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Effects of acute heat stress on lipid metabolism of bovine primary adipocytes

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ABSTRACT

Heat stress (HS) affects numerous physiological processes including nutrient partitioning and lipid metabolism. Objectives of this study were to evaluate how acute HS affects lipid metabolism in subcutaneous adipose tissue of dairy cattle. Adipose tissue biopsies were performed on Holstein cows for bovine primary adipocyte isolation and cultured at either 42°C (HS) or 37°C (thermal neutral, TN). Adipocytes were incubated with increasing isoproterenol (ISO), and with increasing concentrations of insulin in the presence of ISO to evaluate changes in lipolysis. Incorporation of radioactive acetate into lipids was measured as an indicator of lipogenesis. Abundance and phosphorylation of several lipolytic and lipogenic proteins were also measured. Adipocytes exposed to HS had an elevated maximal response to ISO and were more sensitive to lipolytic stimulation by ISO compared with cells cultured at TN. Thermal treatment did not affect the antilipolytic effects of insulin in the presence of ISO. Lipogenesis measured as acetate incorporation was not altered by HS, but a temperature by insulin interaction was observed for the regulation of acetyl CoA carboxylase, such that the presence of insulin resulted in a reduction in phosphorylation of acetyl CoA carboxylase in adipocytes cultured at TN but not HS conditions. Results of this study demonstrate that acute HS has a direct effect on the regulation of lipolysis and the rate-limiting enzyme of lipogenesis in isolated bovine adipocytes.
Key words: lipolysis, lipogenesis, beta-adrenergic receptor

INTRODUCTION

Heat stress (**HS**) is a physiological condition that occurs when the core body temperature of an animal exceeds a safe upper limit. Periods of HS result from a total heat load that is more than the animals' capacity for heat dissipation, thus prompting physiological and behavioral responses to reduce the strain (Bernabucci et al., 2010). In the United States alone, HS costs the dairy industry an estimated $1 billion annually (St-Pierre et al., 2003).

In the past decade, alterations in the endocrine and metabolic status under hot environments have been reported in dairy cattle (Ronchi et al., 2001; Bernabucci et al., 2002, 2006; Rhoads et al., 2009). Ronchi et al. (1999) demonstrated that HS has a direct effect on lipid metabolism and liver enzymatic activities of Holstein heifers. In addition, changes in the oxidative status and circulating adipocytokine levels were observed in periparturient cows exposed to hot environments (Bernabucci et al., 2006). Furthermore, reduced feed intake only partially explains the decrease in milk yield during HS (Rhoads et al., 2009). In a series of pair-feeding experiments, plasma NEFA concentration in lactating dairy cows exposed to HS failed to increase, suggesting that these animals did not mobilize as much adipose tissue as their pair-fed, thermal neutral (**TN**) counterparts despite decreased DMI and loss of BW. Also, plasma insulin concentrations gradually increased in lactating cows and growing steers exposed to HS relative to pair-fed TN counterparts. Additionally, it has been demonstrated that heat-stressed cows have an increased insulin response to a glucose tolerance test (O'Brien et al., 2010; Wheelock et al., 2010), which may be essential in their adaptation mechanism to HS. Therefore, it is not clear if the blunted adipose tissue mobilization during HS is a direct result of hyperthermia on the adipocyte or indirectly due to heat-induced insulinemia.

In recent years, adipose tissue has received much attention due to the discovery of its role as a complex and highly active metabolic and endocrine organ (Kershaw and Flier, 2004). An accurate understanding of the biological mechanism(s) by which thermal stress affects adipose tissue metabolism and its effect on milk synthesis is critical for developing novel approaches (i.e., genetic, managerial, nutritional, or a combination of these) to optimize dairy cow performance, particularly during the summer months. Therefore, the objective

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of this study was to evaluate the direct effect of HS on lipid metabolism in the lactating dairy cow, particularly on the molecular regulators of lipolysis and lipogenesis in cultured bovine adipocytes. In this study, we investigated adipocyte sensitivity to lipolytic and antilipolytic signals to assess if HS (1) alters their lipolytic response to β-adrenergic receptor (**BAR**) agonists, and (2) enhances inhibition of lipolysis by insulin. We also investigated changes in the rate of incorporation of radioactive acetate in bovine cells to determine if HS enhances insulin-stimulated lipogenesis.

MATERIALS AND METHODS

Animals and Adipose Tissue Biopsy

The maintenance of the animals and the experimental procedures performed on them were carried out in accordance with the Iowa State University Animal Care and Use Committee guidelines and regulations.

Experiments were conducted during the spring months (late February until April) of 2012–2014 when cows were not exposed to environmental HS. Second to third lactation Holstein cows (230 to 400 d postpartum) with a BCS $>$ 3.75 were randomly selected for the studies to facilitate biopsy of an adequate quantity of adipose tissue. Subcutaneous adipose tissue biopsies (approximately 40 g) were taken from between the pin and tail bone (ischial tuber and coccyx, respectively) of each cow using a minimally invasive procedure under local anesthesia (Faylon et al., 2014). Adipose tissue was placed in buffered saline solution with glucose, kept at 37°C, and transported to the laboratory.

Isolation of Adipocytes

Primary adipocytes were harvested from adipose tissue biopsies. Samples were subjected to 2 rounds of 40-min collagenase digestion at 37°C in a water bath with shaking (100 cycles/min). Adipocytes were isolated using nylon filter mesh and washed with warmed cocktail buffer [Krebs-Ringer solution (20 m*M* sodium bicarbonate, 20 m*M* HEPES, 20 m*M* D-glucose), 1 *M* sodium bicarbonate, 1 *M* HEPES, 1 *M* glucose, 6% BSA, 1 mg/mL collagenase type 1a from *Clostridium histolyticum* (Sigma Aldrich)]. After removal of the infranatant, adipocytes were suspended in warmed explant medium (1,000 mg/L of low glucose Dulbecco's modified Eagle medium, 4.4 m*M* sodium bicarbonate, 5 m*M* HEPES, 3% BSA), and approximately 900 μL of this cell suspension was aliquoted into 20-mL scintillation vials.

Experimental Treatments

Lipolysis Experiments. The cell suspensions were exposed initially and at 2-h intervals to a mixture of 95% air and 5% CO_2 and incubated in a gyratory incubator at 37°C. Following a 1-h acclimation period, cells were incubated under 2 experimental conditions for 1 h: 42°C (acute HS) and 37°C (TN). Core body temperature of lactating dairy cows is approximately 38°C. A slightly lower temperature was used to represent TN conditions because adipocytes were isolated from the subcutaneous adipose depot, which is expected to be maintained at a temperature slightly less than the core body temperature. Culturing cells at 42°C was done to represent a severe HS event in lactating cattle. This temperature was not reduced, as was done for the TN treatment, because extreme in vivo environmental conditions could exceed this temperature, potentially maintaining the elevated temperature in subcutaneous adipose depots during extreme HS. Subsequently, isoproterenol (**ISO**; $1.0 \times 10^{-5.5}$ *M* to 1.0×10^{-9} *M*) was added to the culture medium and cells were incubated for 90 min under HS or TN conditions. Each ISO dose was evaluated in 3 vials of cells for each thermal treatment, and this experimental protocol was replicated using adipocytes from 5 different cows. The incubation medium was aspirated after a 90-min incubation with ISO and stored at −20°C until analysis. Approximately 400 μL of protein homogenization buffer [10% SDS, 1 *M* sodium fluoride, 0.5 *M* EDTA, 1 *M* HEPES, 1 μL/mL protease inhibitor (Sigma), 1 μL/mL phosphatase inhibitor (Sigma)] was added to the remaining cells and kept at −80°C for subsequent protein extraction.

The lipolytic response of isolated primary adipocytes was also evaluated in the presence of insulin. As with the previous experiment, cells were exposed to the 2 temperature treatments (37°C and 42°C for TN and HS condition, respectively) following an hour-long acclimation period at 37°C. Insulin (0 to 2.5 mU) was administered 30 min after exposure to the thermal treatments. After 30-min incubation with insulin, ISO (4.5×10^{-7} *M*) was added into the cell suspension, and the adipocytes were incubated for 90 min before cell harvest. This concentration of ISO is that required to achieve half of the maximal lipolytic response, as determined for cells cultured under TN conditions in the previous experiment. The incubation medium was carefully removed and kept at −20°C until further analysis. Protein homogenization buffer was added to the remaining cells and kept at −80°C until protein extraction. Each insulin-temperature treatment combination was evaluated in triplicate vials of cells, and this experiment was repeated independently across 5 different cows.