7-2022

Beta-adrenergic agonists alter oxidative phosphorylation in primary myoblasts (Short Communication)

Renae Sieck
University of Nebraska - Lincoln, renaesieck@yahoo.com

Leah K. Treffer
Nebraska Wesleyan University

Anna M. Fuller
University of Nebraska-Lincoln, anna.fuller@unl.edu

Martonio Ponte Viana
University of Nebraska-Lincoln

Oleh Khalimonchuk
University of Nebraska-Lincoln, okhalimonchuk2@unl.edu

See next page for additional authors

Short Communication:

Beta-adrenergic agonists alter oxidative phosphorylation in primary myoblasts

Renae L. Sieck,[1] Leah K. Treffer,[2] Anna M. Fuller,[1]
Martonio Ponte Viana,[3] Oleh Khalimonchuk,[3]
Ty B. Schmidt,[1] Dustin T. Yates,[1]
and Jessica L. Petersen[1]

1 Department of Animal Science, University of Nebraska-Lincoln, Lincoln, NE 68583-0908, USA
2 Biology Department, Nebraska Wesleyan University, Lincoln, NE 68504, USA
3 Department of Biochemistry, University of Nebraska-Lincoln, Lincoln, NE 68588-0664, USA

Corresponding author — J. Peterson, jessica.petersen@unl.edu

Abstract

Beta-adrenergic agonists (β-**AA**s) are widely used supplements in beef and pork production to improve feed efficiency and increase lean muscle mass, yet little is known about the molecular mechanism by which β-AAs achieve this outcome. Our objective was to identify the influence of ractopamine HCl and zilpaterol HCl on mitochondrial respiratory activity in muscle satellite cells isolated from crossbred beef steers (*N* = 5), crossbred barrows (*N* = 2), Yorkshire-cross gilts (*N* = 3), and commercial weather lambs (*N* = 5). Real-time measurements of oxygen consumption rates (**OCR**s) were recorded using extracellular flux analyses with a Seahorse XFe24 analyzer. After basal OCR measurements

Published in *Journal of Animal Science*, 2022, Vol. 100, No. 8.
doi:10.1093/jas/skac208

Submitted March 31, 2022; accepted June 2, 2022 published 1 August 2022.

were recorded, zilpaterol HCl, ractopamine HCl, or no β-AA was injected into the assay plate in three technical replicates for each cell isolate. Then, oligomycin, carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone, and rotenone were injected into the assay plate sequentially, each inducing a different cellular state. This allowed for the measurement of OCR at these states and for the calculation of the following measures of mitochondrial function: basal respiration, non-mitochondrial respiration, maximal respiration, proton leak, adenosine triphosphate (ATP)-linked respiration, and spare respiratory capacity. Incubation of bovine cells with either zilpaterol HCl or ractopamine HCl increased maximal respiration ($P = 0.046$) and spare respiratory capacity ($P = 0.035$) compared with non-supplemented counterparts. No difference ($P > 0.05$) was observed between zilpaterol HCl and ractopamine HCl for maximal respiration and spare respiratory capacity in bovine cell isolates. No measures of mitochondrial function (basal respiration, non-mitochondrial respiration, maximal respiration, proton leak, ATP-linked respiration, and spare respiratory capacity) were altered by β-AA treatment in ovine or porcine cells. These findings indicate that β-AAs in cattle may improve the efficiency of oxidative metabolism in muscle satellite cells by modifying mitochondrial respiratory activity. The lack of response by ovine and porcine cells to β-AA incubation also demonstrates differing physiological responses to β-AA across species, which helps to explain the variation in its effectiveness as a growth supplement.

Lay Summary

Beta-adrenergic agonists (β-**AA**s) are supplemented to pigs and cattle to improve growth performance, carcass weight, and loin muscle area. Little is known about the mechanism taking place within individual cells by which β-AAs achieve this outcome. Previous work reported that β-AA supplementation improves the efficiency in which cells use glucose as an energy source and alters the expression of genes related to mitochondrial function, a key component of cellular energy production. To further our understanding of the impact of β-AA supplementation on these cellular functions, our objective was to identify the influence of two β-AAs used in livestock production, ractopamine HCl and zilpaterol HCl, on the mitochondrial respiratory activity of cells collected from the loin muscle and grown in culture. We isolated cells from cattle, pig, and sheep muscle and measured the oxygen consumption of the cells after treatment with ractopamine HCl, zilpaterol HCl, or with no supplement. We found that both ractopamine HCl and zilpaterol HCl enhance the efficiency of cellular energy production during a state of cellular stress in bovine muscle cells. There was no appreciable effect of the supplement on the energy production of pig or sheep cells. These data indicate that β-AA supplementation in cattle may increase the muscle cell energy production capacity compared with non-supplemented cells. This study also demonstrates that the efficiency

of cell energy production is one plausible mechanism underlying species differences in the response to β-AA supplementation.

Key words: mitochondria, oxidative metabolism, ractopamine HCl, respiratory efficiency, zilpaterol HCl

Abbreviations: β-AR, β-adrenergic receptor; β-AA, β-adrenergic agonists; DMEM, Dulbecco's Modified Eagle Medium; ECAR, extracellular acidification rate; FCCP, carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone; OCR, oxygen consumption rate; PBS, phosphate-buffered saline

Introduction

Beta-adrenergic agonists (**β-AA**s) are Food and Drug Administration-approved supplements utilized in pigs and cattle to improve growth performance, carcass weight, and muscle mass (Lean et al., 2014). Previous studies by our group have focused on understanding molecular changes in skeletal muscle due to β-AA supplementation. That work reported that β-AA supplementation altered the expression of genes involved in glucose metabolism and mitochondrial respiratory activity (Kubik et al., 2018; Reith et al., 2022). Skeletal muscle transcriptomics of lambs supplemented zilpaterol HCl revealed the upregulation of mitochondrial solute carrier *SLC25A25* (Kubik et al., 2018). SLC25A25 is a Ca^{2+}-sensitive ATP-Mg^{2+}/Pi inner mitochondrial membrane solute transporter that is reported to influence physical endurance and metabolic efficiency in mice (Anunciado-Koza et al., 2011). Furthermore, Ingenuity Pathway Analysis with transcriptome data indicated that zilpaterol HCl supplementation of beef steers for 3 d resulted in dysregulation of pathways related to mitochondrial function and oxidative phosphorylation providing further evidence that β-AA supplementation may alter mitochondrial respiratory efficiency (Reith et al., 2022). From a physiological perspective, β-AA supplementation increased glucose oxidation by skeletal muscle isolated from thermoneutral and heat-stressed lambs and from rats (Cadaret et al., 2017; Barnes et al., 2019; Swanson et al., 2020). These effects were independent of insulin signaling and glucose uptake, indicating direct alterations in intracellular metabolic processes. Due to the role of the mitochondria in skeletal muscle metabolism, the objective of this study was to understand how β-AAs affect mitochondrial respiratory activity in skeletal muscle satellite cells isolated from

cattle, pigs, and sheep. We hypothesized that β-AAs would improve the efficiency and ATP production capacity of muscle satellite cells by favorably modifying mitochondrial respiratory activity.

Measurement of the oxygen consumption rate (**OCR**) of cells provides insight into the amount of ATP produced via oxidative phosphorylation because, at a basal state of respiration, oxygen consumption and ATP production are strongly correlated. Direct comparisons cannot be made between cellular OCR and whole animal performance due to large variability in mitochondrial efficiency between individuals, populations, environments, and within a single individual over time (Salin et al., 2015). However, these data still provide valuable insight into whole animal performance because mitochondria provide over 90% of cellular ATP; therefore, animal performance is affected by mitochondrial efficiency (Bottje and Carstens, 2009). Furthermore, beef steers with a low residual feed intake had greater coupling between the electron transport chain and ATP production than steers with a high residual feed intake, thereby indicating a link between mitochondrial efficiency and animal feed efficiency (Kolath et al., 2006).

Materials and Methods

This study was approved by the Institutional Animal Care and Use Committee at the University of Nebraska-Lincoln, which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. Skeletal muscle was collected from ~16-mo-old crossbred beef steers (N = 5), ~7-mo-old crossbred barrows (N = 2), and ~6-mo-old Polypay lambs (N = 5) at the University of Nebraska-Lincoln. Additionally, skeletal muscle tissue was collected surgically from ~150 lb Yorkshire-cross gilts (N = 3) from control animals in other studies at the University of Nebraska-Lincoln. Primary satellite cells were isolated and cultured using the protocol described by Yates et al. (2014), with previously described modifications (Sieck, 2021). Connective tissue, fat, and membranes were dissected away from the muscle and the muscle (~5 g) was minced. The tissue was digested in a buffer of protease from *Streptomyces griseus* (Sigma-Aldrich, St. Louis, MO) in a 37 °C water bath for 1 h. Myoblasts were separated from tissue via serial centrifugation. The supernatant was collected and filtered with 100 μm filter conical

tubes (Millipore Sigma). Filtered fluid was centrifuged at 1,500 × *g* for 5 min. The supernatant was aspirated off, and pelleted satellite cells were suspended in 15 mL media (90% Dulbecco's Modified Eagle Medium [**DMEM**; Thermo Fisher Scientific, Waltham, MA] and 10% fetal bovine serum; Atlanta Biologicals, Flowery Branch, GA) and incubated on Cell Culture Microplates (Agilent Technologies) at 37 °C and 5% CO2 for 2 h to remove fibroblasts. The media and non-adhered myoblast mixture was collected into a 50-mL conical tube. Plates were washed with an additional 10 mL of phosphate-buffered saline (**PBS**), and the PBS was added to the conical tube. Tubes were centrifuged at 1,500 × *g* for 5 min. The supernatant was aspirated off, and pelleted cells were resuspended in 15 mL growth media (78.5% DMEM, 20% fetal bovine serum, 1% AbAm; Thermo Fisher Scientific, Waltham, MA), and 0.5% gentamycin (Thermo Fisher Scientific, Waltham, MA) per 15 cm culture plate. Cells were seeded onto poly-l-lysine and fibronectin-coated cell culture plates and incubated at 37 °C and 5% CO2. Satellite cells were lifted from their culture plates once they reached approximately 90% confluency, prior to differentiation, for splitting into assay plates using Accutase (Innovative Cell Technologies, Inc., San Diego, CA). The cells were collected into a conical tube, and plates were washed with additional warm PBS that was also added to the conical tube. Cells were pelleted by centrifuge at 1,500 × *g* for 5 min. The supernatant was aspirated, and pelleted cells were resuspended in 37 °C PBS and counted using a hemocytometer. Conical tubes were again centrifuged at 1,500 × *g* for 5 min, and the supernatant was aspirated. Seeding density optimization trials were performed as recommended by the manufacturer (Characterizing Your Cells Using OCR Values to Determine Optimal Seeding Density, 2017). Based on the results, bovine cells were resuspended in growth media at a concentration of 1,000,000 cells/mL. Ovine and porcine cells were resuspended in growth media at a concentration of 1,250,000 cells/mL. Cells (100,000 for bovine and 125,000 for ovine and porcine) were added to 20 of the wells in a poly-l-lysine and fibronectin-coated 24-well Cell Culture Microplate (Agilent, Santa Clara, CA) and incubated at 37 °C and 5% CO2 for 10 min. Each microplate had four wells filled with 250 µL growth media but without cells to serve as a blank control. After incubation, 150 µL of growth media was added to each well, and plates were incubated at 37 °C and 5% CO2 overnight. To determine the purity of myoblast isolates, a subsample of 50,000 cells from each isolate was stained for the

myoblast marker pax7 (mouse anti-pax7, 1:50; AbCam, Cambridge, MA, USA) as previously described (Yates et al., 2014). Myoblast isolates averaged 90.1 ± 2.0% pax7+ nuclei.

The assay for OCR was performed using a Seahorse XFe24 Analyzer (Agilent, Santa Clara, CA) following the manufacturer's protocol (Seahorse XF Cell Mito Stress Test Kit User Guide, 2019) with the following modification: Seahorse DMEM Media were supplemented with 0.01% sodium pyruvate (100 mM), 0.01% glutamine (200 mM), and 0.01% glucose (2 M). A carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone (**FCCP**) titration was performed (0 to 2 μM). FCCP, oligomycin, and rotenone were prepared by combining stock solution and Seahorse DMEM Media at a 1:10 ratio. Oligomycin solution (55 μL) was added to port A of the injection plate, FCCP solution (65 μL) was added to port B, and rotenone solution (70 μL) was added to port C. For each species, dose–response curves for zilpaterol HCl (0.05 to 0.2 μM) and ractopamine HCl (1 to 4 μM) were performed. Based on the results, a zilpaterol HCl concentration of 0.1 μM and a ractopamine HCl concentration of 2 μM were selected for all species. Each β-AA was diluted in Seahorse DMEM Media. The respective β-AA was added to port D. Media without β-AA were injected for unsupplemented incubations. Three replicates were performed under each incubation condition for cells isolated from each animal.

Following the Seahorse assay, media were aspirated from the plate, and 75 μL of radioimmunoprecipitation assay buffer (Thermo Fisher Scientific, Waltham, MA) was added to each well and triturated to isolate protein. Protein concentration for each cell lysate was determined using a or a Pierce Coomassie Plus (Bradford) Assay Kit (Thermo Fisher Scientific, Waltham, MA) for the ractopamine and zilpaterol treatments of all bovine cell lysates and the control treatment of two bovine cell lysates following the manufacturer's protocol. Due to the availability of machinery, a Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA) was used to determine protein concentration for the control treatment of three bovine cell lysates and all cell lysates from pigs and sheep following the manufacturer's protocol. OCR data were normalized to the estimated protein concentration of each well in the Seahorse Wave desktop software (Agilent, Santa Clara, CA).

Individual well and individual measurement outliers were excluded based on previously described criteria (Yépez et al., 2018). OCR values

for each well were normalized to that well's average baseline values and are presented as fold changes from baseline. Considerable variation was observed in time point 1 readings, attributed to variation during machine calibration. Therefore, time point 1 was excluded from evaluation in all plates and was not used in the calculation of baseline OCR used for normalization. OCR metrics were calculated based on the manufacturer's user guide (Seahorse XF Stress Test Report Generator User Guide, 2016) with the following modifications (**Figure 1**). Basal OCR was calculated by subtracting the non-mitochondrial OCR from 1. ATP production was calculated by subtracting the minimum rate after oligomycin injection from 1. Due to a small number of biological replicates within each species, our statistical model did not analyze differences between individual cell isolates. Instead, cell isolates within each species were treated as technical replicates. Each OCR metric was analyzed via one-way analysis of variance (ANOVA) with the GLIMMIX procedure in SAS (SAS Institute) for the effect of supplement (i.e., No β-AA, zilpaterol HCl, or ractopamine HCl). Cell isolate was the experimental unit. Data were analyzed separately for each species.

Results

The change in OCR between the time points before and the time points after the addition of β-AAs did not differ among species ($P > 0.05$) or among β-AA conditions ($P > 0.05$). Therefore, baseline OCR was calculated by combining the readings collected before (i.e., time points 2 and 3) and after (i.e., time points 4 to 6) the injection of β-AAs, which provided a greater number of technical replicates. Extracellular acidification rate (**ECAR**) data were also an output of this assay but were not analyzed as part of this study because ECAR readings in this study fell outside of the optimal range recommended by the manufacturer.

Incubation of cells with either zilpaterol HCl or ractopamine HCl increased maximal respiration ($P = 0.046$) for bovine cells compared with those incubated with no β-AA, while no difference in maximal respiration ($P > 0.05$) was observed in porcine or ovine cells (Figure 2). Spare respiratory capacity was greater ($P = 0.035$) for bovine satellite cells incubated with zilpaterol HCl or ractopamine HCl than for those incubated with no β-AA—this was not significant in either of the other

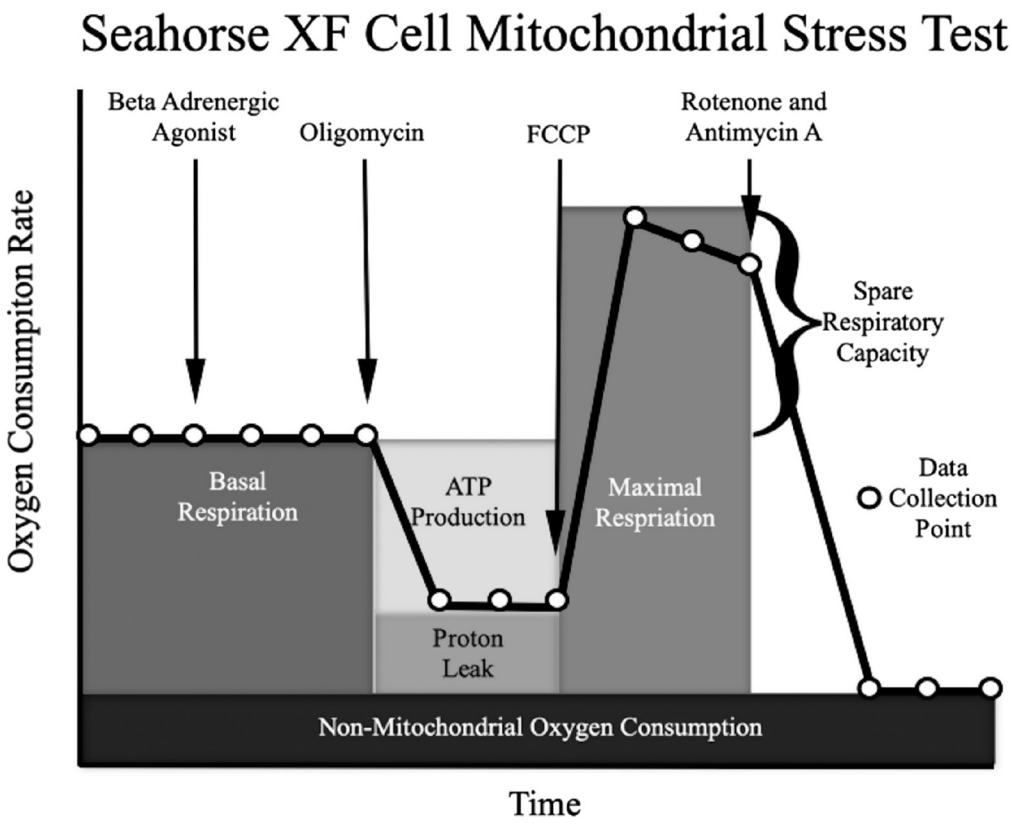

Figure 1. Experimental design for Seahorse XF Cell Mitochondrial Stress Test. Oxygen consumption rate (OCR; y-axis) is measured at 15 time points (open circles) before and after the addition of beta-adrenergic agonists (β-AA; or no/control treatment) and three drugs. The expected response of the cell is shown, and measures of mitochondrial function are illustrated (Seahorse XF Stress Test Report Generator User Guide, 2016). Non-mitochondrial respiration is calculated as the minimum OCR of time points 13 to 15. Basal respiration is 1 minus the non-mitochondrial respiration. Maximal respiration is calculated as the maximum OCR for time points 10 to 12 minus the non-mitochondrial respiration. Proton leak is the minimum OCR of time points 7 to 9 minus the non-mitochondrial respiration. ATP-linked respiration is calculated as 1 minus the minimum OCR of time points 7 to 9. Spare respiratory capacity is maximal respiration minus basal respiration.

species (Figure 2). No difference was observed between zilpaterol HCl and ractopamine HCl ($P > 0.05$) for any measure or within any species. No differences were observed among treatments in any species for basal respiration, ATP-linked respiration, proton leak, or non-mitochondrial respiration due to β-AA treatment ($P > 0.05$; Figure 2).

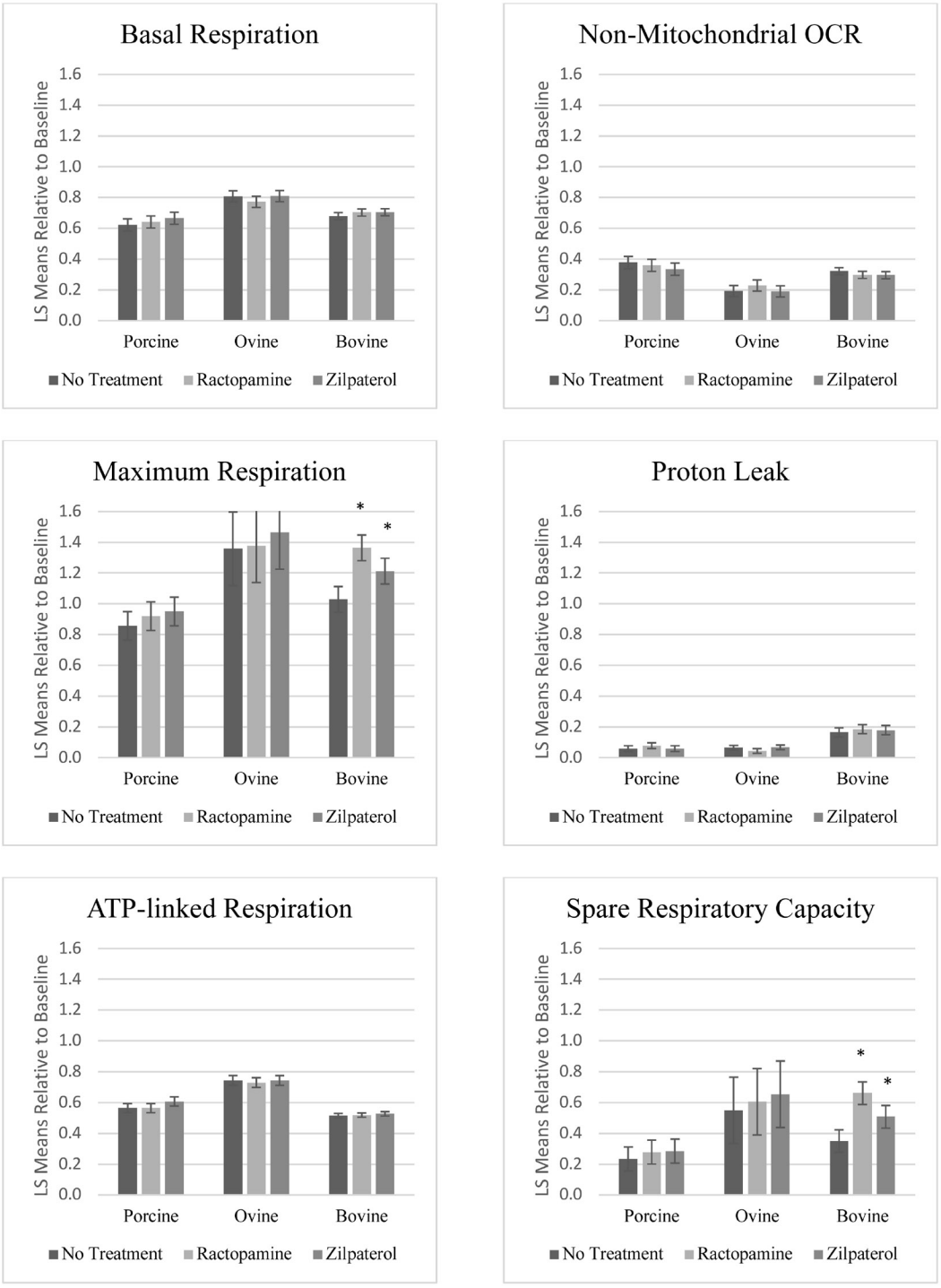

Figure 2. Oxygen consumption rate (OCR) measures of mitochondrial function. Least squared means (±SE) for OCR during each incubation condition relative to baseline. * Signifies a difference ($P < 0.05$) from incubations with no β-adrenergic agonist.

Discussion

In this study, we found that acute exposure to the β1-adrenergic agonist ractopamine HCl or to the β2-adrenergic agonist zilpaterol HCl enhanced the spare respiratory capacity and the maximum respiration of primary bovine myoblasts. However, no such effects of either β-AA were observed for primary porcine or ovine myoblasts. These findings provide further insight into the role that mitochondria play in β-adrenergic regulation of skeletal muscle oxidative metabolism. Increased maximal respiration and spare respiratory capacity in ractopamine HCl-and zilpaterol HCl-treated bovine cells indicates that increased β-adrenergic tone allowed these cells to be better equipped to cope with an ATP-depleted environment due to an increase in mitochondrial functional capacity. We speculate that this would lead to more efficient utilization of available energy substrates by muscle cells of β-AA-supplemented animals and thus more efficient energy production. This study also demonstrates that variation in mitochondrial efficiency is a potential mechanism underlying the differential physiological responses to β-AAs observed among species (Mersmann, 1998). Further characterization of the effects of β-AA on mitochondrial respiratory capacity in muscle cells and tissues is warranted to better understand how they contribute to efficient metabolism and growth of skeletal muscle in livestock.

One mechanism by which β-AAs improve cattle production outcomes is by increasing the efficiency of glucose metabolism and apparent mitochondrial respiratory activity. Previous studies by our group have reported increased glucose oxidation in primary skeletal muscle isolated from lambs that had been supplemented with oral zilpaterol HCl for 21 to 30 d and in primary rat muscle acutely stimulated with zilpaterol HCl in vitro (Cadaret et al., 2017; Barnes et al., 2019; Swanson et al., 2020). Improved glucose oxidation in those studies was independent of insulin signaling and glucose uptake, indicating direct enhancement of intracellular oxidative processes and leading us to investigate the role mitochondria play in β-adrenergic-induced changes in cellular respiratory efficiency. Our results indicate that acute stimulation with β-AAs increased maximum, but not basal respiration rates for bovine myoblasts. We speculate that the increased maximal respiratory capacity is due to an increased ability of the β-AA-treated cells to oxidize substrates compared with cells not stimulated with β-AAs. At a basal state

of respiration, oxygen consumption is coupled to ATP production. During an event, such as a muscle contraction, which causes a quick increase in the oxygen demand of the cell, the oxygen being consumed is less than that required by the tissue (oxygen deficit). In a time of oxygen deficit, the proton-electromotive force across the inner mitochondrial membrane is disrupted, resulting in the uncoupling of oxygen consumption from ATP production. Uncoupling agents such as FCCP used in the present study can mimic the physiological energy demand typical of a muscle during exercise (Adhihetty et al., 2003) by dissipating the proton-electromotive force across the inner mitochondrial membrane. This uncoupling allows for maximal flow of electrons along the electron transport chain as the cell works to reestablish the ion gradient across the inner mitochondrial membrane. To reestablish this ion gradient, skeletal muscle cells must be capable of rapid oxidation of substrates such as sugars, fats, or amino acids to return to a basal state of respiration. In our present study, acute stimulation by β-AAs increased the capacity of bovine satellite cells to oxidize these substrates. However, the lack of an effect of β-AA on basal respiration rates in bovine, porcine, or ovine myoblasts leads us to believe that β-AAs did not play a role in the electron transport chain when cells were in a resting state. This substantiates our prior data demonstrating increases in glucose oxidation, but not glucose uptake in β-AA-stimulated cells. When cells are in a state of rest, their mitochondria utilize only the amount of substrate necessary for the maintenance of basal functions, regardless of the amount of substrate available. Therefore, a difference in basal mitochondrial respiratory activity between cells stimulated with β-AA and unstimulated cells would not be anticipated.

Spare respiratory capacity, the difference between basal and maximal respiration, reflects the cells' ability to respond to increased energy demands by increasing their oxidative metabolic output (Divakaruni et al., 2014). Cells with a greater spare respiratory capacity typically have decreased metabolic demands at resting states and increased mitochondrial biogenesis at different stages of the cell cycle (Divakaruni et al., 2014). Mitochondrial biogenesis in muscle is characterized by an increase in mitochondrial density and enzymatic activity within cells (Adhihetty et al., 2003). Since cells in this study were stimulated with β-AAs for only a few minutes before spare respiratory capacity was measured, it is unlikely that the response observed was due to increased

mitochondria number. Instead, it is likely that β-AA stimulation increased enzymatic activity within the oxidative phosphorylation pathways. Previous work by our group reported transcriptomic evidence that acute supplementation of zilpaterol HCl to beef steers resulted in upregulation of the oxidative phosphorylation pathway in adipose tissue (Reith et al., 2022). Similar transcriptomic changes in myoblasts would help explain the present findings. The increase in enzymatic activity would presumably result in more efficient ATP production by the cell, which would improve animal performance and efficiency measures such as gain-to-feed ratios. As the cells were acutely stimulated, the response of the mitochondria over time remains to be seen as β-adrenoceptors are desensitized with prolonged exposure (Moloney et al., 1991; Spurlock et al., 1994).

Cattle, pigs, and sheep have different responses to β-AA supplementation as illustrated by varying changes in feed efficiency and in muscle and fat deposition (Mersmann, 1998). Therefore, species-specific differences in the responsiveness to β-AA at a cellular level are unsurprising. β-AA doses tested prior to this study were between 0.05 and 0.2 μM for zilpaterol HCl and between 1 and 4 μM for ractopamine HCl. Therefore, species differences may be attributed in part to our selection of a dose too low to elicit a response in pig and sheep myoblasts. The differential responses among species may have also been associated with differences in β-adrenergic receptor (**β-AR**) profiles. Cattle skeletal muscle has predominantly β2 receptors, but some β1 and β3 receptors are also present (Sillence and Matthews, 1994). Alternatively, pig skeletal muscle has a substantially greater relative proportion of β1 receptors and fewer relative β2 and β3 receptors (McNeel and Mersmann, 1999). The differential effects observed between cattle and lambs in this study were more surprising. Lambs are often used as a physiological model for cattle because of their similar metabolic and growth-regulating systems (Barnes et al., 2019). However, this study provided evidence of metabolic differences between these species at a cellular level. Receptor subtype abundance has not been explicitly studied in sheep, but feeding trials in lambs resulted in a more robust response to β2-AA than β1-AA (Lopez-Carlos et al., 2011). Therefore, sheep skeletal muscles were presumed to have primarily the β2-AR subtype, similar to cattle.

There are many possible sources for variation in mitochondrial respiratory activity between individual animals of the same breed and

species. A positive correlation between mitochondrial efficiency and feed efficiency has been reported for broilers, pigs, and beef cattle (Bottje and Carstens, 2009; Grubbs et al., 2013). Therefore, cells isolated from animals with high vs. low feed efficiency would be expected to have corresponding differences in mitochondrial respiratory activity. Furthermore, mitochondrial respiratory activity varies across breeds of livestock. Differences have been reported between Angus and Brahman cattle, which potentially contributes to the greater heat tolerance of the latter (Ramos et al., 2020). Our study did not necessarily control for differences in breed composition or feed efficiency, which may have contributed to some variation in species effects. Evaluation of these variables in future studies could help characterize intra-species variation.

Our findings allow us to conclude that acute stimulation by zilpaterol HCl or ractopamine HCl increases maximal respiration and spare respiratory capacity in bovine muscle stem cells. This indicates that β-AAs increase the ability of these cells to respond to cellular stresses and increased energy demands. Our OCR data provide evidence that favorable modification of mitochondrial capacity is one mechanism by which β-AA supplements increase metabolic efficiency and, in turn, muscle growth efficiency in livestock. However, the lack of an effect on mitochondrial respiratory activity for β-AA-stimulated porcine and ovine cells indicates that additional physiological mechanisms contribute to the improvement of feed efficiency in livestock animals.

* * * *

Acknowledgments We thank Eileen Marks-Nelson for assistance with laboratory protocols. This project is based on research that was partially supported by the Nebraska Agricultural Experiment Station with funding from the Hatch Multistate Research capacity funding program (accession number 1011055 and 1009410) from the USDA National Institute of Food and Agriculture.

Conflict of interest The authors declare no real or perceived conflicts of interest.

References

Adhihetty, P. J., I. Irrcher, A.-M. Joseph, V. Ljubicic, and D. A. Hood. 2003. Plasticity of skeletal muscle mitochondria in response to contractile activity. *Exp. Physiol*. 88:99–107. https://doi.org/10.1113/eph8802505

Anunciado-Koza, R. P., J. Zhang, J. Ukropec, S. Bajpeyi, R. A. Koza, R. C. Rogers, W. T. Cefalu, R. L. Mynatt, and L. P. Kozak. 2011. Inactivation of the mitochondrial carrier SLC25A25 (ATP-Mg^{2+}/Pi Transporter) reduces physical endurance and metabolic efficiency in mice. *J. Biol. Chem*. 286:11659–11671. https://doi.org/10.1074/jbc.M110.203000

Barnes, T. L., C. N. Cadaret, K. A. Beede, T. B. Schmidt, J. L. Petersen, and D. T. Yates. 2019. Hypertrophic muscle growth and metabolic efficiency were impaired by chronic heat stress, improved by zilpaterol supplementation, and not affected by ractopamine supplementation in feedlot lambs. *J. Anim. Sci*. 97:4101–4113. https://doi.org/10.1093/jas/skz271

Bottje, W. G., and G. E. Carstens. 2009. Association of mitochondrial function and feed efficiency in poultry and livestock species. *J. Anim. Sci*. 87:E48–E63. https://doi.org/10.2527/jas.2008-1379

Cadaret, C. N., K. A. Beede, H. E. Riley, and D. T. Yates. 2017. Acute exposure of primary rat soleus muscle to zilpaterol HCl (β2 adrenergic agonist), TNFα, or IL-6 in culture increases glucose oxidation rates independent of the impact on insulin signaling or glucose uptake. *Cytokine* 96:107–113. https://doi.org/10.1016/j.cyto.2017.03.014

Characterizing your cells using OCR values to determine optimal seeding density. 2017. Agilent Technologies, Inc. Available from https://www.agilent.com/cs/library/technicaloverviews/public/5991-7994EN.pdf Accessed February 28, 2022.

Divakaruni, A. S., A. Paradyse, D. A. Ferrick, A. N. Murphy, and M. Jastroch. 2014. Analysis and interpretation of microplate-based oxygen consumption and pH data. In: Murphy, A. N., D. C. Chan, editors. *Methods in enzymology*. Vol. 547. Amsterdam, Netherlands: Elsevier; p. 309–354. Available from https://doi.org/10.1016/B978-0-12-801415-8.00016-3. Accessed January 30, 2021.

Grubbs, J. K., A. N. Fritchen, E. Huff-Lonergan, J. C. M. Dekkers, N. K. Gabler, and S. M. Lonergan. 2013. Divergent genetic selection for residual feed intake impacts mitochondria reactive oxygen species production in pigs. *J. Anim. Sci*. 91:2133–2140. doi:10.2527/jas.2012-5894

Kolath, W. H., M. S. Kerley, J. W. Golden, and D. H. Keisler. 2006. The relationship between mitochondrial function and residual feed intake in Angus steers. *J. Anim. Sci*. 84:861–865. https://doi.org/10.2527/2006.844861x

Kubik, R. M., S. M. Tietze, T. B. Schmidt, D. T. Yates, and J. L. Petersen. 2018. Investigation of the skeletal muscle transcriptome in lambs fed β adrenergic agonists and subjected to heat stress for 21 d. *Transl. Anim. Sci*. 2:S53–S56. https://doi.org/10.1093/tas/txy053

Lean, I. J., J. M. Thompson, and F. R. Dunshea. 2014. A meta-analysis of zilpaterol and ractopamine effects on feedlot performance, carcass traits and shear strength of meat in cattle. B. Kaltenboeck, editor. *PLoS One* 9:e115904. https://doi.org/10.1371/journal.pone.0115904

Lopez-Carlos, M. A., R. G. Ramirez, J. I. Aguilera-Soto, A. Plascencia, H. Rodriguez, C. F. Arechiga, R. M. Rincon, C. A. Medina-Flores, and H. Gutierrez-Bañuelos. 2011. Effect of two beta adrenergic agonists and feeding duration on feedlot performance and carcass characteristics of finishing lambs. *Livest. Sci*. 138:251–258. https://doi.org/10.1016/j.livsci.2010.12.020

McNeel, R. L., and H. J. Mersmann. 1999. Distribution and quantification of Beta1-, Beta2-, and Beta3-adrenergic receptor subtype transcripts in porcine tissues. *J. Anim. Sci*. 77:611–621. https://doi.org/10.2527/1999.773611x

Mersmann, H. J. 1998. Overview of the effects of beta-adrenergic receptor agonists on animal growth including mechanisms of action. *J. Anim. Sci*. 76:160–172. https://doi.org/10.2527/1998.761160x

Moloney, A. P., P. Allen, R. Joseph, and V. Tarrant. 1991. Influence of beta-adrenergic agonists and similar compounds on growth. In: Pearson, A. M., and T. R. Dutson, editors. *Growth regulation in farm animals. Advances in meat research*. Vol. 7. New York (NY): Elsevier Applied Science; p. 455–513.

Ramos, P. M., C. Li, M. A. Elzo, S. E. Wohlgemuth, and T. L. Scheffler. 2020. Mitochondrial oxygen consumption in early postmortem permeabilized skeletal muscle fibers is influenced by cattle breed. *J. Anim. Sci*. 98:1–10. https://doi.org/10.1093/jas/skaa044

Reith, R. R., R. L. Sieck, P. C. Grijalva, R. M. Swanson, A. M. Fuller, D. E. Diaz, T. B. Schmidt, D. T. Yates, and J. L. Petersen. 2022. Transcriptome analyses indicate that heat stress-induced inflammation in white adipose tissue and oxidative stress in skeletal muscle is partially moderated by zilpaterol supplementation in beef cattle. *J. Anim. Sci*. 100. https://doi.org/10.1093/jas/skac019

Salin, K., S. K. Auer, B. Rey, C. Selman, and N. B. Metcalfe. 2015. Variation in the link between oxygen consumption and ATP production, and its relevance for animal performance. *Proc. R. Soc. B*. 282:20151028. https://doi.org/10.1098/rspb.2015.1028

Seahorse XF Stress Test Report Generator user guide. 2016. Agilent user manuals. Agilent Technologies, Inc. Available from https://www.agilent.com/cs/library/usermanuals/public/XF_Stress_Test_Report_Generator_User_Guide.pdf . Accessed January 19, 2021.

Seahorse XF Cell Mito Stress Test Kit user guide. 2019. Agilent User Manuals. Agilent Technologies, Inc. Available from https://www.agilent.com/cs/library/usermanuals/public/XF_Cell_Mito_Stress_Test_Kit_User_Guide.pdf . Accessed January 19, 2021.

Sieck, R. 2021. Variation in the genome and transcriptome associated with beef cattle production and investigation of the metabolic consequences of beta-adrenergic agonist supplementation. Lincoln, Nebraska: University of Nebraska. Available from https://digitalcommons.unl.edu/animalscidiss/214/ . Accessed on February 12, 2022.

Sillence, M. N., and M. L. Matthews. 1994. Classical and atypical binding sites for β-adrenoceptor ligands and activation of adenylyl cyclase in bovine skeletal muscle and adipose tissue membranes. *Br. J. Pharmacol*. 111:866–872. https://doi.org/10.1111/j.1476-5381.1994.tb14818.x

Spurlock, M. E., J. C. Cusumano, S. Q. Ji, D. B. Anderson, C. K. Smith II, D. L. Hancock, and S. E. Mills. 1994. The effect of ractopamine on beta-adrenoceptors density and affinity in porcine adipose and skeletal muscle tissue. *J. Anim. Sci*. 72:75–80. https://doi.org/10.2527/1994.72175x

Swanson, R. M., R. G. Tait, B. M. Galles, E. M. Duffy, T. B. Schmidt, J. L. Petersen, and D. T. Yates. 2020. Heat stress-induced deficits in growth, metabolic efficiency, and cardiovascular function coincided with chronic systemic inflammation and hypercatecholaminemia in ractopamine-supplemented feedlot lambs. *J. Anim. Sci*. 98. https://doi.org/10.1093/jas/skaa168

Yates, D. T., D. S. Clarke, A. R. Macko, M. J. Anderson, L. A. Shelton, M. Nearing, R. E. Allen, R. P. Rhoads, and S. W. Limesand. 2014. Myoblasts from intrauterine growth-restricted sheep fetuses exhibit intrinsic deficiencies in proliferation that contribute to smaller semitendinosus myofibres. *J. Physiol*. 592:3113–3125. https://doi.org/10.1113/jphysiol.2014.272591

Yépez, V. A., L. S. Kremer, A. Iuso, M. Gusic, R. Kopajtich, E. Koňaříkova, A. Nadel, L. Wachutka, H. Prokisch, and J. Gagneur. 2018. OCR-Stats: robust estimation and statistical testing of mitochondrial respiration activities using Seahorse XF analyzer. *PLoS One* 13:1–18. https://doi.org/10.1371/journal.pone.0199938