Meat and Muscle Biology™

Influence of Bovine Myosin Heavy Chain Isoforms and Muscle Fiber Cross-Sectional Area on the Eating Quality and Connective Tissue Characteristics of 11 Different Beef Muscles

Sara R. Hene, Jordan T. Looper, Emily Verrill, Peang A. Hammond, Colin K. Y. Chun, Travis G. O'Quinn, and Michael D. Chao*

Department of Animal Sciences and Industry, Kansas State University, Manhattan, KS 66506, USA
*Corresponding author. Email: mdchao@ksu.edu (Michael D. Chao)

Abstract: The objective of this study was to determine the impact of muscle fiber type, cross-sectional area (CSA), and diameter on the eating quality of 11 different beef muscles. Eleven different beef muscles were utilized in 2 separate studies. In the 2 studies, *triceps brachii*, *rectus abdominus*, *rectus femoris*, *supraspinatus*, *gluteus medias*, *pectoralis profundi*, *semitendinosus*, *longissimus thoracis*, *longissimus lumborum*, *tensor fascia latae*, and *gastrocnemius* were collected from 10 USDA Choice carcasses ($N = 110$). To determine muscle fiber type, myofibrillar proteins were extracted and separated via gel electrophoresis and immunoblot, while muscle fiber CSA and diameter were determined using a dystrophin antibody stain via fluorescence microscopy. Pearson correlation analysis was performed to determine the relationship between muscle fiber type, CSA, diameter, and the eating quality of the 11 beef cuts from previously reported studies. Muscles from both studies showed distinct differences in the relative percentage of type I and type IIA muscle fiber types, CSA, and diameter ($P < 0.05$). Correlation analysis from study 1 demonstrated positive correlations between type I fibers and many positive attributes of eating quality such as tenderness, juiciness, and lipid flavor intensity, while negative correlations were found between type IIA fibers and those attributes ($P < 0.01$). Interestingly, results from study 2 showed that increasing type I fiber percentage may also contribute to greater connective tissue content and collagen crosslink density ($P < 0.01$). Finally, a negative correlation was found between muscle fiber CSA and diameter with connective tissue amount ($P < 0.05$), and a positive correlation was found between muscle fiber CSA and diameter with tenderness measurements ($P < 0.05$) in both studies. Overall, muscles with greater type I fiber % delivered a more favorable eating experience than those with more glycolytic metabolism. Notably, increased CSA and fiber diameter did not diminish eating quality and were found to have a muscle-specific relationship with tenderness.

Key words: muscle fiber type, eating quality, beef, connective tissue, cross-sectional area, correlation analysis
Meat and Muscle Biology 8(1): 18325, 1–18 (2024) **doi:10.22175/mmb.18325**
Submitted 29 August 2024 Accepted 22 October 2024

Introduction

Skeletal muscle is composed of heterogeneous muscle fiber types differentiated by their contractile and metabolic differences (Aberle et al., 2012). In beef, the major muscle fiber types are type I (slow oxidative), type IIA (intermediary oxido-glycolytic), and type IIX (fast glycolytic) (Chikuni et al., 2004; Scheffler et al., 2018). The unique characteristics of each fiber type and their relative distribution in the muscle can influence the kinematic characteristics and morphology of each muscle, and these unique metabolic and physical properties can impact the eating quality of different meat cuts associated with each beef muscle (Mo et al., 2023; Wicks et al., 2019). For example, many studies have shown an increased relative percentage of oxidative muscle fibers is correlated to increased meat quality characteristics like tenderness and juiciness, while more glycolytic fibers

demonstrate a negative relationship to meat quality (Hwang et al., 2010; Anderson et al., 2012). On the other hand, Roy et al. (2024) showed that type I muscle fibers have a positive relationship with biochemical collagen content.

Beyond muscle fiber type, meat quality is also impacted by muscle fiber size. The cross-sectional area (CSA) of a muscle fiber represents the amount of myofibrillar substances the consumer's teeth must bite through during chewing. Therefore, it is logical to expect CSA to be negatively correlated to meat tenderness. Seideman et al. (1988) found that proportional muscle fiber size was negatively correlated with sensory tenderness and positively correlated with shear force; however, some studies have speculated that the interaction between fiber CSA and tenderness may be muscle specific (Hammond et al., 2020). Finally, although many studies have suggested muscle fiber size is directly influenced by muscle fiber type (Picard et al., 2002), Oury et al. (2010) demonstrated that this notion does not always hold true for all beef muscles.

Despite extensive research into the connection between fiber type and size with eating quality, the exact relationship has yet to be fully established. This is largely because the traditional immunohistochemical method of determining muscle fiber type is labor intensive, time consuming, and prone to a high degree of human error. Additionally, the small sections of muscle stained for fiber typing are not usually representative of the metabolic profile of the whole muscle. Therefore, Scheffler et al. (2018) proposed a protocol to determine myosin heavy chain isoforms (MyHC) via gel electrophoresis. The sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot for muscle fiber typing method utilize extracted myofibrillar proteins from homogenized samples that are holistically representative of the whole muscle and subjected to less sampling bias. However, there are still notable disadvantages with the SDS-PAGE and Western blot methods such as consistencies in loading, transfer efficiency, and antibody affinity (Taylor et al., 2013). Although densitometry can be used to estimate band intensities, these measurements are only a semi-quantitative analysis and may not capture the full range of fiber-type variations in mixed muscle samples.

In 2 prior studies from our lab (Hammond et al., 2022; Chun et al., 2020), we conducted a comprehensive analysis of the biochemical and physical properties of 11 beef muscles to identify the factors that may influence their eating quality. Unfortunately, these studies did not account for the significant role of muscle fiber types and size in delivering a consistently satisfying eating experience. Therefore, the objective of this study was to apply a more efficient immunoblotting procedure to identify muscle fiber types and to assess how fiber type and size influence the eating quality of the same 11 beef muscles previously examined in Hammond et al. (2022) and Chun et al. (2020).

Materials and Methods

The use of human subjects in sensory panel evaluations was approved by the Kansas State University (KSU) Institutional Review Board (IRB #7440).

Sample collection and preparation

Eleven different beef muscles from 2 previous studies were utilized. For study 1, the detailed sample collection and fabrication process was described by Hammond et al. (2022). Briefly, *triceps brachii* (TB), *rectus abdominus* (RA), *rectus femoris* (RF), *supraspinatus* (SS), *gluteus medias* (GM), *pectoralis profundi* (PP), *semitendinosus* (ST), and *longissimus thoracis* (LT) were collected from 10 USDA upper 2/3 Choice carcasses ($N = 80$) and fabricated at 2 d postmortem to 8 steaks. Detailed fabrication maps of each muscle for study 1 are shown in figure 1. For study 2, the detailed sample collection and fabrication process was described by Chun et al. (2020). Briefly, *longissimus lumborum* (LL), *tensor fascia latae* (TF), and *gastrocnemius* (GC) were collected from 10 USDA low Choice carcasses ($N = 30$) and fabricated at 5 d postmortem to 8 steaks. Detailed fabrication maps of each muscle for study 2 are shown in figure 2. For both studies, steaks were designated to 3 analysis groups: Warner-Bratzler Shear Force (WBSF), trained sensory analysis, or biochemical analysis. Only the 2 d and 5 d postmortem samples from study 1 and study 2 (represented by steaks #1, #3, and #5), respectively, were utilized in this study.

WBSF and trained panel sensory analysis

The WBSF and trained sensory analysis for study 1 were described in Hammond et al. (2022). The WBSF analysis for study 2 was described by Chun et al. (2021), and the trained sensory analysis for study 2 was described by Chun et al. (2020). For both studies, one 2.54-cm steak from the WBSF analysis

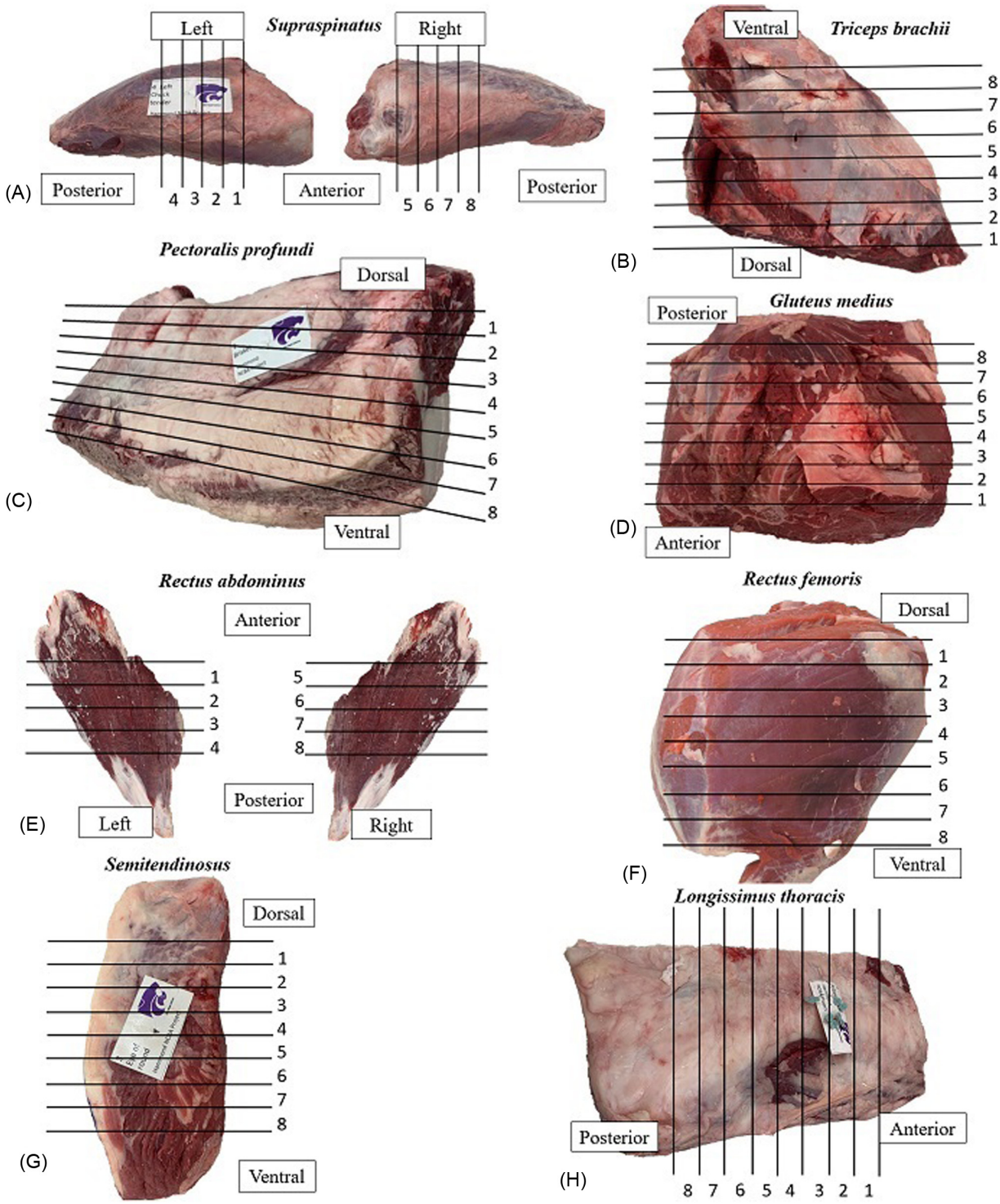

Figure 1. Fabrication maps for all muscles utilized in study 1: A) *supraspinatus*; B) *triceps brachii*; C) *pectoralis profundus*; D) *gluteus medius*; E) *rectus abdominus*; F) *rectus femoris*; G) *semitendinosus*; H) *longissimus thoracis*. For all muscles, steaks 1, 3, and 5 were used in this study for Warner-Bratzler Shear Force, trained panel analysis, and biochemical analysis, respectively at 2 d postmortem.

group (steak #1) and one 2.54-cm steak from the trained sensory panel group (steak #3) were frozen at −40°C immediately upon fabrication. All WBSF and trained panel steaks were thawed at 4°C for 24 h and grilled to an internal temperature of 71°C. Initial juiciness, sustained juiciness, connective tissue content, lipid flavor intensity, myofibrillar tenderness, and overall tenderness were evaluated by trained panelists consisting of faculty, staff, and graduate students.

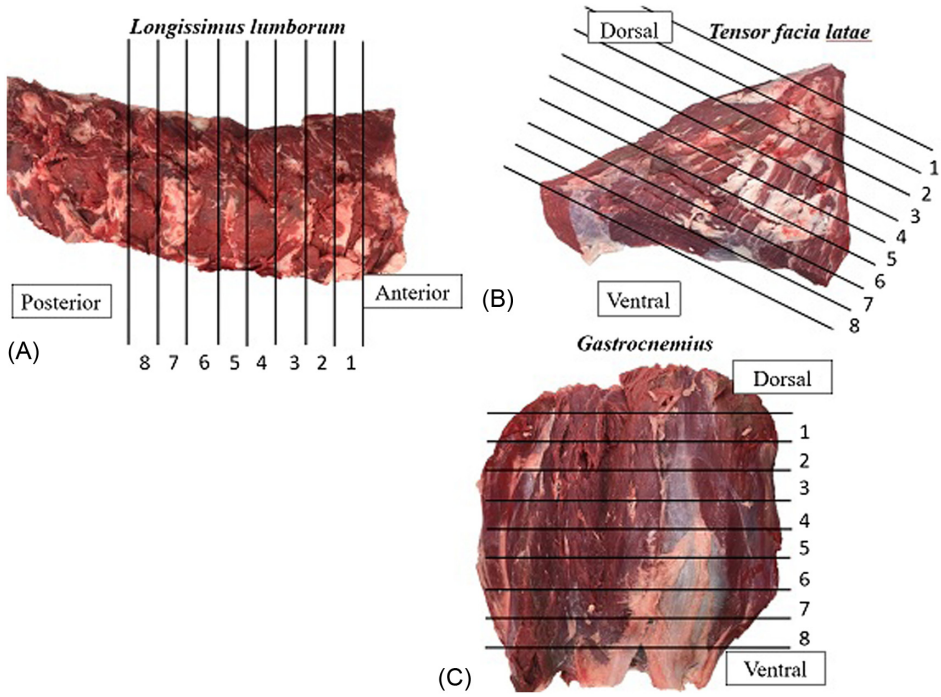

Figure 2. Fabrication maps for all muscles utilized in study 2: A) *longissimus lumborum*; B) *tensor facia latae*; C) *gastrocnemius*. For all muscles, steaks 1, 3, and 5 were used in this study for WBSF, trained panel analysis, and biochemical analysis, respectively at 5 d postmortem.

Biochemical collagen characteristics

The collagen characteristics analysis for study 1 was described by Hammond et al. (2022), and the collagen characteristics for study 2 were described by Chun et al. (2020).

Muscle fiber cross-sectional area and diameter

A 2.54-cm steak from each sample from the biochemical analysis group (steak #5) was designated for muscle fiber typing and cross-sectional area (CSA) analysis ($N = 110$). Three cores with slice faces perpendicular to the muscle fiber direction were obtained from each designated steak immediately following fabrication. The cores were placed with slice face down into a $22 \times 22 \times 20$-mm embedding mold (2219; Epredia, Kalamazoo, MI, USA) and were inundated with optimal cutting temperature tissue embedding media (OCT) (Thermo Fisher Scientific, Waltham, MA, USA). Samples in OCT were frozen in a 2-methyl butane bath cooled by liquid nitrogen.

Muscle fiber CSA and diameter were determined in accordance with the method described by Phelps et al. (2016) with modifications. For each core, two 10-µm cryosections were sliced using a cryostat (Microm HM 550; Thermo Fisher Scientific), transferred to charged microscope slides (Globe Scientific, Mahwah, NJ, USA), and allowed to air dry. Cryosections were traced with a hydrophobic barrier pen (SPM0928, IHC World LLC, Ellicott City, MD, USA) to prevent buffer leakage during washing and incubation. Nonspecific antigen binding sites were inhibited by blocking cryosections in 5% horse serum and 0.2% TritonX-100 in 1X PBS for 30 min. Following blocking, all samples were incubated with 1:50 anti-dystrophin rabbit polyclonal (PA1–37587; Thermo Fisher Scientific) in blocking solution for 1 hr. After primary antibody incubation, cryosections were washed 3 times with 1X PBS and incubated with the secondary antibody (Alexa Fluor 594 goat anti-rabbit H&L; Thermo Fisher Scientific) at 1:1,000 in blocking solution for 30 min. Finally, cryosections were washed 3 times with 1X PBS and a small drop of 9:1 glycerol in 1X PBS was applied with coverslips to the slides prior to imaging. Samples were imaged using a Nikon Eclipse TI-U inverted microscope with 10X working distance magnification (Nikon Instruments Inc., Melville, NY, USA). Five representative photomicrographs per section were captured using a Nikon DS-QiMc digital camera (Nikon Instruments Inc.) that was calibrated to the 10X objective. An average of 400 fibers per sample were analyzed for muscle fiber CSA and diameter using NIS-Elements Imaging Software (Basic Research, 3.3; Nikon Instruments Inc.). A representative cross-section of

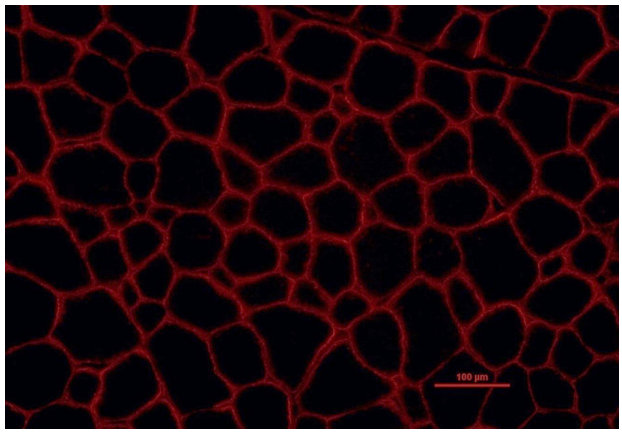

Figure 3. Representative image of dystrophin-stained muscle fiber cross-sectional area (CSA) from *semitendinosus*.

immunohistochemically stained muscle fiber used to determine CSA and fiber diameter is shown in figure 3.

Muscle fiber typing

After cores were removed from the biochemical analysis steak (steak #5), the remainder of the steak designated for muscle fiber typing was cubed, frozen under liquid nitrogen, and pulverized using a commercial blender (model 51BL32, Waring Commercial, Torring, CT, USA). Both the frozen cores and the pulverized samples were stored at −80°C until further analysis.

Myofibrillar proteins were extracted from each sample in congruence with the method described by Pietrzak et al. (1997) with modifications. Briefly, ice-cold ultrapure water was added to pulverized samples and homogenized using a bead homogenizer (D2400 Homogenizer, Benchmark Scientific, Sayreville, NJ, USA) for 30 sec. The homogenate was transferred into a microcentrifuge tube, washed with ultrapure water 3 times to ensure the removal of all sarcoplasmic proteins, and centrifuged at 4,000 × g for 5 min. The pellet was resuspended in a protein extraction buffer (0.1 M Tris HCl, 1.25 mM ethylenediaminetetraacetic acid (EDTA), 2% sodium dodecyl sulfate [SDS]) and centrifuged again at 4,000 × g for 5 min. The supernatant was transferred to a new microcentrifuge tube and considered as the myofibrillar protein stock. Protein stock concentration was determined using a Pierce BCA Protein Assay Kit (Thermo Fisher Scientific). Samples were adjusted to a concentration of 1 mg/mL using a protein extraction buffer.

The protocol for bovine myosin heavy chain isoform isolation and muscle fiber typing was outlined by Scheffler et al. (2018) with modifications. Adjusted protein samples were mixed 1:7 with 1X reducing Laemmli SDS sample buffer and heated at 95°C for 5 min. Exactly 1 µg of sample protein was loaded into each well of a Novex WedgeWell 6% tris-glycine gel (Thermo Fisher Scientific) in a Mini Gel Tank Electrophoresis System (Thermo Fisher Scientific). Electrophoresis was performed at room temperature (21°C) with a constant voltage of 70 V for 3.5 h. Gels were removed from the electrode assembly and transferred to polyvinylidene fluoride (PVDF) membranes (iBlot 2 PVDF Transfer Stack, Thermo Fisher Scientific) using an iBlot 2 Gel Transfer Device (Thermo Fisher Scientific) at 20V for 8 min. Once the transfer was complete, membranes were blocked with 1X OneBlock Western-FL Blocking Buffer (Prometheus Protein Biology Products, Genesee Scientific, San Diego, CA, USA) at 4°C overnight to prevent nonspecific binding.

The Western blot was conducted over the course of 2 d with 2 sets of antibody cocktails. On day 1, membranes were incubated in 5 mL of primary antibody cocktail 1 for 2 h at room temperature (21°C). Cocktail 1 consisted of a mouse IgG2b primary antibody for all MyHC isoforms (MF-20; Developmental Studies Hybridoma Bank, University of Iowa, IA, USA), a mouse IgG1 primary antibody that binds to type I MyHC isoform (M8421; Sigma-Aldrich), and a mouse Immunoglobulin M (IgM) primary antibody that binds to type IIX MyHC isoform (6H1; Developmental Studies Hybridoma Bank), all at an adjusted concentration of 0.5 µg/mL. Following the incubation with the primary antibody cocktail, membranes were washed 3 times for 5 min each with 1X Tris buffered saline (TBS) with 0.1% Tween-20 (TBST). Membranes were then incubated in 5 mL of secondary antibody cocktail for 1 h at room temperature. The secondary antibody cocktail for day 1 consisted of goat anti-mouse Alexa Flour 488 IgG1 (Invitrogen, Waltham, MA, USA), goat anti-mouse Alexa Flour 555 IgM (Invitrogen), and goat anti-mouse Alexa Flour 647 IgG2b (Invitrogen), all at 1:10,000 dilution in the blocking buffer. After secondary antibody incubation, membranes were washed 3 times for 5 min each with 1X TBST and once with 1X TBS. Membranes were then imaged using an iBright FL1500 Imaging System (Thermo Fisher Scientific). Alexa Fluor 488 representing type I MyHC was detected at an excitation of 490 nm and an emission peak at 525 nm, Alexa Flour 555 representing type IIX MyHC was detected with an excitation peak at 555 nm and an emission peak of 580 nm, and Alexa Flour 647 representing all MyHC was detected at excitation of 650 nm and an emission of 665 nm. After imaging, antibodies were stripped off

the membranes using a membrane stripping solution (62.5 mM Tris HCl, 2% SDS, and 0.5% β-mercaptoethanol) in a shaking incubator at 60°C for 30 min. After stripping, membranes were washed 3 times for 5 min each in TBST and re-blocked in OneBlock Western-FL Blocking Buffer (Prometheus Protein Biology Products) overnight at 4°C.

On day 2, membranes were incubated in 5 mL primary antibody cocktail 2 for 2 h at room temperature. In cocktail 2, the primary antibodies consisted of a mouse IgG2b antibody for all MyHC isoforms (MF-20; Developmental Studies Hybridoma Bank) and a mouse IgG1 antibody for type IIA MyHC isoform (SC-71; Developmental Studies Hybridoma Bank) all at an adjusted concentration of 0.5 µg/mL of blocking buffer. Following the incubation of the primary antibodies, membranes were washed 3 times in 5-min increments with 1X TBST. The membranes were then incubated in 5 mL of secondary antibody cocktail for 1 hr at room temperature. The secondary antibody cocktail for day 2 consisted of goat anti-mouse Alexa Flour 488 IgG1 (Invitrogen) and goat anti-mouse Alexa Flour 647 IgG2b (Invitrogen), both at 1:10,000 dilution in blocking buffer. Following secondary antibody incubation, membranes were washed 3 times for 5 min each with 1X TBST and 1 time with 1X TBS for 5 min. Day 2 membranes were imaged using the same imaging system using the same settings as described for day 1, except Alexa Fluor 488 now represented type IIA MyHC.

The relative fiber percentage of each muscle fiber was calculated using the iBright Analysis Software (Thermo Fisher Scientific). For each channel of the composite image, the frame of reference, lanes, and bands were defined. The relative percentages of MyHC type I (detected by M8421), type IIA (detected by SC-71), and type IIX (detected by 6H1) were calculated by dividing the band intensities of each MyHC isoform by the band intensities of all MyHC isoforms (detected by MF-20) within the same lane. Representative Western blot images used to determine muscle fiber type for studies 1 and 2 are shown in Figure 4.

Statistical analysis

All statistical analyses were performed by using the PROC GLIMMIX procedure of SAS (SAS Version 9.4; SAS Inst. Inc., Cary, NC, USA) and treatment comparisons were considered significant with an α of 0.05. For both studies 1 and 2, muscle fiber typing, muscle fiber CSA, and diameter measurements were performed using a completely randomized block design with muscles used as the fixed effect and animals used as the block. The Kenward-Roger approximation was used to estimate the degrees of freedom.

The PROC CORR procedure of SAS was used to determine Pearson's correlation coefficients between muscle fiber characteristics and sensory attributes as evaluated by the trained panel, WBSF, and biochemical collagen characteristics (collagen content and collagen crosslink density) for each of the 11 muscles evaluated. Pearson's correlation coefficients were also determined for the 8 combined muscles from study 1 and the combined 3 muscles from study 2. It is important to note that the primary objective of this study is to explore a measure of association between the muscle fiber characteristics and the various eating quality traits of beef without attempting to infer a predictive or causal relationship.

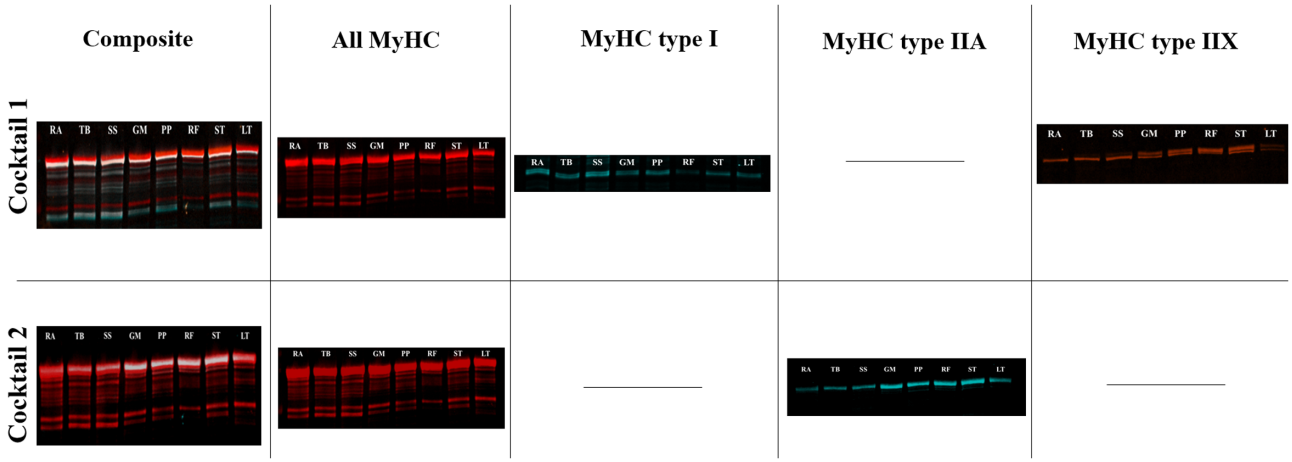

Figure 4. Representative images from study 1 showing the detection of bovine MyHC isoforms relative percentages using 2 antibody cocktails. Cocktail 1 (first row) contains antibodies for all MyHC (MF-20; IgG2b), MyHC type I (M8421; IgG1), and MyHC type IIX (6H1;IgM). Cocktail 2 (second row) contains antibodies for all MyHC (MF-20; IgG2b) and MyHC type IIA (SC71; IgG1). Within each image, lanes 1–8 represent RA = *rectus abdominus*, TB = *triceps brachii*, SS = *supraspinatus*, GM = *gluteus medius*, PP = *pectoralis profundus*, RF = *rectus femoris*, ST = *semitendinosus*, LT = *longissimus thoracis*.

Results

The descriptive statistics for WBSF, trained sensory panel, and biochemical collagen characteristics results were previously reported (Chun et al., 2020; Hammond et al., 2022). Therefore, only the muscle fiber typing, CSA, diameter, and correlation data are reported in this manuscript.

Muscle fiber typing

Relative muscle fiber type percentages of each muscle analyzed in both studies 1 and 2 are presented in Table 1. In study 1, RA (48.87%) and SS (45.60%) had the greatest relative percentage of type I fibers, followed by TB (35.70%) and GM (27.54%), with ST (18.06%) having the lowest relative percentage of all ($P < 0.01$). The PP (24.60%), LT (23.82%), and RF (24.93%) all had similar percentages and did not differ significantly from GM or ST ($P > 0.05$). Furthermore, LT (45.41%), ST (44.39%), RF (39.28%), and GM (37.90%) all had the highest and similar relative percentage of type IIA fibers, followed by TB (30.48%), with SS (20.43%) and RA (18.29%) having the lowest relative percentage of all ($P < 0.01$). The PP (37.31%) did not differ from RF, ST, GM, or TB in relative percentage of type IIA fibers ($P > 0.05$). No difference in the relative percentage of type IIX fibers was found in study 1 ($P > 0.05$); the type IIX muscle percentages for all muscles from study 1 ranged between 30 to 38%.

In study 2, GC (34.50%) had the highest relative percentage of type I fibers among the 3 muscles evaluated ($P < 0.01$), though the relative percentage of type I fibers did not differ significantly between TF (19.25%) and LL (14.19%) ($P > 0.05$). On the other hand, LL (57.63%) had the highest relative percentage for type IIA fibers ($P < 0.05$), followed by TF (49.92%), which did not differ significantly ($P > 0.05$), and finally, GC (44.92%) had the lowest ($P < 0.05$). Similar to study 1, there was no difference in the relative percentage of type IIX fibers among the muscles analyzed in study 2 ($P > 0.05$). The type IIX muscle percentages for all muscles from study 2 ranged from 20 to 30%.

Muscle fiber CSA and diameter

The muscle fiber CSA and diameter of each muscle studied from both studies 1 and 2 are also presented in Table 1. In study 1, RA (3,580.05 μm^2 and 66.69 μm) and LT (3,547.98 μm^2 and 65.42 μm) had the greatest muscle fiber CSA and diameter, followed by GM

Table 1. Relative muscle fiber type percentages, cross-sectional area (CSA), and muscle fiber diameter from both study 1 and study 2.

Muscle ID	Type I, %	Type IIA, %	Type IIX, %	CSA, μm^2	Diameter, μm
Study 1					
RA	48.87^{a}	18.29^{d}	32.83	3580.05^{a}	66.69^{a}
SS	45.60^{a}	20.43^{d}	33.98	2932.82^{b}	59.83^{b}
TB	35.70^{b}	30.48^{c}	33.82	2996.76^{b}	60.01^{b}
GM	27.54^{c}	37.90^{ab}	34.69	3002.94^{b}	60.68^{b}
PP	24.60^{cd}	37.31^{bc}	38.22	2154.73^{c}	51.39^{c}
LT	23.82^{cd}	45.41^{a}	30.91	3547.98^{a}	65.42^{a}
RF	24.93^{cd}	39.28^{ab}	35.80	2743.01^{b}	58.09^{b}
ST	18.06^{d}	44.39^{ab}	37.55	2927.05^{b}	59.83^{b}
[1]**SEM**	3.34	3.07	3.33	164.73	1.60
***P* value**	<0.01	<0.01	0.19	<0.01	<0.01
Study 2					
LL	14.19^{b}	57.63^{a}	25.17	5691.01^{a}	82.74^{a}
TF	19.25^{b}	49.92^{ab}	30.83	3981.75^{b}	69.62^{b}
GC	34.50^{a}	44.92^{b}	20.58	3118.98^{c}	61.65^{c}
[1]**SEM**	2.57	2.78	2.96	296.63	2.52
***P* value**	<0.01	<0.05	0.06	<0.01	<0.01

[a–d]Values within a column without a common superscript differ significantly at $P < 0.05$.

RA = Rectus abdominus; SS = Supraspinatus; TB = Triceps brachii; GM = Gluteus medias; PP = Pectoralis profundi; LT = Longissimus thoracis; RF = Rectus femoris; ST = Semitendinosus LL = Longissimus lumborum; TF = Tensor fasciae latae; GC = Gastrocnemius.

[1]Standard error mean.

(3,002.94 μm^2 and 60.68 μm), TB (2996.76 μm^2 and 60.01 μm), SS (2932.82 μm^2 and 59.83 μm), ST (2927.05 μm^2 and 59.83 μm), and RF (2743.01 μm^2 and 58.09 μm), with PP (2154.73 μm^2 and 51.39 μm) having the smallest muscle fiber CSA and diameter of all 8 muscles ($P < 0.01$). In study 2, LL (5691.01 μm^2 and 82.74 μm) had the greatest muscle fiber CSA and diameter, followed by TF (3981.75 μm^2 and 69.62 μm), with GC (3118.98 μm^2 and 61.65 μm) having the smallest muscle fiber CSA and diameter ($P < 0.01$).

Correlations among muscle fiber characteristics, sensory attributes, and biochemical collagen characteristics for individual muscles

Table 2 shows the correlation data for muscle fiber characteristics and sensory attributes for each individual muscle evaluated in study 1. For PP, there was a negative correlation between the percentage of type IIA fibers and initial juiciness ($r = -0.71$; $P < 0.05$), sustained juiciness ($r = -0.66$; $P < 0.05$), and

Table 2. Correlation coefficient (r) of muscle fiber types (type I, type IIA, and type IIX), cross-sectional area (CSA), and diameter with the sensory attributes evaluated by trained panelists, Warner-Bratzler shear force (WBSF), and biochemical collagen characteristics for the 8 bovine muscles evaluated in study 1.

	Sensory Attributes Evaluated by Trained Panelists						Shear Force	Biochemical Measurements	
Traits for each muscle	Initial Juiciness	Sustained Juiciness	Connective Tissue Content	Lipid Flavor	Myofibrillar Tenderness	Overall Tenderness	WBSF	Collagen Content	Collagen Crosslink Density (Pyridinoline)
GM									
Type I, %	−0.37	−0.41	−0.16	−0.21	−0.28	−0.12	−0.49	−0.16	0.18
Type IIA, %	0.58	0.56	0.1	0.45	0.22	0.12	0.51	0.49	−0.06
Type IIX, %	−0.26	−0.20	0.06	−0.29	0.05	−0.01	−0.06	−0.38	−0.12
CSA, μm^2	0.47	0.40	−0.33	−0.20	0.26	0.33	0.16	−0.31	0.27
Diameter, μm	0.48	0.42	−0.29	−0.16	0.23	0.29	0.23	−0.34	0.30
LT									
Type I, %	−0.09	0.01	0.38	−0.19	−0.03	−0.15	0.15	−0.05	0.52
Type IIA, %	0.51	0.55	−0.11	−0.14	0.23	0.12	−0.08	0.06	−0.23
Type IIX, %	−0.39	−0.47	−0.07	0.21	−0.18	−0.04	−0.002	−0.03	−0.03
CSA, μm^2	−0.52	−0.37	−0.08	−0.17	0.19	0.22	−0.14	−0.15	0.13
Diameter, μm	−0.50	−0.34	−0.06	−0.14	0.16	0.20	−0.19	−0.15	0.13
PP									
Type I, %	0.40	0.28	0.37	0.11	0.32	−0.33	−0.06	0.60	−0.29
Type IIA, %	−0.71**	−0.66**	−0.14	−0.36	−0.87***	−0.23	0.42	0.03	−0.07
Type IIX, %	0.44	0.49	−0.15	0.30	0.67**	0.52	−0.41	−0.53	0.32
CSA, μm^2	−0.32	−0.32	0.16	0.08	−0.29	−0.45	0.45	0.48	−0.58
Diameter, μm	−0.32	−0.32	0.11	0.04	−0.26	−0.41	0.42	0.44	−0.52
RA									
Type I, %	−0.14	0.03	−0.72**	−0.22	0.77***	0.78***	−0.40	−0.05	−0.20
Type IIA, %	−0.09	−0.17	0.63**	−0.42	−0.41	−0.57	0.02	−0.08	0.24
Type IIX, %	0.19	0.04	0.50	0.43	−0.65**	−0.59	0.43	0.09	0.11
CSA, μm^2	−0.37	−0.35	0.35	−0.01	−0.48	−0.45	0.17	0.03	0.16
Diameter, μm	−0.38	−0.35	0.30	−0.03	−0.43	−0.39	0.12	−0.002	0.17
RF									
Type I, %	−0.39	−0.41	−0.59	−0.41	0.08	0.21	−0.09	0.10	−0.18
Type IIA, %	−0.16	−0.14	0.69**	0.38	−0.50	−0.60	0.74**	0.04	0.01
Type IIX, %	0.61	0.62**	0.22	0.23	0.28	0.19	−0.44	−0.15	0.21
CSA, μm^2	−0.04	0.002	0.06	−0.28	−0.24	−0.25	−0.11	−0.37	0.27
Diameter, μm	−0.04	−0.003	0.03	−0.30	−0.22	−0.22	−0.12	−0.35	0.28

Table 2. (*Continued*)

Traits for each muscle	Sensory Attributes Evaluated by Trained Panelists						Shear Force	Biochemical Measurements	
	Initial Juiciness	Sustained Juiciness	Connective Tissue Content	Lipid Flavor	Myofibrillar Tenderness	Overall Tenderness	WBSF	Collagen Content	Collagen Crosslink Density (Pyridinoline)
SS									
Type I, %	−0.12	−0.14	−0.43	0.28	0.02	0.33	−0.11	0.03	0.38
Type IIA, %	0.0001	0.02	0.36	−0.35	−0.12	−0.34	0.08	−0.48	−0.29
Type IIX, %	0.13	0.13	0.15	−0.001	0.08	−0.06	0.05	0.38	−0.15
CSA, μm²	0.14	0.14	0.60	0.06	−0.42	−0.67**	0.70**	−0.35	−0.28
Diameter, μm	0.11	0.12	0.54	0.09	−0.42	−0.64**	0.63**	−0.34	−0.23
ST									
Type I, %	0.27	0.37	−0.31	−0.08	0.44	0.40	−0.35	0.27	−0.22
Type IIA, %	−0.45	−0.44	0.63**	−0.15	−0.41	−0.63**	−0.11	0.004	−0.20
Type IIX, %	0.35	0.29	−0.51	0.18	0.23	0.48	0.03	−0.11	0.29
CSA, μm²	0.43	0.50	−0.62	0.19	0.74**	0.76**	−0.48	−0.07	0.37
Diameter, μm	0.41	0.48	−0.59	0.18	0.71**	0.73**	−0.46	−0.08	0.37
TB									
Type I, %	0.20	0.20	0.25	0.24	0.32	0.22	0.06	0.44	0.02
Type IIA, %	−0.11	−0.11	0.06	−0.29	−0.13	−0.22	0.23	−0.002	0.14
Type IIX, %	−0.12	−0.12	−0.41	0.06	−0.25	−0.01	−0.38	−0.60	−0.22
CSA, μm²	0.49	0.53	0.05	0.57	0.35	0.25	0.45	0.38	−0.28
Diameter, μm	0.48	0.51	0.06	0.55	0.35	0.26	0.43	0.39	−0.27

**$P < 0.05$.

***$P < 0.01$.

GM = gluteus medius, LT = longissimus thoracis, PP = pectoralis profundus, RA = rectus abdominus, RF = rectus femoris, SS = supraspinatus, ST = semitendinosus, TB = triceps brachii.

myofibrillar tenderness ($r = -0.87$; $P < 0.01$). Conversely, the percentage of type IIX fibers in PP had a positive correlation with myofibrillar tenderness ($r = 0.67$; $P < 0.05$). The RA had a negative correlation between type I fiber percentage and connective tissue content ($r = -0.72$; $P < 0.05$), and a positive relationship with both myofibrillar tenderness ($r = 0.77$; $P < 0.01$) and overall tenderness ($r = 0.78$; $P < 0.01$). On the other hand, RA type IIA fiber percentage had a positive correlation with connective tissue content ($r = 0.63$; $P < 0.05$), but RA type IIX fibers had a negative relationship with myofibrillar tenderness (r = −0.65, $P < 0.05$). The RF type IIA relative fiber percentage had a positive relationship with connective tissue content ($r = 0.69$; $P < 0.05$) and WBSF ($r = 0.74$; $P < 0.05$). Additionally, RF type IIX fibers had a positive relationship with sustained juiciness ($r = 0.62$; $P < 0.05$). For SS, muscle fiber CSA and diameter had a negative relationship with overall tenderness ($r = -0.67$ and $r = -0.64$, respectively; $P < 0.05$) and a positive relationship with WBSF ($r = 0.70$ and $r = 0.63$, respectively; $P < 0.05$). The ST had a positive correlation between type IIA fiber percentage and connective tissue content ($r = 0.63$; $P < 0.05$), but a negative relationship with overall tenderness ($r = -0.63$; $P < 0.05$). Interestingly, ST muscle fiber CSA and diameter both had positive relationships with myofibrillar tenderness ($r = 0.74$ and $r = 0.71$, respectively; $P < 0.05$) and overall tenderness ($r = 0.76$ and $r = 0.73$, respectively; $P < 0.05$). All phenotypic correlations between muscle fiber characteristics and sensory attributes for GM, LT, and TB were insignificant ($P > 0.05$). Additionally, no significant relationship was observed between muscle fiber types and either of the biochemical collagen characteristics for any of the 8 individual muscles evaluated in study 1 ($P > 0.05$).

Table 3 shows the correlation data for muscle fiber characteristics and sensory attributes for each individual muscle analyzed in study 2. For LL, the type I muscle fiber percentage had a positive relationship to lipid flavor intensity ($r = 0.66$; $P < 0.05$), while type IIA had a negative relationship ($r = -0.70$; $P < 0.05$). Furthermore, the type IIX relative fiber percentage had a positive correlation with connective tissue content ($r = 0.78$; $P < 0.01$). Muscle fiber CSA and diameter both had positive correlations with connective

Table 3. Correlation coefficient (r) of cross-sectional area (CSA), diameter, and muscle fiber type with the sensory attributes evaluated by trained panelists, Warner-Bratzler shear force (WBSF), and biochemical collagen characteristics for the 3 bovine muscles evaluated in study 2.

	Sensory Attributes Evaluated by Trained Panelists						Shear Force	Biochemical Measurements	
Traits for each muscle	Initial Juiciness	Sustained Juiciness	Connective Tissue Content	Lipid Flavor	Myofibrillar Tenderness	Overall Tenderness	WBSF	Collagen Content	Collagen Crosslink Density (Pyridinoline)
GC									
Type I, %	0.25	0.32	−0.13	−0.35	0.29	0.35	−0.34	0.06	0.26
Type IIA, %	−0.42	−0.47	−0.05	−0.06	−0.36	−0.24	−0.26	0.74**	0.40
Type IIX, %	0.18	0.16	0.17	0.39	0.07	−0.10	0.58	−0.79**	−0.65
CSA, μm²	0.40	0.43	−0.19	0.01	0.45	0.33	−0.42	−0.01	−0.40
Diameter, μm	0.35	0.39	−0.14	0.02	0.43	0.30	−0.44	0.03	−0.42
LL									
Type I, %	0.10	0.12	−0.53	0.66**	−0.17	−0.26	0.48	−0.33	−0.18
Type IIA, %	0.11	0.08	0.23	−0.70**	0.50	0.57	−0.57	0.44	0.09
Type IIX, %	−0.41	−0.40	0.78***	−0.29	−0.46	−0.39	−0.10	−0.03	0.25
CSA, μm²	−0.61	−0.61	0.85**	0.11	−0.89**	−0.83**	0.67	−0.32	0.23
Diameter, μm	−0.63	−0.62	0.83**	0.11	−0.89**	−0.82**	0.67	−0.30	0.21
TT									
Type I, %	−0.03	0.03	0.08	0.38	0.04	0.12	0.01	−0.56	−0.30
Type IIA, %	−0.30	−0.34	0.32	0.20	−0.15	−0.004	0.02	−0.21	−0.44
Type IIX, %	0.25	0.25	−0.29	−0.34	0.09	−0.05	−0.02	0.43	0.49
CSA, μm²	−0.15	−0.07	0.21	−0.04	−0.23	−0.17	0.68**	−0.39	−0.29
Diameter, μm	−0.15	−0.07	0.21	−0.05	−0.22	−0.17	0.69**	−0.37	−0.27

**$P < 0.05$.

***$P < 0.01$.

GC = gastrocnemius, LL = longissimus lumborum, TT = tensor facia latae.

tissue content (r = 0.85 and r = 0.83, respectively; $P <$ 0.05). Moreover, muscle fiber CSA and diameter also showed a negative relationship with myofibrillar tenderness ($r = -0.89$ and $r = -0.89$, respectively; $P <$ 0.05) and overall tenderness ($r = -0.83$ and $r =$ -0.82, respectively; $P < 0.05$). For TF, both muscle fiber CSA and diameter shared a positive correlation with WBSF ($r = 0.68$ and $r = 0.69$, respectively; $P <$ 0.05). For GC, type IIA muscle fiber percentage had a positive relationship with collagen content ($r =$ 0.74; $P < 0.05$), while type IIX has a negative relationship ($r = -0.79$; $P < 0.05$). All other phenotypic correlations between muscle fiber characteristics and sensory attributes for GC were insignificant ($P > 0.05$).

Correlations among muscle fiber characteristics, sensory attributes, and biochemical collagen characteristics for all muscles

Table 4 shows the correlation coefficient of muscle fiber types, muscle fiber CSA, and diameter with sensory attributes for all muscles utilized in studies 1 and 2. In study 1, a positive correlation was observed between type I muscle fibers and initial juiciness ($r = 0.37$; $P < 0.01$), sustained juiciness ($r = 0.39$; $P < 0.01$), lipid flavor ($r = 0.40$; $P < 0.01$) and myofibrillar tenderness ($r = 0.28$; $P < 0.01$). Conversely, there was a negative correlation between type IIA fibers and initial juiciness ($r = -0.41$; $P < 0.01$), sustained juiciness ($r = -0.43$; $P < 0.01$), and lipid flavor ($r = -0.47$, $P < 0.01$). A positive correlation was observed between muscle fiber CSA and sustained juiciness ($r = 0.22$; $P < 0.05$), myofibrillar tenderness ($r = 0.38$; $P < 0.05$) and overall tenderness ($r = 0.39$; $P < 0.01$), while a negative correlation was observed between CSA, connective tissue content ($r = -0.36$; $P < 0.01$), WBSF ($r = -0.25$; $P < 0.05$), and collagen content ($r = -0.30$; $P < 0.01$),. Finally, muscle fiber diameter shared a positive relationship with myofibrillar tenderness ($r = 0.39$; $P < 0.01$) and overall tenderness ($r = 0.40$; $P < 0.01$), but a negative correlation with connective tissue content ($r =$ —0.37; $P < 0.01$), WBSF ($r = -0.26$; $P < 0.05$), and collagen content

Table 4. Correlation coefficient (*r*) of muscle fiber type, cross-sectional area (CSA), and diameter with the sensory attributes evaluated by trained panelists, Warner-Bratzler shear force (WBSF), and biochemical collagen characteristics for all muscles from both study 1 and study 2. The 8 bovine muscles evaluated in study 1 included: *gluteus medius*, *longissimus thoracis*, *pectoralis profundus*, *rectus abdominus*, *rectus femoris*, *supraspinatus*, *semitendinosus* and *triceps brachii*. The 3 bovine muscles evaluated in study 2 included: *gastrocnemius*, *longissimus lumborum*, *tensor facia latae*.

	Sensory Attributes Evaluated by Trained Panelists						Shear Force	Biochemical Measurements	
Traits	Initial Juiciness	Sustained Juiciness	Connective Tissue Content	Lipid Flavor	Myofibrillar Tenderness	Overall Tenderness	WBSF	Collagen Content	Collagen Crosslink Density (Pyridinoline)
Study 1									
Type I, %	0.37***	0.39***	−0.06	0.40***	0.28***	0.16	−0.09	−0.03	0.15
Type IIA, %	−0.41***	−0.43***	−0.03	−0.47***	−0.19	−0.08	0.10	0.05	−0.24**
Type IIX, %	0.01	0.01	0.13	0.07	−0.15	−0.13	0.01	−0.02	0.09
CSA, μm²	0.21	0.22**	−0.36***	0.21	0.38***	0.39***	−0.25**	−0.30***	−0.12
Diameter, μm	0.21	0.22	−0.37***	0.21	0.39***	0.40***	−0.26**	−0.29***	−0.11
Study 2									
Type I, %	−0.23	−0.19	0.38	−0.22	−0.33	−0.37	0.60***	0.58***	0.59***
Type IIA, %	0.01	−0.04	−0.31	0.04	0.28	0.39**	−0.59***	−0.18	−0.21
Type IIX, %	0.26	0.26	−0.14	0.23	0.11	0.06	−0.13	−0.51***	−0.49**
CSA, μm²	0.13	0.12	−0.49**	0.12	0.41**	0.46**	−0.69***	−0.65***	−0.48**
Diameter, μm	0.15	0.15	−0.49**	−0.20	0.41**	0.45**	−0.70***	−0.64***	−0.51**

**$P < 0.05$.

***$P < 0.01$.

($r = -0.29$; $P < 0.01$). In study 1, there was no significant relationship found between type IIX fibers and any of the sensory or biochemical collagen attributes measured.

In study 2, a positive correlation was seen between the relative percentage of type I muscle fiber, WBSF, collagen content, and collagen crosslink density ($r = 0.60$, $r = 0.58$, and $r = 0.59$, respectively; $P < 0.01$). On the other hand, a negative correlation was seen between type IIA fibers and WBSF ($r = -0.59$; $P < 0.01$), and a positive correlation was observed between type IIA fibers percentages and overall tenderness ($r = 0.39$; $P < 0.05$). Type IIX muscle fiber percentage had a negative relationship with collagen content ($r = -0.51$; $P < 0.01$) and collagen crosslink density ($r = -0.49$; $P < 0.05$). Study 2 continued to show the same positive relationships between CSA and myofibrillar tenderness ($r = 0.41$; $P < 0.05$) and overall tenderness ($r = 0.46$; $P < 0.05$) and the same negative relationship between CSA, connective tissue content ($r = -0.49$; $P < 0.05$), WBSF ($r = -0.69$; $P < 0.01$), collagen content ($r = -0.65$; $P < 0.01$), and collagen crosslink density ($r = -0.48$; $P < 0.05$) as seen in study 1. Finally, muscle fiber diameter showed a correlation pattern similar to those from CSA, with myofibrillar tenderness ($r = 0.41$; $P < 0.05$), overall tenderness ($r = 0.45$; $P < 0.05$), connective tissue content ($r = -0.49$; $P < 0.05$), WBSF ($r = -0.70$; $P < 0.01$), collagen content ($r = -0.64$; $P < 0.01$), and collagen crosslink density ($r = -0.51$; $P < 0.05$).

Discussion

Muscle fiber characteristics

In general, it was expected that muscles of support or postural muscles would be more densely comprised of type I fibers as they are specialized for long periods of low-level activities. In contrast, muscles used for locomotion requiring quick bursts of energy for movement would have a high relative percentage of type II fibers (Aberle et al., 2012). Our results broadly reflected this trend. For example, we found that RA comprised close to 50% of type I fibers, while ST only had 18%; however, a portion of the muscle fiber typing data diverged from those reported by others (Kirchofer et al., 2002; Hwang et al., 2010). These discrepancies are likely due to various factors such as different methodologies employed by each study. Hwang et al. (2010) utilized the method proposed by Brooke and

Kaiser (1970), which characterized ATPase activity of the different muscle fiber types. Conversely, Kirchofer et al. (2002) utilized the method described by Ashmore and Doerr (1971), which characterized succinate dehydrogenase activity in the muscle fibers to classify them as either slow-oxidative, fast oxidative glycolytic or fast glycolytic. Peter et al. (1972) later corresponded slow oxidative to type I, fast oxidative glycolytic to type IIA, and fast glycolytic to type IIB fibers. In this study, we followed the method proposed by Scheffler et al. (2018) who used MyHC isoforms as molecular markers to determine muscle fiber types.

Choi et al. (2006) reported that the correlation coefficients for ATPase-based versus electrophoretic-based fiber typing ranged from 0.46 to 0.77 in porcine *longissimus dorsi* muscle. Finally, Lefaucheur et al. (1998) found only 40% type IIB fibers in pig muscles when using more specific molecular probes to identify muscle fiber types compared to the 80% reported by Bee et al. (2004) using classical histochemical methods, further demonstrating how different muscle fiber typing techniques can result in divergences in data. These methodologies inherently assess different characteristics of muscle fibers. We believe that the scientific community should consider renaming the ATPase and succinate dehydrogenase approaches to "muscle fiber characteristics profiling" rather than "muscle fiber typing" to more accurately reflect their purpose and promote consistency in muscle fiber data in the future.

We purposely only stained dystrophin, a protein localized to the cytoplasmic face of the sarcolemma (Gao and McNally, 2015), to evaluate the direct relationship between muscle fiber area and meat quality without the bias of muscle fiber types. Unfortunately, most muscle fiber studies measured the muscle fiber CSA and diameter on a muscle-fiber type basis (Kirchofer et al., 2002; Phelps et al., 2016; Cheng et al., 2020), so the comparisons between overall CSA and diameter for the entire muscle versus those that were separated based on muscle fiber types lack parity. However, the differences in muscle fiber CSA and diameter among muscles can potentially be attributed to variations in the MyHC isoforms they express. Type I fibers are generally recognized as smaller than type II glycolytic fibers. Listrat et al. (2016) and Lefaucheur and Gerrard (2000) ranked muscle fiber size as type I < IIA < IIX < IIB, which coincides with contractile speed. Although this trend was generally observed in our data, there was one major exception—the RA. Despite having the highest percentage of type I fibers, the RA also exhibited one of the largest muscle fiber CSA and diameters among all the muscles evaluated in study 1. Oury et al. (2010) also found that RA had larger mean fiber areas than LT or TB, which both contain more glycolytic muscle fibers than RA. Interestingly, the same study also found that the oxidative muscle fibers in RA displayed larger CSA than the glycolytic muscle fibers. This again highlights that more work is needed to further understand the relationship between muscle fiber types and CSA, particularly for muscles in the abdominal region in beef.

Potential relationship between muscle fiber characteristics and meat quality

Juiciness evaluated by trained panelists. Our correlation data for the combined muscles from study 1 reflected that type I muscle fibers are positively correlated with juiciness, while type IIA muscle fibers are negatively correlated with juiciness. This result was expected for type I muscle fibers as many studies have found similar relationships between type I muscle fibers and water-holding capacity (WHC) (Ryu et al., 2008; Joo et al., 2013; Kim et al., 2018). The generally accepted mechanism for this relationship is that rapid glycolysis in glycolytic muscle fibers can lead to a faster pH decline, triggering extensive myofibril contraction. This process expels water thereby reducing its WHC in muscles with more type II muscle fibers (Mo et al., 2023). Type IIA muscle fibers are unique muscle fibers that possess both glycolytic and oxidative characteristics (Picard and Gagaoua, 2020). Kim et al. (2016) found a negative relationship between type IIA muscle fiber percentages and moisture content in beef, but Lee et al. (2016) found the opposite in pork. Furthermore, we also found a positive relationship between type IIX muscle fiber percentage and juiciness in RF. This was unexpected because type IIX is usually the most glycolytic muscle fiber type found in beef (Scheffler et al., 2018); however, it is important to point out that this general belief that glycolytic muscle fibers have lower WHC is mostly based on pork studies, and the selection within domesticated pigs over the years has resulted in a dominance of type IIB muscle fibers in most pork cuts (Karlsson et al., 1993). Muscle fiber composition within beef is generally more heterogeneous than that in pork (Picard and Gagaoua, 2020). Therefore, utilizing selective knowledge from pork muscle fiber types to explain the results of this study can be potentially misleading. Although the exact mechanism that resulted in the noted relationship is unclear, it is known that muscle fiber types can impact the muscle's ability to retain water and maintain the

structural integrity of the myofilament lattice framework on a muscle-specific basis.

Connective tissue content evaluated by trained panelists and biochemical collagen characteristics

Our correlation data for all muscles combined from studies 1 and 2 revealed a positive correlation between type I muscle fibers and both biochemical collagen content and collagen crosslink density while type II muscle fibers showed a negative relationship with these metrics. This finding disagreed with the commonly accepted notion that locomotive muscles (higher percentage of type II fibers) tend to exhibit greater collagen content and mature collagen crosslink density than postural/support muscles (higher percentage of type I fibers; Rhee et al., 2004; Torrescano et al., 2003). However, our findings aligned with those of Palokangas et al. (1992) and Listrat et al. (2020a), who demonstrated that mature collagen crosslinks are more abundant in muscles with a higher percentage of oxidative fiber types and that total and insoluble collagen content (an indicator of mature collagen crosslink density) are negatively correlated with type IIA fibers in LT muscle, respectively.

It was interesting to note that there was a negative correlation between type I fiber percentage and connective tissue content as assessed by trained panelists for RA muscle, a positive correlation between type IIA fiber percentages and sensory connective tissue content for RA, RF, and ST muscles and a positive association between type IIX fiber percentage and sensory connective tissue content for LL muscle. Despite this, no significant correlations emerged between muscle fiber types and biochemical collagen characteristics in these muscles. Wu et al. (2021) pointed out that the connective tissue content evaluated by trained panelists is mainly influenced by mature collagen crosslinks density in meat prepared with moist heat cookery, but Hammond et al. (2022) showed that this relationship becomes less clear for meat prepared with dry heat cookery. In this study, a dry cookery methodology was utilized. Chun et al. (2020) hypothesized that dry heat cookery is less effective in solubilizing collagen with low mature collagen crosslink density, thus leaving residual noise resulting in poor correlation between biochemical connective tissue characteristics and sensory evaluation of connective tissue content. Perhaps the trained panelists' perception of connective tissue content was influenced by other attributes like meat tenderness (Clark and Lawless, 1994).

Our data also indicated that connective tissue content determined by trained panelists, as well as biochemical collagen content and collagen crosslink density were negatively correlated with muscle fiber CSA and diameters in both studies 1 and 2. Gillies and Lieber (2011) suggested that as muscle fibers grow due to hypertrophy, the extracellular space available for connective tissue deposition and maturation decreases. Our findings corroborated this hypothesis: muscles with larger fiber sizes, such as RA and LL, exhibited lower connective tissue content ratings by trained panelists and reduced biochemical collagen content (Hammond et al., 2022; Chun et al., 2020). Finally, we agree with the perspective of Listrat et al. (2020a) that there is no systematic relationship between the biochemical characteristics of the connective tissue and muscle fiber type in livestock, suggesting this relationship is likely muscle specific. Since few muscles serve solely one function and many are involved in both postural and propulsive roles (Johnson et al., 1986), it may be more valuable for the meat science field to explore the relationship between muscle fiber CSA and collagen characteristics to gain deeper insight into the background toughness of beef.

Lipid flavor intensity evaluated by trained panelists

We found a positive relationship between the percentage of type I muscle fibers and lipid flavor, and a negative relationship between the percentage of type IIA muscle fibers and lipid flavor across all combined muscles from studies 1. In congruence with our data, it is generally accepted that oxidative muscle fibers typically contain higher lipid levels compared to glycolytic muscle fibers due to a greater number of adipose cells in the perimysium and more lipid droplets within the muscle fibers, resulting in a more robust lipid flavor in meat (Essén-Gustavsson et al., 1994; Hwang et al., 2010). From a physiological perspective, it is logical that oxidative fibers would have greater deposition of Intramuscular fat (IMF) as they metabolize lipids through beta oxidation for energy (Zierath and Hawley, 2004); however, this statement does not always hold true. For example, Kim et al. (2016) found no relationship between type I muscle fiber percentages and fat content but did find a positive relationship between type IIA muscle fibers and fat content in 4 beef muscles. Also, many locomotive muscles, such as the SS, contain more oxidative muscle fibers but tend to have lower lipid content than the *longissimus* muscles (Nyquist et al., 2018; Chun et al., 2020). Extensive

selection in livestock focused on increasing IMF content in the loin has resulted in some contrasting examples where glycolytic muscles tend to have higher IMF content than those in oxidative muscles (Silva et al., 2019). In extreme cases, Bonnet et al. (2010) reported that IMF content can be 3 times higher in the white glycolytic than in the red oxidative part of the ST in pigs. This may, in part, explain why a relationship between lipid flavor intensity and fiber type on an individual muscle basis was not observed, with the exception of LL, in study 2.

WBSF and tenderness evaluated by trained panelists

The associations between muscle fiber types and tenderness of beef are complex and rarely consistent (Listrat et al., 2020b). Overall, our correlation data for all muscles combined from study 1 showed that type I muscle fiber percentage were positively correlated to tenderness attributes, primarily driven by the RA. Many others also reported a positive relationship between tenderness and type I fiber proportions (Calkins et al., 1981; Hwang et al., 2010), and some of them speculated that this relationship is due to oxidative fibers typically having smaller CSA (Seideman et al., 1988; Crouse et al., 1991); however, both Oury et al. (2010) and this current study found that RA displayed an exceptionally larger CSA compared to those from the other muscles utilized in the studies, but RA was dominated by type I fibers and exhibited acceptable tenderness.

On the other hand, in study 2, type I muscle fiber percentage was negatively correlated to tenderness attributes, but type IIA was positively correlated to tenderness attributes. Similar to our findings in study 2, Chardulo et al. (2021) showed that type I muscle fibers were more abundant in tough LT, whereas type IIA muscle fibers are elevated in tender LT in Nellore cattle. The differing relationships between muscle fiber types and tenderness observed in studies 1 and 2 can be explained by 2 possible reasons. 1) Muscles with a higher proportion of type IIA fibers are known to have greater calpain activity than those with a higher proportion of type I fibers (Muroya et al., 2010; Xiong, 2004), as oxidative fibers tend to exhibit higher levels of calpastatin, a known endogenous inhibitor of calpains (Ouali and Talmant, 1990; Koohmaraie, 1992). Samples from study 2 were aged for 5 d compared to 2 d for those from study 1, and it is possible that this additional 72 h of aging may have amplified the calpain-driven tenderization effect. 2) Type I fibers generally contain higher levels of IMF content as oxidative muscle fibers metabolize lipids for energy (Zierath and Hawley, 2004), and IMF content is directly related to meat tenderness and juiciness (Frank et al., 2016); however, many studies have focused on enhancing IMF deposition in muscles from the loin area over the years (Wang et al., 2005), leading to enhanced IMF deposition in type II muscles. Furthermore, it is hypothesized that increased deposition of IMF in the perimysium may weaken the structure of connective tissue resulting in enhanced tenderness (Matarneh et al., 2023) Therefore, the positive relationship between type IIA fibers and beef tenderness observed in study 2 was likely influenced by LL, which represented one-third of the samples in that study.

Furthermore, type IIA muscle fiber percentage was negatively correlated with tenderness attributes for PP, RF, and ST, and type IIX muscle fiber percentage was positively correlated with tenderness attributes for PP. The connection between type IIA and IIX fibers and meat tenderness is less clearly defined and subject to debate. Some studies showed type IIA and IIX fiber proportions have a positive relationship with tenderness in various meat cuts (Gagaoua et al., 2018; Chardulo et al., 2021), but many other studies also showed either no relationship or even a negative correlation between the type IIA and IIX muscle fibers and tenderness (Totland et al., 1988; Wright et al., 2018). Picard et al. (2006) pointed out that these differences may be due to hybrid fibers that simultaneously express several MyHCs, particularly between type IIA and IIX (type IIAX). Arguello et al. (2001) and Greenwood et al. (2009) showed that 21% and 7% of all muscle fibers in goat and beef cattle are hybrid muscle fibers, respectively. These transitions can occur due to various factors such as growing conditions, diet, and use of hormones (Picard and Gagaoua, 2020). The presences of hybrid fibers make it difficult to accurately characterize the MyHC fiber composition in a muscle as there are no specific antibodies that have been developed to only identify hybrid fibers.

Our correlation data for all muscles combined from both studies 1 and 2 showed a positive correlation between muscle fiber CSA/diameter and meat tenderness; however, this is simply driven by the muscle selection process as mentioned earlier where the most tender muscles such as RA and LL happened to have largest muscle fiber sizes in this study, and a closer examination of our data confirmed that the relationship between muscle fiber CSA/diameter and meat tenderness is specific to each muscle. As many past studies

have shown, muscle fiber CSA from muscles like SS, LL, and TT from this study have a negative relationship with meat tenderness (Tuma et al., 1962; Seideman et al., 1988). These studies speculated that as the number of myofibrils per unit of mass increased, the amount of force needed to penetrate the meat increased (Seideman et al., 1988); however, some of the muscles in this study, such as ST, displayed the opposite effect. Ouali (1990) stated that muscles that are composed of type II muscle fibers are more susceptible to early postmortem proteolytic degradation than those mainly composed of type I fibers. Perhaps our findings might be explained by the earlier proteolytic degradation in the ST, which leads to muscle fiber fracturing and tearing, thus resulting in a slightly larger muscle fiber CSA and diameter, while making the meat more tender.

Finally, another possible explanation for the inconsistency in the relationship between muscle fiber types/size and quality attributes can be due to location effects. Phelps et al. (2016) showed that the proximal end of beef ST had a lower percentage of type I fibers and a higher percentage of type IIX fibers compared to those from the distal end, which resulted in differences in shear force and myofibrillar protein degradation potential. Furthermore, Rivero et al. (1993) found that type I fiber percentage increased and type IIB fiber percentage decreased when the sampling moved from superficial regions to the deeper parts of GM in horses. Our data also indicated that the cranial portion of the *longissimus* (LT) had more type I fiber and less type IIA fibers compared to the caudal portion (LL), which led to significant differences in muscle fiber CSA/diameter between LT and LL. Therefore, one should not overlook the location effect when investigating the relationship between muscle fiber composition and meat quality.

Conclusion

The impact of muscle fiber type and size on eating quality is complex and multifaceted. Although the relationship between muscle fiber types and CSA/diameter with the eating quality of beef cuts appears to be highly muscle specific, the findings of this study generally suggested that muscles predominated by type I muscle fibers will likely deliver a greater eating quality experience for consumers. Type I fibers were positively correlated with juiciness, lipid flavor, and tenderness but contributed to slightly greater connective tissue content and collagen crosslink density. Interestingly, our data demonstrated that large muscle fiber CSA and diameter do not always diminish the eating quality as previously established due to its negative correlation with connective tissue content and mature collagen crosslink. Like any antibody-based protocol, we recognize that the reported method is not perfect with small concerns of hybrid fibers and minor cross-reactivity; however, our data are comparable to the muscle fiber typing results and correlations from many previous studies. Given its efficacy and efficiency, muscle fiber typing via immunoblotting may prove to be a viable alternative to traditional immunohistochemical methods, but the strengths and weaknesses of each methodology should be considered when comparing results across studies.

Acknowledgements

This study was funded in part by The Beef Checkoff. Salaries and research support were provided by State and Federal Funds appropriated to Kansas State University.

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