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Reduction of Plasma NEFA Concentration by Nicotinic Acid Enhances the Response to Insulin in Feed-Restricted Holstein Cows

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ABSTRACT

The objective was to investigate the relationship between elevated plasma nonesterified fatty acid (NEFA) concentration and insulin resistance in Holstein cows. Six nonlactating, nongestating, ruminally cannulated Holstein cows were blocked by body condition score and randomly assigned to a sequence of 2 treatments in a crossover design. Cows were offered legume and grass hay ad libitum supplemented with minerals and vitamins and were allowed free access to water and a trace mineralized salt block. Mobilization of body reserves was stimulated by withdrawing forage for 48 h before initiation of treatments. Treatments consisted of 11 hourly abomasal infusions of water (control) or nicotinic acid (NA; 6 mg/h per kg of body weight) as an antilipolytic agent. Infusions of NA decreased plasma NEFA concentration from 545 μEq/L to approximately 100 μEq/L within 2 h after initiation of treatments, and differences were maintained throughout infusions. Intravenous glucose tolerance test was performed 8 h after initiation of treatments and was followed by 3 h of blood sampling. The reduction of plasma NEFA concentration led to significantly greater glucose clearance rate (1.9 vs. 1.2%/min) and to decreased glucose half-life (37 vs. 58 min), time to reach basal concentration (81 vs. 114 min) and glucose response area under the curve during 180 min of sampling [6,942 vs. 10,085 (μIU/mL) × 180 min]. Enhanced glucose clearance was achieved when plasma NEFA was reduced by NA, despite lower insulin concentration (70.0 vs. 97.9 ± 13.4 μIU/mL) and a tendency for smaller insulin response area under the curve during 180 min of sampling [7,646 vs. 12,104 ± 2,587 (μIU/mL) × 180 min], reflecting an increased response to endogenous insulin. Based on literature, we do not expect NA to have altered glucose metabolism directly; therefore, this experiment demonstrates a cause and effect relationship between elevated NEFA and insulin resistance in Holstein cows.

Key words: nonesterified fatty acid, insulin resistance, nicotinic acid, bovine

INTRODUCTION

Elevated plasma NEFA concentration is a common feature in dairy cows during the periparturient period and reflects increased reliance on adipose reserves to support energy requirements and milk fat synthesis. However, exaggerated plasma NEFA concentration leads to triacylglycerol accumulation in muscle (Roberts et al., 1981) and liver (Roberts et al., 1981; Grummer, 1993) and is associated with depressed feed intake and metabolic disorders (Grummer, 1993).

Similarly, elevated plasma NEFA concentration causes triacylglycerol accumulation in muscle and liver of nonruminants and induces insulin resistance (Petersen and Shulman, 2006). In vitro incubation of 3T3-L1 adipocytes with just 300 μEq/L of palmitic acid (Van Epps-Fung et al., 1997) or linoleic acid (Gao et al., 2004) is sufficient to impair insulin-stimulated glucose uptake. Insulin resistance results from increased intracellular content of fatty acid metabolism intermediates, such as long-chain acyl-coenzyme A and diacylglycerol, which activate protein kinase C isoenzymes that phosphorylate inhibitory Ser residues of insulin substrate receptor-1, affecting downstream insulin-signaling events (Gao et al., 2004; Petersen and Shulman, 2006).

Insulin resistance in periparturient ruminants serves to prioritize glucogenic nutrients for vital functions, fetal growth, and lactose production and to enhance mobilization of fatty acids and glycerol from adipose tissue (Bell and Bauman, 1997). However, exaggerated insulin resistance in adipose tissue can potentially lead to further increases in plasma NEFA concentration and to the onset of metabolic disorders. Increased plasma NEFA concentration has been associated with insulin resistance in Holstein cows, but a direct link has yet to be demonstrated. For example, feed restriction impaired the response to insulin challenges in Holstein cows, and the degree of insulin resistance was correlated to NEFA concentration before the challenge (Oikawa and Oetzel, 2006). Overconditioned cows had sustained elevation of plasma NEFA postpar-

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tum, impaired clearance of glucose during i.v. glucose tolerance test (**IVGTT**), and greater insulin response when compared with cows calving with lower BCS (Holtenius et al., 2003). These results suggest an insulin-resistant state mediated by elevated plasma NEFA concentration, adiposity, or both. We have shown that induction of hyperlipidemia by i.v. infusion of triacylglycerol emulsion causes insulin resistance in Holstein cows (Pires et al., 2007). The infusion of emulsion increased plasma NEFA, serum triacylglycerol, and glycerol, but we speculate the onset of insulin resistance was mediated by fatty acids derived from emulsion triacylglycerol, which enter the cells of peripheral tissues after lipolysis by lipoprotein lipase (Ferezou and Bach, 1999).

We have developed a model to induce differential concentrations of plasma NEFA in feed-restricted cows, using nicotinic acid (**NA**) as an antilipolytic agent (Pires and Grummer, 2007). This approach allows us to test for a cause-effect relationship between elevated NEFA and insulin resistance in Holstein cows. In the present experiment, we hypothesized that increased plasma NEFA concentration causes insulin resistance in Holstein cows; therefore, reducing plasma NEFA in feed-restricted cows would enhance their response to insulin.

MATERIALS AND METHODS

Animals and Treatments

Six nonlactating, nongestating ruminally cannulated Holstein cows were blocked by BCS (4.0 ± 0.4, mean ± SD; NRC, 2001) and randomly assigned to a sequence of 2 treatments in a crossover design. Twelve days were allowed between experimental periods to avoid potential carryover effects. For logistical reasons, animals were divided in 2 groups, each starting the experimental protocol 1 wk apart.

Cows were fed legume and grass hay ad libitum supplemented with minerals and vitamins to meet or exceed NRC recommendations (NRC, 2001). Feed was provided twice daily, except during feed restriction. Animals were allowed free access to water and a trace mineralized salt block throughout the experiment. Mobilization of body reserves and the consequent increase in plasma NEFA concentration were stimulated by withdrawing forage for 48 h before the infusion of treatments. During feed restriction, cows were supplemented daily with vitamins and minerals that were mixed with wheat middlings as a carrier (total of 1 kg/d) to meet requirements (NRC, 2001). Body weight (786 ± 36 kg; mean ± SD) was recorded on the day before initiation of each period, 5 to 6 h after morning feed was offered and was used to calculate individual treatment and glucose doses.

Administration of treatments started 48 h after initiation of feed restriction and consisted of 11 hourly abomasal infusions of 1 L of water (control) or the same volume of NA solution at a rate of 6 mg of NA/h per kilogram of BW. Nicotinic acid (99.7% purity; Adisseo USA Inc., Alpharetta, GA) was diluted in water with equimolar amounts of sodium bicarbonate to facilitate solubilization. The rate of NA infusion was based on a methodology to induce sustained reductions of plasma NEFA concentration in feed-restricted cows (Pires and Grummer, 2007). Cows were rumen-cannulated to allow infusion of treatments into the abomasum (Gressley et al., 2006) and to prevent ruminal degradation of NA. Each hourly dose was infused over approximately 1 min.

IVGTT and Blood Sampling

Catheters (polyurethane 14-gauge × 13 cm; MILA International Inc., Erlanger, KY) were fitted into the right jugular vein of each cow and attached to an extension set (Baxter International Inc., Deerfield, IL; 86 cm, 3.9-mL vol.) on the day before IVGTT. Patency was maintained by flushing catheters with 8 mL of heparinized saline (100 IU/mL) every 8 h or with diluted heparinized saline (10 IU/mL) during frequent sampling. Cows were given 10,000 IU of penicillin G/d per kilogram of BW (G. C. Hanford Manufacturing Co., Syracuse, NY) following the insertion of catheters as a prophylactic procedure.

Intravenous glucose tolerance test was performed 8 h after initiation of infusions by administering 0.25 g/kg of BW of glucose i.v. (dextrose 50% wt/vol; Phoenix Scientific Inc., St. Joseph, MO) over 4.3 ± 0.4 min (mean ± SD), followed by 50 mL of sterile saline to flush catheters and prevent contamination of subsequent samples. A 14-gauge catheter was chosen to facilitate the flow of glucose and shorten the duration of infusion. Treatments were applied for 8 h before IVGTT to allow comparison of results with a previous experiment in which hyperlipidemia was induced by i.v. infusion of lipid emulsion (Pires et al., 2007).

Blood samples were collected at 0 and 24 h of feed restriction, before each abomasal infusion, and at –15, –5, 7, 10, 15, 20, 30, 40, 50, 60, 75, 90, 120, 150, and 180 min relative to administration of glucose. Hourly infusion of treatments continued throughout the 3 h of IVGTT sampling.

Estrous Synchronization

Estrous cycle was synchronized each period using an intravaginal progesterone-releasing device (controlled internal drug release containing 1.38 g of progesterone;