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Insulin infusion stimulates whole-body protein synthesis and activates the upstream and downstream effectors of mechanistic target of rapamycin signaling in the gluteus medius muscle of mature horses

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ABSTRACT

Little is known about the role insulin plays in regulating whole-body and muscle protein metabolism in horses. The objective of this study was to determine the effects of graded rates of insulin infusion on plasma amino acid concentrations and the activation of factors in the mechanistic target of rapamycin signaling pathway in the skeletal muscle of horses. Isoglycemic, hyperinsulinemic clamp procedures were conducted in 8 mature, thoroughbred mares receiving 4 rates of insulin infusion: 0 $mU \cdot kg^{-1} \cdot min^{-1}$ (CON), 1.2 $mU \cdot kg^{-1} \cdot min^{-1}$ (LOWINS), 3 $mU \cdot kg^{-1} \cdot min^{-1}$ (MEDINS), and 6 $mU \cdot kg^{-1} \cdot min^{-1}$ (HIGHINS). Blood samples were taken throughout the clamp procedures to measure plasma amino acid concentrations, and a biopsy from the gluteus medius muscle was collected at the end of the 2-h clamp to measure phosphorylation of protein kinase B, eukaryotic initiation factor 4E-binding protein 1, and riboprotein S6. Plasma concentrations of most of the essential amino acids decreased ($P < 0.05$) after 120 min of insulin infusion in horses receiving the LOWINS, MEDINS, and HIGHINS treatments, with the largest decreases occurring in horses receiving the MEDINS and HIGHINS treatments. Phosphorylation of protein kinase B, 4E-binding protein 1, and riboprotein S6 increased with all 3 rates of insulin infusion ($P > 0.05$), relative to CON, with maximum phosphorylation achieved with MEDINS and HIGHINS treatments. These results indicate that insulin stimulates whole-body and muscle protein synthesis in mature horses.

1. Introduction

The horse's body is approximately 50% muscle [1], and, as an athletic animal, the factors that regulate the development and maintenance of muscle mass are of particular importance. Protein is the largest nonwater component of muscle, composing approximately 65% of the dry mass of equine skeletal muscle [2]; therefore, net protein balance, or the balance between rates of muscle protein synthesis and breakdown, is a large determinant of muscle mass. Until recently, little research was conducted into the factors that regulate the signaling pathways of muscle protein synthesis and breakdown in equine skeletal muscle, although research in other species has found the central role of the

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mechanistic (formerly mammalian) target of rapamycin (mTOR) signaling pathway in muscle protein synthesis.

The mTOR signaling pathway and its roles in muscle protein synthesis have been reviewed extensively elsewhere, including figures of the pathway and its signaling components [3–5]. Briefly, mTOR is phosphorylated and activated through several independent upstream signaling pathways, which can be activated by anabolic stimuli such as insulin, through protein kinase B (Akt) phosphorylation, exercise, and amino acid administration. Once phosphorylated, mTOR phosphorylates its downstream effectors eukaryotic initiation factor (eIF) 4E binding protein 1 (4E-BP1), and p70 riboprotein S6 kinase (S6K1). When 4E-BP1 is phosphorylated, it can no longer bind to eIF4E, and eIF4E is free to associate with eIF4A and eIF4G to form a complex that can bind to the mRNA and form the 43S ribosomal complex [6]. When S6K1 is phosphorylated, it phosphorylates other downstream targets, including riboprotein S6 (rpS6), a component of the 40S ribosomal subunit [7]. Therefore, in the skeletal muscle, the overall effect of activated mTOR signaling is an increase in translation initiation and subsequently protein synthesis [8,9]. Mechanistic target of rapamycin signaling has not been studied extensively in equine skeletal muscle; however, we have previously shown that feeding activates downstream mTOR signaling in yearling, 2-y-old, and mature horses [10,11].

The feeding stimulus used in previous equine trials to study mTOR signaling resulted in increases in both plasma amino acids and insulin concentrations [10,11]. Amino acids and insulin activate mTOR signaling through independent mechanisms [3–5], and, from our previous research [10,11], we were unable to conclude whether the increases in mTOR signaling were due to the increase in plasma insulin concentrations, the increase in plasma amino acid concentrations, or a combination of both. In humans [12,13] and piglets [14], exogenous insulin infusion is able to increase muscle mTOR signaling independent of changes in plasma amino acid concentrations; however, the role of insulin in the regulation muscle protein synthesis has not been previously investigated in horses. By understanding how insulin regulates whole-body and muscle protein metabolism in healthy, mature horses, future studies can be designed to look at the effects of insulin resistance on protein metabolism in horses.

We hypothesized that increasing the rate of insulin infusion would promote whole-body and muscle protein synthesis. Therefore, the purpose of this study was to determine the effects of graded hyperinsulinemia on indicators of whole-body and muscle protein synthesis in mature, healthy horses. In particular, we were interested in the insulin-mediated changes in plasma amino acid concentrations and the phosphorylation of the upstream (Akt) and downstream (4E-BP1 and rpS6) factors in the mTOR signaling pathway in the gluteus medius muscle.

2. Materials and methods

2.1. Animals, housing, and diets

All procedures were approved by the Virginia Tech Institutional Animal Care and Use Committee. Eight nonpregnant, nonlactating mature (mean ± SD; 13.8 ± 3.4 y) thoroughbred mares were obtained from the Middleburg Agricultural Research and Extension Center herd of horses. All mares were healthy, of moderate-to-fleshy body condition (604.5 ± 37.4 kg; body condition score, 5–7 [scale, 1–9] [15]), and were on regular farrier, anthelmintic, and vaccination schedules.

Mares were group housed in a drylot pen, with free access to water and salt at all times. Diets were designed to meet all nutrient requirements of mature, inactive horses [16]. Timothy-orchard grass hay (mean ± SD; 9.8% ± 0.6% crude protein, 33.2% ± 0.8% acid detergent fiber, 55.4% ± 0.5% neutral detergent fiber, 2.7% ± 0.1% fat, and 6.0% ± 0.3% ash) was fed twice daily (7 AM and 2 PM), for a total daily allocation of 2% of body weight. To ensure that protein, vitamin, and mineral requirements were met, 1 kg of a ration balancer cube (mean ± SD; 15.8% ± 1.3% crude protein, 16.0% ± 0.5% acid detergent fiber, 30.1% ± 0.1% neutral detergent fiber, 5.8% ± 1.3% fat, and 8.1% ± 1.1% ash) (Culpeper Farmers Cooperative Inc, Bealeton, VA, USA) was fed at the 2 PM meal. The hay was fed in many separate piles, spread throughout the drylot, and the ration balancer cube was provided in individual dishes on the ground, spaced at least 6 m apart. Horses were monitored while the ration balancer cube was fed to confirm that each horse was able to consume its individual allocation. Horses were adapted to the drylots and diets for a minimum of 2 wk before the start of sample collection. Throughout the study period, core samples of hay and samples of the ration balancer cube were collected and sent to an external laboratory (Dairy One Forage Laboratory, Ithaca, NY, USA) for nutrient analysis.

2.2. Experimental design and procedures

This study was arranged as a completely randomized crossover design, whereby each horse underwent 4 isoglycemic hyperinsulinemic clamp procedures, once while receiving each of 4 different rates of insulin infusion: 0 $mU \cdot kg^{-1} \cdot min^{-1}$ (CON), 1.2 $mU \cdot kg^{-1} \cdot min^{-1}$ (LOWINS), 3 $mU \cdot kg^{-1} \cdot min^{-1}$ (MEDINS), and 6 $mU \cdot kg^{-1} \cdot min^{-1}$ (HIGHINS). Horses received the infusions in a random order, with approximately 2 wk between each study day. Two horses were studied on each sampling day, and during each sampling period an equal number ($n = 2$) of horses received each rate of insulin infusion. The rates of insulin infusion were selected so that the mTOR signaling response in skeletal muscle could be tested over a broad range of insulin infusion rates. The 1.2 $mU \cdot kg^{-1} \cdot min^{-1}$ rate was selected because previous research [17,18] showed that this rate of insulin infusion resulted in plasma insulin concentrations that approximated postprandial concentrations after a high-starch meal [19]. Although the prolonged (48 h) infusion of insulin at 6 $mU \cdot kg^{-1} \cdot min^{-1}$ induced laminitis in clinically normal horses [20], similar effects have not been reported when this rate was used for a shorter (<6 h) length of time [21–23]. Therefore, 6 $mU \cdot kg^{-1} \cdot min^{-1}$ was used as the highest rate of insulin infusion in the present study. The 3 $mU \cdot kg^{-1} \cdot min^{-1}$ rate was selected to be intermediate to the other 2 insulin infusion rates and has also been used in other studies that examined the activation