

## Developmental Relationship of Unsaturated Fatty Acid Composition and Stearoyl-CoA Desaturase mRNA Level in Hanwoo Steers' Muscle

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**ABSTRACT** : This study was conducted to investigate the developmental relationship between fatty acid composition in different lipid fractions and stearoyl-CoA desaturase (SCD) gene expression in steer muscles during growth. Twenty Hanwoo steers were used at 6, 12, 18, 24 and 30 months of age. Fatty acid composition and SCD mRNA level were analyzed. In the total lipid fraction, developmental profiles of C18:1, as the product of SCD enzyme, and SCD mRNA level were significantly increased between 6 months and 12 months of age. During this period, the percentage of C18:1 increased from 31.9% to 49.5% in the total lipid. The increased C18:1 level was maintained until 30 months of age within the range of 44.8- 49.9%. In contrast, the C18:0 composition decreased with age and this decrease was compensated by the increase of the C18:1. However, the sum of C18:0 and C18:1 was changed before and after 12-month old by a 20% increase. Unlike the C18 fatty acids, the C16 fatty acids such as C16:0 and C16:1 did not show a consistent change with age in steers' muscle. On the other hand, C18:2 proportion as a major polyunsaturated fatty acid in muscle was significantly reduced from 21.1% at 6 months of age to 4.4% at 12-months old and then this reduced level was maintained until 30 months within the range of 7.4-11.4%. As in the C18:1 composition during early stages, a 2-fold significant increase was observed in the  $\Delta^9$ -desaturase index of C18 fatty acid as a measure of SCD activity, but not in that of C16 fatty acid. Also, the steady-state level of SCD mRNA reached a peak at 12 months of age. Thus, the positive relationship between the C18:1 composition and the  $\Delta^9$ -desaturase (SCD enzyme) index of C18 fatty acid or SCD mRNA level was demonstrated during growth, but the negative relationship between the C18:2 composition and the above three indices was demonstrated at the same time, indicating that the sharp induction of SCD mRNA may be closely related to the dramatic reduction of C18:2, which is known as a suppressor of SCD gene expression during growth. (*Asian-Aust. J. Anim. Sci.* 2005, Vol 18, No. 4 : 562-566)

**Key Words** : Fatty Acid, Stearoyl-CoA Desaturase, Hanwoo Muscle

### INTRODUCTION

Recently, there has been a lot of interest in increasing unsaturated fatty acids in beef and decreasing saturated fatty acids due to their effects on human health. The consumption of high levels of saturated fat in human diet, in conjunction with obesity, is a major risk factor for the development of vascular and coronary diseases (Department of Health, 1994). Monounsaturated fatty acid lowers serum LDL cholesterol and thereby may have a positive effect on coronary heart disease (Salter et al., 1998). Ruminant animals in general have relatively high ratios of saturated to monounsaturated fatty acids in their lipids (Harfoot and Hazlewood, 1988), which are consumed by humans as marbled meat and milk products. High levels of unsaturated fatty acids in ruminants are desirable for human health (Malau-Aduli et al., 1997). Efforts to increase the unsaturated fatty acid content of ruminant tissues have been tried through dietary supplement or genetic selection. Saturated (C16:0 and C18:0) and monounsaturated (C16:1

and C18:1) fatty acids comprised nearly 90% of Hanwoo muscle lipid, and the C18:1 composition also comprises nearly 90% of the monounsaturated fatty acids (Kim et al., 2002; Lee et al., 2004). Furthermore, 75% of the polyunsaturated fatty acids comprised the essential C18:2 fatty acid (Siebert et al., 1996). These fatty acid compositions, in addition to marbling, are the main factors for meat quality in terms of texture and taste, which are improved by an increase in the ratio of monounsaturated fatty acid to saturated fatty acid (Yang et al., 1999; Kim et al., 2002). A number of reports have been published relating to fatty acids composition in adipose and muscle tissues. Fatty acids composition was influenced by diet (Mandell et al., 1998; Kim et al., 2004), sex (Zembayashi et al., 1995), age (Hecker et al., 1975) and breed (Siebert et al., 1996), and depends on SCD activity, which is responsible for conversion of saturated fatty acids (C16:0 and C18:0) into monounsaturated fatty acids (C16:1 and C18:1) (Ntambi, 1999). Stearoyl-CoA desaturase (SCD) activity and mRNA level are higher in adipose tissue and muscle compared with liver tissue of cattle. Stearoyl-CoA desaturase (SCD) gene expression is very sensitive to nutritional, hormonal and metabolic factors (Ntambi, 1999). From these facts, the involvement of several factors in the regulation of SCD gene expression is expected during physiological

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maturation of cattle, but the mechanism of developmental regulation is still unclear. Certain polyunsaturated fatty acids including C18:2 repress SCD activity in adipose tissue through depressing the expression of SCD gene (Choi et al., 2001). The relationship between the C18:2 and SCD mRNA or desaturation activity has not been evaluated *in vivo* in animals during growth. Therefore, the objectives of the present study were to investigate the relationship between SCD gene expression and fatty acid composition in bovine muscle at different growth stages.

## MATERIALS AND METHODS

### Animals, diet and sampling

Twenty male Korean Hanwoo steers were used in this study, and the animals were castrated at 3-month old. Steers were slaughtered at 6, 12, 18, 24 and 30-months old, respectively. A commercial concentrate was offered at 1.5% and 1.8% of body weight on growing (6-months) and early fattening stage (6-12 months), respectively. Steers were offered *ad libitum* concentrates in the later fattening stage (13-30 months). Steers were fed forage *ad libitum* and had free access to fresh water during the whole period. Muscle samples were taken from *longissimus dorsi* between 5<sup>th</sup> and 6<sup>th</sup> lumbar vertebrae of steers. All samples were immediately frozen in liquid nitrogen and stored at -80°C until analysis.

### Fatty acid analysis

Total lipids were extracted from 200 mg of loin muscle using methods that were previously described (Folch et al., 1957). After extraction of total lipid, individual triglyceride and phospholipids classes were separated by thin layer chromatography utilizing Silica Gel H (Merck, Darmstadt, Germany) with chloroform:methanol:water (45:35:10, v/v/v) as the developing solvent system. Appropriate standards were visualized by exposure to iodine vapor. Adjacent unexposed sample areas were scraped into vials and fatty acid esters prepared as previously described (Litman, 1975). Fatty acid analysis was performed by gas-liquid chromatography (model 437, Chrompack, Claritan, NJ), using a Packard Chrompack equipped with stainless steel column (3 mm×10 ml) packed with chromasorb W-AW 80/100 (Supelco, Inc, Bellefonte, PA, Netherlands). Injector and detector temperatures were 225°C and 215°C, respectively. The flow rate of nitrogen as a carrier gas was 22 ml/min. Lauric acid (C12:0) standard methyl esters as an internal standard was added. Fatty acid peaks were converted to amounts of fatty acids according to calculations described by Solver and Lanza (1979).

### SCD mRNA analysis by RT-PCR

Total RNA was isolated from loin muscle using Trizol

reagent (Life Technologies Inc., Grand Island, NY, USA) according to the manufacturer's instructions. Following RNA isolation, the concentration and purity of the prepared RNA were analyzed by optical density measurements at 260/280 nm. To evaluate the quality and normalize the amount of cDNA from each sample, G3PDH gene was used as an internal control. Using 2 µl of the 20 µl reverse transcription reaction, primers for G3PDH were mixed with 5 U Taq DNA polymerase, 2.5 mM each of dNTP into a total volume of 40 µl, and then divided into 4 tubes of 10 µl each. The PCR for G3PDH and SCD were amplified for 16, 20, 28 and 35 cycles.

Initially, the PCR reaction started at 95°C for 5 min for pre-denaturation, and the condition was set at 95°C for 1 min, 55°C for 1 min and 72°C for 1 min. The quantity of G3PDH and SCD PCR product was estimated with band intensity, and then the optimal condition of PCR cycle was determined.

To quantify the relative amount of gene transcription for each treatment, PCR was carried out in a 20 µl reaction mixture containing 2 µl of first strand cDNA, 2 µl of 10×PCR buffer, 1 µl of dNTP mix, 10 pmol of each primer and 2.5 U Taq DNA polymerase. The specific primers for SCD analysis were designed through the nucleotide sequences of *Bos taurus* SCD mRNA (GenBank accession No. AY241933). The sense and antisense primers were 5'-ATC ATC CTC ATG GCC CTG-3', and 5'-TGG TAG TTG TGG AAG CCC-3', respectively. Glyceraldehyde-3-phosphate dehydrogenase (G3PDH) gene (GenBank accession No. XM228474) was used as an internal control. The primer sequences of sense and antisense were 5'-ATT GAC CTC AAC TAC ATG G-3' and 5'-AAG CAG TTG GTG GTG CAG G-3', respectively.

### Statistical analysis of data

Data were analyzed using the GLM procedure of SAS program (SAS version 8.1; SAS institute, Cary, NC, USA). The analysis of variance was used to indicate significant differences (\*\* p<0.01 and \* p<0.05) among means. When indicated by analysis of variance, means were separated by the Student-Newman-Keuls method (Ott, 1984).

## RESULTS AND DISCUSSION

### Fatty acids composition of different muscle lipid fractions

The muscle contents of fatty acids are expressed as proportion times 100 in Table 1 and 2 respectively. The major fatty acids in total lipid and the triglyceride fraction of each were C16:0, C18:0 and C18:1 which together accounted for around 84% and 90% of total fatty acid, respectively. The three major fatty acids in the present study was relatively higher than in another study which observed

**Table 1.** The fatty acid composition in total lipid and triglyceride fractions of Hanwoo steers' muscle at different growth stages (mean±SD, n=4)

Items	Total lipid					Triglyceride				
	6	12	18	24	30	6	12	18	24	30
	Growth stage (months)					Growth stage (months)				
Fatty acid (%)										
C16:0	24.1±2.03	26.8±0.9	23.9±1.1	26.3±1.3	25±0.7	27.6±0.4	26.5±0.9	27.6±1.2	25.9±1.0	24.9±0.8
C16:1	5.0±0.27	5.2±0.4	5.2±0.7	6.0±0.3	5.5±0.5	4.6±1.3	3.2±0.1	5.2±0.9	5.9±0.4	4.3±0.4
C18:0	17.8±0.81 <sup>a</sup>	14.2±1.6 <sup>b</sup>	14.5±0.7 <sup>b</sup>	9.8±0.9 <sup>b</sup>	11.1±1.0 <sup>b</sup>	23.7±2.6 <sup>a</sup>	20.5±1.0 <sup>b</sup>	16.0±2.3 <sup>b</sup>	9.3±0.3 <sup>c</sup>	14.6±3.0 <sup>b</sup>
C18:1	31.9±2.17 <sup>a</sup>	49.5±2.4 <sup>B</sup>	44.8±1.3 <sup>B</sup>	49.9±1.3 <sup>B</sup>	49.0±1.7 <sup>B</sup>	29.7±2.9 <sup>a</sup>	45.6±1.4 <sup>B</sup>	47.1±4.0 <sup>B</sup>	56.0±0.8 <sup>C</sup>	52.7±2.3 <sup>D</sup>
C18:2	21.1±2.03 <sup>a</sup>	4.4±2.0 <sup>B</sup>	11.4±1.2 <sup>c</sup>	7.4±1.7 <sup>D</sup>	7.4±1.9 <sup>D</sup>	14.6±2.3 <sup>a</sup>	4.3±0.6 <sup>B</sup>	4.7±1.0 <sup>B</sup>	2.9±0.1 <sup>C</sup>	3.4±0.1 <sup>D</sup>
MUFA/SFA	0.88±0.23 <sup>a</sup>	1.33±0.04 <sup>b</sup>	1.30±0.15 <sup>b</sup>	1.55±0.2 <sup>b</sup>	1.50±0.2 <sup>b</sup>	0.7±0.2 <sup>a</sup>	1.04±0.1 <sup>b</sup>	1.26±0.4 <sup>b</sup>	1.76±0.2 <sup>b</sup>	1.47±0.28 <sup>b</sup>
Δ <sup>9</sup> -desaturase (C16)	0.21±0.07	0.19±0.05	0.21±0.3	0.23±0.07	0.22±0.02	0.16±0.1	0.12±0.1	0.19±0.1	0.22±0.1	0.17±0.12
Δ <sup>9</sup> -desaturase (C18)	1.8±0.1 <sup>A</sup>	3.5±0.05 <sup>B</sup>	3.1±0.7 <sup>B</sup>	5.1±1.0 <sup>C</sup>	4.5±0.9 <sup>C</sup>	1.3±0.2 <sup>A</sup>	2.2±0.12 <sup>B</sup>	2.9±0.3 <sup>B</sup>	6.0±0.3 <sup>C</sup>	3.6±0.8 <sup>D</sup>

Percentage of the total peak area of the fatty acids listed.

MUFA: monounsaturated fatty acid, SFA: saturated fatty acid, Δ<sup>9</sup>-desaturase (C16) = C16:1/C16:0, Δ<sup>9</sup>-desaturase (C18) = C18:1/C18:0.

<sup>A, B, C, D</sup> Means in the same row are significantly different from the control at p<0.01.

<sup>a, b, c, d</sup> Mean in the same row are significantly different from the control at p<0.05.

**Table 2.** The fatty acid composition in phospholipid fraction of Hanwoo steers muscle at different growth stages (mean±SD, n=4)

Items	Phosphatidyl choline					Phosphatidyl ethanolamine				
	6	12	18	24	30	6	12	18	24	30
	Growth stage (months)					Growth stage (months)				
Fatty acid (%)										
C16:0	29.6±2.3 <sup>a</sup>	31.7±3.3 <sup>a</sup>	42.2±2.4 <sup>b</sup>	29.8±3.0 <sup>c</sup>	29.7±2.1 <sup>c</sup>	28.2±2.0	25.4±4.3	30.9±4.2	30.9±2.3	30.4±1.42
C16:1	10.1±2.5 <sup>a</sup>	3.7±1.6 <sup>b</sup>	13.2±2.5 <sup>a,c</sup>	6.0±1.9 <sup>d</sup>	6.1±0.8 <sup>d</sup>	6.5±2.8	6.1±3.1	7.1±2.1	4.1±1.54	5.7±2.0
C18:0	11.8±1.3	9.9±3.0	8.8±5.2	10.8±2.0	9.9±1.2	20.6±1.2	18.2±2.0	17.0±1.0	17.3±2.3	15.9±2.1
C18:1	19.0±2.5 <sup>a</sup>	19.4±5.2 <sup>a</sup>	15.5±5.7 <sup>b</sup>	16.9±3.0 <sup>b</sup>	16.4±3.0 <sup>b</sup>	7.9±2.1 <sup>a</sup>	8.5±2.1 <sup>b</sup>	12.8±2.0 <sup>b</sup>	17.7±3.0 <sup>b</sup>	14.4±1.3 <sup>b</sup>
C18:2	29.4±2.4 <sup>a</sup>	20.6±3.0 <sup>b</sup>	14.6±1.7 <sup>c</sup>	28.1±1.2 <sup>a</sup>	33.5±1.3 <sup>ad</sup>	14.1±0.12	14.6±2.7	18.3±2.1	20.1±0.14	24.4±1.5
MUFA/SFA	0.73±0.1	0.59±0.15	0.56±0.15	0.58±0.15	0.59±0.14	0.29±0.04	0.43±0.07	0.42±0.17	0.49±0.21	0.43±0.06
Δ <sup>9</sup> -desaturase (C16)	0.34±0.2	0.11±0.2	0.31±0.15	0.20±0.15	0.21±0.12	0.23±0.1	0.24±0.2	0.22±0.23	0.13±1.0	0.18±1.0
Δ <sup>9</sup> -desaturase (C18)	1.61±0.3	1.95±0.1	1.76±0.2	1.6±0.12	1.6±0.14	0.38±0.14 <sup>a</sup>	0.46±0.23 <sup>a</sup>	0.75±0.1 <sup>b</sup>	1.0±1.1 <sup>c</sup>	0.9±0.7 <sup>c</sup>

Percentage of the total peak area of the fatty acids listed.

MUFA: monounsaturated fatty acid, SFA: saturated fatty acid, Δ<sup>9</sup>-desaturase (C16) = C16:1/C16:0, Δ<sup>9</sup>-desaturase (C18) = C18:1/C18:0.

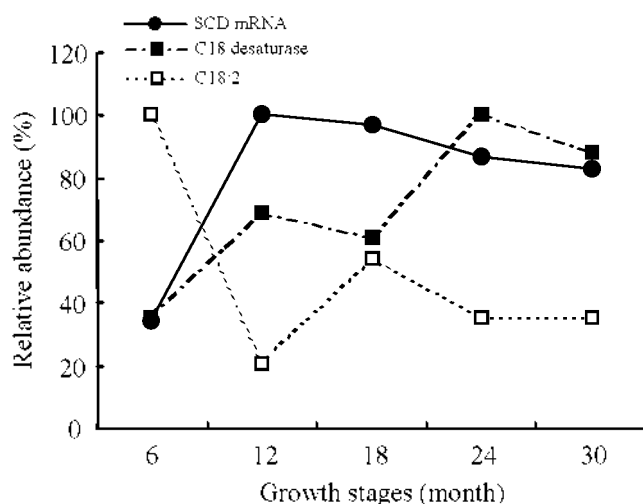
<sup>a, b, c, d</sup> Mean in the same row are significantly different from the control at p<0.05.

80% in Welsh Black and Holstein-Friesian steers (Choi et al., 2000). This may be due to a genetic difference between Hanwoo and western breeds.

The proportions of C16:0 and C16:1 in total lipid and triglyceride fraction were not significantly different across different growth stages. The proportion of C18:0 was significantly higher in total lipid and triglyceride fractions at 6-month old compared with other growth stages (p<0.05). Unlike C18:0, the proportion of C18:1 was significantly lower in total lipid and triglyceride fraction at 6-month old compared with other growth stages (p<0.01). Similarly, Hecker et al. (1975) reported that there is an apparent increase in unsaturation and a corresponding decrease in saturation of fatty acids of muscle as animals grow, suggesting increased activity by fatty acid desaturase with age. The proportion of C18:2 in total lipid was highest at 6-month old, but lowest at 12-month old among different growth stages (p<0.01). In addition, the proportion of C18:2 in the triglyceride fraction was highest at 6-month old, but lowest at 24-month old among different growth stages (p<0.01). In both total lipid and triglyceride fractions, the monounsaturated:saturated fatty acid ratio (MUFA/SFA)

was significantly lower at 6-months old compared with other growth stages (p<0.05). In addition, MUFA/SFA ratio significantly increased after 12-months old and this was due to the increase in the C18:1 rather than changes in the C16:1 and the C16:0. The Δ<sup>9</sup>-desaturase index, as an indirect index of stearoyl-CoA desaturase (SCD) activity, was calculated as C18:1/C18:0 or C16:1/C16:0 (Lee et al., 1996). Stearoyl-CoA desaturase (SCD) enzyme catalyzes the introduction of the first *cis*-double bond in the Δ<sup>9</sup> position (between carbon 9 and 10) of saturated fatty acids, and the preferred substrates are palmitoyl- and stearoyl-CoA (Ntambi, 1999). The Δ<sup>9</sup>-desaturase index of C18 fatty acid increased at 12-months old compared with that at 6-months old by 1.9 fold and reached a maximal level at 24-month old with a 2.8 fold increase in the total lipid (Table 1 and Figure 1). However, the Δ<sup>9</sup>-desaturase level of C16 fatty acid was maintained constantly across the different growth stages.

Phospholipid fraction was separated into phosphatidyl choline and phosphatidyl ethanolamine. The two major phospholipids species in microsome represent about 75% of the total phospholipids by weight (Litman, 1975). The



**Figure 1.** Relative changes of the steady-state SCD mRNA level and  $\Delta^9$ -desaturase index in Hanwoo steers' muscle during stages. The RT-PCR with specific primers of stearoyl-CoA desaturase (SCD) gene and glyceraldehyde-3-phosphate dehydrogenase (G3PDH) gene as an internal control was performed with mRNA isolated from Hanwoo steers' loin muscle at different ages. The  $\Delta^9$ -desaturase index of C18 fatty acid (C18 desaturase) is the ratio of oleic acid/stearic acid in the total lipid fraction. Data expressed relative to the maximum level in each value of SCD mRNA,  $\Delta^9$ -desaturase index of C18 fatty acid and C18:2 composition.

proportions of C16:0 and C16:1 in phosphatidyl choline fraction were highest at 18-month old ( $p < 0.05$ ), but the proportion of C18:0 was not significantly different among different growth stages. Higher proportion of C18:1 in phosphatidyl choline fraction was observed at 6- and 12-months old compared with other growth stages ( $p < 0.05$ ). The proportion of C18:2 in phosphatidyl choline fraction was highest at 30-month old, but lowest at 18-month old among different growth stages ( $p < 0.05$ ). The MUFA/SFA ratio, and  $\Delta^9$ -desaturase indexes of C16 and C18 fatty acid in phosphatidyl choline were constant during the whole growth stages. In phosphatidyl ethanolamine fraction, the proportion of C18:1 was significantly lower at 6-month old compared with other growth stages ( $p < 0.05$ ), but other fatty acids MUFA/SFA ratio were not significantly different across different growth stages. The  $\Delta^9$ -desaturase index of C16 fatty acid in phosphatidyl ethanolamine fraction was constant during the whole growth stages, but its index of C18 fatty acid was significantly increased with increasing age ( $p < 0.05$ ). The C18:1 was concentrated much less in the phospholipids fraction than in the triglyceride fraction (15% vs. 46%). Conversely, the C18:2 was concentrated in the phospholipids fraction and much less in the triglyceride fraction (22% vs. 8%).

#### SCD mRNA level

C18:1 is a key product of the SCD enzyme from *de novo* lipid biosynthesis. The steady-state level of SCD

mRNA in Hanwoo steers' muscle at the different growth stages was examined by RT-PCR analysis. As shown in Figure 1, the SCD mRNA level was not detected at 6-months old, but its level markedly increased by 3-fold by 12-months old. In addition, it was tended to maintain its level until 30-months old. Thus, the present results indicated that the steady-state level of SCD mRNA reached a peak at 12-month old as the result of increased gene transcription. However, the  $\Delta^9$ -desaturase activity in the total lipid and the triglyceride fractions peaked at 24-month old despite the maximal level of SCD mRNA at 12-month old, suggesting the presence of gene regulation in posttranscriptional level. Changes in SCD activity may reflect fatty acid composition in triglyceride and phospholipids of muscle. However, the marked induction of SCD mRNA in muscle of Hanwoo steers during growth did not relate with change of unsaturated fatty acid composition in the phosphatidyl ethanolamine fraction. C18:2 content in muscle may play an important role in the regulation of SCD gene expression. This is because the significant reduction of C18:2 was inversely related to the induction of SCD mRNA during early growth stages. The earlier report of Hecker et al. (1975) showed that C18:2 composition in bovine tissue decreased during later growth stages. In adipocytes, SCD expression is markedly suppressed by polyunsaturated fatty acids, such as C18:2, C18:3 and C20:4 (Choi et al., 2001). In addition, Water and Ntambi (1996) reported that C18:2 inhibits SCD gene transcription. The SCD gene promoter has the sterol-regulatory element which is the binding site of transcription factor and its binding activity can be regulated by hormones and polyunsaturated fatty acids (Ntambi, 2004).

Therefore, the present results showed a negative relationship between SCD gene expression and polyunsaturated fatty acids composition in bovine tissue, particularly at 12-months old.

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