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Effects of the rate of insulin infusion during isoglycemic, hyperinsulinemic clamp procedures on measures of insulin action in healthy, mature thoroughbred mares

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ABSTRACT

The objective of this study was to determine whether the rate of insulin infusion during isoglycemic hyperinsulinemic clamp procedures affected measures of insulin action, including glucose disposal and plasma non-esterified fatty acid, endothelin-1, and nitric oxide concentrations, in mature, healthy horses. Eight thoroughbred mares were studied during a 2-h hyperinsulinemic clamp procedure, conducted at each of 4 rates of insulin infusion: 0 (CON), 1.2 (LOWINS), 3 (MEDINS), and 6 (HIGHINS) $mU \cdot kg^{-1} \cdot min^{-1}$. The infusion rate of a dextrose solution was adjusted throughout the clamp procedures to maintain blood glucose levels within 10% of baseline glucose concentrations. Plasma insulin concentrations were measured throughout the clamp procedures, and used with the rate of glucose infusion to calculate the plasma insulin concentration-to-rate of glucose infusion ratio, a measure of insulin action on glucose disposal. The rate of glucose infusion increased with rate of insulin infusion ($P < 0.05$). The plasma insulin concentration-to-rate of glucose infusion ratio was highest for the LOWINS treatment ($P < 0.05$) and decreased by 62% ($P < 0.05$) and 84% ($P < 0.05$) for the MEDINS and HIGHINS treatments, respectively. Although plasma non-esterified fatty acid concentrations were lower than baseline by t = 30 min of the clamp procedures in the LOWINS, MEDINS, and HIGHINS treatments ($P < 0.05$), the decline was similar for all 3 rates of insulin infusion. Jugular vein plasma nitric oxide and endothelin-1 concentrations were not affected by insulin infusion rate ($P > 0.05$). The data indicate that it is important to standardize insulin infusion rate if data are to be compared between hyperinsulinemic clamp studies.

1. Introduction

Insulin resistance involves the decreased tissue responsiveness to insulin and is of interest in the equine population because of its role in conditions such as equine metabolic syndrome (reviewed by Frank [1]) and the development of pasture-associated laminitis [2,3]. Insulin resistance, as it relates to glucose metabolism, has been well described in horses and has been associated with a variety of conditions, including obesity [4], glucocorticoid administration [5–7], and systemic inflammation [5,8]. Although currently no

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"gold standard" method is globally accepted for diagnosing insulin resistance in a clinical setting, dynamic tests have been used in a research setting to characterize the degree of insulin resistance and to improve our understanding of the effects of insulin resistance on whole-body and tissue metabolism. The hyperinsulinemic clamp method is one such dynamic test and was developed for use in humans in the late 1970s [9] and adapted for use in horses in the early 2000s [10–12]. This method involves the infusion of insulin at a constant rate to create a steady hyperinsulinemic state, and, simultaneously, a glucose solution is infused at a variable rate to "clamp" blood glucose concentration at a predetermined level, either the baseline concentration (isoglycemic clamp) or the accepted fasting concentration (eg, 5 mmol/L; euglycemic). Once a steady rate of glucose infusion is reached, the rate of glucose infusion is assumed to be equivalent to the insulin-mediated rate of glucose uptake and disposal.

Although the hyperinsulinemic clamp (either euglycemic or isoglycemic) method has been widely used in horses to study glucose metabolism under a variety of physiological states [6,13–17], there is no standardized infusion protocol for this procedure. Insulin infusion rates have been variable, with commonly used infusion rates of 1.2 [14], 3 [6], and 6 $mU \cdot kg^{-1} \cdot min^{-1}$ [12]. Although a single study has tried to determine a reference range for normal rates of glucose infusion and glucose clearance, this study used a single rate of insulin infusion (6 $mU \cdot kg^{-1} \cdot min^{-1}$) and only 5 horses [12], and it is unknown whether these same reference ranges would be valid if a different rate of insulin infusion were used. Furthermore, a criticism of this method has been that it often induces a level of hyperinsulinemia far above the physiological range [18], with plasma insulin concentrations of approximately 300 and >1,000 μIU/mL when insulin is infused at 3 and 6 $mU \cdot kg^{-1} \cdot min^{-1}$ [6,13], raising concerns as to whether similar effects on glucose disposal and metabolism would be measured in response to a more physiological hyperinsulinemia, such as that seen after the consumption of a concentrate meal (approximately 50–100 μIU/mL) [19].

In addition to being used to study glucose disposal, hyperinsulinemic clamp procedures have also been used to investigate other insulin-regulated processes such as lipid metabolism [20–22]. Euglycemic hyperinsulinemic clamp studies in humans [20,21] and horses [23] have reported reduced plasma free fatty acid (NEFA) concentrations during clamp procedures. In humans, simultaneous isotopic studies of fatty acid metabolism have shown that the reduction in plasma fatty acid concentrations is due to a reduced plasma rate of appearance of fatty acids [20–22], indicating that insulin infusion under euglycemic procedures suppresses lipolysis. Although in humans the release of palmitate from different adipose tissue deposits is suppressed even by a low level of hyperinsulinemia (0.25–0.5 $mU \cdot kg^{-1} \cdot min^{-1}$ insulin infusion) [22], the effects of insulin infusion rate on changes in plasma fatty acid concentrations have not been studied in horses.

Insulin not only has direct effects on the regulation of metabolic pathways but can also have indirect effects on nutrient utilization through the regulation of blood flow and substrate delivery to the tissues. In particular, 2 vasoactive agents, endothelin-1 and nitric oxide, are released through independent signaling pathways that can both be stimulated by insulin [24]. The effects of circulating insulin concentrations on the circulating levels of these vasoactive agents have not been previously studied in horses.

The objective of this study was to determine the effect of the rate of insulin infusion during isoglycemic hyperinsulinemic clamp procedures on measures of insulin action in mature, healthy horses. These measures of insulin action included measures of glucose disposal, plasma NEFA concentrations, and the circulating concentrations of the vasoactive agents endothelin-1 and nitric oxide.

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 mature thoroughbred mares (mean ± SD; 13.8 ± 3.4 y) were obtained from the Middleburg Agricultural Research and Extension Center herd of horses. Mares were healthy, weighed 604.5 ± 37.4 kg, were of moderate-to-fleshy body condition (score of 5/9 to 7/9 [25]), and received regular farrier and veterinary care.

All mares were housed as a group in a drylot pen and had continuous access to water and salt, and they were adapted to the drylots and diets for 2 wk before the start of study procedures. The provided diet met all nutrient requirements for mature, idle horses [26] and consisted of timothy-orchard grass hay (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) and a ration balancer cube (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). Horses were fed hay at a daily allocation of 2% of body weight, divided between 2 meals (7 AM and 2 PM), and 1 kg of the ration balancer was fed with the 2 PM meal. Horses were monitored during the consumption of the ration balancer cube to ensure that each horse consumed its individual allocation. Samples of hay and 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), in a random order. During each sampling period, 2 horses were studied each day, and 2 horses received each rate of insulin infusion during the period. There were approximately 2 wk between each time an individual horse was studied. The rates of insulin infusion were based on those that have been previously reported in