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ARTICLE

The Relationships between Muscle Fiber Characteristics, Intramuscular Fat Content, and Fatty Acid Compositions in *M. longissimus lumborum* of Hanwoo Steers

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Abstract

The objective of this study was to investigate the relationship between muscle fiber characteristics, intramuscular fat (IMF) content, and fatty acids composition in *longissimus lumborum* (LL) muscle from Hanwoo steers. The LL muscles were obtained from four quality grades (QG) carcasses and subjected to histochemical analysis. There were significant ($p<0.05$) differences in fiber number percentage (FNP) and fiber area percentage (FAP) of muscle fiber types among muscles from four QGs. Both FNP and FAP of type I increased while those of type IIB decreased with increasing QG from QG 2 to QG 1^{++} ($p<0.05$). Also, with increasing QG, the saturated fatty acid (SFA) proportion decreased while monounsaturated fatty acid (MUFA) increased significantly ($p<0.05$). IMF content was positively correlated with both FNP and FAP of type I, but negatively correlated with those of type IIB. The proportions of SFA and MUFA were significantly ($p<0.001$) correlated with both type I and IIB composition. These results implied that muscle fiber type composition is an important factor influencing fatty acid composition in LL muscle of Hanwoo steer.

Keywords muscle fiber characteristics, intramuscular fat content, fatty acid composition, Hanwoo beef quality

Introduction

Hanwoo, a major beef breed in Korea, has been developed to increase the level of marbling over several decades to meet Korean consumer preferences (Joo *et al*., 2017). However, concerns about fat content and fatty acid composition has been increasing, because the consumption of animal fat and saturated fatty acid has been reported to be linked to cardiovascular disease (CVD), obesity and cancer (Micha *et al*., 2010; Pan *et al*., 2012). In this regard, some Korean consumers believe that too much marbling fat in Hanwoo beef may lead to in increased risk of CVD or obesity. These anxieties are based on decades-old information that the fat content and saturated fat in meat has negative health impacts (Joo *et al*., 2017). It was reported that stearic acid (C18:0) has no effect on plasma cholesterol level and also oleic acid (C18:1) has a similar effect as PUFA in lowering serum choles-

terol (Ulbricht and Southgate, 1991). Gilmore *et al*. (2011) showed that the consumption of high-oleic acid beef increases HDL-cholesterol concentration in serum. Our previous study demonstrated that high marbled Hanwoo muscles had higher proportion of MUFA than low marbled muscles due to higher oleic acid proportion (Hwang and Joo, 2016). However, little attention has been paid to the relationships between intramuscular fat (IMF) content, muscle fiber characteristics and fatty acid composition in bovine muscle.

Fat content and fatty acid composition in meat play an important role in not only human health but also meat quality. Especially, IMF content contributes to meat palatability characteristics such as juiciness, tenderness and flavor (Wood *et al*., 2008). Muscle fiber characteristics also strongly influence meat quality because skeletal muscles mainly consist of muscle fibers (Joo *et al*., 2013). Previously we have reported that there was a relationship between the proportion of each fiber type and size, and IMF content in porcine (Kim *et al*., 2013a; Kim *et al*., 2013b) and bovine (Hwang *et al*., 2010) *longissimus* muscle. Consequently, we proposed that meat quality, including IMF content, can be controlled through the manipulation of muscle fiber characteristics (Joo *et al*., 2013). Recently, Jeong *et al*. (2017) reported the relationships between muscle fiber characteristics and fatty acid composition in the *longissimus thoracis* (LT) muscle of pigs reared, according to the same breeding and diet system. However, the relationships in Hanwoo muscles have not yet been studied in depth.

The relationship between IMF content and fatty acid composition should be considered when the quality of Hanwoo beef is controlled through manipulation of the muscle fiber characteristics. It is well documented that IMF content and fatty acid composition, as well as muscle fiber characteristics, can be influenced by genetic and environmental factors such as species, sex, geno-type, diet and muscle location within the same species and breed (Joo *et al*., 2013; Lefaucheur, 2010). Needless to say, a better understanding of the relationships between muscle fiber, IMF, and fatty acid composition would be useful to control the genetic and environmental factors that alter muscle fiber composition in relation to lipid characteristics. Therefore, the objective of this study was to investigate the relationships between IMF content, muscle fiber characteristics, and fatty acid composition in the *longissimus lumborum* (LL) muscle in Hanwoo steers.

Materials and Methods

Samples

A total of 20 LL muscles from Hanwoo carcasses with 4 quality grades (QG) (5 cattle for each QG) were obtained at a commercial meat processing plant in Korea. The QGs (1^{++}, 1^{+}, 1, and 2) were primarily determined by the degree of marbling using Korean Beef Marbling Standard (BMS). Approximately 10 g of each muscle was taken for histochemical analysis within 1 h post-mortem. It was frozen in isopentane chilled with liquid nitrogen. After 24 h of chilling, LL muscles were removed to investigate IMF content and fatty acid composition.

Histochemical analyses

Transverse serial sections of 10 μm in thickness were cut from entire blocks (1.0 × 1.0 × 1.5 cm) with a cryostat microtom (HM525, Microm GmbH, Germany) at -20°C. The sections were subsequently used for histochemical analysis of myosin adenosine triphosphatase (mATPase) following alkaline (pH 10.70) and acid (pH 4.63) preincubation using the method of Brooke and Kaiser (1970) with slight modifications. An image analysis system (Image-Pro®plus 5.1, Media Cybernetics Inc., USA) was used to examine the stained sections. The muscle fibers were classified into fiber type I, IIA, and IIB according to the nomenclature of Brooke and Kaiser (1970). Approximately 500 fibers per sample were counted to analyze the muscle fiber characteristics. Fiber number percentage (FNP) and fiber area percentages (FAP) were determined. FNP refers to the ratio of counted fiber number of each fiber type to the total counted fiber number. FAP was the ratio of total cross-sectional area of each fiber type to total fiber area measured.

Intramuscular fat content

Intramuscular fat content was determined using modified method of Folch *et al*. (1957). Briefly, lipid was extracted from 3 g of homogenized meat sample using 30 mL of Folch solution I (chloroform : methanol = 2:1, v/v). The homogenate was filtered with Whatman no.1 filter paper. Filtered solution was mixed with 0.88% of NaCl by stirring. The solution was then allowed to separate into two layers. After washing the wall of a measuring cylinder with 10 mL of Folch solution II (chloroform : methanol : H_2O = 3:47:50), the final volume of the lower layer was recorded. The upper layer (methanol and water layer) was removed using an aspirator. Then 10 mL of the lower layer

(chloroform containing lipid extracts) was added into a dish to dry at 50°C. The weight of the dish was measured before and after drying. Fat content was computed based on weight difference of the dish.

Fatty acid composition

After the extraction of intramuscular lipids, lipid methyl esters were prepared via saponification with 1.0 N methanolic NaOH and subsequently methylated with boron trifluoride in methanol. Fatty acid methyl esters (FAME) were analyzed using a HP6890N (Hewlett-Packard, USA) gas chromatograph equipped with a HP7683 (Hewlett-Packard) automatic sampler. FAME separations were accomplished using a 100 m SP2560 (Supelco, USA) capillary column (0.25 mm i.d. and 0.20 μm film thickness). For the separation of FAME from the samples, the following temperature program was applied with nitrogen as a carrier gas at a flow rate of 1 mL per min. Column oven temperature increased from 50 to 180°C at 10°C per min, from 180 to 220°C at 5°C per min, 220 to 240°C at 2°C per min, and then held at 240°C for 20 min. The injector and detector were maintained at 250°C. Sample injection volume was 1 μL and the analysis was performed in duplicate. Individual fatty acids were identified by comparison of the retention times with standards (Supelco 37 components FAME Mix, USA). Results were expressed as the percentage of the total fatty acid detected based on the total peak area.

Statistical analysis

Experimental data were analyzed by analysis of variance (ANOVA) procedure of statistical analysis systems (SAS, 2008). Duncan's multiple range test was used to determine significant differences among means at 5% level of significance (SAS, 2008). Correlation analysis was performed using the CORR procedure of SAS to evaluate the correlation between individual fatty acids and the sensory panel traits or the meat quality traits.

Results and Discussion

Muscle fiber characteristics (FNP and FAP) of LL muscle from four QGs of Hanwoo steers are summarized in Table 1. There were significant ($p<0.05$) differences in FNP and FAP among 4 QGs of Hanwoo steers. FNP of type I increased with increasing QG from 2 (30.48%) to 1^{++} (35.04%), but there was no significant ($p>0.05$) difference in FNP of type I between QG 1^{++} and QG 1^{+}. On the contrary FNP of type IIB decreased with increasing QG ($p<0.05$), while FNP of type IIA showed no significant ($p>0.05$) difference between QG 1^{++}, QG 1^{+} and QG 1. Also, there was no significant ($p>0.05$) difference in FNP of type IIA between QG 1^{++} and QG 1^{+}. FAP of three muscle fiber types were similar with FNP results. FAP of type I also increased with increasing QG from 2 to 1^{++}, but FAP of type IIB decreased ($p<0.05$). There were significant ($p<0.05$) differences in FAP of type IIB among all 4 QGs.

The FNP and FAP results in this study clearly showed that muscle fiber characteristics of LL muscle of Hanwoo steers were strongly related with QG. Because QG of Hanwoo carcass was determined by mainly IMF content in LL muscle, results implied that FNP and FAP of type I were increased with increasing of IMF content. This is in agreement with early researches that the αW muscle fiber content was negatively correlated with marbling while at the same time αR muscle fiber content was positively correlated to the marbling (Ashmore *et al*., 1972; Calkins *et al*., 1981). Also, the findings by Iwamoto *et al*. (1991) and Gotoh (2003) support the present results showing that the percentage number and area of type I myofibers increased with increasing of IMF content, while those of type IIB

Table 1. Muscle fiber characteristics of M. longissimus lumborum from four quality grades of Hanwoo steers

Traits	Quality grades			
	1^{++}	1^{+}	1	2
Fiber number (%)				
Type I	35.04±0.48 ^{A}	34.22±0.58 ^{A}	32.34±0.22 ^{B}	30.48±0.28 ^{C}
Type IIA	17.34±0.16 ^{A}	17.22±0.37 ^{A}	16.54±0.40 ^{A}	15.48±0.24 ^{B}
Type IIB	47.62±0.52 ^{C}	48.56±0.83 ^{C}	51.12±0.59 ^{B}	54.04±0.13 ^{A}
Fiber area (%)				
Type I	33.12±0.41 ^{A}	32.50±0.33 ^{A}	31.14±0.18 ^{B}	29.58±0.27 ^{C}
Type IIA	16.62±0.36 ^{A}	16.40±0.23 ^{AB}	15.60±0.29 ^{BC}	15.20±0.39 ^{C}
Type IIB	50.26±0.21 ^{D}	51.10±0.23 ^{C}	53.26±0.15 ^{B}	55.22±0.40 ^{A}

$^{A-D}$Means±SE with different superscripts in the same row are significantly different ($p<0.05$).

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myofibers decreased. However, May *et al*. (1977) reported αR fibers to have significant negative correlation with marbling score and IMF content for several crossbred cattle steers. Ozawa *et al*. (2000) also reported that αR fiber had a negative correlation with marbling score and IMF content in Japanese Black fattening steers. The negative relationship between the αR fiber and the marbling score might be due to the cattle breeds. It is well documented that muscle fiber characteristics and IMF content are influenced by various intrinsic and extrinsic factors including breed (Joo *et al*., 2013).

Fatty acid composition of LL muscle from four QGs of Hanwoo steers are summarized in Table 2. The proportions of SFA and MUFA were significantly (p<0.05) different among 4 QGs due to differences in the majority of fatty acids such as oleic (C18:1 n-9), palmitic (C16:0) and stearic (C18:0) acids. There were significant (p<0.05) differences in SFA proportion among 4 QGs, and the SFA proportion decreased with increasing QG from QG 2 to QG 1^{++}. The proportions of MUFA were also significantly (p<0.05) different between QGs, whereas there were no significant (p>0.05) differences in the PUFA proportions among 4 QGs. The MUFA proportion increased significantly (p<0.05) with increasing QG. Oleic acid comprised the majority of MUFA, and the proportion was significantly (p<0.05) higher in QG 1^{++} than other QGs. On the contrary, the proportion of palmitic acid that comprised the majority of SFA was significantly (p<0.05) higher in QG 2 than other QGs.

These results suggested that IMF content was related to fatty acid composition as well as muscle fiber characteristics in LL muscle of Hanwoo steer. Results also implied that the SFA proportion decreased while the MUFA proportion increased with increasing IMF content in LL muscle. These results were supported by other studies which showed strong relationship between fatty acid composition and IMF content in *longissimus* muscle of Japanese Black steers (Gotoh *et al*., 2011; Sasaki *et al*., 2001; Zembayashi *et al*., 1995). In Japanese Black cattle which contains greater than 30% IMF, the proportion of C18:0 and SFA were much less with higher in the proportion of C18:1 and MUFA compared to Holstein cattle which contains less IMF (Gotoh *et al*., 2014). However, Jeong *et al*. (2017) investigated the relationship between IMF content and fatty acid composition in porcine *longissimus thoracis* (LT) muscle, and reported that the porcine LT muscles with the highest IMF content had the highest SFA and MUFA. This different relationship between bovine and porcine *longissimus* muscle is certainly due to differences in muscle fiber characteristics in relation to energy meta-

Table 2. Fatty acids composition (%) of *M. longissimus lumborum* from four quality grades of Hanwoo steers

Fatty acids (%)	Quality grades			
	1^{++}	1^{+}	1	2
C10:0	0.06±0.00	0.07±0.00	0.06±0.01	0.07±0.02
C12:0	0.06±0.01	0.07±0.00	0.05±0.01	0.07±0.01
C14:0	2.40±0.18^{B}	2.51±0.21^{AB}	2.59±0.12^{AB}	2.72±0.14^{A}
C14:1	0.49±0.06	0.45±0.09	0.50±0.06	0.43±0.08
C16:0	25.53±0.84^{C}	27.19±0.82^{B}	28.04±1.06^{B}	29.62±1.68^{A}
C16:1	4.40±0.19	4.16±0.32	4.30±0.21	4.07±0.32
C17:0	1.31±0.04	1.31±0.15	1.29±0.10	1.36±0.07
C17:1	1.12±0.04^{A}	0.98±0.10^{AB}	0.96±0.19^{AB}	0.86±0.16^{B}
C18:0	9.28±0.59^{C}	10.80±1.05^{B}	11.93±1.02^{B}	13.29±0.85^{A}
C18:1 n-9	50.62±1.01^{A}	48.39±1.20^{B}	44.67±1.30^{C}	40.67±2.61^{D}
C18:2 n-6	2.29±0.03	2.22±0.13	2.18±0.08	2.25±0.16
C18:3 n-3	0.81±0.21	0.97±0.06	0.78±0.18	0.86±0.16
C18:3 n-6	0.12±0.03	0.12±0.01	0.12±0.01	0.13±0.02
C20:0	0.11±0.02^{C}	0.14±0.02^{B}	0.12±0.01^{BC}	0.17±0.02^{A}
C20:1 n-9	0.28±0.07	0.32±0.02	0.27±0.02	0.27±0.03
C20:4 n-6	0.23±0.04	0.20±0.08	0.21±0.05	0.22±0.04
C20:5 n-3	0.06±0.01	0.07±0.02	0.06±0.02	0.07±0.01
C22:6 n-3	0.04±0.02	0.06±0.02	0.05±0.01	0.06±0.02
SFA	38.74±0.98^{C}	42.10±1.71^{B}	43.98±1.93^{B}	47.29±2.58^{A}
MUFA	57.02±0.87^{A}	54.11±0.99^{B}	50.64±1.15^{C}	46.31±2.71^{D}
PUFA	3.55±0.29	3.68±0.12	3.41±0.30	3.59±0.21

$^{A-D}$Means±SE with different superscripts in the same row are significantly different (p<0.05).

Table 3. Correlation coefficients (*r*) between histochemical characteristics, intramuscular fat content and fatty acid percentage in *M. longissimus lumborum* of Hanwoo cattle

Measures	Fiber number percentage			Fiber area percentage		
(%)	Type I	Type II A	Type II B	Type I	Type II A	Type II B
IMF	0.86***	0.69**	-0.85***	0.87***	0.63**	-0.93***
C14:0	-0.60**	-0.65**	0.66**	-0.53*	-0.49*	0.61**
C14:1	0.59**	0.61**	-0.63**	0.47*	0.14	-0.41
C16:0	-0.71***	-0.61**	0.72***	-0.74***	-0.47*	0.76***
C18:0	-0.79***	-0.67**	0.79***	-0.77***	-0.58**	0.82***
C18:1 n-9	0.81***	0.68**	-0.82***	0.85***	0.69**	-0.93***
C18:2	0.26	0.22	-0.27	0.25	-0.16	-0.11
SFA	-0.77***	-0.67**	0.79***	-0.78***	-0.54*	0.82***
MUFA	0.84***	0.71***	-0.84***	0.87***	0.68**	-0.94***
PUFA	0.19	0.28	-0.24	0.11	0.13	-0.13

*p<0.05, **p<0.01, ***p<0.001.

bolic and contractile properties (Joo *et al*., 2013).

Results of correlations between muscle fiber characteristics, IMF content and fatty acid composition are summarized in Table 3. IMF content was positively correlated with FNP of type I (r=0.86) and type IIA (r=0.69) but negatively correlated with type IIB (r=-0.85). Similar tendencies were found between IMF content and FAP of fiber types. The SFA proportion had negative correlation with type I (r=-0.77) but positive correlation with type IIB (r=0.79), whereas the MUFA proportion had positive correlation with type I (r=0.84) but negative correlation with type IIB (r=-0.84). Also, similar tendencies were observed between the proportions of fatty acids and FAP of fiber types. Both type I and IIA had negative correlation with myristic, palmitic and stearic acids, but type IIB showed positive correlation with those SFAs. In contrast, type IIB had negative correlation with oleic acid, but type I and IIA showed positive correlation.

The present data clearly showed that the proportions of SFA and MUFA were significantly (p<0.001) correlated with muscle fiber characteristics in relation to IMF content. The relationship of muscle fiber type composition to IMF content was clarified in this study (Fig. 1). The FNP of type I had a significantly (p<0.001) positive correlation with the IMF content and conversely, the FNP of type IIB had a significantly (p<0.001) negative correlation with

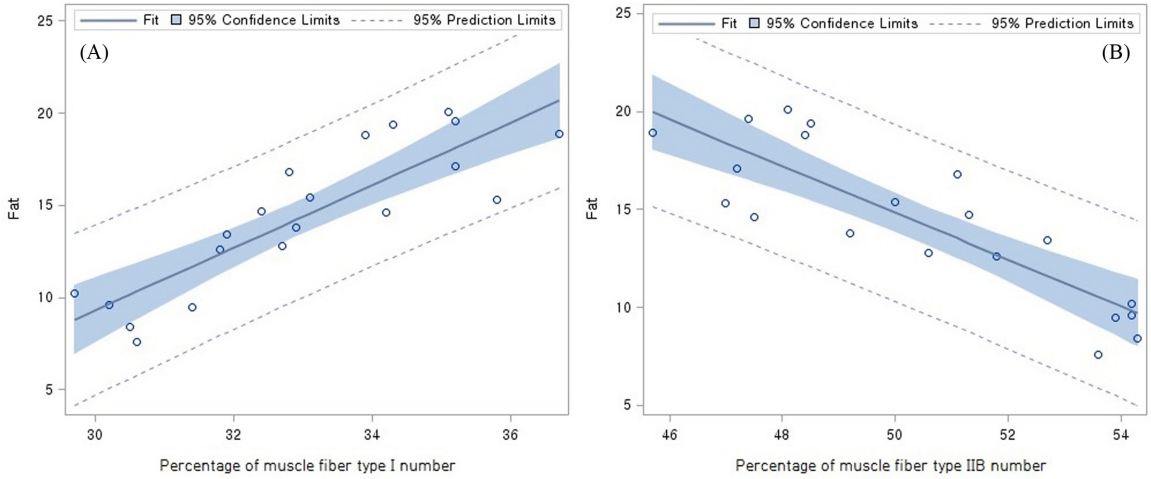

Fig. 1. The interrelationship between the percentage of muscle fiber type composition (type I and IIB) and intramuscular fat content in *M. longissimus lumborum* of Hanwoo steers (n=20). (A) The figure shows correlation between intramuscular fat content and percentage of muscle fiber type I number. The intramuscular fat content (%) = 1.69 × percentage of muscle fiber type I number - 41.56 (p<0.001, r^2 = 0.74). (B) The figure shows correlation between intramuscular fat content and percentage of muscle fiber type IIB number. The intramuscular fat content (%) = -1.19 × percentage of muscle fiber type IIB number + 74.40 (p<0.001, r^2 = 0.73).

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IMF content. In general, the fine network of blood vessels in the muscles of cattle penetrate slightly into primary muscle fiber bundles. The IMF tissue is formed at first around this special part of the arterioles and is spread over its peripheral connective tissue (Hoshino *et al*., 1987). According to Gotoh (2003), the capillary network develops more around type I muscle fibers than type IIB. Therefore, it can be easily anticipated that the overall blood stream could be enhanced and transport more fat and oxygen for energy metabolism in the muscles with a lot of type I muscle fibers. This hypothesis is strongly supported by the present study that demonstrated the significantly positive correlation between the FNP of type I and IMF content in LL muscle of Hanwoo steers. Furthermore, results of correlations between muscle fiber characteristics and fatty acid composition in the present study suggested that muscle fiber type composition is an important factor influencing fatty acid composition in bovine LL muscle.

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