Effect of exercise, training, circadian rhythm, age, and sex on insulin-like growth factor-1 in the horse

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ABSTRACT: Insulin-like growth factor-1 could be a useful marker in the horse for diagnostic, selection, or forensic purposes, provided its physiological regulation is well understood. The objective of this study was to investigate factors, such as acute exercise, fitness training, time of day, sex, and age, that may influence serum IGF-1 in normal, healthy horses. Throughout a 9-wk training program, 6 geldings maintained a mean (±SEM) IGF-1 concentration of 302 ± 29 ng/mL. Moderate or high intensity exercise had no effect on IGF-1 concentrations, when pre- and postexercise values were compared. Over a 24-h period, there was some variation in IGF-1 concentrations but no clear diurnal rhythm. Concentrations of IGF-1 were measured in a large population of thoroughbred horses (1,880) on 3 continents.

Key words: circadian rhythm, exercise, horse, insulin-like growth factor-1, population

INTRODUCTION

Over the past 30 yr, IGF-I has been the subject of many studies and is now understood to be a key regulator of growth (Stewart and Rotwein, 1996) and tissue repair (Mueller et al., 1991), with an additional role in fertility (Roser, 2001). Several binding proteins add to the complexity of measuring IGF-I in blood serum or plasma (Bang et al., 1994), but they also increase its stability relative to pulsatile and less stable proteins such as GH. Accordingly, IGF-I has become a popular diagnostic marker for pituitary function (Rasat et al., 1996), a commercial selection marker for growth and feed efficiency in live-stock (Luxford et al., 1997), a reporter for growth status in pigs (Owens et al., 1997), and more recently, an indicator of GH abuse in racehorses (Noble and Sillence, 2000a). The utility of any hormone as a diagnostic, selection, or forensic marker rests on knowing the natural concentration of that substance in a healthy individual and understanding the physiological factors that can influence this.

The current study was undertaken as part of a project to identify illegal GH administration in racehorses, but our data could be applied to many aspects of equine research. Here, we examined how IGF-I concentrations are influenced by exercise, fitness training, and time of day in a small group of horses and report the effects of sex and age in a large population of thoroughbred horses.

MATERIALS AND METHODS

Animals and Fitness Training

The procedures for the high intensity exercise experiment were approved by the animal care and ethics committee of Charles Sturt University, Australia.
Six sound lighthorse geldings, aged 2 to 13 yr (6.3 ± 1.5 yr of age, BW 460 ± 23.9 kg), obtained from a local horse dealer, were housed individually in 4 × 4-m stables at the Equine Center, Charles Sturt University, Wagga Wagga, New South Wales, Australia. All horses were fed a daily ration of 3 kg of oats, 2 kg of wheat chaff, 3 kg of lucerne chaff, 3 kg of oat hay, 60 g of NaCl, and 60 g of CaCO₃, as-fed. According to chemical analysis (AOAC, 1990), the diet provided 96.3 MJ/d, slightly more than the 95.8 MJ/d recommended for horses in moderate work (NRC, 1989), and was sufficient to maintain their BW.

The horses were allowed 5 d to become acclimatized to their new environment and accustomed to working on a treadmill before the training program began. The horses were unconditioned at the beginning of the experiment, having been kept on pasture for at least 2 mo before the study. They were introduced to an incremental exercise regimen using a high-speed treadmill (Furphy, Ballarat, Victoria, Australia) set at a 3° incline. Horses were exercised 6 d/wk, beginning with 10 min/d, increasing to 30 min/d over a 7-wk period. This was maintained for a further 2 wk, so the horses were in training for a total of 9 wk. The speed of the treadmill was varied to maintain a heart rate of 150 to 160 beats/min. Heart rates were monitored using an equine heart rate monitor (EQB, Unionville, PA). All exercise sessions commenced with a 4-min warm-up period and ended with a 2-min cool-down period at walking pace. Work times were in addition to the warm-up and cool-down periods.

Blood samples were taken from each horse daily at 0700, before feed or exercise, for a total of 60 d, commencing 4 d before the training regimen began. Blood samples were collected into plain glass tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ), allowed to stand for 2 h at room temperature to clot, and then centrifuged at 4°C to eliminate the effects of stress from being cannulated as described above. The first 3 samples were discarded every 30 min for a 26-h period, commencing at 1000, and then centrifuged at 4°C and 3,500 × g for 30 min. Serum was collected and stored at −20°C until assayed for IGF-I concentration.

**Moderate Intensity Exercise**

To examine the effects of moderate intensity exercise on GH and IGF-I concentrations, 8 lighthorse geldings, including 6 from the above experiment, aged 2 to 8 yr (5.6 ± 0.8 yr of age, BW 458 ± 24.0 kg) were subjected to an exercise test, during which the treadmill was run at varying speeds: 1 min at 1.6 m/s, 3 min at 3.4 m/s, 15 min at 5 m/s, then 1 min at 1.6 m/s. This amount of work was similar to each animal’s current exercise levels, and all horses were able to complete the exercise test without difficulty.

Before the exercise test, each horse was fitted with a 14-gauge jugular cannula (Angiocath, Becton Dickinson) attached to a 150-cm length of extension tubing (Tuta, Lane Cove, New South Wales, Australia), and a 3-way stopcock (Becton Dickinson). Blood samples were taken at 5-min intervals commencing 20 min before exercise and continuing through the exercise period. Additional samples were collected at 10-min intervals for up to 40 min after exercise ceased, then 2 final samples were taken 30 min apart. Blood samples (10 mL) were collected according to the protocol described above. For the analysis of GH and IGF-I, 10 mL of blood was collected at each time point into plain glass tubes.

Samples for the analysis of blood lactate concentration were collected into 4-mL glass tubes containing sodium fluoride, EDTA, and potassium oxalate (Vacutainer, Becton Dickinson). Heart rate was measured during exercise using the equine heart rate monitor, and recorded at 5-min intervals, in conjunction with the blood sampling. Blood lactate concentrations were analyzed using a lactate analyzer (YSI Model 23L, Yellow Springs Instruments, Yellow Springs, OH). Serum samples were assayed for IGF-I and GH.

**High Intensity Exercise**

In a separate experiment conducted at HFL, Ltd., Fordham, UK, 4 sound, thoroughbred horses in training (daily work consisted of trotting and cantering for up to 1 h/d and 5 d/wk, BW not recorded) were given fast work on a training track to simulate exercise intensities that may be reached during a race. Two geldings, a stallion, and a mare (10, 12, 7, and 10 yr of age, respectively) were made to gallop 400 m then trot 400 m, 3 times, until each horse had covered a total distance of 2,400 m. A blood sample was taken from each horse 60 min before exercise by jugular venipuncture. After each horse had completed its work, a jugular cannula was fitted, as described above, and commencing 10 min after cessation of exercise, blood samples were drawn every 10 min for 80 min, and then every 30 min for up to 12 h. Blood samples (10 mL) for IGF-I analysis were collected in accordance with the protocol described above.

**Circadian Rhythm**

Twelve lighthorse geldings (including 8 from the moderate exercise study), aged 2 to 13 yr (6.3 ± 3.4 yr of age, BW 460 ± 15.8 kg), were used to determine the 24-h secretory pattern of IGF-I. Because interruptions to the daily routines can alter hormone secretion patterns (Irvine and Alexander, 1994), all horses were allowed 5 d to acclimatize before the study and to become accustomed to a 12-h dark:12-h light cycle. Each horse was fitted with a jugular cannula as described above.

Blood samples (10 mL) were taken from all horses every 30 min for a 26-h period, commencing at 1000, as described above. The first 3 samples were discarded to eliminate the effects of stress from being cannulated and to allow the horses to become accustomed to the sampling procedure. Serum was assayed for IGF-I. Cortisol concentration was also measured as a control hormone that would be expected to show some circadian pattern.
**Age and Sex**

Blood was collected by jugular venipuncture from 1,880 thoroughbred horses, ranging from 1 to 29 yr of age. These horses were located in 3 countries: Australia, South Africa, and the United Kingdom. Horses from South Africa were located in 3 areas: Cape Town, Johannesburg, and Durban. Horses from Australia were located in the Riverina district of New South Wales, and horses from the United Kingdom were from the area surrounding Newmarket, Suffolk. Details such as sex, age, location, and work regimen were recorded, and all horses were declared by their owners to have not received exogenous GH for at least 60 d before testing. Serum was assayed for IGF-I concentrations in 3 different laboratories: Charles Sturt University, Australia; HFL Ltd., UK; and the Laboratory of the National Horseracing Authority of Southern Africa. Although the country of origin for the horses was confounded by the site of assay, the same assay procedure, reagents, and quality control samples supplied by a single manufacturer were used in each laboratory. These assays were also validated and conducted in each laboratory under the direct supervision of 1 investigator.

**Hormone Assays**

Concentrations of IGF-I were measured using the DSL-2800 assay kit (Diagnostics Systems Laboratories, Webster, TX). This nonextraction, 2-site immunoradiometric assay employs \[^{125}\text{I}\]IGF-I as the radiotracer and was originally developed for use in human diagnostics. Although marketed as a nonextraction assay, serum is initially diluted in an acidic solution to dissociate the binding proteins. The assay was validated using equine serum drawn from mares, geldings, and stallions (Noble and Sillence, 2000b) and compares favorably with the accepted standard in measuring IGF-I concentrations with acid-column chromatography (Noble, 2001). Parallelism was assessed by the visual appraisal of graphs produced using serial dilutions of serum (1:4 to 1:200). Recovery (100.5 ± 2.4%) was determined by adding known quantities of human IGF-I to equine serum over the concentration range of 8 to 1,000 ng/mL. Intra- and interassay CV were 7.1 and 11.5%, respectively.

Equine GH was measured by an RIA adapted from a porcine GH assay (Dunshea et al., 2002), as there is a 98% structural homology between these 2 proteins (Zakin et al., 1976). Standards were recombinant eGH (Bresagen, Adelaide, SA, Australia), which was also radiolabeled with \[^{125}\text{I}\] (Amersham, Sydney, New South Wales, Australia) by the chloramine-T method. The first antibody was rabbit anti-porcine GH (Auspep, Melbourne, Victoria, Australia). Separation of antibody-bound and free tracer was achieved by a solid-phase method using cellulose particles coated with anti-rabbit IgG (Sac-Cell, Immunodiagnostics, Boldon, UK). The assay buffer contained 25 mM Tris, pH 7.2 at 24°C (Sigma, St. Louis, MO). The detection limit of the assay was 0.6 ng/mL. The assay demonstrated good parallelism and a recovery of 95.7% when a known quantity of eGH was added to a serum sample. The intraassay CV was 15.5%.

Cortisol concentrations were measured using an inhouse, competitive, protein-binding assay validated for horse serum and adapted from Sillence (1985). Steroids were extracted from the serum using dichloromethane (Sigma), which was evaporated to dryness before the samples were incubated with \[^{3}\text{H}\text{]cortisol (Amersham)} and corticosteroid-binding globulin obtained from a 15-yr-old mare. Separation of binding protein-bound and free tracer was achieved by a solid-phase method using magnesium silicate (Florisil, Sigma). Recovery of added cortisol was 98%. Intra- and interassay CV were 13 and 12%, respectively.

**Statistical Analysis**

All statistical analyses were conducted using SAS (SAS Inst. Inc., Cary, NC). The effect of moderate intensity exercise and fitness training were determined using ANOVA for repeated measures and a paired t-test. Dunnett’s test was used to compare postexercise observations with a single preexercise value for the high-intensity exercise experiment. The effects of age, sex, and work on IGF-I concentrations were determined using ANOVA, with main effects and interactions sought for each individual variable. Tukey’s test was used to separate means where significant interactions occurred. Country of origin was not included in the statistical analysis because it was confounded by site of assay. Tests for normal distribution were also performed using the Shapiro-Wilk univariate test. Results are presented as means ± SEM.

**RESULTS**

**Fitness Training**

The horses tended to lose BW during the initial stages of the training program but showed a moderate cumulative BW gain (9 ± 3.6 kg) for the remainder of the training program, indicating that they were receiving an adequate diet. It was not determined if this BW gain was due to changes in fat, lean body mass, or fluid. The horses maintained a mean IGF-I concentration of 302 ± 29 ng/mL throughout the experiment, as shown in Figure 1. Initially, the horses completed their 10-min exercise at a speed of 4.3 m/s; by the end of the training regimen they were completing the 30-min exercise at speeds between 5.8 and 7 m/sec. Repeated measures ANOVA showed there was no effect of time (reflecting a training effect or possible increase in fitness) on IGF-I concentrations in the horses. Furthermore, there was no difference in mean IGF-I concentrations averaged over 5 d before training (368.4 ± 8.47 ng/mL) compared with IGF-I concentrations averaged over the last 5 d of the training program (381.6 ± 5.40 ng/mL, P = 0.28, paired t-test).
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Figure 1. Mean (±SEM) daily serum IGF-I concentrations in 6 geldings (aged 2 to 13 yr) undergoing daily treadmill exercise 6 d/wk, commencing at 10 min/d and increasing incrementally to 30 min/d over a 9-wk period. Speed of the treadmill was varied to maintain a heart rate of 150 to 160 beats/min.

Moderate Intensity Exercise

Heart rate and blood lactate concentrations showed that exercise intensity was moderate and within the aerobic capacity of the horses, with heart rates varying between 150 and 160 beats/min and blood lactate concentrations not exceeding 2 mM. There was no change in IGF-I concentrations over time (P = 0.14), as shown in Figure 2A.

Concentrations of GH are shown in Figure 2B. Although there seemed to be a large peak in GH 25 min after exercise commenced, this was not statistically significant (P = 0.37). The peak was due to an unusually high GH concentration observed in 1 horse (139 ng/mL), which greatly influenced the mean. Although this observation inflated the SE, it was within 2 SD of the mean and could not be excluded on the basis of a z-test.

High Intensity Exercise

As with the moderate intensity exercise, there was no change in serum IGF-I concentrations with high intensity exercise, when pre- and postexercise values were compared (Figure 3; P = 0.99).

Circadian Rhythm

Although there was some variation in IGF-I concentrations (Figure 4A) over the 24-h period, there was no clear indication of any circadian pattern. In contrast, a diurnal rhythm (P < 0.001) was demonstrated by the measurement of cortisol, with a nadir of 18 ng/mL at midnight, and a zenith of 52 ng/mL at 0730, as shown in Figure 4B.

Figure 2. Mean (±SEM) serum concentrations of IGF-I (A) and GH (B) in 8 geldings (aged 2 to 8 yr). During the 20-min period indicated by the shading, the treadmill was run for 1 min at 1.6 m/s, 3 min at 3.4 m/s, 15 min at 5 m/s, and 1 min at 1.6 m/s.

Population Study: Normal Distribution

Tests for a normal distribution among the entire population, and within groups of each sex, are shown in Table 1. The Shapiro-Wilk statistic shows that the distribution was significantly different than normal (P < 0.001), with a positive test for skewness (P < 0.001) indicating that values above the mean had a greater range than values below the mean (SAS Inst. Inc., Cary, NC). The kurtosis statistic indicates that the distribution plot had slightly heavy tails, confirming that there were more values at the extremes of the distribution.
Figure 3. Mean (±SEM) serum IGF-I concentrations in 4 thoroughbred horses (2 geldings, 1 mare, and 1 stallion) before and after high intensity exercise on a racetrack. Horses were made to gallop for 400 m then trot for 400 m, 3 times until all horses had galloped a total of 2,400 m. Time zero indicates the first sample taken 10 min after exercise; the preexercise sample was taken 60 min earlier.

than would be expected in a normal population. Deviation from a normal distribution occurred stallions, mares, and geldings.

Despite the high level of statistical significance, the degree of deviation from a normal distribution was not large, and a close examination of the normality plots for all the horses (data not shown) showed that this occurred because of a relatively small number of animals (10 horses) with large IGF-I concentrations. The horse with the greatest IGF-I concentration was a 2-yr-old gelding from the United Kingdom. The lowest value was also recorded from a gelding: a 12-yr-old from Australia. The greatest and least concentrations observed in stallions were in a 1-yr-old from Johannesburg and a 5-yr-old from the United Kingdom, respectively. The greatest and least concentrations observed in mares were in a 4- and a 9-yr-old, respectively, both from the United Kingdom, as shown in Table 1.

Effects of Sex

The mean IGF-I concentrations for all horses in the study (global mean) and the mean for each sex are listed in Table 1. There was an effect of sex ($P < 0.001$), with IGF-I concentrations being greatest in stallions ($P < 0.001$, Tukey’s test) but almost identical in mares and geldings.

Figure 4. Mean (±SEM) serum IGF-I (A) and cortisol (B) concentrations in 12 geldings (aged 2 to 13 yr) fitted with jugular cannulas, sampled every 30 min for a 24-h period. Horses were housed in individual stables with a 12-h light:12-h dark cycle, with lights on from 0700.

Effects of Age

The effect of age on mean IGF-I concentrations for all sexes is shown in Table 2. There was an effect of age ($P < 0.001$) as well as an age $\times$ sex interaction ($P < 0.001$). The effect of age for each sex group is illustrated in Figure 5. In mares and geldings, IGF-I concentrations showed a gradual decrease with advancing age. This effect was much less marked in stallions, but the number of stallions sampled above the age of 8 was relatively low, and so this result should be treated with caution.
Table 1. Serum IGF-I concentrations in a population of 1,880 thoroughbred horses, including statistical tests for normality

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Mean age, yr</th>
<th>Mean (±SEM) IGF-I, ng/mL</th>
<th>Greatest concentration, ng/mL</th>
<th>Lowest concentration, ng/mL</th>
<th>99th percentile, ng/mL</th>
<th>Shapiro-Wilk univariate test</th>
<th>P-value</th>
<th>Skewness</th>
<th>Kurtosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>All horses</td>
<td>1,880</td>
<td>3.8</td>
<td>310 ± 2.2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.98</td>
<td>&lt;0.0001</td>
<td>0.58</td>
<td>0.57</td>
</tr>
<tr>
<td>Stallions</td>
<td>307</td>
<td>3.2</td>
<td>336 ± 5.6</td>
<td>627</td>
<td>95</td>
<td>587</td>
<td>0.98</td>
<td>&lt;0.0005</td>
<td>0.50</td>
<td>0.04</td>
</tr>
<tr>
<td>Mares</td>
<td>734</td>
<td>3.5</td>
<td>303 ± 3.4</td>
<td>676</td>
<td>77</td>
<td>547</td>
<td>0.99</td>
<td>&lt;0.0001</td>
<td>0.51</td>
<td>0.57</td>
</tr>
<tr>
<td>Geldings</td>
<td>782</td>
<td>4.3</td>
<td>302 ± 3.2</td>
<td>709</td>
<td>39</td>
<td>546</td>
<td>0.98</td>
<td>&lt;0.0001</td>
<td>0.52</td>
<td>0.57</td>
</tr>
</tbody>
</table>

*Means in a column with different subscripts differ, P < 0.001 (Tukey's test).
1The sum of individual groups does not equal the total population because sex was not recorded for all horses.
2Positive values for kurtosis indicate more extreme values than a normal distribution. Positive values for skewness indicate values above the mean are more spread out than values below the mean.

**Effect of Work**

There was no effect of horses being in work or not on IGF-I concentrations, and no significant interactions occurred between work and sex or age.

**DISCUSSION**

Exercise can influence the concentration of certain hormones, and is an important feature in the life of most horses. Thus, it is important to understand the potential effects of exercise, if equine IGF-I concentrations are to be interpreted accurately. In particular, some workers have observed that GH concentrations increase in response to exercise (Sticker et al., 1995), and this could have an inductive effect on IGF-I.

In the current study, we measured GH as a positive control hormone when examining the effects of moderate intensity exercise. Although GH concentrations seemed to increase at the onset of exercise, there was also a marked variation in GH concentrations at this time, such that a significant increase in mean GH concentration could not be demonstrated. An apparent peak 25 min after the onset of exercise was greatly influenced by a value observed in 1 horse, and likely reflected a sporadic GH pulse. Our results are similar to those of Thompson et al. (1994), who also failed to identify a significant GH response due to a large variation between horses: 3 horses showed no change at all, whereas 2 had very large responses. In contrast, Sticker et al. (1995) found that plasma GH concentrations were increased within 10 min of the initiation of exercise, even though their exercise protocol was less rigorous than that used in the current study. Overall, our results confirm that the measurement and interpretation of GH concentrations is problematic, due to large variations caused in part by its pulsatile secretory pattern.

Some caution should be taken when interpreting the results of the fitness training program because there was no parallel group of untrained horses. Furthermore, the horses used in the high-intensity exercise test were older than most thoroughbred racehorses. Nevertheless, circulating concentrations of IGF-I remained relatively constant during the training program and during the moderate and high-intensity exercise tests. This is in contrast to results obtained by Jackson et al. (1998), who reported that IGF-I concentrations were decreased when horses were given intense treadmill exercise and increased in horses after 40 min walking on a mechanical horse walker. Cappon et al. (1994) observed that 10 min of intense exercise in humans caused IGF-I concentrations to increase and remain significantly elevated for 20 min into recovery. Cappon et al. (1994) also reported that there was no long-term response of IGF-I to exercise, consistent with a number of other human studies that have examined the effects of exercise training (Kraemer et al., 1995; Nicklas et al., 1995) and with the lack of a training effect on equine IGF-I observed in the current study. Notwithstanding the variable effects of exercise reported in the literature, our data, which show no IGF-I response to a bout of exercise even at speeds that reflect race conditions, demonstrates that serum IGF-I is relatively stable in the horse, especially in comparison to GH.

Some hormones exhibit circadian, ultradian, or seasonal rhythms. For example, Irvine and Alexander...
Figure 5. Changes in the mean (±SEM) serum IGF-I concentration with age in 1,880 thoroughbred horses, ranging from 1 to 29 yr. Horses were located in Australia, South Africa, and the United Kingdom, but all samples were assayed using the same IGF-I assay. Numbers above the bars represent sample sizes.

(1994) described a distinct circadian pattern of cortisol secretion in untrained, unperturbed horses at pasture and stabled horses in training. However, they also found that the rhythm could be obliterated by minor disruptions, such as moving a horse to a new environment. The horses in the current study were housed in their stables for 5 d before the experiment, and the presence of a circadian cortisol rhythm in all 12 geldings, with a nadir in the late evening and a zenith early in the morning, was used to confirm that the horses had become accustomed to their new environment.

Having established that the horses exhibited a circadian rhythm for cortisol, we observed that IGF-I had no such rhythm. Although there was some variation in IGF-I throughout the 24-h period, there was no clear nadir or zenith. Two previous studies have attempted to examine whether IGF-I concentrations follow a circadian rhythm in horses (Popot et al., 2001; Jackson et al., 2003) but with less experimental rigor than the current study. Popot et al. (2001) observed no diurnal rhythm for IGF-I but collected samples from only 3 horses every hour from 0500 to 2100, disregarding the influence of nighttime. The study by Jackson et al. (2003) used 6 horses, with an irregular and infrequent sampling regimen of 0, 0.5, 1, 2, 4, 6, 8, 12, 18, and 24 h, commencing in the morning. Jackson et al. (2003) modeled their data using cosinor analysis, which requires that the data can reasonably be considered to take the form of a deterministic cycle with a known period (Lenz, 1990). These workers identified an IGF-I peak at 1730, but the sampling regimen was such that this could not be described safely as the zenith of a circadian cycle. Jackson et al. (2003) also neglected to identify a nadir, which is crucial to the determination of a distinct circadian rhythm (Irvine and Alexander, 1994). Several IGF-I peaks were observed in the current study, but these were small and occurred at irregular intervals.

Minuto et al. (1981) found that IGF-I concentrations in humans are stable during waking hours but decrease after the onset of sleep. The different sleeping patterns of horses and humans may explain why this sleep-wake cycle was not evident in the horse.

In the current study, IGF-I concentrations in the horse were not influenced by the time of day that a blood sample was taken, and therefore, a single sample would be expected to give a reliable indication of IGF-I status in the horse. This is in marked contrast to cortisol, and to GH, which shows an irregular pulsatile pattern of secretion throughout the day (Thompson et al., 1992).

Our study of IGF-I concentrations in a large population of racehorses confirms the findings of Malinowski et al. (1996) that IGF-I concentrations in the horse decline with age. This was observed most clearly in mares and probably reflects an age-related decrease in GH secretion, as is well known to occur in humans (Zadik et al., 1985). The less pronounced decrease in stallions should be interpreted with caution, as there were relatively few older stallions in the population. However, it is possible that IGF-I concentrations decline at a slower rate in male horses due to a counteractive effect of sex steroids, which have been shown to increase IGF-I in other species (Johnson et al., 1996; Clapper et al., 2000). Cymbaluk and Laarveld (1996) found that colt foals have greater serum IGF-I concentrations from birth to 17 wk of age, and it would appear this continues into adulthood because our observation showed the mean IGF-I concentration in stallions was 10% greater than that observed in mares and geldings. The mean age of the horses was similar for stallions, mares, and geldings (3.2, 3.5, and 4.3 yr, respectively), so it is un-
likely that this sex difference reflected the lower mean age of the stallions. Furthermore, IGF-I concentrations are known to be greater in males of other species, including sheep (Gatford et al., 1997) and pigs (Owens et al., 1999).

Age and sex had a predictable influence on IGF-I concentrations, but because there was not an equal representation of horses of all ages and each sex from each location, it was difficult to make a fair assessment of the normality of the population distribution. When this was attempted by applying a Shapiro-Wilk test to the whole data set, a deviation from normal was observed that was small but statistically significant. This deviation was caused by only 10 horses that had greater than expected IGF-I concentrations (in excess of 600 ng/mL), representing 0.5% of the sample population. It is possible these 10 horses had received exogenous GH or GH secretagogues or may have had pituitary tumors. Cymbaluk and Laarveld (1996) found that preweaning nutrition has effects on IGF-I that are detectable up to at least 12 mo of age, so there may have been some underlying nutritional cause.

The greatest concentration of IGF-I observed in the entire population was 709 ng/mL. Because exogenous GH treatment can increase IGF-I concentrations to levels beyond 1,000 ng/mL in horses (Noble and Silence, 2000a), IGF-I could be a useful forensic marker to detect GH abuse, at least as a preliminary screening test. This would require the setting of a threshold value for normal IGF-I concentrations in the horse, which we suggest could be about 800 ng/mL. This is 5 SD greater than the population mean of 310 ng/mL (with an SD of 94) and is 100 ng/mL greater than the preliminary threshold suggested by Popot et al. (2001), based on a much smaller sample size. In the case of a normal distribution, the probability of finding an untreated horse with such a high IGF-I concentration (false positive) would be less than 1 in 1,000,000. Although the true error rate would be slightly greater than this (due to nonnormal distribution), there is still a way to distinguish GH-treated horses from those with naturally high values. A horse that exhibits suspiciously large IGF-I concentrations could be impounded and blood samples taken over several days. Previously, we have reported that IGF-I concentrations decrease markedly within 5 to 10 d after the withdrawal of GH treatment (Noble and Silence, 2000a). In a horse with naturally large IGF-I levels, this decrease would not be seen. In summary, the existence of a small number of horses with naturally large endogenous IGF-I concentrations should not be an insurmountable barrier to the development of a GH forensic test.

Finally, it is known that in other species including humans, nutrition can influence IGF-I concentrations to a large extent, particularly severe changes in dietary protein and energy (Prewitt et al., 1982; Isley et al., 1983). The current study was conducted using horses that were all similar body condition and fed according to their energy requirements. Preliminary studies using diets adjusted for energy and protein within the range likely to be used in commercial racing establishments showed no effect on IGF-I (Noble, 2001). However, further research is needed in this area.

**IMPLICATIONS**

Our studies confirm that serum insulin-like growth factor-I concentrations are relatively stable in the horse, especially in comparison with growth hormone, showing that a meaningful measure of insulin-like growth factor-I status can be obtained from a single daily blood sample. In addition, a large database of equine insulin-like growth factor-I concentrations has been generated. This will enable researchers to identify significant variations in insulin-like growth factor-I concentrations with confidence, for a wide range of purposes.

**LITERATURE CITED**


Equine insulin-like growth factor-1


