Relationship between myofiber type and fatty acid composition in skeletal muscles of Wagyu (Japanese Black) and Holstein cattle

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Abstract — **The purpose of this study was to evaluate the relationship between myofiber type and intramuscular fatty acid (FA) composition in a concentrate feeding system for Wagyu (*n* = 6) and Holstein (*n* = 5) cattle. Animals were intensively fattened with concentrate from 3 months of age until slaughter (26 months of age). After slaughter, 21 skeletal muscles were removed from the animal carcasses, and enzyme and histochemical analysis was carried out. The percentage of intramuscular fat (IMF) content was determined *via* the Soxhlet method. Intramuscular FA compositions were analyzed by gas chromatography. In the 21 muscles analyzed, the percentage of IMF in 13 muscles was significantly higher in Wagyu than in Holstein cattle (*P* < 0.05). The percentage of type I myofibers positively correlated with IMF; conversely, percentages of type IIA and IIB myofibers negatively correlated with IMF. Muscles from Wagyu contained a greater proportion of the FAs C16:1, C18:1, and C20:1, and monounsaturated FAs than did those from Holstein (*P* < 0.001). In Wagyu, the proportion of C18:0 and saturated FA was much lower (*P* < 0.001). The proportion of FAs with shorter chains (<C17) was significantly negatively correlated with those in type I myofibers (C14:0, C16:1, C15:0, and C16:0; *P* < 0.05). FAs with longer chains (>C17) were significantly positively correlated with those in type II (IIA+IIB) myofibers (C18:1, C18:2 n-6, and C20:1; *P* < 0.05). There were no clear trends in Holstein cattle. The IMF in Wagyu tended to be unsaturated compared with that in Holstein cattle.**

Keywords — **cattle muscle, myofiber type, fatty acid composition**

I. INTRODUCTION

Myofiber type compositions of specific muscles are important factors for meat quality because they are closely related to many peri- and postmortal biochemical processes in muscles (Klont et al. 1998). In mammals and birds, more than 200 skeletal muscles have various functions in different parts of the body, for example for locomotion, postural maintenance, and respiration. Heterogeneity has been recognized in fast- and slow-twitch muscles contracting with the use of energy from oxidative and glycolytic metabolism (Peter et al. 1972; Pette & Staron 1990). Fundamentally, myofibers are divided into three major categories depending on histochemical, physiological, and biochemical properties: type I (slow-twitch oxidative; SO), IIA (fast-twitch oxidative glycolytic), and IIB (fast-twitch glycolytic).

Fatty acid (FA) composition in muscle of cattle is an important factor in terms of flavor, fat hardness related to melting point, oxidative stability, and meat quality (Wood et al. 2003). In particular, the ratio of unsaturated FAs to saturated FAs strongly affects fat softness (Wood et al. 2003). It was recently reported that increases in fat and thus FA intake are closely related to induction of chronic diseases in developed countries.

It was reported that myofiber type is closely associated with obesity and weight loss in humans (Tanner et al. 2001). Muscles with different oxidative metabolisms show differences in susceptibility to lipid oxidation in pigs (Andrés et al. 2001). In terms of meat quality, the relationship between myofiber type/FA composition and breed differences remains unclear. Wagyu is a beef-type cattle that has a high potential to accumulate intramuscular fat (IMF). On the other hand, Holstein is a dairy-type cattle. The purpose of this study was to evaluate the relationship between myofiber type and intramuscular FA composition in a concentrate feeding system for Wagyu and Holstein cattle.

II. MATERIALS AND METHODS

Animals and muscle sampling: Japanese Black steers (26 months of age) and Holstein steers (26 months of age) were reared and fattened by a standard fattening system with a considerable amount of grain feed at the Kuju Agricultural Research Center, Kyushu University. Six animals were killed by bleeding from the carotid artery under anesthesia with xylazine hydrochloride. After slaughter, 21 skeletal muscles (*musculus biceps femoris*, proximal part; *m. biceps femoris*, distal part; *m. rectus femoris*; *m. vastus lateralis*; *m. semimembranosus*; *m. semitendinosus*; *m. iliacus*; *m. gluteus accessories*; *m. flexor digitorum superficialis*; *m. serratus ventralis thoracis*; *m. serratus ventralis cervicis*; *m. trapezius*; *m. rhomboideus thoracis*; *m. latissimus dorsi*; *m. longissimus thoracis*; *m. spinalis et semispinalis*; *m. semispinalis capitis*; *m. subscapularis*; *m. infraspinatus*; *m. obliquus externus abdominis*; and *m. psoas major*) were removed from the animal carcasses, and their weights were measured without the peripheral tendon or adipose tissue. Muscle samples for histochemical and biochemical analysis were taken at the center of the muscle.

Histochemical analysis: Serial frozen tissue sections were made transversely and stained by the reactions of acid (pH 4.3) or alkaline (pH 10.5) preincubated myosin ATPase and reduced nicotinamide adenine dinucleotide dehydrogenase activities. On a microscopic photograph taken at the same location of the specimen, myofibers were categorized into Type I, IIA, and IIB according to the nomenclature of Brooke and Kaiser (1970a, b). Myofiber type compositions were calculated and used for analysis.

FA analysis: The percentage of IMF content was determined *via* the Soxhlet method. The lipids extracted from muscle samples were kept with methanol and chloroform in a freezer until analysis. FAs were prepared by extraction from homogenized tissue samples by diethyl ether extraction and saponification with ethanolic KOH at 80°C for 1 h. FA analysis was performed by GC–FID analysis of FA methyl esters (FAME), prepared by methylation of FAs with sulfuric acid:methanol (1:35, v/v) at 55°C for 5 min. FAME were separated on an Omegawax 320 fused-silica capillary column (30 m, 0.32 mm, 0.25 μm; Supelco Japan, Tokyo, Japan) with a GC-17A gas-liquid chromatograph (Shimadzu, Tokyo, Japan), applying a constant-temperature program (200°C). Helium was used as a carrier gas with a 1:30 injector split. The injector and detector temperature was 250°C. Grain Fatty Acid Methyl Ester Mix (Cat. No. 47801; Sigma-Aldrich, Tokyo, Japan), GLC-100 (Cat. No. 1899-1AMP; Sigma-Aldrich, Tokyo, Japan), and c9,t11-18:2 (kindly provided by Assoc. Prof. Koji Nagao and Prof. Teruyoshi Yanagita of Saga University) were used to determine peaks.

Statistical analysis: An unpaired Student's *t*-test was applied to determine FA concentrations of both feeding groups for statistical significance; $P < 0.05$ was considered significant.

III. RESULTS

In the 21 muscles analyzed, the percentage of IMF in 13 muscles was significantly higher in Wagyu than in Holstein cattle ($P < 0.05$). The percentage of type I myofibers positively correlated with IMF; conversely, percentages of type IIA and IIB myofibers negatively correlated with IMF. In their relationships, the equation slopes were stronger in Wagyu (absolute values: 0.63—0.74) than in Holstein cattle (absolute values: 0.41–0.47).

Muscles from Wagyu cattle contained a greater proportion of the FAs C16:1, C18:1, C20:1, and monounsaturated FAs than did those from Holstein cattle ($P < 0.001$) (Table 1). In Wagyu cattle, the proportion of C18:0 and saturated FA was much lower ($P < 0.001$).

Table 1. Comparison of fatty acid compositions between Wagyu and Holstein steers

Fatty acid	Wagyu	Holstein	*P* value
12:0	0.044 ± 0.001	0.033 ± 0.001	< 0.001
14:0	2.454 ± 0.049	2.208 ± 0.059	< 0.01
14:1	0.783 ± 0.030	0.679 ± 0.029	< 0.05
15:0	0.370 ± 0.008	0.337 ± 0.009	< 0.01
15:1	0.034 ± 0.001	0.030 ± 0.001	< 0.05
16:0	25.811 ± 0.197	25.95 ± 0.224	n.s.
16:1	4.476 ± 0.099	3.855 ± 0.097	< 0.001
17:0	0.962 ± 0.019	1.008 ± 0.024	n.s.
17:1	1.015 ± 0.020	0.911 ± 0.027	< 0.01
18:0	10.027 ± 0.160	12.67 ± 0.263	< 0.001
18:1	50.801 ± 0.283	49.17 ± 0.324	< 0.001
18:2 n-6	2.286 ± 0.047	2.492 ± 0.056	< 0.01
18:3 n-3	0.106 ± 0.004	0.122 ± 0.004	< 0.01
CLA 9c,11t	0.276 ± 0.008	0.214 ± 0.007	< 0.001
20:0	0.060 ± 0.002	0.138 ± 0.006	< 0.001
20:1	0.494 ± 0.013	0.187 ± 0.009	< 0.001
ΣSFA	39.728 ± 0.307	42.34 ± 0.398	< 0.001
ΣMUFA	57.603 ± 0.303	54.83 ± 0.373	< 0.001
ΣPUFA	2.669 ± 0.052	2.828 ± 0.057	< 0.05

Values are expressed as mean (%) ± S.E.
SFA: saturated fatty acid. MUFA: monounsaturated fatty acid. PUFA: polyunsaturated fatty acid. Student's *t*-test.

The proportion of FAs with shorter chains (<C17) was significantly negatively correlated with those in type I myofibers in Wagyu cattle (C14:0 and C16:0, $P < 0.05$; C15:0 and C16:0, $P < 0.01$) (Table 2). Conversely, the proportion of FAs with longer chains (>C17) was significantly positively correlated with those in type I myofibers in Wagyu cattle (C18:1, C18:2 n-6, and C20:1; $P < 0.05$) (Table 2). Meanwhile, in Holstein cattle, the proportion of C16:0 was significantly negatively correlated with those in type I myofibers ($P < 0.05$) (Table 2). Conversely, the proportion of C18:0 was significantly positively correlated with those in type I myofibers ($P < 0.05$) (Table 2).

Table 2. Comparison of correlation coefficients between percentage of type I myofibers and fatty acid compositions in Wagyu and Holstein cattle

Fatty acid	Correlation coefficient†					
	Wagyu		Holstein		Wagyu + Holstein	
12:0	-0.221		0.030		-0.146	
14:0	-0.456	*	-0.132		-0.302	
14:1	-0.407		-0.205		-0.320	*
15:0	-0.601	**	-0.126		-0.386	*
15:1	-0.024		-0.162		-0.131	
16:0	-0.629	**	-0.457	*	-0.529	***
16:1	-0.481	*	-0.289		-0.398	**
17:0	0.030		0.263		0.187	
17:1	-0.298		-0.115		-0.230	
18:0	0.383		0.429	*	0.402	**
18:1	0.473	*	0.029		0.180	
18:2 n-6	0.460	*	0.391		0.423	**
18:3 n-3	-0.034		0.035		0.068	
CLA 9c,11t	0.171		-0.297		-0.120	
20:0	0.324		0.339		0.254	
20:1	0.466	*	0.326		0.082	

†Pearson's correlation coefficient. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

FAs with shorter chains (<C17) were significantly positively correlated with those in type II (IIA+IIB) myofibers in Wagyu cattle (C15:0 and C16:0, $P < 0.01$; C16:1, $P < 0.05$). FAs with longer chains (>C17) were significantly negatively correlated with those in type II (IIA+IIB) myofibers in Wagyu cattle (C18:1, C18:2 n-6, and C20:1; $P < 0.05$). Meanwhile, in Holstein cattle, the proportion of C16:0 was positively correlated with those in type II (IIA+IIB) myofibers. The proportion of C18:0 was significantly negatively correlated with those in type II (IIA+IIB) myofibers in Holstein cattle. In Wagyu + Holstein cattle, the proportion of FAs with shorter chains (<C17) was positively correlated with those in type II (IIA+IIB) myofibers (C14:1 and C15:0, $P < 0.05$; C16:0, $P < 0.001$; C16:1, $P < 0.01$). On the other hand, the proportion of FAs with longer chains (<C17) was significantly negatively correlated with those in type II (IIA+IIB) myofibers (C18:0 and C18:2 n-6; $P < 0.01$).

Table 3. Comparison of correlation coefficients between percentage of type II myofibers and fatty acid compositions in Wagyu and Holstein cattle

Fatty acid	Correlation coefficient†					
	Wagyu		Holstein		Wagyu + Holstein	
12:0	0.180		-0.030		0.132	
14:0	0.420		0.132		0.287	
14:1	0.391		0.205		0.313	*
15:0	0.571	**	0.126		0.372	*
15:1	0.031		0.162		0.136	
16:0	0.596	**	0.457	*	0.512	***
16:1	0.486	*	0.289		0.401	**
17:0	-0.053		-0.263		-0.199	
17:1	0.320		0.115		0.241	
18:0	-0.403		-0.429	*	-0.412	**
18:1	-0.431	*	-0.029		-0.158	
18:2 n-6	-0.450	*	-0.391		-0.421	**
18:3 n-3	-0.002		-0.035		-0.082	
CLA 9c,11t	-0.206		0.297		0.111	
20:0	-0.348		-0.339		-0.262	
20:1	-0.440	*	-0.326		-0.067	

†Pearson's correlation coefficient. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

IV. DISCUSSION

In this study, the percentage of type I myofibers was positively correlated with IMF contents. On the other hand, the percentages of type IIA and IIB myofibers were negatively related to IMF contents. These findings suggest that myofiber type metabolism and composition are related

to IMF accumulation in cattle. The results of this study are consistent with those of a previous report in Japanese Black cattle (Gotoh 2003). Meanwhile, in humans, a relationship between muscle fiber type and obesity was reported (Tanner et al. 2001). They observed a reduced percentage of type I and an increased percentage of type IIB myofibers in obese compared with lean individuals. This suggests that myofiber type composition affects not only fat storage in the body, but also IMF accumulation in cattle.

In this study, the percentages of saturated FAs were significantly lower in Wagyu cattle and the percentages of unsaturated FAs were significantly higher in Wagyu compared with Holstein cattle. The IMF in Wagyu cattle tended to be monounsaturated compared with that in Holstein cattle. This trend was stronger in muscles with higher percentages of IMF contents. Stearoyl-CoA desaturase (SCD) catalyzes the synthesis of monounsaturated FAs. The genotype of SCD is related to the FA composition in Japanese Black cattle (Taniguchi et al. 2004a). The levels of SCD mRNA expression in muscle and adipose tissue were significantly higher in Wagyu than in Holstein cattle (Taniguchi et al. 2004b). The breed characteristics of a higher mRNA expression of SCD in Wagyu cattle would be associated with breed differences in the profile of FA composition compared with Holstein cattle.

V. CONCLUSIONS

The current findings suggest that myofiber type compositions in skeletal muscles of cattle are closely related to intramuscular FA compositions. Moreover, their relationships differ between Wagyu and Holstein cattle. The IMF in Wagyu cattle tended to be unsaturated compared with that in Holstein cattle.

VI. REFERENCES

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