Molecular Species Compositions of Triacylglycerols of Subcutaneous Fat, and Their Association with Total Lipid Content of *M. longissimus thoracis* of Fattened Beef Cattle

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Abstract The compositions of fatty acid and molecular species of triacylglycerol of subcutaneous fat of fattened Japanese Black, Japanese Shorthorn and Holstein steers were determined by gas chromatography. There were significant differences in heptadecanoic acid concentration among three breeds, and in six acid (myristic, myristoleic, palmitic, palmitoleic, anteiso-heptadecanoic and stearic acids) concentrations between two breeds of three breeds. The relationship between palmitic and oleic acid concentrations was negative. The major molecular species of subcutaneous triacylglycerol were myristoyl (M)-palmitoyl (P)-oleoyl (O)-glycerol (MPO), PPO, P-Palmitoleoyl (Po)-O-glycerol ([PPoO]), P-stearoyl (S)-O-glycerol (PSO) and POO. POO had the highest concentration in these molecular species of three breeds. Japanese Black had higher concentrations of PSO and SOL than those of Holstein. Relationships between POL and POO, and PPO and POO were positive and negative, respectively. In order to analyze the association of total lipid content of *M. longissimus thoracis* and molecular species composition of triacylglycerol, multiple regression analysis was carried out. In the linear multiple regression analysis, the regression equation of Japanese Black had a good multiple correlation coefficient (0.8110). In discriminant analysis of samples separated according to meat quality score of carcass, the percentage of discrimination were 100% for each group of Japanese Black and Japanese Shorthorn.

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Key words : Triacylglycerol, Molecular species, Subcutaneous fat, Beef

The quantity and the chemical composition of depot fat of meat are important factors affecting the organoleptic qualities of meat^{2, 4, 8, 16, 18, 20-21)}. Characteristics of depot fats are affected by many factors including breed²²⁾, sex²²⁾, age⁶⁾, diet⁷⁾, degree of fatness¹¹⁾ and the others^{10, 19)}. Depot fat consists mainly of triacylglycerol, therefore, characteristics of triacylglycerol may influence meat qualities in many ways. The analysis of triacylglycerol characteristics of beef has been carried out by

fatty acid compositions mainly, and only a few carbon number distribution of triacylglycerol^{12-13, 15)}, while there were no reports for compositions of molecular species of triacylglycerol isolated from subcutaneous fat of Japanese beef cattle. Compositions of molecular species of intramuscular triacylglycerol were determined previously⁵⁾. In the present paper, compositions of the fatty acid and molecular species of triacylglycerol of subcutaneous fat of the fattened beef cattle were

compared, and associations between molecular species compositions of subcutaneous triacylglycerol and total intramuscular lipid content of *M. longissimus thoracis* or meat quality score were analyzed.

Materials and Methods

Samples for the fatty acid and molecular species determinations were obtained from subcutaneous fat at between the tenth and the eleventh rib of carcasses, stored at 2°C for 24 to 30 hours after slaughter, of Japanese Black ($n=10$), Japanese Shorthorn ($n=10$) and Holstein steers ($n=30$). The details of fattening conditions for these steers were unknown. Extraction of total lipid from samples were carried out by the method of Folch *et al.*³⁾. The mean values of total lipid contents with standard deviation in *M. longissimus thoracis* were $30.0 \pm 8.3\%$ for Japanese Black, $11.2 \pm 2.2\%$ for Japanese Shorthorn, and $18.0 \pm 4.8\%$ for Holstein steers. Fractionation of triacylglycerol from total lipid was carried out by thin layer chromatography using Silica gel 60 H and hexan : diethylether : acetic acid (80 : 30 : 1) for the development⁵⁾.

Fatty acid and molecular species compositions of triacylglycerol were determined by gas chromatography described previously⁵⁾.

In order to evaluate the relation of total lipid concentration in *M. longissimus thoracis* and molecular species composition, the stepwise multiple regression analysis was carried out⁴⁾. In addition to this analysis, the stepwise discriminant analysis was carried out to analyze the relation of meat quality score of carcass and molecular species composition. The values of F_{in} and F_{out} of these analyses were 2.0000 and 1.9999, individually.

Results and Discussion

Fatty acid and molecular species compositions of triacylglycerol

Fatty acid compositions of subcutaneous triacylglycerol, and sums and ratios of various

types of fatty acids are shown in Table 1. Sums of major fatty acid (palmitic acid (C16 : 0), stearic acid (C18 : 0) and oleic acid (C18 : 1)) concentrations were approximately 80% for Japanese Black, 85% for Japanese Shorthorn and 80% for Holstein. Significant difference ($P<0.01$) among three breeds was found for heptadecanoic (C17 : 0) acid concentration, and there were significant differences ($P<0.01$) between two of three breeds for myristic (C14 : 0), myristoleic (C14 : 1), palmitic (C16 : 0), palmitoleic (C16 : 1), anteiso-heptadecanoic (a-C17 : 0) and stearic (C18 : 0) acid concentrations. Sums of saturated fatty acids (SFA) of Japanese Black and Holstein steers were lower levels than that of Japanese Shorthorn, while sums of mono-unsaturated fatty acids (MUFA) and unsaturated fatty acids (UFA) of these two breeds were higher than that of Japanese Shorthorn. The ratios of O/S and Po/S for Japanese Shorthorn were lower than those of Japanese Black and Holstein.

The relationship between oleic acid (C18 : 1) and palmitic acid (C16 : 0) concentrations of subcutaneous triacylglycerol of three breeds is shown in Fig. 1. There were negative relationships between C18 : 1 and C16 : 0 in subcutaneous triacylglycerols of three breeds. Leat⁷⁾ showed that the percentage of C18 : 0 in subcutaneous fat of barley-fed Angus and Friesian decreased with age, and was replaced by octadecenoic acid (C18 : 1) and hexadecenoic acid (C16 : 1) in the rearing period. These findings suggested that the relationships between C18 : 0 and C18 : 1 or C16 : 1 were negative. In the present study, the negative relationships between C18 : 0 and C16 : 1 was observed clearly for Japanese Black and Japanese Shorthorn, but not for Holstein. Relationships between C18 : 0 and C18 : 1 were not significant for the three breeds. In addition, there was the negative relationship between C16 : 0 and C18 : 1. Fatty acids having eighteen carbon atoms are formed from C16 : 0 by the fatty acid elongation system. After then,

Table 1. Fatty acid compositions of the subcutaneous triacylglycerols of Japanese Black, Japanese Shorthorn and Holstein steers

Fatty acid	unit : % (w/w)		
	Japanese Black n=10	Japanese Shorthorn n=10	Holstein n=30
C 14 : 0 ¹⁾	2.79 (0.65) ⁴⁾ a	3.10 (0.89) ^{ab}	3.66 (0.73) ^b
C 14 : 1	1.68 (0.66) ^a	0.90 (0.28) ^b	1.98 (0.76) ^a
C 15 : 0	0.52 (0.17)	0.41 (0.10)	0.55 (0.11)
C 16 : 0	23.79 (1.37) ^a	25.99 (2.37) ^{ab}	26.66 (1.32) ^b
C 16 : 1	6.26 (1.35) ^a	4.26 (1.47) ^b	6.19 (1.32) ^a
a ²⁾ -C 17 : 0	0.83 (0.16) ^a	0.67 (0.16) ^a	0.46 (0.14) ^b
i ³⁾ -C 17 : 0	0.89 (0.17)	0.91 (0.10)	0.83 (0.12)
C 17 : 0	1.28 (0.21) ^a	0.78 (0.16) ^b	0.98 (0.20) ^c
C 18 : 0	7.20 (1.79) ^a	13.26 (3.09) ^b	5.07 (2.61) ^a
C 18 : 1	49.66 (3.03)	46.34 (5.14)	47.89 (4.83)
C 18 : 2	1.99 (0.35)	1.94 (0.46)	2.46 (0.68)
SFA ⁵⁾	37.28 (2.04) ^a	44.44 (5.81) ^b	38.20 (3.76) ^{ab}
MUFA ⁶⁾	57.60 (2.49) ^a	51.46 (5.37) ^b	56.05 (4.00) ^a
PUFA ⁷⁾	1.99 (0.35)	1.92 (0.44)	2.46 (0.68)
UFA ⁸⁾	59.59 (2.50) ^a	53.38 (5.43) ^b	58.52 (3.86) ^a
MUFA/SFA	1.55 (0.15) ^a	1.19 (0.26) ^b	1.49 (0.25) ^{ab}
PUFA/SFA	0.054 (0.010) ^{ab}	0.044 (0.011) ^a	0.065 (0.019) ^b
Po+S+O ⁹⁾	63.11 (3.07) ^a	62.99 (3.42) ^a	59.15 (3.32) ^b
O/S ¹⁰⁾	7.27 (1.66) ^a	4.11 (1.53) ^b	7.99 (3.56) ^a
Po/S ¹¹⁾	0.96 (0.37) ^a	0.42 (0.32) ^b	1.08 (0.47) ^a

¹⁾ Chain-length : Number of double bonds ²⁾ anteiso ³⁾ iso ⁴⁾ mean value with standard deviation in bracket ⁵⁾ saturated fatty acid (C 14 : 0+C 15 : 0+C 16 : 0+a-C 17 : 0+i-C 17 : 0+C 17 : 0+C 18 : 0) ⁶⁾ mono-unsaturated fatty acid (C 14 : 1+C 16 : 1+C 18 : 1) ⁷⁾ poly-unsaturated fatty acid (C 18 : 2) ⁸⁾ unsaturated fatty acid (MUFA+PUFA) ⁹⁾ C 16 : 1+C 18 : 0+C 18 : 1 ¹⁰⁾ C 18 : 1/C 18 : 0 ¹¹⁾ C 16 : 1/C 18 : 0
a, b, c : Means in the same row with different superscript letters are significantly different (P<0.01).

C 18 : 1 is formed from C 18 : 0 by the action of fatty acyl-CoA desaturase. Present relationships may suggest that C 18 : 1 may be produced by the systems, described above, from C 16 : 0 during fatty acid synthesis. C 16 : 1 and C 18 : 0 for Japanese Black and Japanese Shorthorn are synthesized competitively from C 16 : 0.

Molecular species compositions of subcutaneous triacylglycerols of Japanese Black, Japanese Shorthorn and Holstein steers are shown in Table 2. Only molecular species present at >1% of the total molecular species of the over twenty five species observed are shown. The

major molecular species were myristoyl (M)-palmitoyl (P)-oleoyl (O)-glycerol ([MPO]), PPO, P-palmitoleoyl (Po)-O-glycerol (PPoO), P-stearoyl (S)-O-glycerol (PSO) and POO. POO had the highest concentration in the molecular species of the three breeds. Japanese Black had higher concentrations of PSO and SOL than those of Holstein, while Holstein had higher concentration of [PoPoPo], P-hexadecanoyl (H)-L-glycerol ([PHL]) and POL than those of Japanese Black. Concentrations of some molecular species of Japanese Shorthorn were medium values between the other breeds. Tsuneishi *et al.*¹⁵⁾ found that C 52 having two

Molecular Species of Subcutaneous Triacylglycerols

Fig. 1. Relationships between oleic acid and palmitic acid (A), and stearic acid and palmitoleic acid (B) concentrations of the subcutaneous triacylglycerols of Japanese Black, Japanese Shorthorn and Holstein steers.

Table 2. Molecular species compositions of subcutaneous triacylglycerols of Japanese Black, Japanese Shorthorn and Holstein steers

Species	unit : % (w/w)		
	Japanese Black n = 10	Japanese Shorthorn n = 10	Holstein n = 30
MMP ¹⁾	2.83 (1.26) ³⁾	2.16 (0.83)	1.63 (0.93)
[MPPo] ²⁾	2.40 (1.14)	2.65 (0.86)	2.91 (1.36)
[MPS]	4.09 (2.22)	3.21 (1.63)	3.86 (1.44)
[MPO]	8.36 (3.82)	8.49 (2.50)	9.02 (2.52)
[PoPoPo]	2.93 (1.65) ^a	4.84 (1.24) ^b	5.12 (1.42) ^b
MSS	3.36 (1.34)	2.76 (1.43)	2.43 (0.79)
PPO	14.83 (3.15)	13.12 (1.74)	15.23 (1.52)
[PPoO]	8.51 (2.29)	10.67 (2.83)	13.00 (1.59)
[PHL]	1.30 (0.54) ^a	1.85 (0.50) ^a	2.49 (0.42) ^b
PSO	12.22 (4.54) ^a	9.88 (5.14) ^{ab}	7.49 (2.39) ^b
POO	25.75 (5.32)	25.70 (4.75)	24.37 (4.61)
POL	2.80 (1.02) ^a	3.31 (1.03) ^{ab}	4.65 (1.06) ^b
SOO	2.46 (1.60) ^a	1.99 (0.72) ^a	<0.5 ^b
SOL	5.24 (3.02) ^a	4.66 (1.33) ^a	2.92 (1.72) ^b
OOO	3.00 (1.79)	3.53 (1.23)	3.23 (1.80)

¹⁾ M : myristoyl, P : palmitoyl, Po : palmitoleoyl, H : heptadecanoyl, S : stearyl, O : oleoyl, L : linoleoyl ²⁾ presumed species ³⁾ mean value with standard deviation in bracket

^{a,b} : Means in the same row with different superscript letters are significantly different (P < 0.01).

(Only molecular species present at >1% of total molecular species for one breed are shown.)

double bonds was the most predominant, and C 50s having one double bond and two double bonds were higher than other molecules in triacylglycerol of subcutaneous fat of beef cattle. POO (C 52) having 52 of total carbon number in the acyl groups, has two double bonds, and PPO (C 50) and [PPoO] (C 50) have one and two double bonds in acyl groups, respectively. The concentration of these molecular species in the present study agreed with the results of Tsuneishi *et al.*¹⁵⁾.

Relationships between POO and PPO, and POO and POL concentrations in subcutaneous triacylglycerols of the three breeds are shown in Fig. 2. There were negative relationships ($p < 0.01$) between POO and PPO of each breed, and positive relationships ($P < 0.01$) between POO and POL of each breed. There were no significant slopes among linear regressions for the three breeds from results of the analysis of covariance, while there were significant differences ($P < 0.05$) of center positions of data and intercepts of regressions between Japanese Black and Holstein in POO-PPO relationships. These relationships were similar to that of the

intramuscular triacylglycerol¹⁶⁾. The concentrations of POO and POL, and POO and PPO in triacylglycerol may affect reciprocally at synthetic stages of triacylglycerol molecule for depot fat.

The coefficients of the pattern similarity^{12,14)}, showing total similarities of fatty acid and molecular species compositions of triacylglycerol, are shown in Table 3. The coefficient between Japanese Black and Japanese Shorthorn was larger than that between Japanese Black and Holstein. These relationships show that fatty acid and molecular species compositions between Japanese Black and Japanese Shorthorn were closer than that between Japanese Black and Holstein on the whole.

Relation of molecular species composition of triacylglycerol and lipid content of *M. longissimus thoracis* or meat quality score of carcass

There were no relationships of the second order regression or the exponential regression between total lipid content of *M. longissimus thoracis* and any molecular species concentrations of subcutaneous triacylglycerol for the

Fig. 2. Relationships between POO and PPO (A), and POO and POL (B) concentrations of the subcutaneous triacylglycerols of Japanese Black, Japanese Shorthorn and Holstein steers.

Molecular Species of Subcutaneous Triacylglycerols

Table 3. Coefficients of pattern similarities among three breeds based on the fatty acid composition and the molecular species composition of the subcutaneous triacylglycerol

(1) Pattern similarity based on the fatty acid composition			
	J. Black	J. Shorthorn	Holstein
Japanese Black	1.00000	0.99855	0.99084
Japanese Shorthorn	0.99855	1.00000	0.98950
Holstein	0.99084	0.98950	1.00000

(2) Pattern similarity based on the molecular species composition			
	J. Black	J. Shorthorn	Holstein
Japanese Black	1.00000	0.99169	0.94713
Japanese Shorthorn	0.99169	1.00000	0.97891
Holstein	0.94713	0.97891	1.00000

three breeds. Therefore, in order to analyze the relation of molecular species composition of subcutaneous triacylglycerol and total lipid content of *M. longissimus thoracis*, multiple regression analysis were carried out⁴⁾. In multiple regression analysis, using total lipid concentrations of *M. longissimus thoracis* for the dependent variable (y) and molecular species concentrations of triacylglycerol for the independent variable, the linear multiple regression equation $y = -2.13 (\text{PPO}) + 7.07 (\text{PHL}) + 52.43$ was obtained for Japanese Black. This regression equation with significant F value ($P < 0.05$) had good multiple correlation coefficient (0.8110) and coefficient of determination (0.6578). The regression equations $y = 0.22 (\text{POO}) + 5.50$ for Japanese Shorthorn, and $y = -0.86 (\text{PPO}) + 27.94$ for Holstein had low multiple correlation coefficients of 0.4635 for Japanese Shorthorn and 0.2648 for Holstein. In these equations, there were no common independent variables selected for three breeds, while PPO was a common variable for Japanese Black and Holstein. Using combined data of Japanese Black, Japanese Shorthorn and Holstein, the regression equation had low multiple correlation coefficient of 0.4691. In multiple regression analysis, using molecular species concen-

trations of intramuscular triacylglycerol in *M. longissimus thoracis*⁵⁾ for the independent variable, the regression equations of Japanese Black and Japanese Shorthorn had high multiple correlation coefficients of 0.9504 and 0.9625 respectively, while the regression equation of Holstein had low coefficient of 0.2368. From these results, molecular species compositions of subcutaneous triacylglycerol of Japanese Shorthorn and Holstein may have no close relationships with total lipid concentration of *M. longissimus thoracis*.

In order to analyze the relation of meat quality score of carcass and molecular species composition of subcutaneous triacylglycerol, stepwise linear discriminant analysis was carried out⁴⁾. In the discriminant analysis, samples were separated into two groups according to meat quality score of grading for carcass. Two groups for Japanese Black were G1 for 3 (1 animal)+4 (3 animals) of meat quality score and G2 for 5 (6 animals) of score. Two groups for Japanese Shorthorn and for Holstein were G1 for 2 (5 animals) of meat quality score and G2 for 3 (5 animals) of score, and G1 for 2 (2 animals)+3 (15 animals) of score and G2 for 4 (13 animals) of score, respectively. Results of the linear discriminant analysis are shown in

Table 4. Percentages of discrimination using the best two discriminant functions were 100% of each group for Japanese Black and Japanese Shorthorn, and 82.4 to 84.6% for Holstein. For Japanese Black, [MPS], [PHO] and POL were

included in the discriminant functions. For Japanese Shorthorn PoPoPo, MSS and [PPoO] were included. Holstein had two discriminant factors of PPO and [PPoO]. [PPoO] was a common factor for Japanese Shorthorn and

Table 4. Discriminant functions and the results of discrimination for Japanese Black, Japanese Shorthorn and Holstein steers using the stepwise linear discriminant analysis procedures

(1) Japanese Black

(a) Discriminant functions

$$G1 = 11.7 \times ([MPS]) + 30.1 \times ([PHO]) + 22.8 \times (POL) - 52.6$$

$$G2 = 14.5 \times ([MPS]) + 43.6 \times ([PHO]) + 30.2 \times (POL) - 89.3$$

(b) Result of discrimination

Group	No. of animals	No. of animals classified		Percent correct
		G 1	G 2	
G 1	4	4	0	100.0
G 2	6	0	6	100.0

(G 1 : 3 and 4 of meat quality score, G 2 : 5 of meat quality score)

(2) Japanese Shorthorn

(a) Discriminant functions

$$G1 = -54.7 \times ([PoPoPo]) + 50.6 \times (MSS) + 75.1 \times ([PPoO]) - 353.7$$

$$G2 = -48.8 \times ([PoPoPo]) + 44.5 \times (MSS) + 66.3 \times ([PPoO]) - 272.6$$

(b) Result of discrimination

Group	No. of animals	No. of animals classified		Percent correct
		G 1	G 2	
G 1	5	5	0	100.0
G 2	5	0	5	100.0

(G 1 : 2 of meat quality score, G 2 : 3 of meat quality score)

(3) Holstein

(a) Discriminant functions

$$G1 = 4.8 \times (PPO) + 4.6 \times ([PPoO]) - 57.9$$

$$G2 = 4.3 \times (PPO) + 5.1 \times ([PPoO]) - 58.5$$

(b) Result of discrimination

Group	No. of animals	No. of animals classified		Percent correct
		G 1	G 2	
G 1	17	14	3	82.4
G 2	13	2	11	84.6

(G 1 : 2 and 3 of meat quality score, G 2 : 4 of meat quality score)

Holstein. In discriminant analyses using combined data of the three breeds, percentages of discrimination were not good in any cases of grouping for four groups (2, 3, 4 and 5 of meat quality score), three groups (2+3, 4 and 5, or 2, 3 and 4+5 of meat quality score) or two groups (2 and 3+4+5, 2+3 and 4+5, or 2+3+4 and 5 of meat quality score). These results suggest that there were no common discriminant factors for the three breeds for discrimination based on meat quality score.

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肥育牛の皮下脂肪トリアシルグリセロールの分子種組成および その胸最長筋全脂質含量との関連性

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肥育された黒毛和種, 日本短角種およびホルスタイン種の皮下脂肪のトリアシルグリセロールの脂肪酸組成および分子種組成をガスクロマトグラフィーによって測定した。いずれかの二品種間には7つの脂肪酸(ミリスチン酸, ミリストレン酸, パルミチン酸, パルミトレイン酸, anteiso-ヘプタデカン酸, ヘプタデカン酸およびステアリン酸)の濃度に差が認められた。パルミチン酸とオレイン酸の濃度間には負の関係があった。皮下脂肪のトリアシルグリセロールの主要分子種は, ミリストイル (M)-パルミトイル (P)-オレオイル (O)-グリセロール (MPO), PPO, P-パルミトレオイル (Po)-O-グリセロール ([PPoO]), P-トテアロイル (S)-O-グリセロール (PSO) および POO であった。これらの中で三品種とも POO が最も多かった。黒毛和種では PSO および SOL の含量がホルスタイン種より多かった。POL と POO 濃度間には正, PPO と POO 間には負の関係があった。胸最長筋の全脂肪含量と分子種組成との関連性を解析するために重回帰分析を行ったところ, 黒毛和種の重回帰式が良好な重相関係数を持っていた。試料を採取した枝肉の肉質等級を二群に分類して行った判別分析では, 黒毛和種および日本短角種で二群とも 100% の正判別率であった。

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