted at least 1 hour, but had decayed away by 2 hours [16]. Moreover, the GH responses to exercise at 2 and 3 hours after TRH administration were greater than the response immediately after saline administration, indicating a rebound-like effect after the inhibition waned. Thus, experiment 5 tested the possibility that TRH pretreatment might serve as a method of normalizing the GH response to exercise. Unlike the results of Pruett et al. [16], we did not observe an increase in GH response due to pretreatment with TRH 2 hours earlier. Nor did pretreatment with TRH alter the variability of responses. It is possible that for some stallions the inhibition persisted past 2 hours, because there was a slight reduction in GH concentrations after TRH pretreatment averaged over all horses.

Christensen et al. [37] reported that horses acclimated to a single grain meal daily experienced a 2- to 5-hour period after eating of no endogenous GH surges; they suggested that secretagogues administered at 5 hours would have the greatest chance of invoking a GH response. Although this is similar to the approach we took with TRH administration herein, we have not observed a meal-induced period of reduced GH secretion for up to 8 hours after a meal [18] like that reported by Christensen et al. [37].

In summary, the prolactin and GH responses to exercise differ under conditions of multiple acute exercise bouts compared to extended exercise bouts, likely due to the difference in the mode

of their hypothalamic regulation. Moreover, the two hormones respond very differently to arginine, which did not alter the GH response to exercise like has been observed in other species. It is assumed that the large variability in GH responses to exercise within given stallions is due in part to the timing of exercise relative to endogenous episodes in GH and somatostatin secretion; however, this assumption needs to be studied further. Pretreatment with TRH was not useful for normalizing the GH response to exercise.

Acknowledgements

This work was supported by the USDA National Institute of Food and Agriculture, Hatch project Accession Number 0224441.

The authors thank A. F. Parlow and the National Institute of Diabetes and Digestive and Kidney Diseases, National Hormone and Pituitary Program, Harbor-University of California Los Angeles Medical Center, Torrance, CA, for reagents.

References

- [1] Colborn, DR, Thompson DL Jr, Rahmanian MS, Roth TL. Plasma concentrations of cortisol, prolactin, LH and FSH in stallions following physical exercise and injection of secretagogue before and after sulpiride treatment in winter. *J Anim Sci* 1991;69:3724-32.
- [2] Thompson DL Jr, Rahmanian MS, DePew CL, Burleigh DW, DeSouza, CJ, Colborn DR. Growth hormone in mares and stallions: pulsatile secretion, response to growth hormone-releasing hormone and effects of exercise, sexual stimulation, and pharmacological agents. *J Anim Sci* 1992;70:1201-7.
- [3] Thompson DL Jr, DePew CL, Ortiz A, Sticker LS, Rahmanian MS. Growth hormone and prolactin concentrations in plasma of horses: sex differences and the effects of acute exercise and

- administration of growth hormone releasing hormone. *J Anim Sci* 1994;72:2911–8.
- [4] Buckler JMH. Exercise as a screening test for growth hormone release. *Acta Endocrinol* 1972;69:219-29.
- [5] Coleman ES, Elsasser TH, Kempainen RJ, Coleman DA, Sartin JL. Effect of endotoxin on pituitary hormone secretion in sheep. *Neuroendocrinology* 1993;58:111-22.
- [6] Rushen J, Schwarze N, Ladewig J, Foxcraft G. Opioid modulation of the effects of repeated stress on ACTH cortisol prolactin and growth hormone in pigs. *Physiol Behav* 1993;53:923-8.
- [7] Shibasaki T, Hotta M, Masuda A, Imaki T, Obara N, Demura H, Ling N, Shizume K. Plasma GH responses to GRH and insulin induced hypoglycemia in man. *J Clin Endocrinol Metab* 1985;60:1265-7.
- [8] Muller EE. Neural control of somatotrophic function. *Physiol Rev* 1987;67:962-1053.
- [9] Volta C, Bernasconi S, Lughetti L, Ghizzoni L, Rossi M, Costa M, Cozzini A. Growth hormone response to growth hormone-releasing hormone (GHRH), insulin, clonidine, and arginine after GHRH pretreatment in obese children: evidence of somatostatin increase? *Eur J Endocrinol* 1995;132:716-21.
- [10] Alba-Roth J, Muller OA, Schopohl J, von Werder K. Arginine stimulates growth hormone secretion by suppressing endogenous somatostatin secretion. *J Clin Endocrinol Metab* 1988;67:1186-9.
- [11] Ghigo E, Groffi S, Nicolosi M, Arvat E, Valente F, Mazza E, Ghigo MC, Camanni F. Growth hormone (GH) responsiveness to combined administration of arginine and GH-releasing hormone does not vary with age in man. *J Clin Endocrinol Metab* 1990;71:1481-5.
- [12] Arvat E, Gianotti L, Grottoli S, Imbimbo BP, Lenaerts V, Deghenghi R, Camanni F, Ghigo E. 1994. Arginine and growth hormone-releasing hormone restore the blunted growth hormone-releasing activity of hexarelin in elderly subjects. *J Clin Endocrinol Metab*

994;79:1440-3.

[13] Johnson AL, Becker SE. Effects of physiologic and pharmacologic agents on serum prolactin concentrations in the nonpregnant mare. *J Anim Sci* 1987;65:1292-7.

[14] Thompson DL Jr, Wiest JJ, Nett TM. Measurement of equine prolactin with an equine canine radioimmunoassay: Seasonal effects on the prolactin response to thyrotropin releasing hormone. *Domest Anim Endocrinol* 1986;3:247-52.

[15] Kennedy SR, Thompson DL Jr, Pruett HE, Burns PJ, Deghenghi R. Growth hormone response to a novel GH releasing tripeptide in horses: Interaction with GnRH TRH and sulpiride. *J Anim Sci* 2002;80:744-50.

[16] Pruett HE, Thompson DL Jr, Cartmill JA, Williams CC, Gentry LR. Thyrotropin releasing hormone interactions with growth hormone secretion in horses. *J Anim Sci* 2003;81:2343-51.

[17] Henneke DR, Potter GD, Kreider JL, Yeates BF. Relationship between condition score physical measurements and body fat percentage in mares. *Equine Vet J* 1983;15:371-6.

[18] DePew CL, Thompson DL Jr, Fernandez JM, Sticker LS, Burleigh DW. Changes in concentrations of hormones, metabolites, and amino acids in plasma of adult horses relative to overnight feed deprivation followed by a pellet hay meal fed at noon. *J Anim Sci* 1994;72:1530-9.

[19] DePew CL, Thompson DL Jr, Fernandez JM, Southern LL, Sticker LS, Ward TL. Concentrations of prolactin, glucose, insulin, urea nitrogen, and total amino acids in plasma of stallions after ingestion of feed or direct gastric administration of feed components. *J Anim Sci* 1994;72:2345-53.

[20] Nadal MR, Thompson DL Jr, Kincaid LA. Effect of feeding and feed deprivation on plasma concentrations of prolactin, insulin, growth hormone, and metabolites in horses. *J Anim Sci* 1997;75:736-44.

[21] Gentry LR, Thompson DL Jr, Stelzer AM. Treatment of seasonally anovulatory mares with

thyrotropin releasing hormone and(or) gonadotropin releasing hormone analog. J Anim Sci 2002;80:208-13.

[22] Colborn DR, Thompson DL Jr, Roth TL, Capehart JS, White KL. Responses of cortisol and prolactin to sexual excitement and stress in stallions and geldings. J Anim Sci 1991;69:2556-62.

[23] Steel RGD, Torrie JH, Dickey DA. Principles and procedures of statistics: a biometrical approach. 3rd ed. McGraw-Hill Book Co New York, NY; 1997.

[24] Gill JL Hafs HD. Analysis of repeated measurements of animals. J Anim Sci 1971;33:331-6.

[25] Guyton AC, Hall JE. Textbook of medical physiology. 12th ed. W.B. Saunders, Philadelphia, PA; p 729–42; 2011.

[26] Johnson AL. Seasonal and photoperiod-induced changes in serum prolactin and pituitary responsiveness to thyrotropin-releasing hormone in the mare. Proc Soc Exper Biol Med 1987;184:118-22.

[27] Thompson DL Jr, Johnson L, St George RL, Garza F Jr. Concentrations of prolactin luteinizing hormone and follicle stimulating hormone in pituitary and serum of horses: effect of sex season and reproductive state. J Anim Sci 1986;63:854-60.

[28] Rabb MH, Thompson DL Jr, Barry BE, Colborn DR, Garza F Jr, Hehnke KE. Effects of sexual stimulation, with and without ejaculation, on serum concentrations of LH, FSH, testosterone, cortisol and prolactin in stallions. J Anim Sci 1989;67:2724-9.

[29] McAtee JW, Trenkle A. Effects of feeding fasting glucose or arginine on plasma prolactin levels in the bovine. Endocrinology 1971;89:730-4.

[30] Davis SL. Plasma levels of prolactin growth hormone and insulin in sheep following the infusion of arginine leucine and phenylalanine. Endocrinology 1972;91:549-55.

[31] Chew B, Eisenman PJR, Tanaka TS. Arginine infusion stimulates prolactin growth hormone insulin and subsequent lactation in pregnant dairy cows. J Dairy Sci 1984;67:2507-18.

- [32] Merimee TJ, Rabinowitz D, Fineberg SE. Arginine-initiated release of human growth hormone. *New England J Med* 1969;250:1434-38.
- [33] Tanaka K, Inoue S, Shiraki J, Shishido T, Numata K, Takamura Y. Age-related decrease in plasma growth hormone: Response to growth hormone-releasing hormone arginine and L-dopa in obesity. *Metabolism* 1991;40:1257-62.
- [34] Sticker LS, Thompson DL Jr, Gentry LR. Pituitary hormone and insulin responses to infusion of amino acids and N- methyl-DL-aspartate in horses. *J Anim Sci* 2001;79:735-44.
- [35] Smith LA, Thompson DL Jr, French DD, Leise BS. Effects of recombinant equine somatotropin on wound healing carbohydrate and lipid metabolism and endogenous somatotropin responses to secretagogues in geldings. *J Anim Sci* 1999;77:1815-22.
- [36] Stewart F, Goode JA, Allen WR. Growth hormone secretion in the horse: Unusual pattern at birth and pulsatile secretion through to maturity. *J Endocrinol* 1993;138:81-9.
- [37] Christensen RA, Malinowski K, Scanes CG, Hafs HD. Pulsatile release of somatotropin related to meal feeding and somatotropin response to secretagogues in horses. *J Anim Sci* 1997;75:2770-7.

Fig. 1. Plasma concentrations of growth hormone (GH; a) and prolactin (b) before and after 5 minutes of exercise at times 0, 60, 120, and 180 minutes in experiment 1. Pooled SEM were 0.69 and 0.49 ng/mL for GH and prolactin, respectively. The p-values in parentheses indicate the significance of the individual responses when analyzed as maximal net change in hormonal concentration from pre-exercise (i.e., the 0, 60, 120, and 180-minute samples).

Fig. 2. Plasma concentrations of growth hormone (GH; top panels) and prolactin (bottom panels) in experiment 2 before and during varying levels of exercise on a treadmill: 1.6 m/s for 5 minutes (warm-up), approximately 3.6 m/s for 10 minutes (heart rate [HR] of 160), approximately 6.0 m/s for 2 minutes (HR of 200), and then again at HR of 160 for 10 minutes and 200 for 2 minutes. Blood samples were drawn before exercise (time 0), at the end of each exercise period, and again at 5, 30, and 60 minutes of recovery (1.6 m/s for 5 minutes and passive thereafter). Means are averaged over treatment and control geldings because there was no effect of β -hydroxy- β -methyl butyrate feeding. The first exercise bout (pre-treatment) was performed on August 19, and the subsequent bouts were performed after 7 and 12 weeks of conditioning. These latter two bouts had an additional 10 minutes of exercise at 3.6 m/s (HR of 160) added immediately prior to recovery. Pooled SEM were 4.4, 3.8, and 5.0 ng/mL for GH and 1.4, 1.0, and 0.35 ng/mL for prolactin for the three bouts, respectively.

Fig. 3. Plasma concentrations of growth hormone (GH; a) and prolactin (b) before and after 5 minutes of exercise at times 0 and 60 minutes in geldings infused with saline (Sal; 2 mL/kg BW) or arginine (Arg; 0.5 g/kg BW in water) immediately before the first exercise bout in experiment 3. Concentrations of GH were affected by time ($P < 0.001$) but not by arginine infusion. There was an interaction ($P < 0.001$) between treatment and time for prolactin concentrations. Means for the

two groups differed ($P < 0.05$) for all time points encompassed by the asterisks and horizontal line in panel b as determined by the least significant difference test. Pooled SEM were 1.0 and 4.2 ng/mL for GH and prolactin, respectively.

Fig. 4. Mean plasma growth hormone (GH) concentrations (a), mean net GH response to exercise (b), and individual net GH increases in response to exercise (c) in stallions exercised for 5 minutes on six separate occasions at least 1 day apart in experiment 4. There was large variation in GH response not only among the six stallions but also within any given stallion for the six exercise bouts. The coefficients of variation associated with the mean net responses for the individual stallions were 102, 112, 94, 106, 88, 203, 95, and 157% for the eight stallions, respectively.

Fig. 5. Plasma concentrations of growth hormone (GH; a) and prolactin (b) before and after 5 minutes of exercise at time 0 in stallions administered saline (0.01 mL/kg BW) or thyrotropin releasing hormone (TRH) at 10 μ g/kg BW in saline 2 hours before onset of exercise in experiment 5. Pooled SEM were 1.1 and 8.4 ng/mL for GH and prolactin, respectively. Asterisks indicate significance of the differences between means for the two groups for GH; for prolactin, means between groups differed ($P < 0.05$) for all time points encompassed by the asterisks and horizontal line in panel b as determined by the least significant difference test.

Fig. 6. Individual net growth hormone (GH) responses in six stallions exercised for 5 minutes on eight separate occasions and pretreated with saline (four occasions) or thyrotropin releasing hormone (TRH; four occasions) at 10 μ g/kg BW in saline 2 hours before onset of exercise in experiment 5. Pretreatment with TRH did not alter ($P > 0.1$) the net GH response to exercise nor the variability among responses within any given stallion. The coefficients of variation associated

with the mean net GH responses ranged from 82 to 180% when stallions were pretreated with saline and 88 to 193% when stallions were pretreated with TRH.