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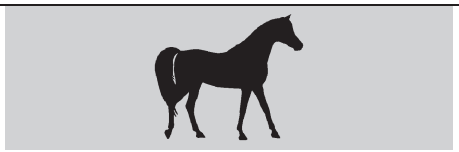

The effects of lipoic acid supplementation on blood glucose and insulin concentrations in pony mares[1]

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ABSTRACT

Lipoic acid has been found to modulate insulin and glucose dynamics in several species; therefore, the objectives of this project were to determine a) blood concentrations of lipoic acid after oral delivery to pony mares and b) whether daily treatments of 10 mg/kg of BW of lipoic acid for 14 d to overweight pony mares would alter insulin and glucose dynamics. In Exp. 1, 3 pony mares were used to determine absorption of lipoic acid into the peripheral blood supply after oral dosing. Jugular catheters were inserted and serial blood samples collected after administration of 10 mg/kg of BW of lipoic acid. Serum concentrations of lipoic acid increased 3,000-fold at 30 min postdosing, providing evidence that orally dosed lipoic acid is absorbed. In Exp. 2, 11 pony mares were blocked by BCS and ovarian status (intact vs. ovariectomized) and randomly assigned to receive daily treatments for 14 d of 10 mg/kg of BW of lipoic acid (LA; n = 6) or to the control group (CON; n = 5). On d 14, CON and LA ponies were fitted with jugular catheters, and i.v. glucose tolerance tests were performed. Insulin area under the curve was greater (P = 0.05) among CON (7,551 ± 1,136) than among LA (4,046 ± 1,037) treated ponies. No differences (P = 0.68) were found in glucose area under the curve between CON (3,961 ± 269) and LA (4,114 ± 246) treated ponies. These data provide evidence that lipoic acid supplementation for 14 d alters insulin dynamics in pony mares.

Key words: pony, glucose, insulin, lipoic acid

INTRODUCTION

Insulin is a biological regulator of energy balance whose primary role is maintenance of glucose homeostasis (Schwartz et al., 2000). Insulin also acts as an acute indicator of metabolic processes, increasing during glucose elevation (e.g., food intake) and decreasing during exercise and stress (Benoit et al., 2004). Insulin resistance is described by Kahn (1978) as existing "whenever normal concentrations of hormone produce a less than normal biologic response." In humans and horses insulin resistance and hyperinsulinemia have been associated with obesity and are recognized as growing health problems in both species (Jeffcott et al., 1986; Bastard et al., 2006; Geor, 2008; Hwang et al., 2012). Additionally, insulin resistance and hyperinsulinemia are associated with an increased risk for development of laminitis in equids (Treiber et al., 2006; de Laat et al., 2010; Johnson et al., 2010).

Lipoic acid is a naturally occurring, 8-carbon compound synthesized by animals and plants (Carreau et al., 1975) that is readily absorbed by the gastrointestinal tract of cattle and rats (Bustamante et al., 1998; Schmidt, 2004). Supplementation with lipoic acid has been found to improve

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insulin activity and glucose use in humans, rodents, and chickens (Saengsirisuwan et al., 2001; Hamano, 2006; Henriksen, 2006). Specifically in rats, lipoic acid supplementation improved insulin response as evidenced by increased expression of insulin receptor substrate-1 (IRS-1) signaling in skeletal muscle, a primary site for glucose uptake and storage (Saengsirisuwan et al., 2004). Supplementation of lipoic acid to horses is limited to its study as an antioxidant where it was found to moderately reduce oxidative stress in mature geldings kept at pasture (Williams et al., 2002).

The objectives of this study were to determine 1) the pharmokinetics of lipoic acid in pony mares after oral delivery and 2) serum concentrations of insulin and glucose as measured by an i.v. glucose tolerance test (**IVGTT**). It was hypothesized that lipoic acid would be readily absorbed by pony mares and that supplementation of lipoic acid for 14 d to overweight pony mares would alter insulin and glucose dynamics.

Table 1. Chemical analysis (DM basis) of hay fed to pony mares at 2% of BW daily throughout the 14-d trial period[1]

Item	%
CP	9.7
ADF	47.1
NDF	66.4
Nonfiber carbohydrate[2]	12.9
Fat	1.7
Ash	9.3
Calcium	0.4
Phosphorus	0.2

[1]Analyses were performed at Custom Laboratory Inc., Golden City, MO.

[2]Nonfiber carbohydrate = 100 − (CP + ash + fat + NDF).

MATERIALS AND METHODS

Animal Management and Blood Collection for Exp. 1

All animal procedures were approved by the University of Missouri Animal Care and Use Committee (#4105). Three pony mares between 8 and 18 yr of age with an average BW of 287 ± 12.1 kg and average BCS of 7.3 were used for Exp. 1. Ponies were removed from a drylot and placed in 3.6 × 3.6 m^2 stalls where they were acclimated for 1 d before the initiation of Exp. 1. Ponies were fed mixed-grass hay (Table 1) from the same source and load as had been fed while in the drylot at 2% of their BW daily split into equal feedings at 0830 h and evening hay at 2030 h with ad libitum access to fresh water and trace-mineral salt blocks. On the day of the study, ponies were fitted with indwelling jugular catheters (Extended Use MILACATH, Florence, KY) beginning at 0700 h. A baseline blood sample (10 mL) was taken at time −5 min (1030 h), and at time = 0 min, a 10 mg/kg of BW dose of lipoic acid was mixed with 30 mL of generic-brand maple syrup and administered as a bolus orally to each animal. Maple syrup was chosen as the carrier to ensure consumption of the bitter-tasting lipoic acid. The mixture was readily accepted by all ponies. Subsequent blood samples (10 mL) were taken at 1, 5, 30, 60, 120, 240, 360, 480, 600, 720, and 1,440 min after lipoic acid dosing. Blood samples were collected into Monoject vacutainer tubes with no additive (Tyco Healthcare Group, Mansfield, MA) and allowed to clot for 1 h at room temperature. Blood samples were then centrifuged at 3,000 × *g* for 20 min at 4°C. Serum was harvested and stored at −80°C for later HPLC analysis.

HPLC Analysis

Samples were prepared using the procedure of Teichert and Preiss (1995). Samples were thawed. A total of 250 μL of serum was placed into a 1.5-mL microtube, and a spiked sample was prepared by adding 100 μL of 1 mg/kg lipoic acid standard to a blank serum. A total of 500 μL of diluted alcalase in buffer was added to samples and spike for enzyme hydrolysis. After vortexing, samples were incubated in a 37°C water bath for 30 min. After incubation, 1 mL of distilled water and 1 mL of 0.2 *M* trichloroacetic acid were added, and the tubes were vortexed. Samples were loaded onto preconditioned (methanol followed by distilled water) Baker SPE (J.T. Baker, Phillipsburg, NJ) phenyl columns and washed with 1 to 2 mL of distilled water, and the columns were dried under vacuum. Lipoic acid was eluted from the columns with 4 mL of methanol and taken to dryness. The samples were reconstituted in 1 mL of HPLC-grade methanol:water (80:20), vortexed, and equilibrated for 30 min before HPLC analysis. Cloudy samples were transferred to microtubes, centrifuged at 13,000 × *g* for 5 min at 4°C, and then transferred to HPLC vials for analysis. Samples were analyzed using a ESA Model 582 pump (ESA Inc., Chelmsford, MA), a Thermoseparation Products autosampler (Thermo Scientific, Waltham, MA), and an ESA CoulArray detector (ESA Inc.; cell potentials of −90 mV, 580 mV, 759 mV, and 850 mV) with a Phenomenex 250 × 4.6 mm reversed phase Luna column (5 μm; Phenomenex, Torrance, CA). The mobile phase was acetonitrile:methanol:50 m*M* sodium acetate buffer (pH 4.8; 26:26:48). The flow rate was 1 mL/min.

Animal Management and Blood Collection for Exp. 2

Eleven pony mares between 8 and 18 yr of age with an initial average BW of 252.5 ± 18.3 kg were used. Ponies were removed from a drylot and placed in 3.6 × 3.6 m^2 stalls where they were acclimated for 2 d before the initiation of Exp. 2. While on test, ponies continued to be fed mixed-grass hay (Table 1) from the same source and load as had been fed while in the drylot. Ponies were maintained