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Effects of heat stress and nutrition on lactating Holstein cows: II. Aspects of hepatic growth hormone responsiveness[1]

M. L. Rhoads,* J. W. Kim,† R. J. Collier,* B. A. Crooker,‡ Y. R. Boisclair,† L. H. Baumgard,* and R. P. Rhoads*[2]
*Department of Animal Sciences, University of Arizona, Tucson 85721
†Department of Animal Science, Cornell University, Ithaca, NY 14850
‡Department of Animal Science, University of Minnesota, St. Paul 55108

ABSTRACT

Heat stress (HS) is a multibillion-dollar problem for the global dairy industry, and reduced milk yield is the primary contributor to this annual economic loss. Feed intake declines precipitously during HS but accounts for only about 35% of the decreased milk synthesis, indicating that the physiological mechanisms responsible for decreased milk production during HS are only partly understood. Thus, our experimental objectives were to characterize the direct effects of HS on the somatotropic axis, a primary regulator of metabolism and milk yield. We recently reported no differences in mean growth hormone (GH) concentrations, GH pulsatility characteristics, or GH response to growth hormone releasing factor in HS versus pair-fed (PF) thermoneutral controls. Despite similarities in circulating GH characteristics, plasma insulin-like growth factor (IGF)-I concentrations were reduced during heat stress conditions but not in PF animals, suggesting that uncoupling of the hepatic GH-IGF axis may occur during HS. We investigated this possibility by measuring proximal indicators of hepatic GH signaling following a GH bolus. Heat stress but not PF decreased abundance of the GH receptor and GH-dependent signal transducer and activator of transcription (STAT)-5 phosphorylation. Consistent with reduced GH signaling through STAT-5, basal hepatic IGF-I mRNA abundance was lower in HS cows. Thus, the reduced hepatic GH responsiveness (in terms of IGF-I gene expression) observed during HS appears to involve mechanisms at least partially independent of reduced nutrient intake. The physiological significance of reduced hepatic GH receptor abundance during HS is unclear at this time. Aside from reducing IGF-I production, it may reduce other GH-sensitive bioenergetic processes such as gluconeogenesis.
Key words: heat stress, somatotropin, hyperthermia

INTRODUCTION

The mechanism by which heat stress affects production is thought to be primarily explained by reduced feed intake (Collier and Beede, 1985). However, other hyperthermically induced physiological changes include an altered endocrine status and increased maintenance requirements (Collier et al., 2005) and both may cause a net decrease in nutrient and energy availability for milk synthesis. Recent studies demonstrated that circulating NEFA are not increased in heat-stressed (**HS**) dairy cattle (Shwartz et al., 2009) despite a reduction in feed intake, and this is especially obvious when HS animals are compared with their pair-fed (**PF**) thermoneutral counterparts (Rhoads et al., 2009). This altered post-absorptive lipid metabolism, independent of associated changes in feed intake, may help explain why reduced feed intake does not fully explain the decrease in milk synthesis during heat stress (Rhoads et al., 2009). In addition to lipid metabolism, heat stress alters carbohydrate homeostasis in dairy cattle and other species (Febbraio, 2001; Jentjens et al., 2002; Wheelock et al., 2010) providing further evidence of a perturbed metabolic milieu that may contribute to production losses during heat stress.

Metabolic and physiological adaptations are coordinated by changes in the concentration and actions of homeorhetic hormones (Bauman and Currie, 1980). Experimental evidence of homeorhetic action is particularly strong for growth hormone (**GH**; Bauman, 2000). Direct physiological effects of GH result from activating its extracellular receptor (**GHR**), which is present in numerous tissues (Le Roith et al., 2001). Direct action examples include promotion of NEFA mobilization and gluconeogenesis from adipose tissue and liver, respectively (Bauman and Vernon, 1993; Etherton and Bauman, 1998). Another target is skeletal muscle where GH decreases glucose utilization and may favor amino acid export by inducing insulin resistance (Bau-

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[2]Corresponding author: rhoadsr@email.arizona.edu

man and Vernon, 1993; Bell and Bauman, 1997). These direct actions provide the mammary gland with critical precursors for milk synthesis by sparing nonmammary tissue use (Bell, 1995; Bauman, 2000). Chronic heat stress appears to reduce circulating GH levels (Mohammed and Johnson, 1985; Igono et al., 1988; McGuire et al., 1991), an effect that may be partially dependent on plane of nutrition (Rhoads et al., 2009). It is unclear if altered circulating GH concentrations underlie a portion of the homeorhetic alterations observed during heat stress.

The majority (~80%) of plasma IGF-I is produced by the liver under the control of GH and represents the indirect actions of GH (Le Roith et al., 2001). However, when body reserves are needed to support various physiological processes, such as milk production, immune function, or the maintenance of essential systems, the GH-dependent hepatic IGF-I production becomes curtailed and discordant changes in the GH-IGF axis ensue (Bauman and Vernon, 1993). Thus, the uncoupling of the hepatic GH-IGF axis serves as a homeorhetic adjustment that facilitates body reserve mobilization (Bauman and Vernon, 1993). Moreover, bST administration during periods of negative energy balance results in blunted plasma IGF-I and galactopoietic responses (Vicini et al., 1991). Rhoads et al. (2009) recently reported that circulating IGF-I levels are modestly depressed during heat stress. This was not caused by a reduction in GH secretion because a similar decline in plasma GH occurred in PF cows without a subsequent reduction in plasma IGF-I (Rhoads et al., 2009). An alternative explanation may be a reduction in hepatic GH responsiveness (in terms of IGF-I production) during heat stress.

The ability of GH to activate a family of proteins known as the signal transducers and activators of transcription (**STAT**) has been extensively studied (Herrington et al., 2000). Upon binding to its receptor, GH predominantly activates STAT5, which leads to the homo- and heterodimerization of STAT molecules and eventually their translocation to the nucleus. Once in the nucleus, STAT molecules initiate transcription by binding a γ-interferon–activated sequence (STAT5a and 5b) or interferon-stimulated response element (STAT1 and 3; Imada and Leonard, 2000). In particular, STAT5 is required for signaling the positive effects of GH on hepatic IGF-I transcription (Davey et al., 2001; Woelfle et al., 2003).

Reduced feed intake accounts for a minor portion of reduced milk synthesis during heat stress (Rhoads et al., 2009), and the physiological basis for production losses during environmentally induced hyperthermia remains poorly understood. Our objective was to determine whether heat stress impairs hepatic GH responsiveness in dairy cows. This was evaluated by assessing GHR abundance and STAT5 activation following a GH bolus.

MATERIALS AND METHODS

Animals and Experimental Design

The animals and experimental design used in this study have been described previously (Rhoads et al., 2009). Briefly, 12 multiparous, lactating Holstein cows (140 ± 13 DIM, 663 ± 68 kg BW; 2 groups of 6 cows) were randomly assigned to individual tie stalls in 1 of 2 environmental chambers at the University of Arizona's William J. Parker Agricultural Research Complex. Throughout the experiment, cows were milked and individually fed a TMR twice daily (0500 and 1700 h). All procedures were reviewed and approved by the University of Arizona Institutional Animal Care and Use Committee.

After adapting to the environmental chambers for 7 d, cows in both treatment groups were exposed to constant thermoneutral conditions [20°C, 20% humidity (temperature-humidity index, **THI** = 64), with 12-h light and dark cycles] and allowed to eat ad libitum for 9 d [experimental period (**P**) 1]. During P1, the 2 groups of cows were managed identically and identified as well-fed (**WF**) if pair-fed (**TNPF**) in P2, or as thermoneutral (**TN**) if heat-stressed (**WFHS**) in P2. Period 1 and P2 were separated by 7 d and cows remained in a similar environment as described in P1 between periods. During P2 (9 d), the TN cows (group 2) were heat stressed, endured cyclical temperatures (to mimic daily variation) ranging from 29.4 to 38.9°C with constant 20% humidity and 12-h light and dark cycles, and were fed ad libitum (WFHS). Between 0000 and 0700 h, the THI remained at 73; thereafter the conditions became increasingly warmer until peaking at a THI of 82 between 1300 and 1500 h. After peak THI, temperatures gradually declined until the THI again reached 73 at 2300 h. During P2, the WF cows (group 1) remained in the same thermoneutral conditions but their daily intake was reduced to match that of the HS cows (TNPF). Decrease in daily feed intake by WFHS cows in P2 was determined as a percentage of their mean daily ad libitum intake in P1 and this percentage reduction was used to decrease intake of the TNPF cows. Throughout the study, all cows were fed the same diet composition and were maintained on the same milking regimen regardless of period or treatment.

Somatotropin Challenge

On d 9 of both periods cows were administered bST (1.85 mg/100 kg of BW; Monsanto, St. Louis, MO)