Cellular development in muscle differs between Angus steers from low and high muscle score selection lines¹

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ABSTRACT: This study assessed cellular characteristics of longissimus lumborum (LL) and semitendinosus (ST) muscles in steers genetically selected for low (Low) or high (High) muscling using live muscle scoring, and High steers with 1 copy of the loss-of-function 821 del11 *MSTN* allele (High^{Het}). We hypothesized High and High^{Het} have altered muscle cellular characteristics and mechanisms influencing muscling compared with Low steers. Angus steers 25 mo old comprising 14 High, 19 Low, and 11 High^{Het} were backgrounded to 20 mo of age, grain finished for 150 d, and then slaughtered. Body and carcass weights did not differ due to muscling line ($P = 0.46$). Weight of LL was 16% greater ($P = 0.004$) and total protein in LL was 18% greater ($P = 0.012$) in High^{Het} than Low steers. ST weight in High^{Het} was 10% and 13% greater than in High and Low steers ($P = 0.007$), respectively, and of total ST protein 12% and 17% greater in High^{Het} than High or Low ($P = 0.002$). Cross-sectional area (CSA) of LL was greater in High^{Het} than in High and greater in High than in Low (85.0 vs. 77.0 vs. 70.4 cm², $P < 0.001$). Apparent number of myofibers and myofibers per unit CSA did not differ between the muscling lines in LL ($P = 0.14$) or ST ($P = 0.47$). Myofiber CSA was greater in the ST of Low than

of High and High^{Het} for type 1 (36% and 31% respectively, $P = 0.005$) and 2A (22% and 25%, $P < 0.001$). High^{Het} steers had greater area of glycolytic (type 2X) relative to more oxidative myofiber types within LL ($P = 0.02$; 11% and 43% more than High and Low, respectively) and ST ($P < 0.001$; 27% and 75%). Concentration of RNA in LL was 13% and 10% greater ($P = 0.005$) in High than in Low and High^{Het}, respectively, and total amount of RNA in LL was 22% greater in High and 20% greater in High^{Het} than in Low ($P < 0.001$). The LL of High steers had less protein to RNA ($P = 0.03$; 57.4 vs. 65.6) and more RNA to DNA ($P = 0.007$; 9.03 vs. 7.83) than Low. High^{Het} steers had 11% more DNA in ST than High ($P = 0.04$) and 19% more RNA in ST than Low ($P = 0.012$). The shift towards glycolytic myofibers was consistent with loadings in a principal component that explained 39% of the variation in LL and 38% in ST. Overall, these findings show that selection for increased muscling using live cattle muscle scoring, and 1 copy of the 821 del11 *MSTN* allele, results in more glycolytic muscle. They also suggest that increased muscling of the High compared with Low steers may be associated with increased translational capacity in the LL.

Key words: beef quality, meat yield, muscle cellularity, muscle score, myofibers, myostatin

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INTRODUCTION

Genetic selection for increased retail yield is important for profitable beef production (Alford et al., 2009). This requires increased proportion of muscle in the carcass and is achievable by genetic selection using assessments of muscling such as live cattle muscle score (McKiernan, 1990, 2007; Robinson et al., 1992, 2014) or eye muscle area (Robinson et al., 2014), or gene markers such as *myostatin* (*MSTN*) gene mutations (Allais et al., 2010; Cafe et al., 2014) that result in double muscling (Arthur, 1995; McPherron and Lee, 1997). Cellular characteristics associated with increased muscling in cattle due to genetic selection using live cattle muscle score have not been reported. Cellular characteristics associated with increased muscling and mechanisms that enhance muscling may vary depending on specific genetic mutations (Warner et al., 2010). Quantitative genetic selection or gene mutations that increase muscling can have adverse effects on meat quality, as shown in sheep (Freking et al., 2004; Warner et al., 2010) and pigs (Rehfeldt et al., 2004). Increased muscling is generally associated with whiter, fast glycolytic myofibers and less oxidative myofiber types, which may result in meat less favorable to consumers (Warner et al., 2010). In the present study, we tested the hypothesis that increased muscling in cattle achieved through genetic selection using live muscle score alters cellular characteristics and mechanisms that increase muscling. We also tested the hypothesis that the presence of 1 copy of the 821 del11 *MSTN* mutation (McPherron and Lee, 1997) would affect muscle cellular characteristics within high muscle score cattle. These hypotheses were tested by assessing myofiber characteristics and indices of cellular development in the longissimus lumborum (LL) and semitendinosus (ST) muscles of Angus cattle selected for low and high muscling using live cattle muscle score, including cattle with 1 copy of the 821 del11 *MSTN* mutation within the high line.

MATERIALS AND METHODS

Use of animals and the procedures performed in this study were approved by the New South Wales (NSW) Department of Primary Industries (DPI) Wollongbar Agricultural Institute Animal Ethics Committee (Approval Number G6/2004).

Animals, Management, and Slaughter Measurements

The steers used in this study were from the NSW DPI Angus muscling herd. Development of the herd is described in detail by Cafe et al. (2018). Briefly, selection based on visual muscle score (McKiernan, 2007) was used from 1992 onwards to create **Low** and **High** muscled Angus cow lines. The 821 del11 myostatin mutation (Grobet et al., 1998) was inadvertently introduced into the High muscle line in about 1998, and animals heterozygous for this mutation were considered as a third (**High^{Het}**) group.

One cohort of 44 steers described in O'Rourke et al. (2009) was used in this study. The steers comprised 14 High and 19 Low steers that were homozygous wildtype at the nucleotide 821 site, and 11 High^{Het} steers that were heterozygous for the 821 del11 mutation. The steers were reared on their dams, weaned, and then backgrounded on improved perennial pastures at NSW DPI Glen Innes Agricultural Research and Advisory Station (GIARAS: latitude 29°44' S, longitude 151°42' E; altitude 1057 m) until approximately 20 mo of age. At the end of backgrounding in 2005, the steers were transported 290 km to Yarranbrook Feedlot at Inglewood, Queensland (latitude 28°24' S, longitude 151°04' E; altitude 282 m) for grain finishing for 150 d. Steers were weighed before being transported 108 km to John Dee Abattoir, Warwick, Queensland on the afternoon before slaughter. The steers were held in a lairage pen as one group with access to water and without feed, and slaughtered the following morning.

The steers were slaughtered at approximately 25 mo of age (mean $\pm$ SD 761 $\pm$ 21 d, ranging from 719 to 793 d) in November 2005, with slaughter BW, and carcass, yield, and objective beef quality characteristics measured as detailed in O'Rourke et al. (2009), who reported carcass, yield, and objective beef quality results for these cattle.

Myostatin Genotyping

Tail hair samples were collected from each of the animals used in the study and DNA extracted as described previously (Dennis and Healy, 2001). Genotyping to determine nucleotide 821 (*MSTN*) allelic status was conducted by real-time PCR

procedure employing dual-labeled probes as detailed in O'Rourke et al. (2009).

Based on the results of the genotyping, animals that did not have any *MSTN* 821 del11 mutation were classified as homozygous wildtype. Animals with 1 copy of the *MSTN* 821 del11 allele were classified as heterozygous for this mutation. Belgian Blue cattle homozygous for this mutation display extreme muscle hypertrophy (Kambadur et al., 1997; McPherron and Lee, 1997; Grobet et al., 1998).

Muscle and Cellular Characteristics

Muscle weights. The striploin and eye round (ST) cuts of meat were removed from the carcass during commercial bone-out. The LL was then excised from the striploin and weighed, and the ST trimmed of any external fat and weighed. These muscles were chosen for study because of their contrasting structural, functional, and metabolic characteristics. The LL is an axial, lumbar muscle with a more postural function, and is of higher market value. The LL comprises more mixed myofiber types and hence oxidative and glycolytic capacity, with a propensity to deposit intramuscular fat and marbling. The ST is an upper hind limb muscle with locomotor function that primarily comprises fast glycolytic myofibers, has high connective tissue content and low marbling capacity, and is of lower economic value.

Myofiber characteristics. A tissue sample of approximately 1 cm² × 2 cm deep was removed from the superficial (dorsal) surface of the LL and ST of each carcass the day after slaughter, at the same sites for each animal. Any subcutaneous fat and fascia and approximately 5 mm of muscle were removed from the superficial surface, leaving a block of muscle approximately 1 cm³ for immunohistochemistry and myofiber analyses. During sample preparation, the alignment of the sample was maintained to allow cryosections to be cut perpendicular to the direction of the muscle cells, commencing from the cranial surface of the tissue block. Each muscle sample was mounted using gum tragacanth (Sigma Chemical Company, St Louis, MO: prepared 5% wt/vol in ddH₂O) onto a cork block, with muscle fibers running perpendicular to the cork block. Samples were frozen by immersion in iso-pentane cooled to approximately -160 °C in liquid nitrogen, before storage at -70 °C.

Myofiber staining and classification were performed as detailed in Greenwood et al. (2009). Three serial sections (10 µm) were cut from each muscle block and stained for myosin heavy chain (MyHC) composition using antibodies against type 1 (clone

WB-MHCs), type 2 (clone MY-32), and types 1, 2X, and 2B (clone S5-8H2) as described by Picard et al. (1998), but with modifications for bright field microscopy detailed by Greenwood et al. (2006a, 2006b, 2009). Rabbit anti-laminin, affinity isolated antibody was also included in the solution containing anti-type 1 MHC to allow cellular margins to be delineated (Figure 1). Myofibers were classified into 5 subtypes (1, 2C, 2A, 2AX, and 2X) based on their staining characteristics for the 3 antibodies against MyHCs (Figure 1, Greenwood et al., 2009). Cross-sectional area (CSA) of each classified myofiber was measured using image analysis by tracing the outlines of individual myofibers, and the average myofiber CSA for each myofiber type determined. The total area of each myofiber type relative to the total myofiber area, and the overall average CSA of myofibers, was then calculated from the percentage and average size of the myofiber types. Number of myofibers in LL and ST muscle cross sections (apparent number of myofibers) was calculated from the average number of myofibers per field of view and the muscle CSA for each animal.

Myofiber types for each muscle sample were classified from 2 microscopic fields of view across the 3 serial stained sections. Fields of view were selected to avoid muscle fibers that were not sectioned perpendicular to their length (Figure 1). There were (mean ± SD) 474 ± 109 muscle cells across the 2 fields of view for the LL and 587 ± 93 muscle cells across the 2 fields of view for the ST used to determine the number of myofibers per mm². A total of 214 ± 46 myofibers were classified and measured for CSA per LL sample within 2 fields of view. The ferret ratio (ratio of maximum to minimum ferret diameters of a cell) for all classified and measured LL myofibers was 0.63 ± 0.05 across all the steer samples. The average SD between duplicate fields within LL sample for percentage myofiber types was 2.9% for type 1, 0.9% for type 2C, 3.2% for type 2A, 1.0% for type 2AX, and 3.9% for type 2X. The average SD between duplicate fields within LL sample for myofiber CSA was 333 µm² for type 1, 575 µm² for type 2C, 446 µm² for type 2A, 815 µm² for type 2AX, and 588 µm² for type 2X. A total of 255 ± 49 myofibers were classified and measured for CSA per ST sample. The ferret ratio for all classified and measured myofibers in ST samples was 0.68 ± 0.03 across all steer samples. The average SD between duplicate fields within ST sample for percentage myofiber types was 2.1% for type 1, 0.4% for type 2C, 3.5% for type 2A, 1.2% for type 2AX, and 4.2% for type 2X. The average SD between duplicate fields within ST sample for myofiber CSA was 400 µm² for type 1, 739 µm² for type 2C, 275 µm² for type 2A, 646 µm² for type 2AX, and 521 µm² for type 2X.

Figure 1. Immunocytochemical staining of myosin heavy chains (MyHC) and laminin in bovine longissimus muscle. Representative myofibers are indicated: s, type 1 ($\equiv$ slow oxidative); c, type 2C ($\equiv$ type 1-type 2A intermediate); a, type 2A ($\equiv$ fast oxidative-glycolytic); x, type 2X ($\equiv$ fast glycolytic); ax, type 2AX ($\equiv$ type 2A-type 2X intermediate). Classification of type 2AX myofibers was based on positive staining for the type 2 MyHC antibody, intermediate staining for the types 1, 2B, and 2X MyHC antibody, and negative staining for the type 1 MyHC antibody. Classification of type 2C myofibers was based on positive or intermediate staining for all 3 MyHC antibodies.

Indices of muscle cellular development. Samples (approximately 5 g) from the LL and ST were excised from standardized sites on the carcasses

within 30 min following slaughter, and subcutaneous fat and fascia trimmed from the sample, and the remaining muscle was snap frozen in liquid nitrogen. The sample of LL was obtained from the superficial (dorsal) region adjacent to the 13th rib, and the samples of ST from the superficial, proximal region. Sampling of the ST was performed ensuring the tapering portion at the end of the muscle was avoided. Care was also taken to standardize the sampling site for each muscle to avoid potential for variation in cellular characteristics that may be associated with differing locations within the muscle.

Concentrations of DNA, RNA, and protein in the muscle samples were determined by extraction, solubilization, and assay, essentially as described by [Greenwood et al. \(2000\)](#). The procedures for extraction or solubilization of RNA, DNA, and protein in the wet muscle tissue were performed gravimetrically using muscle homogenates.

Muscle sample homogenates were prepared from the stored, frozen (-80°C) muscle samples pulverized following cooling in liquid nitrogen. Approximately 2.5 g of frozen, pulverized muscle was weighed (mean $\pm$ SD 2.544 ± 0.111 g) and approximately 7.5 mL of homogenizing buffer added (7.599 ± 0.125 g) to produce approximately 10 g of muscle homogenate (10.143 ± 0.144 g). RNA and DNA were extracted in triplicate from approximately 1 g aliquots of the muscle homogenate (1.039 ± 0.027 g) pipetted using a positive displacement pipette into siliconized culture tubes using a perchloric acid (PCA) extraction procedure based on the original procedures of [Schmidt and Thannhauser \(1945\)](#). The following procedures were also applied to RNA standards prepared from calf liver RNA and to DNA standards prepared from calf thymus DNA. Cold 0.2N PCA was used to precipitate tissue protein, phospholipids and nucleic acids, and the precipitate obtained by centrifugation at 4°C for 12 min at 2,600 rpm. This homogenization, precipitation, and centrifugation procedure was performed and repeated twice more to ensure removal of interfering substances such as low molecular weight compounds including sugars, free nucleotides, and organic and inorganic phosphate compounds that interfere with subsequent nucleic acid determination. Solubilization of RNA without degradation of DNA was then achieved by incubation of the precipitate in 2.0 mL of hot (100°C) 0.3N NaOH for 20 min, and of DNA and protein by addition of 1.5 mL 2.3N PCA. The solution was cooled on ice, and the solubilized RNA collected in the supernatant following centrifugation. The DNA was extracted from the DNA and protein pellet by incubation with 1.5 mL of hot

(100 °C) 0.4N PCA for 20 min. This solution was cooled on ice and the supernatant collected following centrifugation.

Concentration of RNA was determined by reaction of orcinol with pentoses and of DNA by reaction with diphenylamine. Triplicate RNA extracts (1 mL) including the RNA standards (1 mL) in the range 0 to 240 µg/mL were reacted with 2 mL of orcinol reagent and measured at 600 nm. Triplicate DNA extracts (1 mL), including the DNA standards (1 mL) in the range 0 to 60 µg/mL, were reacted with 2 mL of diphenylamine reagent to produce ω -hydroxylevulinyl aldehyde which condenses with diphenylamine to yield products with maximum absorption near 600 nm.

Protein concentration was determined using triplicate aliquots (0.498 ± 0.057 g) of the muscle homogenates. Aliquots were solubilized in 0.5% lauryl sulfate/0.6 N NaOH by heating at 60 °C for 30 min. The concentration of protein in samples diluted 1 in 40, and standards prepared from bovine serum albumin in the range of 0 to 250 µg/mL, was measured using the bicinchoninic acid (BCA) protein assay. The BCA detection reagent (2 mL) was added to 1 mL of the diluted solubilized samples and the standards, placed on ice for 5 min, and absorbance read at 562 nm.

The RNA, DNA, and protein assay results were corrected for weights of tissue and buffer in homogenates and weight of tissue homogenates used in extraction and solubilization procedures. The RNA assay had within and between assay coefficients of variation of 5.7% and 6.7%, respectively, the DNA assay 5.5% and 9.6%, and the protein assay 4.0% and 3.0%.

Ratios of protein to DNA (an index of cell mass within syncytial tissue such as muscle, or DNA unit [Cheek, 1985]), protein to RNA (index of translational activity), and RNA to DNA (index of transcriptional activity) were calculated for each muscle. The total amount of DNA (index of transcriptional capacity), RNA (index of translational capacity), and protein (index of muscle hypertrophy) in LL and ST were calculated by multiplying the concentration of each component by the weight of the respective muscle.

Statistical Analyses

Steer BW, carcass and muscle weights, myofiber characteristics, and muscle cellular indices were analyzed using the residual maximum likelihood method of linear mixed modeling by applying the statistical model reported by O'Rourke et al. (2009). A term combining the selection line and nucleotide 821

MSTN genotype was created and used as the only fixed effect in the model, and the effect of sire was included in the model as a random term. The combined term for selection line resulted in the steers being categorized into 3 distinct groups: a low muscle selection line homozygous normal for *MSTN* 821 (Low), a high muscle selection line homozygous normal for *MSTN* 821 (High), and a high muscle selection line with 1 copy of the *MSTN* 821 del11 allele (High^{Het}). The significance of differences of pairwise comparisons between muscle selection lines was determined using least significant differences (LSDs), calculated as $2 \times \text{SED}$ for each pairwise comparison.

To determine the consistency of correlations between muscles (LL and ST) prior to principal component analyses, a matrix of Pearson's correlation coefficients within each muscle were used to assess relationships of cellular characteristics with retail beef yield (weight and percent cold carcass weight). Retail beef yield was chosen for these analyses because the live muscle score phenotype is used to increase and is correlated with retail beef yield (Perry et al., 1993). It is also independent of other individual muscle-specific traits used to grade or assess the commercial value of carcasses, such as marbling which is assessed in the longissimus muscle. There was variation in the significance of correlation coefficients between the 2 muscles; hence, separate principal component analyses were performed using the correlation matrix for each muscle, rather than one principal component analysis using pooled within-muscle residuals as in Hocquette et al. (2012). The variables analyzed within the principal component analyses were percent area of type 1, 2A and 2X myofibers, apparent myofiber number, and protein, DNA and RNA concentrations.

All statistical analyses were performed using GENSTAT release 10 (VSN International: Hemel Hempstead, United Kingdom). Statistical significance for all analyses was accepted at $P < 0.05$, and a tendency towards a significant difference at $P < 0.10$.

RESULTS

Age, Body Weight, Carcass Weight, Retail Beef Yield, and Muscle Weights and CSA

Differences were not apparent due to muscling line in age ($P = 0.99$), BW ($P = 0.46$), and carcass weight ($P = 0.97$; Table 1). Retail yield as % of cold carcass weight was greater in High^{Het} than High and Low steers ($P < 0.001$), although kg of retail meat produced did differ between muscle lines ($P = 0.15$).

Table 1. Body weight, carcass weight, retail beef yield, and longissimus lumborum and semitendinosus muscle weights and cross-sectional areas (CSAs) of low and high muscling Angus steers

Variable	Muscling genotype			Average s.e.d.	P-value
	Low	High	High ^{Het}		
Number of steers	19	14	11		
Age, d	761	761	762	8.1	0.991
Body weight, kg	666	659	638	24.1	0.459
Carcass weight, kg	359	360	357	18.7	0.972
Retail beef yield, kg	218	225	225	6.0	0.150
Retail beef yield, % CCW ¹	62.2 ^a	63.5 ^a	67.0 ^b	0.72	<0.001
Longissimus lumborum weight, kg	5.106 ^a	5.654 ^{ab}	6.107 ^b	0.294	0.004
Longissimus lumborum CSA, cm ²	70.4 ^a	77.0 ^b	85.0 ^c	3.24	<0.001
Semitendinosus weight, kg	2.250 ^a	2.317 ^a	2.542 ^b	0.097	0.007
Semitendinosus CSA, cm ²	84.6	86.8	88.4	6.19	0.814

^{a,b}Within rows, mean values with different letters differ ($P < 0.05$).

¹CCW = cold carcass weight.

Weight of LL was greater in High^{Het} than in Low steers ($P = 0.004$). The LL CSA was smaller in Low than in High steers which had smaller LL CSA than High^{Het} steers ($P < 0.001$). The High^{Het} steers had a heavier ST than the High and Low steers ($P = 0.007$). Differences in ST CSA were not apparent between muscling lines ($P = 0.81$).

Myofiber Characteristics

Longissimus lumborum. Apparent number of myofibers ($P = 0.14$) and myofibers/mm² ($P = 0.14$) in LL did not differ due to muscling line (Table 2). The percentage of type 1 myofibers was greater in Low than in High and High^{Het} steers ($P = 0.02$). The ratio of fast to slow myofibers was less in Low than in High and High^{Het} steers ($P = 0.04$). Differences in myofiber CSA in the LL were not evident ($P > 0.11$) due to muscling line for any of the myofiber types or overall. The relative area of type 1 myofibers was greater in Low than in High and High^{Het} steers ($P < 0.001$). The relative area of type 2X myofibers was lower in Low than in High and High^{Het} steers ($P = 0.03$). The Low steers had a lower ratio for the relative area of fast to slow myofibers than the High and High^{Het} steers ($P = 0.002$). The High^{Het} steers had a higher ratio for the relative area of glycolytic to more oxidative myofiber types than Low steers ($P = 0.02$).

Semitendinosus. The apparent number of myofibers ($P = 0.47$) and myofibers/mm² ($P = 0.38$) in the ST did not differ due to muscling line (Table 3). The percentage of type 2X myofibers tended to be greater and the ratio for the percentage of glycolytic to more oxidative myofibers was greater in High^{Het} than in Low steers ($P = 0.05$). The Low steers had type 1 ($P = 0.005$) and type 2A ($P < 0.001$)

myofibers with a greater CSA than the High and High^{Het} steers. The relative area of type 1 myofibers was greater in Low than in High^{Het} steers ($P = 0.02$) and was greater in Low than in High and High^{Het} steers for type 2A myofibers ($P < 0.001$). The relative area of type 2X myofibers and the ratios for the relative areas of fast to slow and of glycolytic to more oxidative myofiber types were greater in High^{Het} than in Low steers (both $P < 0.001$).

Indices of Muscle Cellular Development

Longissimus lumborum. The total amount of protein in the LL was lower ($P = 0.01$) in the Low than in the High^{Het} steers (Table 4). The concentration of RNA in the LL was greater in the High than in the Low and High^{Het} steers ($P = 0.005$). The total amount of RNA in the LL was greater in the High and High^{Het} steers than in the Low steers ($P < 0.001$). The High steers had lower ratio of protein to RNA ($P = 0.03$) and a higher ratio of RNA to DNA ($P = 0.007$) in the LL than the Low steers.

Semitendinosus. The High^{Het} steers tended to have a higher concentration ($P = 0.08$) and had more protein in total ($P = 0.002$) in the ST than the High and Low steers (Table 5). The High^{Het} steers had more total DNA in the ST than the High steers ($P = 0.04$), and more total RNA in the ST than the Low steers ($P = 0.01$).

Correlations With Retail Beef Yield

Longissimus lumborum. Weight of retail beef was positively correlated with the total amounts of protein ($P < 0.001$), DNA ($P < 0.001$), and RNA ($P < 0.001$) in the LL (Table 6). The apparent

Table 2. Myofiber characteristics of the longissimus lumborum muscle of low and high muscling Angus steers

Variable	Muscling genotype			Average s.e.d.	P-value
	Low	High	High ^{Het}		
Number of steers	19	14	11		
Apparent number of myofibers $\times 10^{-6}$	1.12	1.25	1.47	0.141	0.135
Myofibers/mm ² CSA ¹	168.9	163.4	171.9	16.21	0.865
% myofibers ²					
Type 1	30.6 ^b	25.7 ^a	25.1 ^a	2.31	0.020
Type 2C	1.71	1.87	1.97	0.660	0.909
Type 2A	23.6	24.2	25.1	2.27	0.778
Type 2AX	1.93	2.16	2.67	0.828	0.639
Type 2X	42.2	46.1	45.2	2.90	0.279
Fast:Slow	2.4 ^a	3.0 ^b	3.1 ^b	0.32	0.037
Gly:Ox	0.76	0.87	0.86	0.098	0.371
Myofiber CSA, μm^2					
Type 1	3358	3173	3143	241.9	0.559
Type 2C	2687	2888	2786	356.1	0.807
Type 2A	3965	3913	3252	370.8	0.113
Type 2AX	4628	4433	3890	538.2	0.333
Type 2X	5234	5515	5609	487.1	0.279
Overall	4265	4442	4295	340.2	0.828
% myofiber area					
Type 1	24.3 ^b	18.3 ^a	18.4 ^a	1.85	<0.001
Type 2C	1.14	1.12	1.24	0.438	0.959
Type 2A	21.6	21.3	19.1	2.17	0.466
Type 2AX	2.00	2.17	2.48	0.822	0.829
Type 2X	50.9 ^a	57.2 ^b	58.7 ^b	2.86	0.027
Fast:Slow	3.28 ^a	4.71 ^b	4.68 ^b	0.484	0.002
Gly:Ox	1.08 ^a	1.39 ^{ab}	1.54 ^b	0.176	0.021

^{a,b}Within rows, mean values with different letters differ ($P < 0.05$).

¹CSA = cross-sectional area.

²Type 1, type 1 myosin heavy chain (MyHC) $\equiv$ slow oxidative; Type 2A, type 2A MyHC $\equiv$ fast oxidative-glycolytic; Type 2X, type 2X MyHC $\equiv$ fast glycolytic; Type 2C, type 1-type 2A intermediate; Type 2AX, type 2A-type 2X intermediate; Fast:Slow = ratio of fast (type 2) to slow (type 1) myofiber types; Gly:Ox = ratio of fast glycolytic (type 2X) to more oxidative (types 1, 2C, 2A, and 2AX) myofiber types.

number of myofibers ($P = 0.09$) and the concentration of protein ($P = 0.07$) in the LL tended to be positively correlated with weight of retail beef.

Apparent number of myofibers ($P = 0.03$), ratio of fast to slow myofiber percentages ($P = 0.04$), ratio of relative area comprising fast to slow myofibers ($P = 0.006$), ratio of glycolytic to more oxidative myofiber types ($P = 0.03$), and the total amounts of protein ($P = 0.002$), DNA ($P = 0.006$), and RNA ($P = 0.004$) in the LL were positively correlated with retail beef yield as a percentage of cold carcass weight (Table 6). Percentage of type 1 myofibers ($P = 0.02$), percentage area comprising type 1 myofibers ($P = 0.01$), and CSA of type 2A myofibers ($P = 0.01$) were correlated negatively with retail beef yield as a percentage of cold carcass weight. Percentage area comprising type 2X % myofibers in LL tended to be positively correlated with retail beef yield as a percentage of cold carcass weight ($P = 0.08$).

Semitendinosus. Apparent number of myofibers ($P = 0.03$), concentration of protein ($P = 0.02$), and total amounts of protein ($P < 0.001$), DNA ($P < 0.001$), and RNA ($P < 0.001$) in ST were positively correlated with weight of retail beef (Table 6). Percent area comprising type 2X myofibers ($P = 0.08$), percent area of fast relative to slow myofibers ($P = 0.08$), percent glycolytic to more oxidative myofibers ($P = 0.09$), and protein to DNA ($P = 0.09$) in ST tended to be correlated with weight of retail beef.

Retail beef yield as a percentage of cold carcass weight was positively correlated with amounts of protein ($P = 0.002$), DNA ($P = 0.03$), and RNA ($P = 0.03$), tended to be correlated positively with percent area of type 2X myofibers ($P = 0.08$), and tended to be correlated negatively with type 2A myofiber CSA ($P = 0.08$), percent area of type 1 myofibers ($P = 0.09$), and protein concentration ($P = 0.07$) in ST (Table 6).

Table 3. Myofiber characteristics of the semitendinosus muscle of low and high muscling Angus steers

Variable	Muscling genotype			Average SD	P-value
	Low	High	High ^{Het}		
Number of steers	19	14	11		
Apparent number of myofibers $\times 10^{-6}$	1.721	1.878	1.766	0.144	0.473
Myofibers/mm ² CSA ¹	204.2	217.9	201.9	13.06	0.375
% myofibers ²					
Type 1	14.0	13.3	11.3	2.11	0.401
Type 2C	0.88	0.76	0.63	0.321	0.705
Type 2A	21.8	20.0	18.1	1.91	0.115
Type 2AX	3.1	3.5	2.4	0.80	0.350
Type 2X	60.1 ^a	62.4 ^{ab}	67.6 ^b	3.21	0.051
Fast:Slow	7.7	7.6	9.3	1.62	0.503
Gly:Ox	1.62 ^a	1.79 ^{ab}	2.24 ^b	0.262	0.042
Myofiber CSA, μm^2					
Type 1	3519 ^b	2585 ^a	2677 ^a	362	0.005
Type 2C	2011	1812	1831	482	0.875
Type 2A	3178 ^b	2606 ^a	2544 ^a	209	<0.001
Type 2AX	3482	3116	3298	429	0.569
Type 2X	4525	4464	4445	324	0.960
Overall	3986	3784	3852	272	0.681
% myofiber area					
Type 1	11.7 ^b	9.3 ^{ab}	7.7 ^a	1.53	0.019
Type 2C	0.52	0.43	0.32	0.225	0.645
Type 2A	17.4 ^b	13.9 ^a	12.1 ^a	1.69	<0.001
Type 2AX	2.7	3.0	2.2	0.76	0.568
Type 2X	67.8 ^a	73.4 ^{ab}	77.7 ^b	2.78	<0.001
Fast:Slow	8.6 ^a	12.2 ^{ab}	14.1 ^b	2.06	0.013
Gly:Ox	2.24 ^a	3.10 ^{ab}	3.93 ^b	0.480	<0.001

^{a,b}Within rows, mean values with different letters differ ($P < 0.05$) or tend to differ (italics, $P < 0.10$).

¹CSA = cross-sectional area.

²Type 1, type 1 myosin heavy chain (MyHC) = slow oxidative; Type 2A, type 2A MyHC = fast oxidative-glycolytic; Type 2X, type 2X MyHC = fast glycolytic; Type 2C, type 1-type 2A intermediate; Type 2AX, type 2A-type 2X intermediate; Fast:Slow = ratio of fast (type 2) to slow (type 1) myofiber types; Gly:Ox = ratio of fast glycolytic (type 2X) to more oxidative (types 1, 2C, 2A, and 2AX) myofiber types.

Table 4. Cellular characteristics of the longissimus lumborum muscle of low and high muscling Angus steers

Variable	Muscling genotype			Average SD	P-value
	Low	High	High ^{Het}		
Number of steers	19	14	11	—	—
Protein, mg/g	146.8	149.0	145.0	2.76	0.346
DNA, $\mu\text{g/g}$	292.5	289.7	277.3	10.83	0.323
RNA, $\mu\text{g/g}$	2286 ^a	2591 ^b	2358 ^a	110.6	0.005
Protein:DNA	505	515	527	18.4	0.412
Protein:RNA	65.6 ^b	57.4 ^a	62.4 ^{ab}	3.59	0.034
RNA:DNA	7.83 ^a	9.03 ^b	8.55 ^{ab}	0.446	0.007
Protein, g	752 ^a	835 ^{ab}	886 ^b	49.4	0.012
DNA, g	1.498 ^a	1.613 ^{ab}	1.686 ^b	0.089	0.076
RNA, g	11.90 ^a	14.57 ^b	14.32 ^b	0.815	<0.001

^{a,b}Within rows, mean values with different letters differ ($P < 0.05$) or tend to differ (italics, $P < 0.10$).

Principal Component Analyses

The loadings for the first principal component in the LL and the ST were similar for the myofiber types, but had differing loadings for apparent

myofiber number and concentrations of DNA and RNA, as detailed below and shown in [Figure 2](#). The second principal component in both muscles had the highest loadings for concentrations of protein,

Table 5. Cellular characteristics of the semitendinosus muscle of low and high muscling Angus steers

Variable	Muscling genotype			Average s.e.d.	P-value
	Low	High	High ^{Het}		
Number of steers	19	14	11	—	—
Protein, mg/g	149.5 ^a	149.5 ^a	154.3 ^b	2.32	0.077
DNA, µg/g	270	256	264	10.2	0.283
RNA, µg/g	2215	2344	2375	102.7	0.185
Protein:DNA	556	591	590	21.0	0.102
Protein:RNA	68.1	64.6	66.0	2.91	0.375
RNA:DNA	8.26 ^a	9.25 ^b	9.09 ^{ab}	0.55	0.084
Protein, g	335 ^a	349 ^a	392 ^b	17.0	0.002
DNA, g	0.605 ^{ab}	0.590 ^a	0.670 ^b	0.033	0.040
RNA, g	5.05 ^a	5.42 ^{ab}	6.03 ^b	0.341	0.012

^{a,b}Within rows, mean values with different letters differ ($P < 0.05$) or tend to differ (italics, $P < 0.10$).

Table 6. Correlations for relationships between longissimus lumborum (LL) and semitendinosus (ST) muscle cellular characteristics and retail beef yield

Variable	Retail beef yield, kg		Retail beef yield, % CCW ¹	
	LL	ST	LL	ST
Myofiber characteristics				
Myofibers $\times 10^{-6}$	0.27 ^a	0.34 ^a	0.38 ^a	0.08
Myofibers/mm ² CSA ²	0.04	0.00	0.16	0.10
Type ³ 1, %	−0.05	−0.15	−0.36 ^a	−0.15
Type 2A, %	0.12	−0.15	0.16	−0.06
Type 2X, %	−0.02	0.25	0.12	0.14
Fast:Slow, %	−0.01	0.12	0.33 ^a	0.15
Glycolytic:oxidative, %	−0.02	0.22	0.16	0.15
Type 1, CSA	−0.01	−0.16	−0.25	−0.17
Type 2A, CSA	0.04	−0.18	−0.40 ^a	−0.28 ^a
Type 2X, CSA	0.19	−0.16	0.00	0.08
Mean CSA	0.15	−0.13	−0.12	0.01
Type 1, % area	−0.22	−0.24	−0.39 ^a	−0.27 ^a
Type 2A, % area	0.08	−0.16	−0.10	−0.25
Type 2X, % area	0.09	0.28 ^a	0.28 ^a	0.28 ^a
Fast:Slow, % area	0.09	0.28 ^a	0.42 ^a	0.24
Glycolytic:oxidative, % area	0.07	0.27 ^a	0.35 ^a	0.24
Cellular indices				
Protein, mg/g	0.29 ^a	0.38 ^a	0.01	0.29 ^a
DNA, µg/g	−0.13	−0.11	−0.06	−0.03
RNA, µg/g	−0.01	0.04	0.10	0.05
Protein:DNA	0.31 ^a	0.27 ^a	0.09	0.14
Protein:RNA	0.15	0.08	−0.13	0.09
RNA:DNA	0.07	0.09	0.12	0.03
Protein, g	0.67 ^a	0.72 ^a	0.46 ^a	0.46 ^a
DNA, g	0.51 ^a	0.52 ^a	0.42 ^a	0.35 ^a
RNA, g	0.50 ^a	0.50 ^a	0.44 ^a	0.34 ^a

^{a,b}Correlation coefficients followed by a are significant ($P < 0.05$), and followed by *a* tend towards significance ($P < 0.10$).

¹CCW = cold carcass weight.

²CSA = cross-sectional area.

³Type 1, type 1 myosin heavy chain (MyHC) $\equiv$ slow oxidative; Type 2A, type 2A MyHC $\equiv$ fast oxidative-glycolytic; Type 2X, type 2X MyHC $\equiv$ fast glycolytic; Type 2C, type 1-type 2A intermediate; Type 2AX, type 2A-type 2X intermediate; Fast:Slow = ratio of fast (type 2) to slow (type 1) myofiber types; Glycolytic:oxidative = ratio of fast glycolytic (type 2X) to more oxidative (types 1, 2C, 2A, and 2AX) myofiber types.

DNA, and RNA, which differed in magnitude between muscles, the LL having the highest loading for

RNA concentration and the ST for protein and DNA concentrations, and there were differences in the

Figure 2. Principal component analyses showing relationships between muscle characteristics performed on correlation matrices for variables within the longissimus lumborum muscle (left biplot) and the semitendinosus muscle (right biplot). The variables are the proportions (percent of total myofiber area) of the myofibers with major myosin heavy chain (MyHC) isoforms, type 1 (MyHC 1), type 2A (MyHC 2A), and type 2X (MyHC 2X), the apparent number of myofibers (No. Myofibers), and the concentrations of protein (Protein conc.), DNA (DNA conc.), and RNA (RNA conc.) in the muscles.

contribution of the other variables between muscles, as also detailed below and shown in Figure 2.

Longissimus lumborum. The first principal component explained 39% of the variation within the correlation matrix for the LL (Figure 2). It had the highest, but opposed loadings for the percent area of type 2X (−0.58), and types 1 (0.45) and 2A (0.43) myofibers, followed by concentrations of DNA (0.34), protein (0.29), and apparent myofiber number (−0.28). The second principal component explained 20% of the variation within the correlation matrix for the LL (Figure 2). It had the highest loadings for concentrations of RNA (0.64), protein (0.48) and DNA (0.38), and percent area of type 1 myofibers (−0.34) and apparent myofiber number (0.29).

Semitendinosus. The first principal component explained 38% of the variation within the correlation matrix for the ST (Figure 2). It had the highest loading for the percent area of type 2X (−0.59), followed by opposite loadings for type 1 (0.55) and type 2A (0.51) myofibers. The second principal component explained 19% of the variation within the correlation matrix for the ST. It had the highest loadings for concentrations of protein (0.67), DNA (0.66), and RNA (0.27).

DISCUSSION

The results of this study show that selection for increased muscling and retail beef yield in Angus

cattle using live cattle muscle scoring and 1 copy of the *MSTN* 821 del11 allele result in shifts towards larger muscles comprised of more glycolytic myofibers and less of the more oxidative myofiber types for both the LL and the ST. They also suggest that increased muscling in the LL of the High compared with Low steers may be associated with increased translational capacity.

We determined myofiber types, CSAs, and number of myofibers per unit area and per muscle, using histological techniques. The muscle histological sample collection site on the carcass, tissue orientation during mounting and freezing, and avoidance of measurements of myofibers that were sectioned at oblique angles (see Myofiber characteristics subsection of Materials and Methods) were undertaken to minimize error in determinations of myofiber types, size, and numbers. However, it is recognized that the number of myofibers apparent in a muscle cross section will differ from the number of myofibers in the entire anatomical muscle due to intrafascicular termination of myofibers, and that the size of myofibers, in particular of fast glycolytic (type 2X or 2B) myofibers, may be underestimated using histological techniques compared with newer volumetric methods (Kim et al., 2018). However, the methods used in the present study provide meaningful comparisons between the muscling lines, as in our previously published studies using these techniques (Greenwood et al., 2006a, 2006b, 2009).

The finding that relative muscle hypertrophy as a result of selection using live cattle muscle scoring

was associated with a shift towards whiter, more glycolytic myofiber types is consistent with results of other studies in cattle (Wegner et al., 2000; Hocquette et al., 2012) and other species including sheep (Warner et al., 2010) selected for increased muscling. A similar shift to that observed in the present study was shown previously in cattle heterozygous for double muscling (Holmes and Ashmore, 1972). However, the present findings show that average CSA of myofibers was similar between the muscle lines, whereas Allais et al. (2010) found reduced CSA of myofibers in cattle heterozygous for a myostatin muscle hypertrophy allele. In the present study, the CSA of the more oxidative type 1 and type 2A myofibers was reduced in the ST of both the High and High^{Het} steers compared with the Low steers.

Muscle and myofiber type specific effects of myostatin have been demonstrated in studies using rodents. Myostatin reduced protein turnover in the predominantly fast glycolytic (type 2B) myofiber type extensor digitorum longus in rats through increasing glycolysis as a result of enhanced expression of genes that regulate metabolism of glucose, but had no effect on protein turnover in the soleus muscle which is predominantly of slow oxidative (type 1) myofiber type (Manfredi et al., 2017). *MSTN* knock-out mice exhibit muscle hypertrophy, whereas in normal mice *MSTN* gene expression has been shown to be highest in fast glycolytic (type 2b MyHC) myofibers, implying other pathways influence size of myofibers, rather than a direct effect of myostatin on myofiber size (Carlson et al., 1999). It has also been proposed that myofiber types within muscle are regulated by myostatin through its influence on myocyte enhancer factor 2 and MyoD during myogenesis (Hennebry et al., 2009).

Overall, effects on myofiber characteristics were more pronounced in the High^{Het} steers with the High steers intermediate between the Low and High^{Het} genotypes. However, this finding and those of others including Wegner et al. (2000) and Hocquette et al. (2012) are at odds with those of McGilchrist et al. (2016) who compared myofibers and oxidative and glycolytic enzyme activities in Low and High steers from the same herd of origin as in the present study, and found High steers to have more muscles with more oxidative myofibers and enzyme capacity than Low steers. In the present study, the Low steers had muscle with more oxidative myofiber types than the High steers. Our findings possibly differ those of McGilchrist et al. (2016) due to cattle in the present study having more time on concentrate feed and being older and heavier animals.

In relation to the indices of cellular development, the LL of the High steers had greater concentration of RNA than in the Low and the High^{Het} groups of steers, which resulted in the High steers having the highest ratio of RNA to DNA, lower protein to RNA, and more RNA in total than the Low steers. This finding suggests, at least in the LL muscle, that the cattle selected for increased muscling using quantitative genetics have more ribosomes, which contain most of the RNA in muscle (Burleigh, 1974), and hence greater muscle translational capacity. However, the finding of lower protein to RNA in the High steers compared with the other lines suggests reduced translational activity relative to the number of ribosomes in these steers. This may relate to lesser differences between the lines in transcriptional capacity as indicated by amount and concentration of DNA at the time of slaughter. In the ST, High^{Het} steers had more protein, DNA, and RNA than the Low steers, and there were lesser or no differences between the lines of cattle for protein to DNA, protein to RNA, and RNA to DNA than in the LL. These findings do not appear to concur with those of Ashmore and Robinson (1969) who showed homozygous normal, heterozygous, and homozygous double muscled cattle did not differ in indices of cellular development. This difference between studies may be explained by the differences in the groups of cattle that were compared. Effects more specific to the double muscling condition were assessed using the groups of cattle studied by Ashmore and Robinson (1969).

Myostatin regulates the number and postnatal size of muscle fibers, and nonfunctional myostatin is associated with increased myoblast proliferation, delayed myoblast differentiation, and increased satellite cell cycle activity, which can result in more myofibers and myonuclei in support of muscle hypertrophy (Lee, 2004). In cattle that were heterozygous for the *MSTN* 821 del11 allele from the same herd as the present study, it has been shown that there is an overall up-regulation of expression of *MSTN* genes compared with homozygous wild-type *MSTN* steers, presumably due to negative feedback, but not to a level to fully compensate for loss of 1 copy of wild-type *MSTN* (Parnell et al., 2009). Although the relative levels of the functional myostatin protein arising in these homozygotes and heterozygotes were not determined, partial compensation in production of functional myostatin in the heterozygotes may account for the relatively small degree of muscle hypertrophy and myofiber responses to 1 copy of *MSTN* 821 del11. This could explain the small increase only in muscle

hypertrophy and associated shifts in muscle cellular characteristics in heterozygous compared with homozygous double muscled cattle (Holmes and Ashmore, 1972). As discussed above, there were no significant shifts in the total number of myofibers due to muscling line or presence of the *MSTN* 821 del11 allele in the present study, with only a tendency towards more myofibers in the LL, but not in the ST, in the *MSTN* 821 del11 heterozygotes. This finding is generally consistent with earlier research on normal and heterozygous cattle (Holmes and Ashmore, 1972; Allais et al., 2010), but is in sharp contrast to homozygous double muscled cattle which have more myofibers in affected muscles due to increased hyperplasia in muscle during fetal life (Ashmore et al., 1974; Swatland and Kieffer, 1974).

Although objective assessments of meat quality of the muscling selection line steers in the present study did not differ (O'Rourke et al., 2009), and cattle with myostatin muscle hypertrophy alleles can have improved muscle eating quality characteristics (Wheeler et al., 2001; Allais et al., 2010), it has previously been shown that continued quantitative genetic selection for increased muscularity can have adverse affects on meat quality (Rehfeldt et al., 2004; Warner et al., 2010), and that specific myofiber characteristics can affect eating quality of beef (e.g., Waritthitham et al., 2010; Lee et al., 2018). This is particularly the case if the selection pressure on specific alleles of genes reduces intramuscular fat content and/or is associated with reduced proteolysis resulting in reduced degradation of muscle protein, and hence, reduced tenderization associated with aging of meat (Warner et al., 2010). Although the results for the myofiber characteristics from the present study support the notion of whiter meat due to more glycolytic myofibers being associated with muscle hypertrophy as a result of quantitative genetic selection and/or 1 copy of the *MSTN* 821 del11 heterozygotes a nonfunctional *MSTN* allele, this was not borne out in the findings of O'Rourke et al. (2009) for meat color and other meat quality characteristics. Our results are not informative, however, about protein degradation. In relation to our findings for the High^{Het} steers, it has been suggested that reduced protein turnover, and associated declines in calpain1, calpastatin, and cathepsin B and L levels may be a primary mechanism by which muscle hypertrophy occurs in double muscled cattle (Uytterhaegen et al., 1994). However, these findings appear to contrast with those of Wheeler et al. (2001) who showed greater ease of myofiber fragmentation across a range of muscles, despite muscle and myofiber type specific

effects of myostatin on protein turnover including degradation having been shown (Manfredi et al., 2017), suggesting protein degradation was not reduced with increasing number of *MSTN* alleles that produce nonfunctional myostatin in cattle.

Double muscled cattle have been shown to have less and/or finer muscle collagen, generally resulting in beef eating quality that is acceptable compared with normal muscled cattle (Bailey et al., 1982; Uytterhaegen et al., 1994) due to effects on connective tissue specific to *MSTN* alleles that do not result in production of functional myostatin (Wheeler et al., 2001; Allais et al., 2010). This latter effect may have contributed to the trend towards higher protein concentration measured in the ST of High^{Het} compared with the other lines of cattle in the present study, due to a potential shift in proportions of more soluble myofibrillar proteins relative to the less soluble connective tissue proteins, and reflected in a tendency towards lower compression in the ST of High^{Het} steers reported by O'Rourke et al. (2009). Hence, differences between muscling lines in solubility of collagen may have affected the protein assay results in the present study due to the solubilization procedure used. This, coupled with a trend towards lower intramuscular fat in these steers (O'Rourke et al., 2009), could have contributed to this trend towards higher protein concentration in the ST of High^{Het} compared with the other steer lines.

Finally, it is important that meat quality be considered within selection indices that include assessment of muscling using live muscle scoring, carcass conformation scores, or retail yield of cattle. This is particularly so given that automated imaging techniques are being developed to live muscle score cattle to enhance uptake by industry (McPhee et al., 2017), and the trends observed at the myofiber level within the present study and previously observed in pigs (Rehfeldt et al., 2004) and sheep (Greenwood et al., 2006a, 2006b; Gardner et al., 2006; Warner et al., 2007) selected using muscling phenotypes. Such monitoring of meat quality should include sensory characteristics, given shifts in myofiber, muscle compositional, metabolic, and carcass characteristics that may occur with shifts towards more glycolytic muscle associated with selection for increased muscling, which have potential to affect consumer perceptions of beef tenderness, juiciness, flavor and/or color, and hence, acceptability.

CONCLUSIONS

Overall, the findings of the present study show that Angus steers with 1 copy of the *MSTN* 821 del11

allele (High^{Het}) have muscles that were more glycolytic than High Angus steers. In turn, the High Angus steers had muscles that tended to be more glycolytic than the Low Angus steers as a result of quantitative genetic selection using muscle scoring. The results for the indices of cellular development also suggest that increased muscling in LL of the High compared with Low steers was associated with increased translational capacity, but lower translational activity per ribosome, with these effects less evident for the ST. The shift towards more glycolytic myofiber types in the higher muscling steers selected using muscle scoring compared with the lower muscling steers suggests value in on-going objective and sensory evaluations of beef quality if muscle hypertrophy due to genetic and/or genomic selection increases in Angus and other breeds of cattle. Index-based genetic selection that includes beef quality traits is recommended to ensure beef quality is maintained during continued selection for higher levels of muscling.

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