Published September 21, 2017

Meat and Muscle Biology™

Zilpaterol Hydrochloride affects Cellular Muscle Metabolism and Lipid Components of 10 Different Muscles in Feedlot Heifers

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Abstract: This study determined if zilpaterol hydrochloride (ZH) altered muscle metabolism and lipid components of 10 muscles. Crossbred heifers were either supplemented with ZH ($n = 9$) or not (Control; $n = 10$). Muscle tissue was collected (*adductor femoris*, *biceps femoris*, *gluteus medius*, *infraspinatus*, *latissimus dorsi*, *longissimus dorsi*, *pectoralis profundi*, *semitendinosus, subscapularis*, *trapezius*) immediately following carcass splitting. The mRNA abundance of AMPkα, IGF-I, MHC-I, IIA and IIX, β1-adrenergic receptor (βAR) and β2AR was determined, as well as, cross-sectional area and proportion of myosin isoforms, β1AR, β2AR, β3AR, nuclei, and satellite cell density. Furthermore, neutral (NL) and polar lipid (PL) fatty acids (FA) were quantified (mg/g). Zilpaterol hydrochloride decreased MHC-IIA mRNA ($P = 0.007$). In addition, ZH decreased total nuclei and β1AR and increased MHC-IIX cross-sectional area ($P \leq 0.021$). Quantity of NL FA were not affected by ZH ($P \geq 0.173$). However, among PL FA the ratio of PUFA:SFA was greater with ZH ($P = 0.048$). Muscle type impacted mRNA concentration of AMPkα, IGF-I, MHC-I, IIA, IIX, and β1AR mRNA concentration ($P \leq 0.037$). Furthermore, the fiber type proportion, fiber cross-sectional area, and the densities of nuclei, β1AR, β2AR, β3AR, and satellite cells were influenced by muscle type ($P \leq 0.030$). Total NL FA were affected by muscle ($P \leq 0.046$). Meanwhile, total PL FA did not differ due to muscle ($P = 0.242$). However, prominent PL FA,18:0, 18:1 *trans*, and 18:2 n-6 were each greater ($P < 0.05$) among the oxidative *subscapularis* compared with glycolytic *semitendinosus* and *adductor femoris*. Overall, these data reveal that ZH impacts muscle metabolism and myogenic activity that establishes protein deposition. Meanwhile, ZH did not alter triglyceride content (NL), but cell membrane saturation (PL) was influenced, in accordance with alterations to muscle fiber type. Muscle also influenced muscle fiber type and lipid components. Therefore, muscle biology is greatly influenced by muscle but also through dietary inclusion of ZH.

Keywords: beef cattle, β-adrenergic receptor, fatty acids, myosin heavy chain, zilpaterol hydrochloride
Meat and Muscle Biology 1:192–206 (2017) **doi:10.22175/mmb2017.02.0013**
Submitted 15 Feb. 2017 Accepted 26 June 2017

Introduction

Zilpaterol hydrochloride (ZH) is a β-adrenergic agonists (βAA) that is commonly used in beef cattle to increase lean tissue deposition on the carcass while simultaneously improving feedlot performance and profitability. Beta-adrenergic agonists have been reported to increase protein synthesis and decrease protein degradation while simultaneously decreasing lipogenesis and increasing lipolysis (Mersmann, 1998). Zilpaterol hydrochloride was approved for use in cattle in 2006 under the trade name of Zilmax (Merck Animal Health, Summit, NJ) and is fed at a rate of 6.8 g/t for the last 20 to 40 d of the finishing phase with a 3 d withdrawal prior to slaughter. Rathmann et al. (2012) as well as other studies reported that ZH improves feed

Supported by the Gordon W. Davis Regent's Chair in Meat Science and Muscle Biology Endowment at Texas Tech University and Merck Animal Health.

to gain, hot carcass weight, dressing percentage and LMA while decreasing back fat thickness (Scramlin et al., 2010; Strydom et al., 2009; Vasconcelos et al., 2008). Furthermore, the effect of ZH on cellular muscle metabolism has been study extensively on the *longissimus dorsi, gluteus medius*, and *semimembranosus* and is known to cause increased mRNA expression of MHC-IIX and fiber cross-sectional area (Knobel, 2014; Baxa et al., 2010; Kellermeier et al., 2009; Rathmann et al., 2009).

Intramuscular fat content is known to vary significantly between muscles (Jeremiah et al., 2003). Intramuscular fat is composed of 2 primary lipid fractions: the polar lipids (PL) containing phospholipids and the neutral lipid (NL) triglycerides. Previously intramuscular fat content was determined to influence the content and composition of NL and PL fatty acids (FA; Legako et al., 2015). Recently muscle was determined to influence both NL and PL FA (Hunt et al., 2016). However, it is presently unclear if muscle fiber or metabolism is related with NL and/or PL FA. Additionally, no known studies have determined the influence of βAA on NL and PL FA.

Therefore, the objective of this study was to determine how ZH alters muscle metabolism in muscles from the chuck, loin and round in crossbred heifers. Furthermore, this study sought to determine the influence of ZH and muscle type on NL and PL FA.

Materials and Methods

All procedures involving the use of live animals were conducted within the guidelines of and approved by the University of Nebraska Institutional Animal Care and Use Committee (IACUC #902).

Experimental design and treatments

A total of 19 ovariectomized English-influenced cross bred heifers (initial BW = 556 ± 7 kg) were utilized and randomly assigned to 1 of 2 treatments: 1) a finishing diet containing no ZH (CON; n = 10) or a finishing diet supplemented with ZH at 8.33 mg/kg BW on a DM basis (ZH; n = 9). Treatment diets were individually mixed and administered to heifers individually once daily at 0800 for 20 d followed by a 3 d mandatory ZH withdrawal. Heifers were harvested on d 25, 26, and 27 of the study. This resulted in an extension of withdrawal days (4, 5, and 6 d after ZH supplementation depending on harvest day). The heifers were randomly assigned to harvest day, and were harvested on an alternating basis of treatment.

All heifers were from a dual corticotropin-releasing hormone and arginine vasopressin challenge study conducted by Buntyn et al. (2016). The challenge phase was conducted to evaluate response to an acute moderate stress period for control animals compared with ZH. Following the challenge animals were allowed ample time to recover indicated by cortisol levels returning to baseline level. The stress undergone during the challenge phase would be considered similar to common stressors, such as loading or transportation. The authors of this study see no overriding impact from the challenge phase on the results reported here.

Harvest and collection of muscle tissue

Heifers were transported from the University of Nebraska Agriculture Research and Development Center feedlot to the Loeffel Meat Laboratory at the University of Nebraska-Lincoln, Lincoln, NE and subsequently harvested under USDA-FSIS inspection. Muscle tissue (500 g) was collected from the anterior portion of the center of the *infraspinatus* (IF), *pectoralis profundi* (PP), *subscapularis* (SS), *latissimus dorsi* (LA), *longissimus dorsi* (LD), *trapezius* (TP), *adductor femoris* (AD), *biceps femoris* (BF), *gluteus medius* (GM), and *semitendinosus* (ST) of carcasses. The muscle samples were all collected at the same time, after the carcass had been split. Muscles were collected from the right side of the carcass. These muscles were chosen to attempt to accurately profile the differences in muscles throughout the carcass that are not commonly evaluated. Two 10-g samples were cut from the anterior portion of the 500 g of muscle tissue collected from the carcass to avoid contamination. Samples were then placed into a whirl-pack for either RNA or protein analysis, flash frozen in liquid nitrogen, and placed in a cooler of dry ice for shipment to Texas Tech University. For immunohistochemical analysis, a 2.5-cm^2 sample of muscle tissue was cut parallel to the muscle fiber and placed in clear frozen section compound (VWR International, West Chester, PA), frozen using 2-methylbutane, chilled with dry ice, and then placed in a cooler of dry ice for shipment to Texas Tech University.

Rna isolation and real time quantitative reverse transcription polymerase chain reaction

Ribonucleic acid from muscle and adipose tissue was isolated with ice-cold buffer containing TRI Reagent (Sigma Aldrich Corp., St. Louis, MO). Approximately 300 mg of frozen tissue was homogenized with 3 mL of TRI Reagent. The homogenate was then pipetted into 2 microcentrifuge tubes (1-mL sample per tube), 200 μL

chloroform was added to each tube, vortexed for 30 s, and incubated for 5 min. The sample was then centrifuged at 15,000 × *g* for 15 min separating the sample into 3 layers. The top supernatant layer was pipetted off and placed into a new microcentrifuge tube. Ice cold isopropyl alcohol (250 µL) was added to the supernatant, shaken, and incubated for 10 min at 25°C. The samples were then centrifuged at 15,000 × *g* for 10 min. The supernatant was poured off, the RNA pellet at the bottom of each tube was allowed to dry, and 500 µL of 75% ethanol was added to each tube to rinse and suspend the RNA pellet. Samples were then placed in a –80°C freezer until needed (no longer than 3 mo). Samples were then removed from the freezer and thawed on ice. Samples were then centrifuged at 15,000 × *g* for 10 min, ethanol was poured off, and the pellet was air dried. Nuclease free water (30 µL) was then added to each sample to dissolve the RNA pellet. The concentration of RNA was determined with a spectrophotometer at an absorbance of 260 nm using a NanoDrop 1000 (Thermo Fisher Scientific, Inc., Waltham, MA). The 260/280 ratios ranged from 1.33 to 1.97, averaging 1.83 and 260/230 ratios ranged from 0.12 to 2.84 averaging 1.36. Samples were then treated with DNase to remove any DNA contaminants using a DNA-free kit (Life Technologies, Grand Island, NY). The RNA was then subjected to reverse-transcription to produce cDNA. The cDNA was synthesized using separate components purchased through Thermo Fisher [10x RT buffer (AB4376212), 25 mM MgCl (AM9530G), dNTP (100004893), random hexamers (ABN8080119), Multiscribe (AB4311235)] for a total reaction volume of 50 µL, was then used for real-time quantitative reverse transcription-PCR (RT-qPCR) to measure the abundance of AMP-activated protein kinase α (AMPKα), insulin-like growth factor one (IGF-I), β 1 adrenergic receptor (β1AR), β 2 adrenergic receptor (β2AR), myosin heavy chain-I (MHC), MHC-IIA and MHC-IIX mRNA relative to the abundance of ribosomal protein subunit 9 (RPS9) mRNA in total RNA isolated from muscle tissue. Bovine primers and probes for AMPKα, IGF-I, β1AR, β2AR, MHC-I, MHC-IIA and MHC-IIX are presented in Table 1 (Rathmann et al., 2009). Assays were performed in the GeneAmp 7900HT Sequence Detection System (Applied Biosystems, Life Technologies) using thermal cycling parameters recommended by the manufacturer (40 cycles of 15 s at 95°C and 1 min. at 60°C).

Immunohistochemical analysis

Muscle fiber distribution, area, β adrenergic receptor (βAR) and satellite cell abundance was determined (Hergenreder et al., 2016; Knobel, 2014; Paulk et al., 2014; Gonzalez et al., 2007). Twenty-4 h prior to sectioning, embedded muscle samples were moved from –80°C to a –20°C freezer to thaw. Muscle fiber distribution, area, βAR and satellite cell abundance was determined on 10-µm-thick cross-sections. The sections were cut at –20°C using a Leica CM1950 cryostat (Lieca Biosystems, Buffalo Grove, IL) from the embedded muscle samples. The sections were then mounted on positively charged glass slides (5 slides per sample/3 cryosections per slide; Superfrost Plus; VWR International, Radnor, PA). Cryosections were fixed using 4% paraformaldehyde (Thermo Fisher Scientific, Fair Lawn, NJ) for 10 min at 25°C followed by 2 brief rinses and a single 5 min rinse in phosphate buffered saline (PBS). Cryosections were incubated with 5% horse serum (Invitrogen, Grand Island, NY), 2% bovine serum albumin (MP Biomedical, Solon, OH), 0.2% Triton-X100 (Thermo Fisher Scientific) in PBS for 30 min at 25°C to block non-specific antibody binding. Cryosections were then incubated for 1 h at 25°C in the following primary antibodies: Slide 1-1:100 α-dystrophin, rabbit, IgG (PA137587: Thermo Fisher Scientific); 1:100 supernatant anti-myosin heavy chain (MHC) type 1, IgG2b (BA-D5; Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA); and supernatant anti-MHC (all but type IIX IgG1; BF-35, Developmental Studies Hybridoma Bank); Slide 2-1:750 α-β 1 AR, rabbit, IgG (ab85037: abcam, Cambridge, MA); 1:750 α-β 2 AR, chicken, IgY (ab13989: abcam); 1:500 α-β 3 AR, goat, IgG (ab77588: abcam); Slide 3- 1:10 supernatant anit-Paired Box 7, mouse α-chicken (Pax7; Developmental Studies Hybridoma Bank); 1:100 anti-Myogenic factor 5, rabbit, IgG (Myf5; SC-302: Santa Cruz Biotechnology, Dallas, TX). Slides were then rinsed 3 times for 5 min in PBS. Cryosections were incubated for 30 min at 25°C in opaque boxes in the following secondary antibodies: Slide 1- 1:1,000 goat α-rabbit, IgG, Alexa-Fluor 488 (A-11008: Invitrogen); 1:1,000 goat α-mouse, IgG1, Alexa-Fluor 546 (A-21123: Invitrogen); 1:1,000 goat α-mouse, IgG2b, Alexa-Fluor 633 (A-21146: Invitrogen); Slide 2- 1:1,000 goat α-chicken, IgY, H & L, Alexa-Fluor 488 (ab150173: abcam); 1:1,000 donkey α-rabbit, IgG, Alexa-Fluor 546 (A-10040: Invirtogen); 1:1,000 donkey α-goat, IgG, Alexa-Fluor 633 (A-21082: Invitrogen); Slide 3- 1:1,000 goat α-rabbit, IgG, Alexa-Fluor 488 (A-11008: Invitrogen); 1:1,000 goat α-mouse, IgG1, Alexa-Fluor 546 (A-21123: Invitrogen). Slides were then rinsed 3 times for 5 min in PBS. Finally, cryosections were incubated in 1 µg/mL 4',6-diamidino-2-phenylindole (DAPI, Thermo Fisher Scientific) for 1 min followed by 2 brief PBS rinses. Slides were cov-

Table 1. Sequence of bovine-specific PCR primers and TaqMan probes to be used for determination of expression of mRNA of AMPKα, MHC-I, MHC-IIA, MHC-IIX, IGF-I, β1AR and β2AR, and RPS9[1]

Primer	Sequence (5' to 3')
AMPkα (accession #NM_001109802)	
Forward	ACCATTCTTGGTTGCTGAAACTC
Reverse	CACCTTGGTGTTTGGATTTCTG
TaqMan probe	6FAM-CAGGGCGCGCCATACCCTTG-TAMRA
MHC-I (accession #AB059400)	
Forward	CCCACTTCTCCCTGATCCACTAC
Reverse	TTGAGCGGGTCTTTGTTTTTCT
TaqMan probe	6FAM-CCGGCACGGTGGACTACAACATCATAG-TAMRA
MHC-IIA (accession #AB059398)	
Forward	GCAATGTGGAAACGATCTCTAAAGC
Reverse	GCTGCTGCTCCTCCTCCTG
TaqMan probe	6FAM-TCTGGAGGACCAAGTGAACGAGCTGA-TAMRA
MHC-IIX (accession # AB059399)	
Forward	GGCCCACTTCTCCCTCATTC
Reverse	CCGACCACCGTCTCATTCA
TaqMan probe	6FAM-CGGGCACTGTGGACTACAACATTACT-TAMRA
IGF-I (accession #X15726)	
Forward	TGTGATTTCTTGAAGCAGGTGAA
Reverse	AGCACAGGGCCAGATAGAAGAG
TaqMan probe	6FAM-GCCCATCACATCCTCCTCGCA-TAMRA
β1AR (accession #AF188187)	
Forward	GTGGGACCGCTGGGAGTAT
Reverse	TGACACACAGGGTCTCAATGC
TaqMan probe	6FAM-CTCCTTCTTCTGCGAGCTCTGGACCTC-TAMRA
β2AR (accession #NM_174231)	
Forward	CAGCTCCAGAAGATCGACAAATC
Reverse	CTGCTCCACTTGACTGACGTTT
TaqMan probe	6FAM-AGGGCCGCTTCCATGCCC-TAMRA
RPS9 (accession #DT860044)	
Forward	GAGCTGGGTTTGTCGCAAAA
Reverse	GGTCGAGGCGGGACTTCT
TaqMan probe	6FAM-ATGTGACCCCGCGGAGACCCTTC-TAMRA

[1]AMPKα = AMP-activated protein kinase alpha, MHC-I = myosin heavy chain-I, MHC-IIA = myosin heavy chain-IIA, MHC-IIX = myosin heavy chain-IIX, IGF-I = insulin-like growth factor-I, β1AR = beta 1 adrenergic receptor, β2AR = beta 2 adrenergic receptor and RPS9 = ribosomal protein S9.

er-slipped with mounting media (Aqua Mount; Lerner Laboratories, Pittsburgh, PA) and thin glass cover slips (VWR International), and dried at 4°C for 24 h. All slides were imaged within 48 h of staining.

The slides were imaged at 20 times working difference magnification using an inverted fluorescence microscope (Nikon Eclipse, Ti-E; Nikon Instruments Inc., Mellville, NY) equipped with a UV light source (Nikon Intensilight Inc., Melville, NY; C-HGFIE). The images were captured by a CoolSnap ES^2 monochrome camera, and artificially colored and analyzed using NIS Elements Imaging software.

Five random images were taken of cryosections from each slide. All fibers positive for BAD-5 and BF-35 antibodies were labeled as type I, fibers positive for BF-35 antibody only were labeled as type IIA, and fibers with no stain or weakly stained with BF-35 were labeled as type IIX. Myosin heavy chain type I, IIA, and IIX muscle fibers in each image were identified and expressed as a percentage of the total number of muscle fibers. The cross-sectional area of each fiber in each image was measured using NIS Elements software (Nikon Instruments Inc.) and expressed on a μm^2 basis. The total number of DAPI-stained cells in each image were enumerated to determine the nuclear density on a per mm2 basis. All β-AR, Pax7, Myf5, and dual expressing Pax7:Myf5 satellite cells were identified on the respective slides stained for them, counted, and densities are reported on a per mm^2 basis. The β-AR were classified as β-AR or internalized β-AR. The classification of the β-AR was determined by the location of the stained β-AR on the fiber cross-section. The β-AR located on

the sarcolemma were considered normal, functioning β-AR, and the β-AR located within the fiber cross-section were considered internalized β-AR.

Fatty acid analysis

Fatty acids were determined for the PL and NL of each muscle tissue sample (Legako et al., 2015). Muscle tissue was cubed, flash-frozen in liquid nitrogen, and homogenized into a fine powder. All tissue homogenates were stored at –80°C until subsequent analysis. Total lipids were extracted from 0.5-g tissue homogenates by a chloroform:methanol extraction (Folch et al., 1957). Extracted lipids were fractionated using a Resprep silica gel cartridge (Restek Corporation, Bellefonte, PA), where NL were initially eluted with chloroform and PL were subsequently eluted with methanol (Juaneda and Rocquelin, 1985). Fatty acids of the NL were saponified and derivatized to fatty acid methyl esters (FAME) using sodium methoxide in methanol (Li and Watkins, 2001). Saponification and derivatization of PL FA was performed with methanolic potassium hydroxide (Maxwell and Marmer, 1983). Tridecanoic acid methyl ester (CAS # 1731–88–0, Sigma-Aldrich) was used as the internal standard during derivatization. Analysis of FAME was performed by an Agilent Technologies (Santa Clara, CA) 7890 gas chromatograph equipped with an HP-88 capillary column (30 m × 250 µm × 0.2µm; Agilent Technologies, Santa Clara, CA) and a flame ionization detector. Identity of FAME was determined by comparison with authentic FAME standards (Supelco 37 Component FAME Mix, Sigma-Aldrich, St Louis, MO) and quantified by an internal standard calibration. Individual FA were calculated as milligram per g of muscle tissue. Percentages of FA groups (Saturated, monounsaturated, and polyunsaturated; SFA, MUFA, and PUFA, respectively) were determined by dividing the group FA concentration (mg/g muscle tissue) by the corresponding total FA concentration (mg/g muscle tissue) then multiplying by 100.

Statistical analysis

All data were analyzed using the GLIMMIX procedure of SAS (version 9.3, SAS Inst. Inc., Carey, NC). The model was a split-plot design where treatment group (ZH or control) served as the whole-plot and muscle was the sub-plot. Harvest date was included in the model as a covariate. Heifer served as the experimental unit. The Kenward-Roger adjustment was used to correct degrees of freedom. Means were separated using the LSMEANS procedure with the PDIFF option and considered different when $P \leq 0.05$. Tendencies for differences among treatment means were declared when $0.05 > P \leq 0.10$.

Results and Discussion

Zilpaterol hydrochloride

No interactions were determined between treatment and muscle type ($P > 0.05$). However, tendencies were present for two NL FA (20:0, $P = 0.054$; 20:2, $P = 0.052$) and two PL FA (20:3 n-6, $P = 0.098$; 22:0, $P = 0.085$). It is unclear what biological significance these tendencies hold. Therefore, the ZH effect will be discussed here. The muscle effect will be discussed in a subsequent section.

Zilpaterol hydrochloride had no effect ($P \geq 0.394$) on AMPkα, IGF-I, β1AR, β2AR, and MHC-I and abundance (Table 2). Control heifers had an increased ($P = 0.007$) abundance of MHC-IIA mRNA compared to ZH heifers. Furthermore, there was tendency ($P = 0.080$) for greater MHC-IIX mRNA abundance among ZH heifers. Several studies support our findings reporting little to no changes in IGF-I, β1AR, β2AR, MHC-I and MHC-IIX mRNA abundance of cattle supplemented ZH or ractopamine HCl (RH: Parr et al., 2014; Miller et al., 2012; Baxa et al., 2010; Walker et al., 2010; Rathmann et al., 2009). Furthermore, cattle supplemented a βAA had decreased MHC-IIA mRNA abundance (Baxa et al., 2010; Walker et al., 2010; Rathmann et al., 2009).

Zilpaterol HCl increased ($P = 0.017$) the MHC-IIX, fiber cross-sectional area (Table 3). Zilpaterol HCl had

Table 2. Effect of zilpaterol hydrochloride on relative mRNA abundance of AMPKα, IGF-I, MHC-I, MHC-IIA, MHC-IIX, β1AR, and β2AR genes in muscle tissue[1]

	Treatment			
Gene[2]	Control	Zilpaterol	SEM[3]	*P*-value
AMPkα	1.71	1.57	0.133	0.441
IGF-I	2.41	2.10	0.306	0.465
β1AR	100.65	100.66	16.230	0.999
β2AR	1.56	1.74	0.234	0.584
MHC-I	3.71	3.20	0.423	0.394
MHC-IIA	4.03	2.38	0.393	0.007
MHC-IIX	0.98	1.33	0.135	0.080

[1]AMPKα = AMP-activated protein kinase alpha, IGF-I = insulin-like growth factor-I, MHC-I = myosin heavy chain-I, MHC-IIA = myosin heavy chain-IIA, MHC-IIX = myosin heavy chain-IIX, β1AR = beta 1 adrenergic receptor, and β2AR = beta 2 adrenergic receptor.

[2]Relative abundance of the AMPKα, IGF-I MHC-I, MHC-IIA, MHC-IIX, β1AR and β2AR genes were normalized with the ribosomal protein S9 endogenous control by using the change in cycle threshold (ΔΔCT).

[3]Pooled standard error of the mean.

Table 3. Effect of zilpaterol hydrochloride on myosin heavy chain (MHC)-I, IIA and IIX cross-sectional area and distribution in muscle tissue

	Treatment			
Item	Control	Zilpaterol	SEM[1]	*P*-value
MHC-I*	3430	3652	148.9	0.297
MHC-IIA*	3857	4476	256.22	0.101
MHC-IIX*	4248	5499	359.8	0.017
MHC-I**	37.55	35.24	0.012	0.198
MHC-IIA**	42.86	44.77	0.011	0.227
MHC-IIX**	17.69	18.22	0.092	0.823

*Fiber cross-sectional area in μm^2.

**Percentage of myosin heavy chain type I, IIA and IIX.

[1]Pooled standard error of the mean.

Table 4. Effect of zilpaterol hydrochloride on nuclei and β-adrenergic receptor and satellite cell density in muscle tissue

	Treatment			
Item[1], mm^2	Control	Zilpaterol	SEM[2]	*P*-value
Total Nuclei	576.39	504.89	14.960	0.003
Myofiber Nuclei	351.87	341.40	22.020	0.735
β1AR	344.89	313.18	8.608	0.017
β1AR-internalized	2.50	3.66	1.151	0.500
β2AR	337.57	319.20	11.749	0.274
β2AR-internalized	5.22	10.11	1.805	0.048
β3AR	91.27	70.39	10.531	0.171
β3AR-internalized	–	2.05	0.253	0.281
Pax7	11.25	10.40	0.581	0.292
Myf5	210.53	150.99	22.210	0.071
Pax7:Myf5	11.56	11.79	0.610	0.791

[1]β1AR = beta 1 adrenergic receptor, and β2AR = beta 2 adrenergic receptor, Pax7 = paired box 7, Myf5 = myogenic factor 5, Pax7:Myf5 = dual expressing paired box 7 and myogenic factor 5.

[2]Pooled standard error of the mean.

no effect ($P = 0.297$ and 0.101, respectively) on MHC-I and MHC-IIA fiber cross-sectional area. The ZH treatment had no effect ($P \geq 0.198$) on the proportion of MHC-I, IIA and IIX fibers (Table 3). The efficacy of βAA on fiber cross-sectional area and proportion seem to be dependent on the muscle. Zilpaterol HCl has been reported to increase fiber cross-sectional area and the increase the proportion of MHC-IIX fibers in the LD (Knobel, 2014; Kellermeier et al., 2009). Furthermore, ZH decreased fiber cross-sectional area and proportion of MHC-I and increased fiber cross-sectional area and proportion of MHC-IIX fibers in the GM (Knobel, 2014). Ractopamine HCl increased the fiber cross-sectional area of MHC-I in the IF and LD and MHC-IIA in the IF and SM (Gonzalez et al., 2008; Gonzalez et al., 2007). Furthermore, RH increased the proportion of MHC-I fibers in the IF (Gonzalez et al., 2008) and had no effect on the proportion of MHC-I and II fibers in the LD (Gonzalez et al., 2007). Conversely, Gonzalez et al. (2010) reported no change in cross-sectional area of the LD, AD, VL, SM, *gracilis,* and *rectus femoris*.

Zilpaterol HCl decreased ($P \leq 0.017$) the density of total nuclei and β1AR (Table 4), and increased ($P = 0.048$) the density of internalized β2AR. Zilpaterol HCl had no effect ($P \geq 0.171$) on myonuclei, β2AR, β3AR, internalized β1AR and β3AR, Pax7, and dual expressing Pax7:Myf5 densities (Table 4). However, Myf5 tended to be decreased ($P = 0.071$) in ZH heifers. There has been mixed results on how βAA affect nuclei density. Knobel (2014) and Gonzalez et al. (2007) reported no change in nuclei density of cattle supplemented a βAA while Gonzalez et al. (2008) reported RH decreased nuclei density. Beta-adrenergic agonists work through an interaction with the βAR (Avendaño-Reyes et al., 2006, Abney et al., 2007) which are responsible for binding endogenous catecholamines (Mills and Mersmann, 1995). Zilpaterol HCl affects myofiber hypertrophy by binding to the β2AR, which is the predominant βAR found in beef cattle muscle and adipose tissue (Mersmann, 1998). The increased density of internalized β2AR in ZH cattle may have been caused by the overstimulation of the β2ARs by ZH. Overstimulation of the βARs by βAA has been reported to result in receptor desensitization (Lohse et al., 1990; Waldo et al., 1983). Receptor desensitization elicits downregulation of adenylate cyclase catalytic activity, thereby resulting in a reduction of cAMP synthesis and protein kinase A activation (Pippig et al., 1993). When the βARs become desensitized, they are sequestered within an intracellular vesicle and lose the ability to propagate the signal transduction pathway (Lohse et al., 1990; Waldo et al., 1983). Once the βARs become desensitized a βAA will no longer have an effect on muscle hypertrophy. Muscle hypertrophy is supported by satellite cell infusion into the muscle cell providing new myonuclei to the myofibers accounting for increased DNA accumulation (Di Marco et al., 1987; Moss and Leblond, 1971). Studies have reported no change in satellite cell densities due to β-AA supplementation (Knobel, 2014; Gonzalez et al., 2007). Satellite cells respond to intrinsic and extrinsic factors to leave quiescence and to repair, maintain, or grow muscle mass. Beta adrenergic agonists maybe supplemented for too short of a duration to activate satellite cells out of quiescence resulting in decreased nuclei density due to increased fiber cross-sectional area.

Among NL FA no ZH effect was determined ($P \geq 0.154$; Table 5). However, among PL FA the ratio of PUFA:SFA was greater ($P = 0.013$) in ZH compared

Table 5. Effect of zilpaterol hydrochloride on neutral and polar lipid fatty acids (mg/g muscle tissue) among ten beef muscles[1]

	Neutral lipid				Polar lipid			
	Treatment				Treatment			
Fatty acid	Control	Zilpaterol	SEM[2]	*P*-value	Control	Zilpaterol	SEM[2]	*P*-value
SFA	14.95	13.29	0.92	0.223	4.09	3.73	0.18	0.172
14:0	1.12	1.02	0.08	0.387	0.22	0.21	0.02	0.832
15:0	0.19	0.16	0.02	0.339	0.04	0.03	0.01	0.089
16:0	8.35	7.47	0.56	0.294	1.94	1.79	0.11	0.347
17:0	0.49	0.41	0.05	0.314	0.09	0.07	0.01	0.036
18:0	4.25	3.67	0.27	0.159	1.29	1.10	0.06	0.040
20:0	0.03	0.03	0.01	0.739	0.04	0.04	0.01	0.691
21:0	0.09	0.09	0.02	0.974	0.08	0.10	0.01	0.459
22:0	0.31	0.32	0.06	0.905	0.25	0.30	0.05	0.452
23:0	0.11	0.11	0.02	0.847	0.10	0.11	0.01	0.432
MUFA	17.09	15.95	1.34	0.554	3.95	3.66	0.28	0.478
14:1	0.46	0.44	0.04	0.765	0.14	0.15	0.02	0.713
15:1	0.08	0.08	0.02	0.839	0.08	0.10	0.01	0.231
16:1	1.31	1.22	0.10	0.522	0.26	0.23	0.02	0.422
17:1	0.46	0.40	0.04	0.375	0.11	0.09	0.01	0.040
18:1 *trans*	1.18	0.98	0.24	0.565	0.17	0.16	0.01	0.313
18:1 *cis*-9	13.29	12.01	1.03	0.395	2.61	2.26	0.17	0.170
20:1	0.35	0.38	0.04	0.539	0.16	0.19	0.03	0.503
22:1	0.26	0.28	0.05	0.795	0.21	0.25	0.04	0.491
24:1 n-9	0.17	0.18	0.03	0.859	0.22	0.24	0.02	0.628
PUFA	1.39	1.40	0.11	0.977	2.98	3.21	0.10	0.139
18:2 n-6	0.65	0.65	0.04	0.961	1.51	1.59	0.08	0.455
18:2 *trans*	0.08	0.08	0.01	0.965	0.04	0.08	0.01	0.085
18:3 n-3	0.21	0.22	0.03	0.742	0.17	0.19	0.02	0.438
18:3 n-6	0.07	0.08	0.01	0.729	0.07	0.08	0.01	0.523
20:2	0.03	0.03	0.01	0.739	0.04	0.04	0.01	0.691
20:3 n-6	0.22	0.21	0.04	0.870	0.32	0.34	0.03	0.530
20:4 n-6	0.03	0.03	0.01	0.794	0.69	0.69	0.04	0.968
20:5 n-3	0.19	0.18	0.04	0.702	0.21	0.23	0.03	0.529
Total fatty acids	33.48	30.67	2.28	0.397	11.08	10.64	0.51	0.538
Total n-3	0.35	0.36	0.05	0.918	0.37	0.42	0.05	0.519
Total n-6	1.04	1.04	0.07	0.965	2.61	2.78	0.10	0.213
n6:n3	5.62	5.16	0.53	0.563	8.64	8.81	0.84	0.888
PUFA:SFA	0.10	0.12	0.01	0.306	0.76	0.89	0.03	0.013
SFA, %	44.96	42.97	0.95	0.154	36.75	35.06	0.32	0.001
MUFA, %	50.64	52.13	0.79	0.200	35.07	33.39	1.17	0.311
PUFA, %	4.32	4.83	0.37	0.337	27.82	31.14	1.16	0.058

[1]*adductor femoris*, *biceps femoris*, *gluteus medius*, *infraspinatus*, *latissimus dorsi*, *longissimus dorsi*, *pectoralis profundi*, *subscapularis*, *semitendinosus*, *trapezius*.

[2]Pooled standard error of the mean.

with control (Table 5). This proportional shift was due to an increased ($P = 0.001$) percentage of SFA among control cattle compared with ZH cattle. Interestingly, total PL FA did not differ ($P = 0.538$) between treatment groups, indicating a compositional shift to lower PL SFA percent with ZH. Cell membrane phospholipids are the primary component of PL. In human trials exercise has been shown to decrease the saturation of phospholipid FA, while not impacting triglyceride FA (Andersson et al., 1998; Helge et al., 2001). Furthermore, exercise induced alterations to muscle fibers have occurred simultaneously with decreases in SFA and overall greater unsaturation of phospholipid FA, while triglyceride FA were unchanged (Helge et al., 2001). This result is in agreement with our present study where muscle fiber components (MHC-IIX fiber cross-section area and MHC-IIA

mRNA) were altered and PL FA saturation was altered while NL FA were unaffected. A shift in PL saturation affects membrane fluidity and permeability (Hagve, 1988; Stubbs and Smith, 1984). Presently it is unclear how membrane fluidity and permeability is related with ZH. However, it has been concluded that changes to membrane fluidity may influence metabolic processes such as insulin-mediated glucose uptake (Andersson et al., 1998). It may be speculated that increased membrane fluidity plays a role in supplying glucose to glycolytic MHC-IIX fibers of greater cross-sectional area.

Muscle

Muscle affected ($P \leq 0.007$) the abundance of MHC-I, IIA and IIX mRNA (Table 6). The greatest ($P < 0.05$) abundance of MHC-I mRNA was in the TP and the least ($P < 0.05$) in ST. The greatest ($P < 0.05$) abundance of MHC-IIA mRNA was in the AD and greatest ($P < 0.05$) MHC-IIX mRNA was in the LA, with the lowest ($P < 0.05$) abundance of MHC-IIA mRNA in the GM and lowest ($P < 0.05$) MHC-IIX in the SS. The abundance of β1AR mRNA was the greatest ($P < 0.05$) in the TP and the least ($P < 0.05$) in the AD. Muscle affected ($P = 0.037$) the abundance of AMPkα mRNA with the most ($P < 0.05$) in the LD and least ($P < 0.05$) in the IF. The abundance of IGF-I was also affected by muscle ($P = 0.030$), with the least ($P < 0.05$) in the AD and BF, and the greatest ($P < 0.05$) abundance in IF. Muscle did not affect ($P = 0.541$) the abundance of β2AR mRNA.

Muscle also affected ($P \leq 0.023$) the density of nuclei, β1AR, β2AR, β3AR, internalized β2AR and satellite cells (Table 7). The SS had the greatest ($P < 0.05$) density of nuclei and the TP had the greatest ($P < 0.05$) total cells. The LD had the least ($P < 0.05$) nuclei and the LD and ST had the least ($P < 0.05$) total cells. Furthermore, the TP had the greatest ($P < 0.05$) density of β1AR and β2AR, and the GM had the greatest ($P < 0.05$) density of β3AR. The ST had the least ($P < 0.05$) β1AR, β2AR and β3AR. The BF had the greatest ($P < 0.05$) density of internalized β2AR and the GM had the least ($P < 0.05$). The greatest ($P < 0.05$) density of satellite cells expressing Myf5 and both Pax7:Mfy5 was in the TP and the ST had the least ($P < 0.05$) satellite cells expressing Myf5 and Pax7:Myf5.

Moreover, muscle affected ($P < 0.001$) the proportions of MHC-I, IIA and IIX, and the fiber cross-sectional areas of MHC-I, IIA and IIX ($P = 0.018$; Table 8). The SS had the greatest ($P < 0.05$) proportion of MHC-I and the ST had the lowest ($P < 0.05$) proportion. The AD had the greatest ($P < 0.05$) proportion of MHC-IIA and the PP had the lowest ($P < 0.05$) proportion. The ST had the greatest ($P < 0.05$) proportion of MHC-IIX, and the SS had the lowest ($P < 0.05$) proportion. In regards to fiber cross-sectional area the TP and SS had the greatest ($P < 0.05$) cross-sectional area of MHC-I and LA had the smallest ($P < 0.05$) area (Table 8). The LD and SS had the largest ($P < 0.05$) MHC-IIA fiber cross-sectional area and least ($P < 0.05$) area was in the TP. Myosin heavy chain-IIX fiber cross-sectional area was the greatest ($P < 0.05$) in the SS and LD and the smallest ($P < 0.05$) in the TP.

Hunt and Hedrick (1977) evaluated the proportion and area of β red (βR; equal to MHC-I), α red

Table 6. Effect of muscle on relative mRNA abundance of AMPKα, IGF-I, MHC-I, MHC-IIA, MHC-IIX, β1AR, and β2AR genes[1]

	Muscle[2]											
Gene[3]	AD	BF	GM	IF	LA	LD	PP	SS	ST	TP	SEM[4]	*P*-value
AMPkα	1.64^{abc}	1.40^{c}	1.46^{bc}	1.22^{c}	1.81^{abc}	2.16^{a}	1.80^{abc}	1.31^{c}	1.56^{abc}	2.00^{ab}	0.245	0.037
IGF-I	1.51^{c}	1.56^{c}	2.03^{bc}	3.42^{a}	1.83^{bc}	2.96^{ab}	2.46^{abc}	2.05^{bc}	1.93^{bc}	2.74^{ab}	0.492	0.030
β1AR	42.85^{d}	47.26^{cd}	65.14^{bcd}	53.86^{cd}	155.09^{ab}	132.21^{abc}	143.25^{ab}	153.14^{ab}	48.06^{cd}	165.66^{a}	37.534	0.003
β2AR	1.29	1.46	1.94	1.65	1.76	1.56	1.46	1.76	1.83	1.82	0.277	0.541
MHC-I	2.34^{cd}	1.99^{d}	4.57^{ab}	2.83^{bcd}	2.90^{bcd}	3.74^{bc}	3.84^{bc}	4.20^{b}	1.91^{d}	6.18^{a}	0.739	< 0.001
MHC-IIA	5.16^{a}	2.89^{bc}	2.18^{c}	3.35^{bc}	3.15^{bc}	3.89^{ab}	2.88^{bc}	2.78^{bc}	2.66^{bc}	3.13^{bc}	0.580	0.007
MHC-IIX	1.48^{a}	1.38^{ab}	1.04^{bc}	0.89^{c}	1.61^{a}	1.52^{a}	0.92^{c}	0.44^{d}	1.54^{a}	0.77^{cd}	0.174	< 0.001

$^{a–d}$Means in the same row having different superscripts are significant at $P \leq 0.05$.

[1]AMPKα = AMP-activated protein kinase alpha, IGF-I = insulin-like growth factor-I, MHC-I = myosin heavy chain-I, MHC-IIA = myosin heavy chain-IIA, MHC-IIX = myosin heavy chain-IIX, β1AR = beta 1 adrenergic receptor, and β2AR = beta 2 adrenergic receptor.

[2]AD = *adductor femoris*, BF = *biceps femoris*, GM = *gluteus medius*, IF = *infraspinatus*, LA = *latissimus dorsi*, LD = *longissimus dorsi*, PP = *pectoralis profundi*, SS = *subscapularis*, ST = *semitendinosus*, TP = *trapezius*.

[3]Relative abundance of the AMPKα, IGF-I MHC-I, MHC-IIA, MHC-IIX, β1AR, and β2AR genes were normalized with the ribosomal protein S9 endogenous control by using the change in cycle threshold (ΔΔCT).

[4]Pooled standard error of the mean.

Table 7. Effect of muscle on nuclei and β-adrenergic receptor and satellite cell density

	Muscle[1]											
Item[2], mm[2]	AD	BF	GM	IF	LA	LD	PP	SS	ST	TP	SEM[3]	*P*-value
Total Nuclei	516.49^{d}	509.82^{de}	558.28^{c}	565.91^{bc}	543.08^{cd}	456.60^{f}	539.49^{cd}	642.35^{a}	473.83^{ef}	602.59^{ab}	17.470	< 0.001
Myofiber Nuclei	334.64^{bc}	339.40^{bc}	392.18^{a}	372.00^{ab}	348.14^{bc}	271.67^{d}	324.65^{c}	404.48^{a}	335.39^{bc}	343.80^{bc}	20.970	< 0.001
Total cells	223.74^{bcd}	200.50^{ef}	213.55^{de}	234.67^{abc}	220.27^{cd}	189.83^{f}	197.77^{ef}	240.67^{ab}	189.41^{f}	244.82^{a}	8.859	< 0.001
β1AR	328.14^{c}	316.44^{cde}	328.83^{c}	341.44^{abc}	335.23^{bc}	301.11^{de}	323.55^{cd}	360.62^{ab}	291.80^{e}	363.16^{a}	11.137	< 0.001
β1AR-internalized	9.37	2.65	2.43	2.35	2.36	1.68	3.72	1.85	1.00	3.38	3.101	0.321
β2AR	328.72^{b}	320.27^{b}	329.44^{b}	337.61^{ab}	323.26^{b}	311.83^{b}	325.98^{b}	363.90^{a}	278.29^{c}	364.54^{a}	12.776	< 0.001
β2AR-internalized	8.92^{ab}	12.60^{a}	3.68^{c}	6.11^{bc}	9.63^{ab}	7.06^{bc}	7.74^{bc}	5.68^{bc}	8.09^{bc}	7.14^{bc}	2.103	0.023
β3AR	77.15^{cd}	76.71^{cd}	100.40^{a}	81.45^{bcd}	67.69^{de}	79.38^{cd}	80.21^{cd}	98.84^{ab}	57.94^{e}	87.54^{abc}	9.576	< 0.001
β3AR-internalized	2.00	2.28	2.59	2.08	–	1.48	1.19	1.49	1.46	1.46	0.729	0.754
Pax7	10.16	9.16	11.73	13.28	10.67	9.54	10.02	11.89	10.06	11.74	1.182	0.202
Myf5	170.72^{cd}	159.28^{de}	153.03^{de}	180.57^{cd}	179.42^{cd}	172.70^{cd}	202.25^{bc}	221.95^{ab}	126.81^{e}	240.85^{a}	19.152	< 0.001
Pax7:Myf5	10.27^{c}	9.97^{c}	11.62^{bc}	11.94^{bc}	11.64^{bc}	11.45^{bc}	13.64^{ab}	11.47^{bc}	10.08^{c}	14.68^{a}	1.028	0.009

$^{a–f}$Means in the same row having different superscripts are significant at $P \leq 0.05$.

[1]AD = *adductor femoris*, BF = *biceps femoris*, GM = *gluteus medius*, IF = *infraspinatus*, LA = *latissimus dorsi*, LD = *longissimus dorsi*, PP = *pectoralis profundi*, SS = *subscapularis*, ST = *semitendinosus*, TP = *trapezius*.

[2]β1AR = beta 1 adrenergic receptor, and β2AR = beta 2 adrenergic receptor, Pax7 = paired box 7, Myf5 = myogenic factor 5, Pax7:Myf5 = dual expressing paired box 7 and myogenic factor 5.

[3]Pooled standard error of the mean.

Table 8. Effect of muscle on myosin heavy chain (MHC)-I, IIA and IIX cross-sectional area and distribution

	Muscle[1]											
Item	AD	BF	GM	IF	LA	LD	PP	SS	ST	TP	SEM[2]	*P*-value
MHC-I**	3335^{bcde}	3298^{cde}	3488^{bcd}	3216de	3030^{e}	3625^{bc}	3667^{b}	3173de	4298^{a}	4278^{a}	162.9	< 0.001
MHC-IIA**	4081^{bc}	4536^{b}	4213^{bc}	3568^{d}	4014^{cd}	5417^{a}	5102^{a}	3733^{cd}	4070^{bcd}	2932^{e}	251.6	< 0.001
MHC-IIX**	4427^{bc}	5403^{bc}	4744^{bc}	3746^{bc}	5032^{bc}	6033^{b}	5330^{bc}	9639^{a}	5586^{bc}	3079^{c}	1381.9	0.018
MHC-I***	22.90^{f}	30.44^{e}	49.09^{b}	31.66^{e}	37.37^{d}	31.97^{e}	40.92^{c}	53.63^{a}	20.36^{g}	52.97^{a}	0.011	< 0.001
MHC-IIA***	56.96^{a}	46.81^{c}	37.47^{f}	49.14^{b}	41.84^{e}	46.89^{c}	33.99^{g}	43.79^{de}	45.29^{cd}	36.90^{f}	0.011	< 0.001
MHC-IIX***	19.51^{d}	22.04^{c}	15.11^{e}	18.92^{d}	20.16^{d}	20.42^{cd}	24.02^{b}	6.11^{g}	33.33^{a}	11.71^{f}	0.015	< 0.001

$^{a–g}$Means in the same row having different superscripts are significant at $P \leq 0.05$.

**Fiber cross-sectional area in μm^2.

***Percentage of myosin heavy chain type I, IIA, and IIX.

[1]AD = *adductor femoris*, BF = *biceps femoris*, GM = *gluteus medius*, IF = *infraspinatus*, LA = *latissimus dorsi*, LD = *longissimus dorsi*, PP = *pectoralis profundi*, SS = *subscapularis*, ST = *semitendinosus*, TP = *trapezius*.

[2]Pooled standard error of the mean.

(αR; equal to MHC-IIA), and α white (αW; equal to MHC-IIX) fibers in the LD, *psoas major* (PM), GM, outer *semitendinosus* (OST), inner *semitendinosus*, outer *semimembranosus* (OSM), and inner *semimembranosus*. The PM had the greatest proportion of βR (MHC-I) fibers and the least αW (MHC-IIX) fibers of all the muscles (Hunt and Hedrick, 1977). The OST had the greatest proportion of αW (MHC-IIX) fibers, and the ISM had the least βR (MHC-I) fibers (Hunt and Hedrick, 1977). The LD and GM muscles were intermediate to the PM and the SM and ST in terms of proportions of βR (MHC-I), αR (MHC-IIA), and αW (MHC-IIX) fibers (Hunt and Hedrick, 1977). The αW (MHC-IIX) fibers had the greatest area in all the muscles, and the αR (MHC-IIA) fibers had the least area in all muscles except for the ISM (Hunt and Hedrick, 1977). We found that the MHC-IIX fibers had the greatest area in all the muscles with the exception of the TP. The fibers with the greatest area in the SS were MHC-IIX and MHC-I in the TP. Kirchofer et al. (2002) did more extensive work identifying the fiber type composition of 38 bovine muscles (12 from the round and 26 from the chuck) from 4 A-maturity, Select-grade carcasses, including the 10 muscles that we sampled. Kirchofer et al. (2002) classified these muscles as red, white, or intermediate, when greater than 40% of the fibers within a muscle was βR (MHC-I), αW (MHC-IIX), or αR (MHC-IIA) fiber

types. Nine muscles from the round were classified as white and 3 were classified intermediate (Kirchofer et al., 2002). The AD, BF, GM, and ST were all classified as white muscles (Kirchofer et al., 2002). This is contradictory to the current data, as none of these muscles would have classified as white (Table 8). The AD, BF, and ST would have classified as intermediate and the GM as red in the current study. In the chuck 10 muscles were red, 9 were intermediate, and 7 were white (Kirchofer et al., 2002). The IF and TP were classified as red, the deep pectoral and SS as intermediate, and the LA and LD as white. In the current study the PP, SS, and TP would have classified as red, and the IF, LA, and LD as intermediate. These differences may be due to the fact all the samples in the current study came from heifers, and Kirchofer et al. (2002) randomly chose chuck and rounds from a commercial abattoir based on weight and yield grade disregarding gender. However, there has been limited research comparing gender differences in skeletal muscle fiber type composition of cattle. The methods used for muscle section staining by Kirchofer et al. (2002) were different than the methods we used to identify fiber type. Kirchofer et al. (2002) first stained samples for succinate dehydrogenase activity, then incubated them in an acid incubate, and finally stained for acid-active adenosine tri-phosphatase activity to determine fiber type. We used poly and monoclonal antibodies against myosin preparations. Furthermore, Kirchofer et al. (2002) had a much smaller sample size, only evaluating muscle from 4 animals.

The function of an individual muscle plays an enormous role in the fiber type composition of that muscle. A greater proportion of oxidative, slow twitch fibers are mostly found in muscles involved in posture, like the muscles that rest over joints to resist flexion (Ogata and Yamasaki, 1985). The muscles used for locomotion and rapid acceleration have a greater proportion of glycolytic, fast twitch fibers (Johnson et al., 1986). The change in fiber composition from chuck to round is shown in Fig. 1. Muscles in the chuck have smaller cross-sectional areas, more total cells and are comprised of more MHC-I and IIA fibers. As muscles progressed to the round fibers had greater cross-sectional areas, less MHC-I fibers and more MHC-IIX fibers. Armstrong et al. (1987) reported that the deepest muscles of the limbs typically have the greatest percentage of Type I fibers, and the superficial muscles have the greatest percentage of glycolytic fibers in pigs. In the *semimembranosus* of cattle Brandstetter et al. (1997) reported a decrease in glycolytic activity and increased oxidative activity toward the distal extremity. The proportion of Type I fibers became more abundant from the proximal to the distal regions (Brandstetter et al., 1997). Livestock animal's muscles usually have posture and locomotive roles, and the proportion and arrangement of muscle fibers varies from muscle to muscle (Aalhus et al., 2009). Typically, the greatest proportions of slow twitch, oxidative fibers are found deeper in the muscle nearest to the joint in which the muscle controls and the closest to the blood supply (Aalhus et al., 2009). The greatest proportions of fast twitch, glycolytic fibers are found toward the outer edges of the muscle for use during intense activity to generate more power (Aalhus et al., 2009).

Intramuscular NL FA were greatly affected by muscle type (Table 9). Total NL FA content was greatest ($P < 0.05$) among the IF compared will all other muscles. The TP had lower ($P < 0.05$) content compared with the IF, however, the TP had greater ($P < 0.05$) content in comparison with all other muscles. The BF, GM, LA, LD, and PP each had similar ($P > 0.05$) total NL FA content. The total NL FA content of the LA and LD were each greater ($P < 0.05$) than the AD, SS, and ST. However, total NL FA contents of the BF, GM, and PP did not differ ($P > 0.05$) from the AD, SS, and ST. The total NL FA contents in this study are in agreement with pervious muscle total fat content (mg/g) data reported by Jeremiah et al. (2003). The one exception being the PP which was ranked lower among muscles in our study compared with Jeremiah et al. (2003). It should be noted that the previous work utilized a minimum of 200 g of sample for each muscle, while in our study smaller samples were collected and fractionated prior to analysis. Overall a strong relationship was seen between total NL FA and total fat contents reported by others. This result is supported by previous work, which indicated that FA accumulation occurs primarily in the NL triglycerides (Legako et al., 2015). In general, the content of individual NL FA followed the order described above for total NL FA (Table 9).

Total PL FA content is primarily derived of cell membrane components and thus may have some relationships with muscle ultrastructure. Previously red oxidative muscle fiber type was determined to have greater phospholipids in comparison with white glycolytic muscle fiber types (De Smet et al., 2004). Among the PL FA there was no difference ($P = 0.264$) in the total content of muscles (Table 10). However, the content of several individual PL FA (14:0, 17:0, 23:0, 18:1 *trans*, 18:2 n-6, 20:5 n-3) differed due to muscle ($P \leq 0.049$). Furthermore, PL stearic acid (18:0) tended to differ ($P < 0.057$) due to muscle. Interestingly, content of many of these individual FA appeared to be related with muscle fiber type distribution. Specifically,

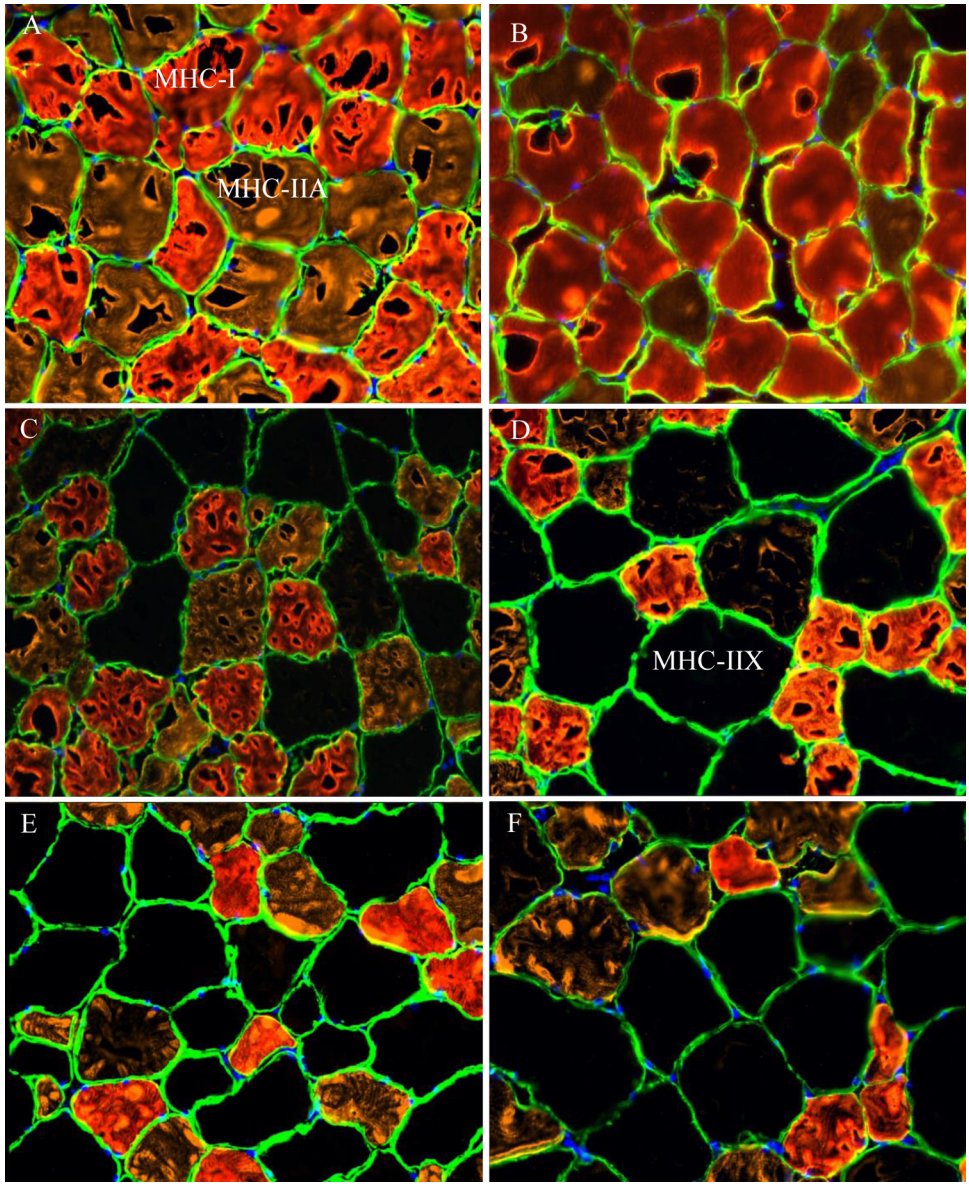

Figure 1. Immunohistochemical detection on muscle cross-sections from control heifers and heifers administered zilpaterol HCl, demonstrates sarcolemma by dystropin in green, nuclei in blue, myosin heavy chain type I positive muscle fibers in red, myosin type IIA in orange, and myosin type IIX in black (negative for myosin heavy chain type I and IIA). (A) *trapezius* of a control heifer, (B) *trapezius* of a heifer administered zilpaterol HCl, (C) *longissimus dorsi* of a control heifer, (D) *longissimus dorsi* of a heifer administered zilpaterol HCl, (E) *semitendinousus* of a control heifer, (F) *semitendinousus* of a heifer administered zilpaterol HCl.

among muscles with the most extreme distribution, the SS compared with the ST and AD (Table 8). The fiber distribution of the SS was dominated by oxidative MHC-I and had the smallest distribution of glycolytic MHC-IIX fibers of all muscles. Meanwhile, the ST and AD had a low distribution of MHC-I compared with SS and greater distributing of MHC-IIX compared with the SS. Furthermore, the content of PL FA, 18:1 *trans* and 18:2 n-6 were each greater in SS compared with ST and AD ($P < 0.05$). Having greater PL FA content among a predominately oxidative muscle compared with predominately glycolytic is in agreement with several past works (Ruff-Morrison and Campbell, 1971; Allen et al., 1967; Luddy et al., 1970). Oxidative muscle fibers are well known to be smaller in size leading to greater cell membrane surface area, additionally, oxidative muscle fibers contain greater numbers of membrane bound organelles compared with glycolytic fibers. This data indicates that shifts in muscle fiber metabolism and muscle ultrastructure significantly influence PL FA contents in extreme cases (SS vs. ST and AD).

Table 9. Effect of muscle on neutral lipid fatty acids (mg/g muscle tissue) from cattle receiving zilpaterol hydrochloride (ZH) and cattle not receiving any ZH

	Muscle[1]											
Fatty acid	AD	BF	GM	IF	LA	LD	PP	SS	ST	TP	SEM[2]	*P*-value
SFA	7.57^{f}	12.33^{cde}	11.91^{cdef}	28.73^{a}	14.31^{c}	13.03^{cd}	11.38^{cdef}	9.51^{def}	8.53^{ef}	23.72^{b}	1.74	< 0.001
14:0	0.60^{d}	0.88^{bcd}	0.90^{bcd}	2.02^{a}	1.16^{b}	1.02^{bc}	0.84^{bcd}	0.69^{cd}	0.62^{d}	1.89^{a}	0.17	< 0.001
15:0	0.09^{c}	0.15^{bc}	0.13^{bc}	0.34^{a}	0.17^{b}	0.16^{b}	0.13^{bc}	0.12^{bc}	0.10^{bc}	0.34^{a}	0.03	< 0.001
16:0	4.31^{d}	7.12^{bc}	6.77^{bcd}	15.62^{a}	8.03^{b}	7.39^{bc}	6.52^{bcd}	5.22^{cd}	4.90^{cd}	13.21^{a}	1.01	< 0.001
17:0	0.22^{d}	0.40^{bc}	0.38^{bcd}	0.91^{a}	0.42^{bc}	0.45^{b}	0.34^{bcd}	0.32^{bcd}	0.27^{cd}	0.81^{a}	0.07	< 0.001
18:0	1.82^{d}	3.19^{bcd}	3.17^{bcd}	8.25^{a}	3.78^{bc}	3.90^{b}	3.26^{bcd}	2.83^{bcd}	2.40^{cd}	6.94^{a}	0.57	< 0.001
20:0	0.03^{bcd}	0.03^{bcd}	0.03^{bcd}	0.08^{a}	0.04^{b}	0.03^{bcd}	0.02^{cd}	0.02^{d}	0.02^{bcd}	0.04^{bc}	0.01	< 0.001
21:0	0.09^{bc}	0.10^{bc}	0.09^{bc}	0.26^{a}	0.12^{b}	0.08^{bc}	0.07^{bc}	0.05^{c}	0.06^{bc}	0.09^{bc}	0.03	< 0.001
22:0	0.29^{b}	0.33^{b}	0.37^{b}	0.85^{a}	0.36^{b}	0.21^{b}	0.15^{b}	0.16^{b}	0.16^{b}	0.25^{b}	0.10	< 0.001
23:0	0.09^{bc}	0.12^{b}	0.12^{bc}	0.29^{a}	0.11^{bc}	0.09^{bc}	0.07^{bc}	0.06^{c}	0.06^{bc}	0.08^{bc}	0.03	< 0.001
24:0	0.17^{b}	0.20^{b}	0.20^{b}	0.48^{a}	0.30^{ab}	0.17^{b}	0.12^{b}	0.08^{b}	0.13^{b}	0.16^{b}	0.10	0.043
MUFA	9.57^{e}	14.25^{cde}	13.89^{cde}	33.14^{a}	17.10^{c}	15.72^{cd}	15.67^{cd}	10.33^{de}	9.63^{e}	25.90^{b}	2.09	< 0.001
14:1	0.29^{cd}	0.39^{bcd}	0.38^{bcd}	0.85^{a}	0.49^{b}	0.39^{bc}	0.36^{bcd}	0.28^{cd}	0.25^{d}	0.76^{a}	0.06	< 0.001
15:1	0.07^{bc}	0.08^{bc}	0.10^{b}	0.21^{a}	0.09^{bc}	0.07^{bc}	0.05^{c}	0.04^{c}	0.05^{c}	0.07^{bc}	0.03	< 0.001
16:1	0.77^{d}	1.08^{bcd}	0.98^{bcd}	2.26^{a}	1.27^{bc}	1.13^{bcd}	1.33^{b}	0.84^{cd}	0.71^{d}	2.25^{a}	0.17	< 0.001
17:1	0.23^{c}	0.38^{bc}	0.34^{bc}	0.82^{a}	0.35^{bc}	0.41^{b}	0.41^{bc}	0.30^{bc}	0.25^{bc}	0.79^{a}	0.07	< 0.001
18:1 *trans*	0.80^{cd}	0.70^{cd}	0.63^{cd}	1.90^{ab}	2.15^{a}	0.93^{bcd}	0.69^{cd}	0.67^{cd}	0.55^{d}	1.76^{abc}	0.44	0.021
18:1 *cis*-9	6.78^{c}	10.88^{bc}	10.70^{bc}	25.25^{a}	11.94^{b}	12.18^{b}	12.21^{b}	7.79^{bc}	7.49^{bc}	21.29^{a}	1.87	< 0.001
20:1	0.26^{cd}	0.32^{bcd}	0.37^{bc}	0.82^{a}	0.36^{bc}	0.33^{bcd}	0.31^{bcd}	0.24^{d}	0.22^{d}	0.43^{b}	0.05	< 0.001
22:1	0.23^{bc}	0.27^{bc}	0.36^{b}	0.69^{a}	0.31^{bc}	0.22^{bc}	0.15^{c}	0.14^{c}	0.15^{c}	0.21^{bc}	0.08	< 0.001
24:1 n-9	0.15^{bc}	0.18^{bc}	0.16^{bc}	0.47^{a}	0.20^{b}	0.15^{bc}	0.11^{bc}	0.09^{c}	0.11^{bc}	0.15^{bc}	0.04	< 0.001
PUFA	0.93^{cd}	1.27^{cd}	1.22^{cd}	3.41^{a}	1.32^{bcd}	1.36^{bc}	0.97^{cd}	0.87^{d}	0.85^{d}	1.75^{b}	0.18	< 0.001
18:2 n-6	0.37^{d}	0.54^{cd}	0.47^{cd}	1.44^{a}	0.55^{cd}	0.68^{c}	0.51^{cd}	0.50^{cd}	0.39^{d}	1.05^{b}	0.09	< 0.001
18:2 *trans*	0.06^{de}	0.07^{cde}	0.08^{cde}	0.19^{a}	0.09^{c}	0.08^{cd}	0.06^{de}	0.05^{e}	0.05^{e}	0.12^{b}	0.01	< 0.001
18:3 n-3	0.17^{bcd}	0.20^{bcd}	0.25^{b}	0.53^{a}	0.23^{bc}	0.19^{bcd}	0.13^{cd}	0.12^{d}	0.13^{cd}	0.22^{bcd}	0.04	< 0.001
18:3 n-6	0.06^{bc}	0.07^{bc}	0.10^{b}	0.18^{a}	0.06^{bc}	0.07^{bc}	0.04^{c}	0.04^{c}	0.04^{c}	0.06^{bc}	0.02	< 0.001
20:2	0.03^{bcd}	0.03^{bcd}	0.03^{bcd}	0.08^{a}	0.04^{b}	0.03^{bcd}	0.02^{cd}	0.02^{d}	0.02^{bcd}	0.04^{bc}	0.01	< 0.001
20:3 n-6	0.18^{b}	0.23^{b}	0.19^{b}	0.58^{a}	0.24^{b}	0.19^{b}	0.13^{b}	0.11^{b}	0.13^{b}	0.19^{b}	0.06	< 0.001
20:4 n-6	0.02^{b}	0.04^{b}	0.02^{b}	0.08^{a}	0.02^{b}	0.03^{b}	0.03^{b}	0.02^{b}	0.02^{b}	0.03^{b}	0.01	< 0.001
20:5 n-3	0.15^{b}	0.16^{b}	0.16^{b}	0.52^{a}	0.21^{b}	0.16^{b}	0.10^{b}	0.08^{b}	0.10^{b}	0.14^{b}	0.06	< 0.001
Total fatty acids	18.08^{e}	27.88^{cde}	27.06^{cde}	65.35^{a}	32.77^{c}	30.44^{cd}	28.06^{cde}	20.71^{de}	19.04^{e}	51.37^{b}	3.93	< 0.001
Total n-3	0.25^{bc}	0.31^{bc}	0.39^{b}	0.98^{a}	0.40^{b}	0.32^{bc}	0.21^{bc}	0.15^{c}	0.20^{bc}	0.33^{bc}	0.09	< 0.001
Total n-6	0.68^{d}	0.95^{cd}	0.83^{cd}	2.43^{a}	0.93^{cd}	1.04^{c}	0.76^{cd}	0.71^{d}	0.65^{d}	1.43^{b}	0.12	< 0.001
n6:n3	4.29^{d}	4.68^{cd}	5.18^{cd}	4.44^{d}	5.14^{cd}	5.77^{bc}	5.22^{bcd}	6.53^{ab}	4.91^{cd}	7.73^{a}	0.60	< 0.001
PUFA:SFA	0.14^{a}	0.13^{ab}	0.11^{abc}	0.14^{a}	0.09^{bc}	0.12^{ab}	0.09^{bc}	0.10^{abc}	0.11^{abc}	0.08^{c}	0.01	0.015
SFA, %	41.61^{cd}	43.55^{bcd}	44.14^{bc}	43.62^{bcd}	44.57^{abc}	43.37^{bcd}	40.94^{d}	45.52^{ab}	44.70^{ab}	47.63^{a}	1.27	0.004
MUFA, %	52.82^{ab}	51.17^{bc}	51.21^{bc}	50.58^{bc}	51.43^{bc}	51.83^{b}	55.35^{bc}	49.99^{bc}	50.68^{bc}	48.78^{c}	1.21	0.011
PUFA, %	5.50^{a}	5.17^{ab}	4.59^{abc}	5.67^{a}	3.92^{bc}	4.72^{abc}	3.65^{c}	4.43^{abc}	4.55^{abc}	3.51^{c}	0.53	0.011

[a–f]Means in the same row having different superscripts are significant at $P \leq 0.05$.

[1]AD = *adductor femoris*, BF = *biceps femoris*, GM = *gluteus medius*, IF = *infraspinatus*, LA = *latissimus dorsi*, LD = *longissimus dorsi*, PP = *pectoralis profundi*, SS = *subscapularis*, ST = *semitendinosus*, TP = *trapezius*.

[2]Pooled standard error of the mean.

Conclusions

The objective of this study was to determine if ZH alters muscle metabolism and lipid components of 10 beef muscles. Among the components measured, response to ZH was not muscle dependent. Overall, ZH decreased MHC-IIA mRNA, total nuclei, β1AR, and PL PUFA:SFA ratio. Meanwhile, ZH increased MHC-IIX cross-sectional area. Alteration of muscle fiber components (MHC-IIX fiber cross-section area and MHC-IIA mRNA) and PL FA saturation is in agreement with other research. Presently it is unclear if this change in PL FA serves to influence metabolic processes, such as

Table 10. Effect of muscle on polar lipid fatty acids (mg/g muscle tissue) from cattle receiving zilpaterol hydrochloride (ZH) and cattle not receiving any ZH

	Muscle[1]											
Fatty acid	AD	BF	GM	IF	LA	LD	PP	SS	ST	TP	SEM[2]	*P*-value
SFA	3.55	3.96	4.40	4.06	3.99	4.28	3.40	3.94	3.48	4.04	0.32	0.142
14:0	0.19^{abc}	0.23^{ab}	0.23^{ab}	0.22^{ab}	0.23^{ab}	0.25^{a}	0.14^{c}	0.19^{abc}	0.18^{bc}	0.24^{b}	0.03	0.043
15:0	0.03	0.03	0.03	0.04	0.03	0.04	0.02	0.04	0.03	0.04	0.01	0.164
16:0	1.76	1.90	2.02	1.84	1.98	2.19	1.52	1.80	1.67	1.92	0.19	0.070
17:0	0.07^{c}	0.08^{bc}	0.09^{abc}	0.09^{ab}	0.08^{abc}	0.10^{a}	0.07^{c}	0.09^{abc}	0.07^{c}	0.09^{abc}	0.01	0.046
18:0	0.97	1.08	1.30	1.26	1.15	1.25	1.27	1.42	1.03	1.27	0.11	0.057
20:0	0.05	0.05	0.05	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.01	0.542
21:0	0.10	0.11	0.12	0.09	0.09	0.09	0.07	0.07	0.09	0.08	0.02	0.558
22:0	0.28	0.38	0.35	0.31	0.31	0.21	0.22	0.19	0.27	0.27	0.06	0.103
23:0	0.13^{ab}	0.13^{abc}	0.14^{a}	0.11^{abcd}	0.10^{abcd}	0.10^{abcd}	0.08^{d}	0.09^{d}	0.09^{cd}	0.10^{bcd}	0.02	0.049
MUFA	3.55	3.97	4.03	3.90	3.93	4.24	3.25	3.88	3.41	3.95	0.38	0.441
14:1	0.15	0.18	0.18	0.15	0.15	0.14	0.10	0.12	0.13	0.16	0.02	0.106
15:1	0.11	0.11	0.11	0.09	0.08	0.08	0.07	0.08	0.08	0.08	0.02	0.373
16:1	0.22	0.26	0.25	0.24	0.26	0.28	0.21	0.26	0.20	0.26	0.03	0.288
17:1	0.08	0.09	0.09	0.12	0.10	0.11	0.11	0.12	0.09	0.09	0.01	0.136
18:1 *trans*	0.12^{d}	0.16^{abcd}	0.16^{abcd}	0.19^{a}	0.18^{abc}	0.17^{abc}	0.13^{cd}	0.21^{a}	0.14^{bcd}	0.19^{ab}	0.02	0.014
18:1 *cis*-9	2.19	2.40	2.46	2.47	2.51	2.85	2.16	2.63	2.14	2.56	0.26	0.317
20:1	0.19	0.22	0.21	0.18	0.18	0.17	0.13	0.13	0.17	0.17	0.03	0.147
22:1	0.28	0.32	0.30	0.22	0.24	0.22	0.15	0.16	0.22	0.22	0.05	0.060
24:1 n-9	0.23	0.27	0.27	0.24	0.24	0.22	0.19	0.18	0.23	0.22	0.03	0.158
PUFA	2.82	3.40	3.67	3.12	3.12	2.77	3.12	3.16	2.90	2.85	0.24	0.082
18:2 n-6	1.34^{b}	1.52^{ab}	1.85^{a}	1.59^{ab}	1.53^{ab}	1.42^{b}	1.61^{ab}	1.82^{a}	1.39^{b}	1.45^{b}	0.14	0.038
18:2 *trans*	0.05	0.15	0.06	0.05	0.05	0.04	0.10	0.04	0.04	0.04	0.03	0.239
18:3 n-3	0.20	0.22	0.22	0.18	0.19	0.17	0.13	0.14	0.18	0.17	0.03	0.060
18:3 n-6	0.09	0.09	0.09	0.08	0.07	0.07	0.05	0.05	0.07	0.07	0.01	0.110
20:2	0.05	0.05	0.05	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.01	0.543
20:3 n-6	0.35	0.39	0.39	0.34	0.33	0.30	0.28	0.27	0.32	0.30	0.04	0.089
20:4 n-6	0.55	0.78	0.78	0.69	0.71	0.61	0.79	0.70	0.67	0.61	0.07	0.077
20:5 n-3	0.24^{abc}	0.28^{a}	0.27^{ab}	0.22^{abcd}	0.23^{abc}	0.20^{bcd}	0.17^{cd}	0.16^{d}	0.23^{abcd}	0.19^{cd}	0.03	0.017
Total fatty acids	9.97	11.39	12.13	11.14	11.07	11.35	9.92	11.01	9.84	10.89	0.82	0.264
Total n-3	0.44^{abc}	0.50^{a}	0.50^{ab}	0.41^{abcd}	0.42^{abcd}	0.35^{cd}	0.30^{d}	0.29^{d}	0.40^{abcd}	0.36^{bcd}	0.06	0.021
Total n-6	2.37	2.91	3.17	2.72	2.70	2.42	2.83	2.87	2.48	2.48	0.22	0.073
n6:n3	7.12^{c}	7.44^{c}	8.56^{bc}	8.63^{bc}	7.99^{c}	8.91^{bc}	10.56^{ab}	11.64^{a}	7.67^{c}	8.59^{bc}	1.04	0.002
PUFA:SFA	0.81^{abcd}	0.92^{ab}	0.87^{abc}	0.82^{abcd}	0.79^{bcd}	0.72^{d}	0.94^{a}	0.83^{abcd}	0.86^{abc}	0.74^{cd}	0.06	0.038
SFA, %	35.66^{bc}	34.64^{c}	36.19^{abc}	35.95^{abc}	36.23^{abc}	37.41^{a}	34.61^{c}	35.60^{bc}	35.48^{c}	37.29^{ab}	0.64	0.013
MUFA, %	35.18	33.57	32.25	34.13	34.88	35.85	32.57	35.04	33.57	35.30	1.57	0.473
PUFA, %	28.71	31.39	31.23	29.46	28.55	26.38	32.45	29.05	30.54	27.04	1.80	0.093

$^{a–d}$Means in the same row having different superscripts are significant at $P \leq 0.05$.

[1]AD = *adductor femoris*, BF = *biceps femoris*, GM = *gluteus medius*, IF = *infraspinatus*, LA = *latissimus dorsi*, LD = *longissimus dorsi*, PP = *pectoralis profundi*, SS = *subscapularis*, ST = *semitendinosus*, TP = *trapezius*.

[2]Pooled standard error of the mean.

insulin-mediated glucose uptake. However, it may be concluded through our results that there is a relationship between muscle fiber components and PL FA.

In agreement with past research myofibrilar components (fiber type proportion, fiber cross-sectional area, the densities of nuclei, β1AR, β2AR, β3AR, satellite cells, and mRNA concentration of AMPkα, IGF-I, MHC-I, IIA, IIX, and β1AR mRNA) varied between muscles. Also, as expected NL FA varied with muscle type in agreement with each muscles disposition toward adipose accretion. The PL FA were less varied due to muscle, however, PL 18:1 *trans*, and 18:2 n-6 were each greater among the oxidative SS compared with glycolytic ST and AD. These results further indicate that PL FA are related with muscle fiber components, such as myofibrilar metabolism.

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