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Pituitary pars intermedia dysfunction does not necessarily impair insulin sensitivity in old horses

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ABSTRACT

Equine pituitary pars intermedia dysfunction (PPID) has been associated with reduced insulin sensitivity in comparison with younger adult horses; however, the difference in insulin sensitivity between horses with PPID and aged-matched controls has not been well characterized. The objective of the study was to determine if aged horses with PPID had reduced insulin sensitivity and alterations in the insulin-mediated signaling pathways in the skeletal muscle when compared with healthy aged horses. Isoglycemic hyperinsulinemic clamp procedures were conducted in 12 horses that were classified as either PPID (n = 6; age: 25.0 ± 2.5 yr; mean ± standard deviation) or non-PPID, aged-matched controls (control) (n = 6; age: 25.7 ± 2.0 yr). Blood samples were taken before and during the clamp procedures to measure plasma glucose, insulin, and amino acid concentrations, and 2 muscle biopsies were collected from the gluteus medius muscle, one in the basal state and the second at the end of the clamp procedure (insulin-stimulated state). Plasma insulin concentrations increased ~9-fold during the clamp compared with basal conditions ($P < 0.001$) in both groups. During the last 30 min of the clamp, the rate of glucose infusion required to maintain isoglycemia in horses with PPID was similar to that in the control horses ($P = 0.67$). The plasma concentrations of most indispensible amino acids were lower in the insulin-stimulated state than the basal state ($P < 0.05$). PPID status did not have an effect on the activation of factors associated with protein synthesis and breakdown; however, factors associated with protein synthesis had increased phosphorylation in the insulin-stimulated state, compared with basal. The results from this study provide evidence that PPID is not always associated with impairments in insulin sensitivity.

1. Introduction

Pituitary pars intermedia dysfunction (PPID), also known as equine Cushing's disease, is believed to affect 15% to 30% of aged horses [1,2]. The disease is caused by a hypertrophy, hyperplasia, or adenoma formation on the pars intermedia of the pituitary gland and typically occurs in horses older than 15 yr of age [3]. Clinical signs of PPID can be highly variable among horses diagnosed with PPID [1] and include hirsutism [4], muscle atrophy [5], laminitis [6], increased secondary infections [7], and decreased insulin sensitivity [8].

Insulin resistance is defined as a condition in which normal concentrations of insulin produce a subnormal physiological response [9]. Currently, 2 studies have investigated the effects of PPID on dynamic measures of insulin sensitivity in the horse [8,10] and did detect differences in glucose and insulin dynamics between PPID and non-PPID horses. However, in both studies, the PPID horses were not age matched with the control horses, with one study having PPID horses >12 yr of age and control horses between 3 and 13 yr of age

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[10] and the second study having a mean age of PPID horses of 21 yr, versus a mean age of 10 yr in the control group [8]. Insulin sensitivity has been shown to decrease with age [11] and therefore, it is likely that age was a confounding factor in both of these studies [8,10]. It has not been fully elucidated as to whether insulin sensitivity is different between PPID and non-PPID, aged horses, although a large epidemiologic study recently found that aged horses diagnosed with PPID were more likely to be hyperinsulinemic (basal plasma insulin concentrations greater than 20 μIU/mL) than aged horses without PPID [1]. However, because only 32% of the PPID horses in this study were hyperinsulinemic [1], it appears that a PPID diagnosis does not necessarily imply insulin resistance in the aged equine.

PPID has also been associated with muscle atrophy [1,5], with 48% of horses diagnosed with PPID showing a wasted topline in a previous study [1]. Muscle atrophy occurs when the rate of protein degradation exceeds the rate of protein synthesis. The signaling pathways associated with both protein accretion and breakdown are affected by insulin [12,13], as well as other anabolic signals including amino acids and exercise [12,14]. The signaling pathways involved in muscle protein synthesis and degradation have been reviewed extensively elsewhere [14,15]. Briefly, it has been demonstrated that insulin, amino acids, and exercise all independently increase the phosphorylation of factors in the mechanistic target of rapamycin (mTOR) signaling pathway, which is responsible for the initiation of muscle protein synthesis [14]. A previous study determined that there was an increase in the activation of mTOR signaling factors of the skeletal muscle of mature (~14 yr old) horses in response to hyperinsulinemia [16]; however, there has been only limited research regarding the activation of the signaling pathways associated with muscle protein degradation in the horse [17]. Therefore, alterations in the insulin-responsive signaling pathways in the skeletal muscle could be a contributing factor to the mechanism behind the muscle atrophy that occurs in horses with PPID. However, because the incidence of muscle wasting in horses with PPID appears to be greater than the incidence of hyperinsulinemia (an indicator of insulin resistance) in these horses [1], it is likely that there are also other contributing factors to the loss of muscle mass that occurs in horses with PPID.

The objective of this study was to compare insulin sensitivity in horses with PPID compared with age-matched non-PPID controls using a dynamic test and proxies to measure insulin sensitivity. Furthermore, the study aimed to characterize differences in the activation of the signaling pathways associated with muscle protein synthesis and breakdown in response to insulin administration between the PPID and age-matched non-PPID control horses.

2. Materials and methods

The University of Kentucky Institutional Animal Care and Use Committee approved all procedures in this study. Horses were obtained from an already established herd of aged horses housed at the Department of Veterinary Science's Spindletop Farm at the University of Kentucky.

2.1. Animals, housing, and diets

Twelve horses, older than 20 yr of age, were classified as either PPID (n = 6; 25.0 ± 2.5 yr; mean ± standard deviation [SD]) or non-PPID age-matched control (control) (n = 6; 25.7 ± 2.0 yr). The selected horses consisted of 10 thoroughbreds (PPID: n = 5; control: n = 5), one mixed breed horse (control: n = 1) and one quarter horse (PPID: n = 1). Both the PPID and control group comprised 4 mares and 2 geldings of moderate body condition scores (PPID: 5.5 ± 0.5; control: 5.0 ± 0.7; [scale 1–9] [18]). Horses classified as PPID exhibited a variety of the classic physical signs associated with the disease including hirsutism, hyperhidrosis, muscle atrophy, a history of laminitis, and hair coat abnormalities [19]. All horses that were allocated to the PPID group displayed at least hirsutism and some degree of muscle atrophy (subjectively assessed), and PPID classification was confirmed based on a resting adrenocorticotropic hormone (ACTH) greater than 35 pg/mL and serum cortisol greater than 1 μg/dL 19 to 20 h following dexamethasone administration. Blood for the plasma ACTH hormone assay was collected in April using EDTA tubes (Vacutainer; Becton-Dickinson, Franklin Lakes, NJ) and sent to an external laboratory for analysis (Animal Health Diagnostic Center; Cornell University, Ithaca, NY) that used a chemiluminescent immunoassay to measure plasma ACTH. Plasma ACTH concentrations were well above the 35 pg/mL cut-off for PPID horses (103.6 ± 73.7 pg/mL) and much lower in the control horses (23.3 ± 8.2 pg/mL). Horses in the PPID group had plasma ACTH concentrations ranging from 50 to 243 pg/mL. Dexamethasone suppression tests were performed in May according the previously described procedures [20]. Blood samples were collected into glass evacuated tubes containing no additive (Vacutainer; Becton-Dickinson) before and 19 to 20 h following an intramuscular injection of dexamethasone at a dose of 0.04 mg/kg (DexaJect; Butler Schein Animal Health, Dublin, OH). Serum cortisol concentrations were determined by an external laboratory (Animal Health Diagnostic Center; Cornell University), which used a chemiluminescent immunoassay to measure cortisol concentrations. Plasma concentration of cortisol was suppressed in the control horses (0.46 ± 0.32 μg/dL), but not in the PPID horses (2.27 ± 0.92 μg/dL), consistent with a diagnosis of PPID. Horses were returned to the Department of Veterinary Science's herd of horses at the end of the study, and therefore it was not possible to perform a postmortem examination for a more definitive diagnosis of pituitary pars intermedia abnormalities.

All horses were housed in 1 paddock with free access to mixed grass hay (mean ± SD, as fed; 10.1% ± 0.1% crude protein, 33.0% ± 0.6% acid detergent fiber, 50.9% ± 0.6% neutral detergent fiber, 2.7% ± 0.4% fat, and 6.5% ± 0.4% ash), water, and salt blocks. Horses were fed a commercial concentrate (mean ± SD, as fed; 14.1% ± 0.1% crude protein, 22.5% ± 0.7% acid detergent fiber, 37.2% ± 1.3% neutral detergent fiber, 6.2% ± 0.2% fat, and 7.4% ± 0.2% ash) individually in stalls twice daily (12 g/kg BW/d), divided equally between meals at 7 AM and 3 PM. Feed and hay samples were collected throughout the study by random sampling and analyzed by Dairy One Forage Laboratory (Ithaca, NY). Horses were adapted to the diets and housing for a minimum of 2 wk before the isoglycemic hyperinsulinemic clamp procedures.