Proceedings, Western Section, American Society of Animal Science

Vol. 68, 2017

Identifying hyperthermia in heat-stressed lambs and its effects on β agonist-stimulated glucose oxidation in muscle.[1]

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ABSTRACT: Heat stress is known to decrease value and production efficiency in food animals. Conversely, β agonists increase value due to increased muscle growth efficiency, but it is unknown how each of these factors impacts the other. In this study, we sought to determine how heat stress and β agonists affect glucose oxidation in muscle independently and in combination. Crossbred lambs were fed high-energy diets for 21 days containing one of three dietary β agonist treatments: no supplement, ractopamine HCl (β1 agonist), or zilpaterol HCl (β2 agonist). In addition, lambs were housed under one of two environmental conditions: thermoneutral (25°C, 15% RH) or heat stress (40°C, 35% RH). On the last day of treatment, two alternative temperature-measuring devices (infrared (**IR**) thermometer gun and IR camera) were compared to core body temperatures measured by rectal thermometer. Lambs were harvested on day 22 and intact soleus muscle strips were used to measure ex vivo glucose oxidation under basal and insulin-stimulated conditions. We found that ear and eye temperatures recorded with the IR camera and skin temperatures (sheared and unsheared) recorded with the IR thermometer guns (at higher emissivity) consistently correlated to core body temperatures measured with the rectal thermometer (r = ~0.6 to 0.7) and may represent non-invasive alternatives to rectal temperature for detecting hyperthermia in sheep. Surprisingly, we did not observe interactions among environmental treatment, dietary supplement, and incubation media for glucose oxidation rates. Exposure to heat stress for 21 days decreased ($P < 0.05$) skeletal muscle glucose oxidation by ~21%, dietary supplementation of β2 agonist for 21 days increased ($P < 0.05$) muscle glucose oxidation by ~15%, and addition of insulin to media during ex vivo incubation of muscle strips increased ($P < 0.05$) glucose oxidation by ~25%. Interestingly, dietary supplementation of β1 agonist had no discernable effect on muscle glucose oxidation. These findings show that heat stress reduces muscle glucose oxidation and β2 agonist increases it, although neither altered the impact of the other. Moreover, these effects were present 24 hours after treatments ended, which shows that heat stress and β agonist supplementation have lasting metabolic effects.
Keywords: metabolic regulation, growth efficiency

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INTRODUCTION

Heat stress and β adrenergic agonists both elicit responses in tissues by activating adrenergic pathways. In livestock, heat stress is known to decrease growth and metabolic efficiency, and β agonist supplementation has been shown to improve growth performance and efficiency (Buntyn et al., 2016). However, little is known about how these two activators of the adrenergic system interact with each other. Catecholemines, such as epinephrine, are the natural ligand of the adrenergic system. These compounds interact with two classes of receptors, α adrenergic receptors and β adrenergic receptors (Mersmann, 1998). In recent years, supplementation of β-specific adrenergic agonists have benefited the livestock industry due to the increase in lean muscle mass and increase in total body weight that the supplements induce in feedlot animals (Elam et al., 2009; Montgomery et al., 2009). Two β agonist supplements are presently FDA-approved; the β1 agonist, ractopamine HCl, and the β2 agonist, zilpaterol HCl (Delmore et al., 2010; Boler et al., 2012) Boyd et al. (Boyd et al., 2015) hypothesized that the increase in muscle mass in zilpaterol-fed animals may lead to greater heat stress signals such as increased respiration and panting in cattle. However, after analyzing average and maximum body temperatures of these animals, they found that zilpaterol-fed animals actually maintained lower average body temperatures than control animals. These findings show that there is still much to be learned about how these compounds affect muscle metabolic function and growth. Additionally, it is important to understand how environmental stressors such as heat stress affect the efficacy of the supplements. Because of their contrasting individual effects, we hypothesized that heat stress and β agonist supplementation would have interacting influences on skeletal muscle glucose oxidation, a key determinant of metabolic efficiency. Our objective was to determine the impact that heat stress, β1 agonists, and β2 agonists have on muscle-specific glucose metabolism and how the effects of these factors interact. Furthermore, we sought to test the ability of alternative temperature-measuring devices to detect hyperthermia in heat stressed animals.

MATERIALS AND METHODS

Animals and experimental design.

This study was approved by the Institutional Animal Care and Use Committee at the University of Nebraska-Lincoln. Studies were performed at the UNL Animal Science Complex, which is accredited by the Association

for Assessment and Accreditation of Laboratory Animal Care.

Columbia-Suffolk crossbred lambs averaging 11 months of age were purchased commercially. The study was performed in 2 blocks of 24 lambs each. After a 3-week acclimation period, all lambs were individually penned and fed identical high-energy base diets for 21 days and were housed under either thermoneutral (25°C, 15% RH) or heat stress (40°C, 35% RH) conditions. Additionally, each lamb received 1 of 3 dietary supplements: no supplement, ractopamine HCl (0.03996 g/hd/d), or zilpaterol HCl (0.025 g/hd/d) delivered in 200g ground corn added to the ration. Lambs were slaughtered on day 22.

Body temperature measurements.

Ambient temperature and humidity in each pen were measured with a Hobo (Onset Computer Corporation, Bourne, MA) at the time of temperature measurements. Two identical rectal thermometers (ReliOn, Bentonville, AR) were used to measure core body temperature, and the readings were averaged.

Two infrared (IR) thermometer guns, designated gun A (Model FB61354, Fisher Scientific, Pittsburgh, PA) and gun B (Model TN418LD, Metris Instruments, Los Gatos, CA), and an IR camera (Model A655sc, FLIR Systems Inc., Wilsonville, OR) were used to measure outer body temperatures to compare to rectal temperatures. The guns were held ~2ft from the animal and 10-second average temperatures were recorded at three different locations: the center of nose between the nostrils, the sheared loin area of the back over the $14/15^{th}$ ribs, and a non-sheared area (~3cm wool length) directly cranial to the sheared area. Temperature at each area was measured across a range of emissivity values, from 0.40-1.00. Images were captured with the IR camera at two different distances: 3-5 feet and 6-8 feet. Three forward-facing images were taken from each distance of each sheep. Images were analyzed using FLIR ResearchIR Max (FLIR Systems Inc.), and temperatures at the center of the nose between the nostrils, the center of the eye, and the center of the inside of the ear were averaged across the three images.

Soleus muscle isolation.

Soleus muscles were collected tendon-to-tendon from the left hindlimb at harvest and intact longitudinal strips were used to measure glucose oxidation. Muscle was washed in ice-cold phosphate buffered saline (PBS), dissected longitudinally, and strips were pre-incubated for 1 h at 37° C in gassed (95% O_2, 5% CO_2) Krebs-Henseleit bicarbonate buffer (KHB) containing 0.1% bovine serum albumin (Gibco Life, Grand Island, NY). Media was spiked with either nil (basal) or 5 mU/ml insulin (Humulin-R; Ely Lilly), and 5mM glucose. Strips were then washed for 20 minutes in treatment-spiked KHB with no glucose.

Glucose oxidation.

Rates for glucose oxidation were determined by oxidation of [^{14}C-U]-D-glucose as previously described (Cadaret et al., 2016)(Cadaret, 2016) with some modifications. Muscle strips were placed in sealed dual-well chambers and incubated for 2 h at 37°C in treatment-spiked KHB with 5 mM [^{14}C-U]D-glucose (0.25 µCi/mmol). The adjacent well contained 2M NaOH to capture CO_2. Following incubation, chambers were cooled at -20°C for 2 min, 2M HCl was injected into the media through the rubber seal to release media-bound CO_2, and the chambers were incubated for 1 hr at 4°C. Following incubation, muscle strips were weighed and NaOH was collected and mixed with UltimaGold scintillation fluid to determine specific activity of $^{14}CO_2$ using liquid scintillation with a Beckman-Coulter 1900 TA LC counter (Brea, CA). Specific activity of the media was determined from three 10-µl aliquots mixed with 500µl distilled water and scintillation fluid. Radioactive compounds and scintillation fluids were purchased from Perkin-Elmer (Waltham, MA).

Statistical analysis.

Temperature data were analyzed by one-way ANOVA for differences between thermoneutral and heat-stressed lambs using the GLM procedure of SAS (SAS Institute, Cary, NC). Pearson's correlation values between IR temperatures and rectal temperatures were also calculated using the CORR procedure of SAS. Glucose oxidation data were analyzed as a 2x3x2 factorial design by ANOVA using the GLM procedure of SAS, with environmental treatment (n = 24) and dietary supplement (n = 16) in the main plot and incubation media (n = 24) and all interactions in the subplot. For all outputs, lamb was the experimental unit. Data are presented as means ± standard error.

RESULTS

Hyperthermic measurements.

Temperatures measured by rectal thermometer, IR thermometer guns, and IR camera were higher ($P < 0.05$) in heat stressed lambs than in thermoneutral lambs (Figure 1). Typically, IR temperature measurements taken at the ear, eye, and back correlated well with rectal temperatures. However, IR temperatures measured on the nose of the animal were less correlative with rectal temperatures. Temperatures recorded by IR camera for each area did not differ due to distance from the animal, and correlations to rectal temperature were slightly greater for the ear than for the eye. For temperatures measured by IR thermometer gun, the greatest correlations were observed at higher emissivity levels (above 0.70), and correlations were not significant below 0.50 emissivity for either gun. Surprisingly, correlations to rectal temperatures were similar between sheared and unsheared areas of the back.

Glucose oxidation

No interactions were observed between environmental treatment, dietary supplement, and incubation media, and thus only main effects are presented. As expected, incubation of muscle strips with insulin increased ($P < 0.05$) glucose oxidation rates in all muscle strips compared to incubation without insulin

(Figure 2). Exposure to heat stress for 21 days decreased ($P < 0.05$) glucose oxidation rates in muscle collected at slaughter the day after ending the environmental treatment. Dietary supplementation of ractopamine for 21 days did not affect muscle glucose oxidation rates, but dietary supplementation of zilpaterol for 21 days increased ($P < 0.05$) muscle glucose oxidation.

DISCUSSION

In this study, we show that hyperthermia can be detected in chronically heat-stressed livestock by infrared devices and that it is detrimental to metabolic efficiency. Surprisingly, chronic heat stress did not affect the metabolic benefit of β2 agonist supplementation, as muscle glucose oxidation was similarly decreased in heat stressed animals regardless of whether they received the dietary supplement or not. Likewise, β2 agonist supplementation improved muscle glucose oxidation in thermoneutral and heat stressed animals alike. Moreover, the respective effects of heat stress and β2 agonist supplementation on muscle glucose oxidation were observed under both basal and insulin-stimulated conditions. Together, these findings show that hyperthermic animals exhibit less metabolic efficiency, which helps to explain poorer growth performance under heat stress conditions. Moreover, β2 agonists are effective promoters of metabolic efficiency even in heat stressed animals, which contributes to their value as growth promoters.

Heat stress, like most physiological stressors, activates the adrenergic system, and thus it would be reasonable to postulate that animals experiencing chronic heat stress would be less responsive to β adrenergic supplements. However, we show that there was no interaction between heat stress and β agonist supplementation on skeletal muscle glucose metabolism. These surprising results indicate that the inhibitory and stimulatory effects of heat stress and β2 agonist supplementation, respectively, on muscle glucose oxidation occur through independent mechanisms. Although it is safe to assume that the β2 agonist is functioning solely through β adrenergic pathways, it is unclear whether the effects of heat stress are mediated by other components of the adrenergic system or by other regulatory systems altogether. It is important to note that the effects from heat stress and zilpaterol were observed 24 hours after ending both treatments. Although the possible effects of residual zilpaterol in the animal's system cannot be dismissed, it would appear from these findings that both factors have lasting effects on muscle glucose metabolism, which is not surprising when the adaptability of skeletal muscle is considered. Future studies may be able to show additional mechanisms by which heat stress and β2 agonist supplementation work to influence metabolic efficiency in muscle.

In order to evaluate the effects of hyperthermia on metabolic function in livestock, it is important to reliably identify hyperthermic animals. Core body temperature is traditionally estimated by measuring rectal temperature with a thermometer. However, safety concerns and animal disturbance may limit the use of this technique. In this study, we show that hyperthermia can be reliably detected in sheep by measuring body surface temperatures with infrared devices. In general, temperatures measured at the eye, ear, and loin area of the back by IR camera as well as the more affordable IR thermometer guns correlated well with rectal temperatures. Importantly, temperatures measured at the nose were highly inconsistent and did not correlate well with rectal temperatures, and thus should not be considered as appropriate measurements of body temperature. We speculate that the poor results from the nose temperatures were due to the movement of air around the nose and differing amounts of moisture on the nose itself. Nonetheless, these IR devices are potentially useful tools to detect hyperthermia in livestock without entering the animal's pen or making physical contact. Moreover, additional research to normalize surface body temperature to core body temperature, these devices could be used as an alternative to rectal thermometers in clinical or production settings, which could improve safety and reduce animal stress.

IMPLICATIONS

In this study, we show that infrared devices can be used to detect hyperthermia in heat stressed animals and that hyperthermic animals exhibit reduced skeletal muscle glucose oxidation, which helps to explain performance deficits in hotter conditions. Moreover, β2 agonists improve muscle glucose oxidation but do not offset the heat stress-induced deficits, meaning that neither chronic heat stress or β2 agonist supplementation affects the animal's metabolic response to the other factor.

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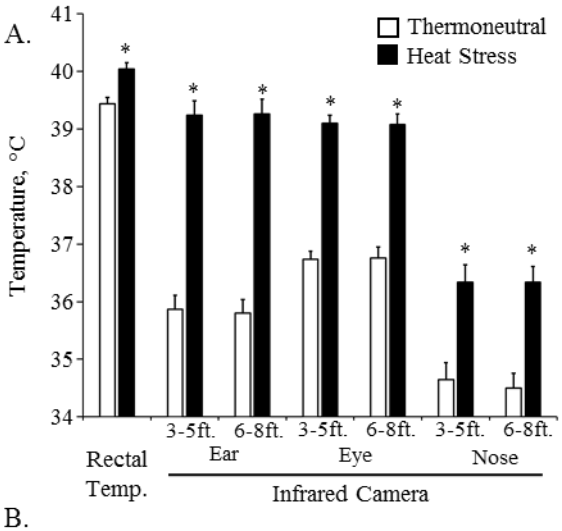

B.

Area	Distance (ft)	Correlation w/ Rect. Temp (r)	*P*-val
Ear	3-5	0.678	<0.01
	6-8	0.660	<0.01
Eye	3-5	0.604	<0.01
	6-8	0.611	<0.01
Nose	3-5	0.381	0.06
	6-8	0.354	0.08

C.

Emissivity	Area	Correlation w/ Rectal Temp (r)	*P*-val
0.60	Sheared Back	0.600	<0.01
	Unsheared Back	0.649	<0.01
	Nose	-	NS
0.75	Sheared Back	0.715	<0.01
	Unsheared Back	0.707	<0.01
	Nose	0.517	<0.01
0.90	Sheared Back	0.705	<0.01
	Unsheared Back	0.700	<0.01
	Nose	0.575	<0.01

Figure 1. Body temperatures in control and heat-stressed lambs. A. Differences in temperature measured at the ear, eye, and nose of lambs by infrared camera. *Denotes differences ($P < 0.05$) between thermoneutral and heat stressed lambs for each area/distance. B. Pearson correlation coefficients between rectal temperature and temperatures measured by infrared camera. C. Pearson correlation coefficients between rectal temperature and temperatures measured by infrared thermometer.

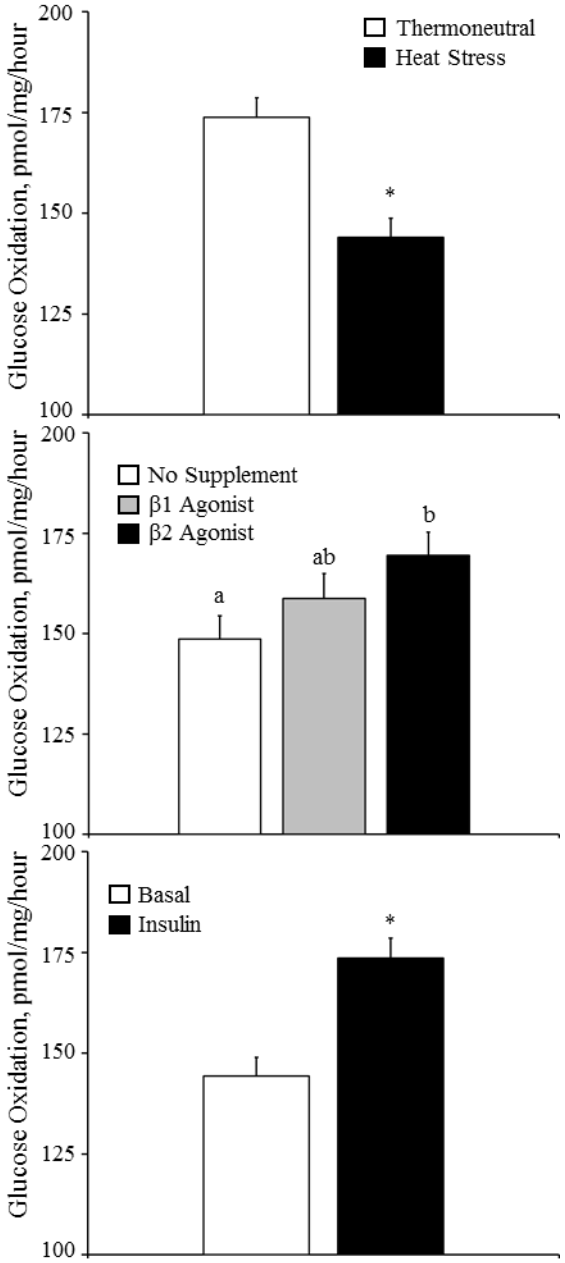

Figure 2. Glucose oxidation in primary soleus muscle from lambs house under thermoneutral or heat stressed (40°C) conditions and fed a diet supplemented with β1 or β2 agonists. *Denotes differences ($P < 0.05$) between thermoneutral and heat stressed lambs in the top box and between basal and insulin-spiked media in the bottom box. [a,b]Denote differences ($P < 0.05$) among dietary supplements.