Effects of heat stress and nutrition on lactating Holstein cows: II. Aspects of hepatic growth hormone responsiveness¹

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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 somatotrophic 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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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 respon-

siveness 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)

Table 1. Oligonucleotide primers used for real-time PCR analysis (SYBR Green assay)

Transcript	Sequence ¹	Reference
Growth hormone receptor 1A	F: CCAGCCTCTGTTTCAGGAGTGT R: TGCCACTGCCAAGGTCAAC	Rhoads et al., 2008
Total growth hormone receptor	F: GGTATGGATCTCTGGCAGCTG R: CTCTGACAAGGAAAGCTGGTGTG	Rhoads et al., 2008
IGF-I	F: TTGGTGGATGCTCTCCAGTTC R: GCACTCATCCACGATTCCTGT	Rhoads et al., 2008
Suppressors of cytokine signaling-3 (SOCS3)	F: CCCCCAGGAGAGCCTATTAC R: ACGGTCTTCCGACAGAGATG	Rhoads et al., 2007
Ribosomal protein S9 (RPS9)	F: CCTCGACCAAGAGCTGAAG R: CCTCCAGACCTCACGTTTGTTTC	Janovick-Guretzky et al., 2007

¹For each transcript, the sequence of the forward (F) and reverse (R) primer is given in the 5' to 3' direction.

via the jugular catheter. Blood was collected from the intravenous catheter at −30, −20, −10, 0, 2.5, 5.0, 7.5, 10, 15, 20, and 30 min relative to bST administration. Plasma was harvested and stored as described for the daily blood samples (Rhoads et al., 2009).

Liver tissue was harvested by needle biopsy at −10 and 30 min relative to bST administration as described previously (Rhoads et al., 2007) with minor modifications. An area (approximately 40 cm² in size) within the 11th intercostal space on the cow's right side was clipped and disinfected with an iodine scrub solution, followed by 70% ethanol. Local anesthesia (10 mL of 2% lidocaine hydrochloride solution, Vedco Inc., St. Joseph, MO) was administered subcutaneously at the incision site. Once the area was properly anesthetized, a 1-cm-long incision was made at the center of the intercostal space. Liver tissue was collected with a 12-gauge biopsy needle (15 cm long with a 20-mm notch; US Biopsy, Franklin, IN), placed in a screw-cap microcentrifuge tube, and immediately snap-frozen in liquid nitrogen and stored at −80°C until RNA or protein extraction.

Analysis of Gene Expression

Total cellular RNA was isolated from liver tissue using the TRIzol reagent (Invitrogen, Carlsbad, CA) and Phase Lock Gels (Eppendorf AG, Hamburg, Germany) according to the manufacturers' instructions. The RNA was cleaned with the RNeasy Mini Kit (Qiagen, Valencia, CA) and DNase treated with the RNase-Free DNase kit (Qiagen). The RNA content of each sample was calculated based on absorbance at 260 nm measured by spectrophotometry (Nanodrop 1000, Thermo Fisher Scientific Inc., Wilmington, DE). The RNA quality was evaluated by calculating the ratio of absorbance at 260 nm and 280 nm, followed by Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA) analysis. The RNA was stored at −80°C. The SuperScript First Strand Synthesis System for reverse transcription-PCR (Invitrogen) was used to reverse transcribe 2.1 µg of RNA to cDNA.

Real-time quantitative PCR using Sybr Green I dye (Bio-Rad, Hercules, CA) was used to evaluate GHR1A, total GHR (**tGHR**), IGF-I, suppressors of cytokine signaling-3 (**SOCS3**), and ribosomal protein S9 (**RPS9**) gene expression in the liver samples as described previously (Rhoads et al., 2008). Primers for each gene of interest were designed using the Primer Express Software (Applied Biosystems, Foster City, CA; Table 1). The PCR reactions were performed in triplicate in a 25-µL volume using iQ SYBR Green Supermix (Bio-Rad). The PCR reactions were performed and fluorescence was quantified with the ABI Prism 7300 Sequence Detector (Applied Biosystems). For each assay, 40 PCR cycles were run and a dissociation curve was included to verify the amplification of a single PCR product. Analyses of amplification plots were performed with the Sequence Detection Software (Applied Biosystems). Data were analyzed using a 5-point relative standard curve generated using serial 10-fold dilutions of cDNA prepared and pooled from experimental samples. Each assay plate contained negative controls and the standard curve to determine amplification efficiency of the respective primer pair. The fold differences between an internal control sample and the samples of interest were calculated with the amplification efficiencies of each respective target gene [amplification efficiency taken to the power of the control threshold cycle (C_T) minus the sample C_T]. The fold differences for GHR1A, tGHR, IGF-I, and SOCS3 were calculated using the 25-ng pool dilution as the internal control.

Western Immunoblot Analysis

Total cellular protein extracts were prepared as described previously (Rhoads et al., 2007). Protein content of extracts was measured with the bicinchoninic acid protein assay (Pierce, Rockford, IL). Fixed protein amounts were electrophoresed on 10% discontinuous SDS-polyacrylamide gels and transferred overnight to nitrocellulose membranes (Schleider and Schuell Inc., Keene, NH). The membranes were blocked and incu-

bated with primary antibodies validated for bovine extracts as described previously (Rhoads et al., 2007). Antibodies were rabbit anti-human GHR (Jiang et al., 1998; Zhang et al., 2001), rabbit anti-mouse STAT5 (#SC-835, Santa Cruz Biotechnology Inc., Santa Cruz, CA), and rabbit anti-mouse phospho-STAT5 (Tyr694, Cell Signaling Technology Inc., Danvers, MA). Signals were detected by chemiluminescence (LumiGLO, KPL, Gaithersburg, MD) and quantified by densitometry using NIH Image software (<http://rsb.info.nih.gov/nih-image>).

Plasma Analyses

Plasma GH concentrations were determined in triplicate by radioimmunoassay as described previously (Weber et al., 2007). The minimal detectable GH concentration was 0.7 ng/mL of standard or sample added to the assay tubes. Intra- and interassay CV were 3.1 and 6.8%, respectively.

Statistical Analyses

Protein and gene expression data were statistically analyzed separately based on group using the PROC MIXED procedure (SAS Institute, Cary, NC) accounting for treatment (TN vs. WFHS or WF vs. TNPF) as a fixed effect and animal as the random effect. For plasma GH, each cow was analyzed using repeated measures with an autoregressive covariance structure and time as the repeated effect. The model contained covariate, treatment, and time and a treatment $\times$ time interaction. Data are reported as least squares means and considered significant if $P < 0.05$ or a trend if $P < 0.1$.

RESULTS

Because plasma IGF-I originates almost completely from the liver in a GH-dependent fashion (Le Roith et al., 2001), reduced hepatic IGF-I production in HS animals could be caused by the deficit in circulating GH or a product of impaired hepatic GH responsiveness. To investigate the latter, we measured proximal indicators (i.e., GH receptor and STAT-5 abundance and STAT-5 phosphorylation) of GH signaling in response to an acute bST challenge during heat stress and malnutrition. As shown in Figure 1, circulating GH increased >60 -fold ($P < 0.05$) immediately (2.5 min) following the intravenous bST bolus regardless of treatment period and, at 30 min post-bolus, remained more than 20-fold greater than pre-bolus plasma GH levels ($P < 0.05$). Heat stress but not TNPF decreased ($P < 0.05$) basal abundance of the GH receptor (measured

Figure 1. Profile of circulating somatotropin following intravenous bST administration (1.85 mg/100 kg of BW). Dairy cattle were maintained in constant thermoneutral conditions and fed ad libitum for 9 d (TN and WF) followed by exposure to cyclical temperatures ranging from 29.4 to 38.9°C with constant 20% humidity and were fed ad libitum (WFHS) or exposed to constant thermoneutral conditions and pair-fed (TNPF) with heat-stressed (HS) counterparts. Vertical arrows indicate time of liver biopsies (−10 and 30 min relative to intravenous bST infusion). The pooled SE was 18.3 ng/mL. There was no effect of period, treatment, or their interaction ($P > 0.2$).

in liver samples before the bST challenge; Figures 2A and 3A). Neither WFHS nor TNPF altered STAT5 abundance; however, WFHS decreased ($P < 0.05$) and TNPF tended to decrease ($P = 0.08$) STAT-5 phosphorylation (Figures 2A and 3A).

Consistent with the WFHS-related decline in GHR abundance, hepatic GHR1A gene expression was reduced by $\sim 33\%$ ($P < 0.05$; Figure 3B). Similarly, total GHR mRNA abundance declined during WFHS ($P < 0.05$; Figure 3B). The TNPF cows tended to exhibit an increase in GHR1A mRNA abundance ($P < 0.1$; Figure 2B) and a nonsignificant numerical increase in total GHR gene expression.

Next, we sought to determine if the reduction in proximal GH signaling through STAT5 was associated with reduced basal hepatic IGF-I mRNA abundance. Hepatic IGF-I gene expression decreased approximately 25% ($P < 0.05$) in WFHS cows, and a similar numerical but not statistically significant reduction of approximately 18% ($P = 0.14$) occurred in TNPF cows (Figure 4).

Neither WFHS nor TNPF altered SOCS3 gene expression (pre-bST challenge values; Figure 5). In contrast, acute bST administration increased SOCS3 gene expression 1.5- to 3-fold, regardless of environment ($P < 0.05$; Figure 5).

DISCUSSION

Dairy cows consume less feed and reduce milk production when exposed to temperatures above their

thermal comfort zone, and the extent of nutrient intake depression is thought to determine the overall decline in milk yield. We reexamined this idea in experiments conducted with dairy cows exposed to heat stress conditions or maintained in a thermoneutral environment but on a similar plane of nutrition. These studies demonstrate that the reduction in milk yield caused by heat stress is approximately 50% greater than that anticipated from feed reduction alone (Rhoads et al., 2009). Initially, we hypothesized that altered aspects of circulating GH (i.e., a reduction in GH action) may explain the discrepancy between feed intake and milk yield, especially given the critical role of GH in galac-

topoiesis and maintenance of metabolic homeostasis in energy-deficient dairy cows (Bell and Bauman, 1997; Bauman, 2000). Some aspects of circulating GH appeared to mirror changes in metabolism during heat stress; for example, heat-stressed dairy cattle do not appear to mobilize adipose tissue reserves despite being in a moderate negative energy balance (Rhoads et al., 2009; Schwartz et al., 2009). In addition, we observed a modest depression in plasma IGF-I during HS that was not evident in the pair-fed cohort animals, suggesting that HS animals may exhibit a mild form of hepatic GH resistance (Rhoads et al., 2009). Herein, we present cellular data demonstrating that the ability of the liver

Figure 2. Effect of plane of nutrition and bST challenge on hepatic components of growth hormone (GH) signaling and GH receptor (GHR) gene expression. Six lactating dairy cows were exposed to constant thermoneutral conditions and allowed to eat ad libitum for 9 d (well fed; WF) followed by exposure to constant thermoneutral conditions and pair-fed to the feed intake of heat-stressed counterparts (TNPF). On d 9 of each period, bST (1.85 mg/100 kg of BW) was administered with liver biopsies obtained at -10 and 30 min relative to bST administration. A) Left panel: liver protein extracts (50 µg) were analyzed by Western immunoblotting for the abundance of GHR, signal transducer and activator of transcription (STAT)5, and phosphorylated STAT5 (P-STAT5). The GHR antibody detected signals of ~130 and 140 kDa corresponding to mature and immature forms of the receptor (Kim et al., 2004); STAT5 and P-STAT5 signals have molecular size of ~95 kDa; data are from one representative cow. Middle and right panels: Bars represent means ± SE of GHR (before bST bolus) and P-STAT (post-bST bolus) protein abundance. B) Abundance of GHR1A and total GHR mRNA (before bST bolus) analyzed by real-time PCR. Results are expressed relative to expression in liver of WF cows. Bars represent the means ± SE of GHR1A and total GHR. Bars with different letters (y, z) differ at $P < 0.10$.

to respond to GH appears to be less in heat-stressed compared with thermoneutral dairy cows maintained on a similar plane of nutrition.

In dairy cattle, the liver-specific GHR1A transcript is regulated by physiological state, is positively associated with hepatic IGF-I gene expression, and appears to be the primary determinant underlying GH receptor abundance (Lucy et al., 2001). For these reasons, numerous investigations have used GHR1A gene expression, particularly during the transition period, to estimate the fidelity of the hepatic GH-IGF axis (Butler et al., 2003; Radcliff et al., 2003; Rhoads et al., 2004). In the current study, the positive relationships among GHR

abundance, GHR1A mRNA, and IGF-I gene expression remained intact (i.e., all decreased to a similar extent) in HS cows. In contrast, a moderate reduction in plane of nutrition tended to reduce basal hepatic IGF-I mRNA abundance despite a tendency for increased GHR1A gene expression. The lack of a moderate nutritional insult on GHR1A mRNA abundance has been reported by others (Kobayashi et al., 2002; Rhoads et al., 2007) and differs from the transition period where GHR1A abundance is depressed. The mechanism(s) for these discordant changes in GHR1A gene expression between stressful states, such as the transition period and heat stress and other periods of nutritional insufficiency,

A

B

Figure 3. Effect of heat stress and bST challenge on hepatic components of growth hormone (GH) signaling and GH receptor (GHR) gene expression. Six lactating dairy cows were exposed to constant thermoneutral conditions and allowed to eat ad libitum for 9 d (TN) followed by exposure to cyclical temperatures ranging from 29.4 to 38.9°C with constant 20% humidity and were fed ad libitum (WFHS). On d 9 of each period, bST (1.85 mg/100 kg of BW) was administered with liver biopsies obtained at -10 and 30 min relative to bST administration. A) Left panel: liver protein extracts (50 μ g) were analyzed by Western immunoblotting for the abundance of the GHR, signal transducer and activator of transcription (STAT)5, and phosphorylated STAT5 (P-STAT5). The GHR antibody detected signals of ~130 and 140 kDa corresponding to mature and immature forms of the receptor (Kim et al., 2004); STAT5 and P-STAT5 signals have molecular size of ~95 kDa; data are from one representative cow. Middle and right panels: Bars represent means $\pm$ SE of GHR (before bST bolus) and P-STAT (post-bST bolus) protein abundance. B) Abundance of GHR1A and total GHR mRNA (before bST bolus) analyzed by real-time PCR. Results are expressed relative to expression in liver of cows fed ad libitum. Bars represent the means $\pm$ SE of GHR1A and total GHR. Bars with different letters (a, b) differ at $P < 0.05$.

Figure 4. Effect of plane of nutrition (panel A) or heat stress (panel B) on hepatic IGF-I gene expression. Abundance of IGF-I mRNA analyzed by real-time PCR. Results are expressed relative to expression in liver of well-fed (WF) or thermoneutral (TN) cows. Bars represent the means $\pm$ SE of IGF-I (before bST bolus). Bars with different letters differ at $P < 0.05$ (a, b). When cows transitioned from WF to being maintained in thermoneutral conditions and pair-fed to the level of feed intake in heat-stressed cows (TNPF), there was a numerical reduction ($P = 0.14$) in hepatic IGF-I gene expression.

have not been resolved. One possibility may relate to increased levels of stress hormones (e.g., epinephrine, cortisol) typically observed during both the periparturient period (Bell, 1995) and elevated environmental temperatures compared with a reduced plane of nutrition (Collier and Beede, 1985); that is, certain physiological states (periparturient period and heat stress)

but not others (reduced feed intake) are associated with elevated stress hormones. However, Kobayashi et al. (2002) indicated that a single injection of epinephrine had no effect on GHR1A mRNA abundance, but the short duration of this catabolic signal may not have been sufficient to mimic the chronic nature (i.e., days) of exposure observed during the transition period or the

Figure 5. Effect of bST on hepatic suppressors of cytokine signaling-3 (SOCS3) gene expression in cows subjected to a reduced plane of nutrition (panel A) or exposed to heat-stress conditions (panel B). Twelve lactating dairy cows were exposed to constant thermoneutral conditions and ad libitum feed intake for 9 d (TN and WF) followed by exposure to cyclical temperatures ranging from 29.4 to 38.9°C with constant 20% humidity and were fed ad libitum (WFHS) or exposure to constant thermoneutral conditions and pair-fed to the feed intake of heat-stressed counterparts (TNPF). On d 9 of each period, bST (1.85 mg/100 kg of BW) was administered with liver biopsies obtained at -10 (pre) and 30 min (post) relative to bST administration. Abundance of SOCS3 mRNA was analyzed by real-time PCR; results are expressed relative to expression in liver of TN or WF cows. Bars represent the means $\pm$ SE of the SOCS3 signal normalized to the ribosomal protein S9 (RPS9) signal. The significant effect of bST is reported.

heat load utilized in this study. Future experiments are warranted to determine the long-term effect of stress hormones on hepatic GHR gene expression.

In the current study, we used an *in vivo* protocol consisting of a bST challenge surrounded by liver biopsies to measure activation of hepatic STAT5 molecules as an index for hepatic GH responsiveness. Such a protocol is necessary to capture proximal GH signaling because baseline activation of the janus kinase (**JAK**)-STAT pathway, indicated by phosphorylated molecules, is undetectable (Beauloye et al., 2002; Rhoads et al., 2007). By exposing each animal to this experimental protocol during control (TN conditions and WF) and treatment periods (HS or PF), we observed that heat stress reduced the ability of bST to activate proximal signaling (hepatic P-STAT5 protein) emanating from the GHR, and TNPF appeared to induce a similar response. Despite this similarity, heat stress appears to reduce intracellular GH signaling via a reduction in abundance of both GHR mRNA and protein, whereas undernutrition increased GHR-1A mRNA without altering GHR protein and therefore appears to alter postreceptor events. We hypothesized that members of the SOCS family of proteins might provide a possible mechanism for attenuation of GH signaling during intervals of insufficient intake imposed by WFHS or reduced feeding. Current evidence, from *in vitro* data, suggests a role for SOCS3 in the downregulation of GH-dependent transcriptional activation and suppression of JAK2 activity (Adams et al., 1998; Davey et al., 1999; Hansen et al., 1999). Although hepatic SOCS3 mRNA abundance did not differ among the WF, TNPF, TN, and WFHS cows before bST administration, bST markedly increased SOCS3 gene expression consistent with the response in nonruminant species (Adams et al., 1998; Davey et al., 1999; Hansen et al., 1999). This information supports a role for bovine SOCS3 in the downregulation of GH-dependent transcriptional activation and suppression of JAK2 activity, although this possibility requires direct examination.

Modest reductions in feed intake do not affect plasma IGF-I concentrations in lactating dairy cows (McGuire et al., 1992; Rhoads et al., 2009), but insufficient intake and the increased demand for nutrients by the mammary gland during early lactation result in increased plasma GH and decreased plasma IGF-I; in other words, an uncoupling of the GH-IGF axis. The slight reductions in plasma IGF-I concentrations in our HS cows (Rhoads et al., 2009) supported the notion that the metabolic milieu might favor uncoupling of the GH-IGF axis during heat stress. Our current results indicate that hepatic regulation of the GH-IGF axis might be more coupled in WFHS cows (decreased GHR mRNA and protein and decreased IGF-I mRNA) than in cows that only experi-

ence severe reductions in feed intake (increased GHR mRNA, decreased GHR protein and IGF-I mRNA). Although the mechanisms for reduced IGF-I mRNA, and presumably IGF-I protein, might differ, this might not be unexpected because the physiological objectives differ. During early lactation, uncoupling of the GH-IGF axis can be advantageous because substantial mobilization of body reserves is needed to meet lactation demands (Bauman and Currie 1980; Bell, 1995). In contrast, it appears the objective of GH-IGF axis uncoupling during HS may reduce mammary nutrient utilization and partition nutrients toward peripheral tissues (i.e., skeletal muscle) to help combat the increased thermal load and remain euthermic.

Emerging evidence indicates that heat-stressed growing (O'Brien et al., 2008) and lactating dairy cattle (Itoh et al., 1998; Wheelock et al., 2010) have increased basal insulin concentrations despite decreased feed intake and reduced plasma glucose levels. In addition, we observe greater glucose disposal (rate of glucose entry into cells) following a glucose tolerance test in HS compared with PF thermoneutral control animals (R. P. Rhoads and L. H. Baumgard; unpublished observations). Glucose disposal differences are associated with a weaker insulin response to the glucose bolus by the PF thermoneutral control cows whereas the insulin response remains unchanged during HS when compared with the thermoneutral period. Although we did not measure plasma insulin in this study, we would expect plasma insulin to increase from thermoneutral to heat-stress periods as we have demonstrated in our other heat stress studies (O'Brien et al., 2008; Wheelock et al., 2010; R. P. Rhoads and L. H. Baumgard; unpublished observations). If true, it is surprising that WFHS cattle exhibited indices of hepatic GH resistance given that insulin increases or restores hepatic IGF-I production during some periods of positive and negative energy balance, respectively (Butler et al., 2003; Rhoads et al., 2004). Recently, Meier et al. (2008) demonstrated that irreversible glucose loss caused by phloridizin treatment resulted in a partial uncoupling of the GH-IGF axis that could not be recapitulated with a similar level of undernutrition. Significantly, the effect of phloridizin treatment on the GH-IGF axis occurred without a change in plasma insulin, indicating that the liver may become insulin insensitive during periods when the interaction between insulin and glucose is weakened. It is tempting to speculate that HS dairy cattle experience a similar phenomenon, emphasizing the need to further investigate the role of insulin and glucose homeostasis in the regulation of liver function and the hepatic GH-IGF axis during heat stress.

Although not the focus of this study, a last key point relates to the mobilization of body reserves and mainte-

nance of milk production during periods of nutritional insufficiency and physiological stress. During heat stress, lactating dairy cows do not mobilize adipose tissue despite a moderate decrease in feed intake. In contrast, pair-fed thermoneutral control animals immediately begin to mobilize adipose tissue, which increases progressively as nutrient restriction continues (Rhoads et al., 2009; Wheelock et al., 2010). The heat-stressed and pair-fed controls both have a similar and marked decrease in calculated energy balance and both lose substantial amounts of body weight, indicating that the differential adipose tissue mobilization is independent of energetic state. Normally during periods of negative energy balance, GH promotes NEFA export from adipose tissue by accentuating the lipolytic response to β -adrenergic signals and by inhibiting insulin-mediated lipogenesis and glucose utilization (Bauman and Vernon, 1993; Etherton and Bauman, 1998). Direct GH actions such as these provide the mammary gland with critical precursors for milk synthesis by sparing nonmammary tissue use (Bell, 1995; Bauman, 2000) and if attenuated may prevent attempts to maintain milk production during periods of energy and nutrient insufficiency. We were not able to measure GH responsiveness in adipose tissue or other tissues in this study, and further investigations are needed to identify the presence and extent of peripheral tissue GH resistance.

Overall, these data indicate that variation in hepatic GHR abundance cannot be explained by considering only changes in GHR gene expression. Similarly, changes in tissue GH responsiveness do not always correlate with changes in GHR abundance, indicating that abundance or activity of intracellular signaling proteins is also regulated. Although our study demonstrates that abundance of hepatic STAT5 is almost invariant, future studies are needed to consider whether the activities of STAT5, JAK2, or other GH signaling proteins play a role in the effect of physiological state, such as heat stress and plane of nutrition, on GH responsiveness.

CONCLUSIONS

During periods of heat stress, dairy cattle use several physiological adaptations in an attempt to maintain a state of homeostasis. These adaptations are employed at the expense of milk production, and our understanding of these adaptations remains insufficient. The fact that only 35% of the precipitous decrease in milk production during heat stress is caused by reduced feed intake indicates that heat stress is directly acting through other mechanisms to alter production. During this experiment, we measured heat stress-induced alteration of hepatic GH responsiveness because of the central role the somatotrophic axis plays in dairy cattle

metabolism and production. Physiological adaptation to insufficient intake in early lactation includes an uncoupling of the GH-IGF axis. Lactating cows exposed to heat stress reduce their intake but the associated physiological adaptations differ because although circulating IGF-I is reduced, hepatic components of the GH-IGF axis appear to remain coupled. Although heat stress alone does not appear to be solely responsible for changes in STAT5 phosphorylation or SOCS3 gene expression, heat stress did significantly decrease hepatic GH receptor abundance and IGF-I mRNA abundance independent of reduced feed intake. Future experiments are warranted to investigate the physiological significance of these observations in an attempt to broaden our understanding of HS physiology in lactating dairy cattle.

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