DOI:10.1021/jf902276r

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

# Gene Expression Profiling of Metabolism-Related Genes between Top Round and Loin Muscle of Korean Cattle (Hanwoo)

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Using differential display reverse transcriptase polymerase chain reaction, we detected 11 differentially expressed genes between top round and loin muscle in Korean cattle (Hanwoo). In the loin muscle, the lightness ($L^*$) value ($P < 0.01$) and marbling fat content ($P < 0.01$), which are important factors in determining meat quality, were higher than in top round muscle. Three of the 11 genes were validated as significant genes between two types of muscle by real-time polymerase chain reaction ($P < 0.05$). To determine whether the three genes were associated with meat quality traits, a regression analysis was preformed. The result demonstrated that two genes (NADH dehydrogenase 2 and cytochrome oxidase III), which are involved in oxidative phosphorylation in mitochondria, were significantly correlated with marbling fat content in the loin muscle ($P < 0.01$), while two genes were not significant with marbling fat content in top round muscle. No significant effects for two genes on other meat quality traits such as meat color (redness and yellowness value), Warner−Bratzler shear force, and water-holding capacity were detected in this study.

**KEYWORDS: Differential display RT-PCR; differentially expressed genes; intramuscular fat content; Korean cattle**

## INTRODUCTION

Intramuscular fat (marbling) has been highlighted in the Korean beef industry because it is important for determining beef meat quality (*1*). Intramuscular fat in cattle is affected by many factors, such as breed, genotype, gender, age, and muscle type (*2*, *3*). Recently, Pethick et al., (*4*) suggested that intramuscular fat development within muscle was a biochemical feature associated with fatty acid oxidation and adenosine triphosphate (ATP) synthesis arising in muscle tissue. In particular, a study comparing intramuscular fat content and lipogenic and mitochondrial protein content in two extremely different muscle types across three breeds showed that intramuscular fat content was strongly correlated with fatty acid binding protein 4, cytochrome *c* oxidase, and isocitrate dehydrogenase protein activity and content (*5*).

Some previous studies have described a correlation between myosin heavy chain (MyHc) isoforms and meat qualities (*6*−*8*). MyHc isoforms are divided into four types (2A, 2B, 2X, and slow) depending on the metabolic properties (*9*). The slow isoform is most oxidative among other isoforms. With this knowledge, we examined the mRNA levels of two types of MyHc isoforms 2A (glycolitic-oxidative) and slow (oxidative) using real-time polymerase chain reaction (PCR). The mRNA of the slow (oxidative) isoform was 6.7 times more highly expressed in the loin muscle than in the top round muscle ($P < 0.01$, data not shown). This result indicated that loin muscle is a more oxidative type than the top round muscle. Additionally, loin muscle exhibits a higher intramuscular fat content as compared to top round muscle. Different muscles with different metabolic properties may be a good biological model for identifying intramuscular fat-related genes in cattle.

Recent gene expression technology enables rapid and large-scale analysis of transcriptional responses in varying biological conditions (*10*). One of these technologies, differential display reverse transcriptase PCR (DDRT-PCR), is a relatively fast and sensitive technique suitable for small amounts of RNA; therefore, it is widely used to detect differentially expressed genes (DEGs) among different types of samples (*11*). However, it should be mentioned that DDRT-PCR is not quantitative (*12*). Therefore, this technology needs the other assay such as real-time PCR, which is used to identify the mRNA expression levels of each gene, for validation of the results of DDRT-PCR. We investigated DEGs between different types of muscle tissues of Korean cattle (Hanwoo) using DDRT-PCR and then performed real-time PCR for validation of gene expression level of DEGs

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Published on Web 10/29/2009
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and identified their potential as biomarkers for the fat content of cattle.

## MATERIALS AND METHODS

**Animals and Sample Preparation.** We used eight steers (28−30 months old) with grade 1++ beef. Eight animals were transported to the National Institute of Animal Science (NIAS) abattoir and fasted for approximately 12 h but given access to water prior to slaughter. The animals were stunned by a captive bolt pistol, hanged, and bled. The carcasses were hung by their Achilles tendon and chilled at 2 °C for 48 h. Samples of top round and loin muscle tissue were taken from each carcass, ground to a fine powder under liquid nitrogen with a mortar and pestle, and stored at −80 °C. All experimental procedures and care of animals were conducted in accordance with the guidelines of the Animal Care and Use Committee of the NIAS in Korea.

**Measurement of Meat Quality.** The fat contents of each muscle were determined using a microwave solvent extraction method (method 985.15) described by the Association of Official Analytical Chemists (AOAC) (*13*). Objective meat color (CIE *L*, *a*, and *b*) was determined using a Minolta Chromameter CR300 (Minolta, Japan) on freshly cut surfaces of meat samples after a 30 min blooming at 1 °C. The water-holding capacity (WHC) was determined using the filter paper press method (*14*). The Warner−Bratzler shear force (WBS) (kg) was measured by Wheeler et al. (*15*). The meat blocks (6 cm × 5 cm × 2.54 cm thick) in zipper bags were cooked in the preheated water bath at 80 °C for 60 min or until the core temperature reached 70 °C and were subsequently cooled in running water (ca. 18 °C) for 30 min to reach a core temperature below 30 °C. Eight cores of 1.27 cm diameter were made for each sample, and the peak force was determined using a V-shaped shear blade with a cross-head speed of 400 mm/min.

**DDRT-PCR.** Total RNA was prepared from each tissue sample (100 mg) using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA) according to the manufacturer's instructions. To synthesize cDNAs, 3 μg of RNA was reverse transcribed in a 20 μL reaction volume using standard methods (*16*) and the GeneFishingTM DEG kit (Seegene, Seoul, Korea). The synthesized cDNA samples were diluted in 80 μL of diethylene pyrocarbonate-treated water. The PCR reaction was performed in a 20 μL reaction volume consisting of 10 μm of 120 different arbitrary annealing control primers (ACPs), 10 μm of dT-ACP2, 10 μL of 2× SeeAmp ACP Master mix, and 4 μL of first-strand cDNA using a GeneAmp PCR System 9700 (Applied Biosystems, Warrington, United Kingdom). The PCR amplification was performed for one cycle at 94 °C for 5 min, 50 °C for 3 min, and 72 °C for 1 min; this was followed by 40 cycles at 94 °C for 40 s, 65 °C for 40 s, and 72 °C for 40 s; a final cycle was run at 72 °C for 5 min. The PCR products were separated by electrophoresis on 2.0% agarose gels, and the DEGs were extracted from the gel and sequenced using the AB3730xl (Applied Biosystems). The sequences were analyzed by searching for similarities using the BLAST program (GenBank, NCBI).

**Quantitative Real-Time PCR.** The mRNA levels were analyzed by real-time PCR using gene-specific primer sets (**Table 1**). Total RNA was prepared from each tissue sample (100 mg) with TRIzol reagent (Invitrogen). For real-time PCR analysis, 2 μg of RNA was reverse transcribed in a 20 μL reaction volume using random primers (Promega, Madison, WI) and reverse transcriptase (SuperScript II Reverse Transcriptase, Invitrogen). Reactions were incubated at 65 °C for 5 min, 42 °C for 50 min, and then 70 °C for 15 min to inactivate the reverse transcriptase.

Real-time PCR was performed using the 2X Power SYBR Green PCR Master mix (Applied Biosystems) with the 7500 Real Time PCR system (Applied Biosystems) using a 10 pM concentration of each primer. The PCR was run for 2 min at 50 °C and 10 min at 95 °C, followed by 40 cycles of 95 °C for 10 s, and then 60 °C for 1 min. Following amplification, a melting curve analysis was performed to verify the specificity of the reactions. The end point used in the real-time PCR quantification, Ct, was defined as the PCR threshold cycle number. The ΔCt value was determined by subtracting the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) Ct value for each sample from the target Ct value (*17*). To confirm whether the GAPDH gene was suitable for internal housekeeping gene in this experiment, we tested gene suitability of this gene. The gene expression stability value is less than 0.05, which met the stability requirement to be a housekeeping gene (*18*). The real-time PCR data were used to calculate the normalized expression values ($2^{-\Delta Ct}$) for statistical analysis.

**Table 1.** Primer Sequences for the PCR Amplification of Specific Genes[a]

| gene | | sequence (5′−3′) | product size (bp) |
|---|---|---|---|
| TM2 | forward | AGCTGATGAGAGCGAGAGAGGAAT | 84 |
| | reverse | TGCATCTCCTGCAGCTCCATCTTT | |
| LRP16 | forward | CATATCCACTGGAGTGTTCGGGTA | 92 |
| | reverse | TGTCTTTGTGCTGCTCTAGCCACT | |
| HSPB6 | forward | GCATTTCTCGGTGTTGCTGGATGT | 82 |
| | reverse | AACTTCCACGTGGTCACCAACCA | |
| ND2 | forward | AATTCCACCACCACTACCCTGTCA | 81 |
| | reverse | GAGAGTGGCAAGAATTAGGACGGT | |
| ND1 | forward | ACCCGCTACATCTTCAGCCTCAAT | 185 |
| | reverse | TGGAAGCTCAGCCTGATCAGAGAA | |
| UQCR | forward | GCGCCATCGAACCAAGAAGGAAAT | 196 |
| | reverse | TGACCCATGGCAAGGGCAGTAATA | |
| COX3 | forward | ACGTCATCATTGGGTCCACCTTCT | 91 |
| | reverse | GCTTCAAAGCCGAAGTGGTGGTTA | |
| ATP6 | forward | GCCCACTTTCTACCACAAGGAACA | 98 |
| | reverse | ACGGCTAGGGCTACTGGTTGAATA | |
| MYOZ1 | forward | AGGACAGCAATGCCTTATGGTGGA | 139 |
| | reverse | TGAAAGAAGGCCTGTTGGAGAGGT | |
| RPL12 | forward | AAGGCAACTGGTGATTGGAAGGGT | 85 |
| | reverse | AAGGTACCACCTCAATCTGGGCTT | |
| NDUFS3 | forward | GTTGGCTCAAGAGTTCCGCAAGTT | 145 |
| | reverse | AGGATCCACATGCCTTCCCTGAAA | |
| 2A-F | forward | AAGGGACTATCCAGAGCAG | 133 |
| Slow-F | forward | AGGCCACAATTTTGTCTCG | 133 |
| MyHc-R | reverse | ATGGCCATGTCCTCGATCTT | |
| GAPDH | forward | GGGTCATCATCTCTGCACCT | 176 |
| | reverse | GGTCATAAGTCCCTCCACGA | |

[a] LRP16, macro domain-containing protein 1; HSPB6, heat shock protein, α-crystallin-related B6; ND2, NADH dehydrogenase subunit 2; ND1, NADH dehydrogenase subunit 1; UQCR, ubiquinol-cytochrome *c* reductase; COX3, cytochrome oxidase subunit III; ATP6, ATP synthase F0 subunit 6; MYOZ1, myozenin 1; RPL12, ribosomal protein L12; NDUFS3, NADH dehydrogenase Fe−S protein 3; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

**Statistical Analysis.** For identifying the DEGs between top round and loin muscle, statistical analysis was performed by an analysis of variance (ANOVA) model using the MIXED procedure in an R statistical package for animals nested within age as the random effect. We also examined their least-squares means (LSM) for testing if there was significant difference between top round and loin muscles using Student's *t* test. Then, a regression model was used to examine the association between meat quality traits and normalized expression values ($2^{-\Delta Ct}$) within muscle types. This resulted in the following equation:

$$\text{expression} = \mu + \text{trait} + \text{age} + \text{residual}$$

where expression is a normalized gene expression value ($2^{-\Delta Ct}$) and $\mu$ is an overall mean, trait is the meat quality trait of each animal, and age is the slaughtering age (months) as a covariate.

## RESULTS AND DISCUSSION

We used top round and loin muscle to identity DEGs using DDRT-PCR. In the loin muscle, lightness ($L^*$) value and fat content, which are important factors in determining the meat quality, were higher than in top round muscle (**Table 2**). To confirm the differences in muscle types, we used ANOVA analysis by the MIXED procedure of R statistical package for animals nested within age as the random effect. As shown in **Table 2**, the lightness ($L^*$) value ($P < 0.01$), fat content ($P < 0.001$), and WBS ($P < 0.01$) were significantly difference between muscle types. We investigated DEGs in different muscle types, top round and loin muscle, using DDRT-PCR. In the DDRT-PCR analysis,

**Table 2.** Characteristics of Meat Quality Traits between Top Round (T) and Loin (L) Muscles (Means ± Standard Errors) in Korean Cattle (Hanwoo) Steers[a]

| | CIE | | | | | |
|---|---|---|---|---|---|---|
| muscle type | *L* | *a* | *b* | fat (%) | WBS (kg) | WHC (%) |
| T (*n* = 8) | 38.5 ± 0.21 | 23.6 ± 0.63 | 11.2 ± 0.39 | 9.2 ± 1.36 | 5.3 ± 0.28 | 55.0 ± 0.67 |
| L (*n* = 8) | 42.8 ± 0.91** | 24.9 ± 0.24 | 13.0 ± 0.47 | 27.7 ± 2.14*** | 2.3 ± 0.34** | 57.3 ± 1.66 |

[a] Fat, marbling fat content. ** and ***, significant differences ($P < 0.01$, and 0.001) between T and L muscles determined using the mixed ANOVA module.

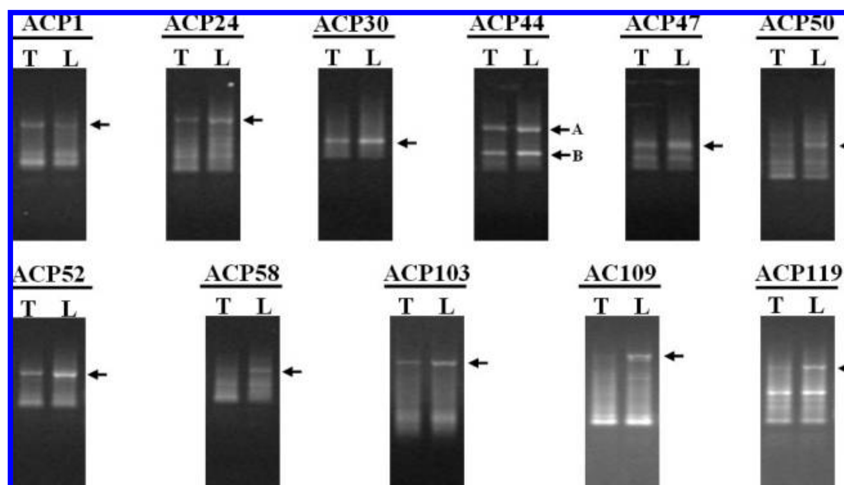


**Figure 1.** Expression patterns of differentially expressed bands between top round (T) and loin (L) muscles. The DDRT-PCR products were electrophoresed on 2.0% agarose gels and stained with ethidium bromide. The arrow indicates the differentially expressed bands between top round and loin muscles.

**Table 3.** Characterizations and Expression Patterns of DEGs between Top Round (T) and Loin (L) Muscles

| | | | | expression pattern (L/T) | |
|---|---|---|---|---|---|
| ACP no. | gene name | GenBank accession no. | expect value | DDRT-PCR | real-time PCR |
| ACP1 | TM2 | AAI04539 | $8.00 \times 10^{-4}$ | down | up |
| ACP24 | LRP16 | AAI12880 | $5.00 \times 10^{-33}$ | up | up |
| ACP30 | HSPB6 | AAI26730 | $1.00 \times 10^{-37}$ | up | up |
| ACP44-A | ND2 | AAP47868 | $3.00 \times 10^{-28}$ | up | up |
| ACP44-B | ND1 | AAM08335 | $3.00 \times 10^{-39}$ | up | up |
| ACP47 | ND1 | AF493541 | $4.00 \times 10^{-36}$ | up | up |
| ACP50 | UQCR | BC133632 | $8.00 \times 10^{-20}$ | up | down |
| ACP52 | COX3 | AAM08332 | $2.00 \times 10^{-48}$ | up | up |
| ACP58 | ATP6 | ABP63007 | $6.00 \times 10^{-6}$ | up | up |
| ACP103 | MYOZ1 | AAI22690 | $3.00 \times 10^{-67}$ | up | up |
| ACP109 | RPL12 | BAC56456 | $8.00 \times 10^{-32}$ | up | up |
| ACP119 | NDUFS3 | NP777244 | $7.00 \times 10^{-30}$ | up | up |

12 bands were differentially expressed between loin and top round muscle (**Figure 1**). After the DDRT-PCR analysis, differentially expressed bands were extracted from the agarose gels. Isolated DNA was directly sequenced and annotated by a BLAST search, as presented in **Table 3**. These genes, except for the tropomyosin 2 (TM2) gene (ACP1 band), were highly expressed in loin than top round muscle (**Figure 1** and **Table 3**). They were classified into five categories according to their biological functions: oxidative metabolism (NADH dehydrogenase 1, ND1; NADH dehydrogenase 2, ND2; ubiquinol-cytochrome *c* reductase, UQCR; cytochrome oxidase subunit III, COX3; ATP synthase F0 subunit 6, ATP6; and NADH dehydrogenase Fe−S protein 3, NDUFS3), myogenesis and muscle development (trophomyosin 2, TM2; and myozenin 1, MYOZ1), protein synthesis (ribosomal protein L12, RPL12), signal transduction (macro domain-containing protein 1, LRP16), and protein binding (heat shock protein α-crystallin-related B6, HSPB6).

To confirm 11 DEGs generated from the DDRT-PCR experiment, real-time PCR analysis was carried out. Gene expression of GAPDH was first confirmed for consistency in two different muscle types. Consistency of GAPDG gene expression was 0.04 in this experiment, which value met the requirement to be a housekeeping gene (*18*). The GAPDH was therefore used as an internal housekeeping gene for data normalization. The gene expression level of DEGs was analyzed and then was normalized to the GAPDH mRNA expression levels. Finally, we transformed expression levels to the $2^{-\Delta Ct}$ value for further analysis (*17*). Overall, real-time PCR expression patterns for each gene, except for the TM2 and UQCR genes, closely mirrored those obtained from DDRT-PCR analysis (**Table 3**).

**Table 4** displays the differences of individual gene expression on the muscle types. Among the DEGs, the gene expression of the three genes (TM2, ND2, and COX3) was significantly different between top round and loin muscles ($P < 0.001$, 0.01, and 0.05, respectively). The mRNA level of TM2 gene in loin muscle, which involved myofibril protein, was approximately 2.4 times higher than that in the top round muscle, and the other two genes (ND2 and COX3) also were 2.0 and 1.8 times higher in the loin muscle.

**Table 4.** ANOVA Table of DEGs between Top Round and Loin Muscles[a]

| gene | source | Df | sum sq. | mean sq. | *F* value | Pr(>*F*) |
|---|---|---|---|---|---|---|
| TM2 | tissue | 1 | 28.6594 | 28.6594 | 34.275 | $7.784 \times 10^{-5}$*** |
| | residuals | 12 | 10.034 | 0.8362 | | |
| MYOZ1 | tissue | 1 | 0.09077 | 0.09077 | 1.9697 | 0.1858 |
| | residuals | 12 | 0.55298 | 0.04608 | | |
| RPL12 | tissue | 1 | 0.015407 | 0.015407 | 1.7747 | 0.2076 |
| | residuals | 12 | 0.104179 | 0.008682 | | |
| NDUFS3 | tissue | 1 | 0.00006006 | 0.00006006 | 0.8612 | 0.3717 |
| | residuals | 12 | 0.00083696 | 0.00006975 | | |
| LRP16 | tissue | 1 | 0.024406 | 0.024406 | 1.5363 | 0.2389 |
| | residuals | 12 | 0.190631 | 0.015886 | | |
| HSPB6 | tissue | 1 | 0.004655 | 0.004655 | 0.6032 | 0.4524 |
| | residuals | 12 | 0.092604 | 0.007717 | | |
| ND2 | tissue | 1 | 38.81 | 38.81 | 14.885 | 0.002277** |
| | residuals | 12 | 31.289 | 2.607 | | |
| ND1 | tissue | 1 | 33.946 | 33.946 | 3.1618 | 0.1007 |
| | residuals | 12 | 128.837 | 10.736 | | |
| UQCR | tissue | 1 | 0.0001 | 0.0001 | 1.1115 | 0.3125 |
| | residuals | 12 | 0.00107964 | 0.00008997 | | |
| COX3 | tissue | 1 | 40.206 | 40.206 | 8.5092 | 0.01291* |
| | residuals | 12 | 56.7 | 4.725 | | |
| ATP6 | tissue | 1 | 0.011572 | 0.011572 | 2.6267 | 0.131 |
| | residuals | 12 | 0.052868 | 0.004406 | | |

[a] *, **, and ***, significant differences ($P < 0.05$, 0.01, and 0.001) between T and L muscles determined using the mixed ANOVA module.

**Table 5.** Least Squares Means (±Standard Errors) and *t* Statistics for Gene Expressions between Top Round and Loin Muscle Types

| | least square mean | | | |
|---|---|---|---|---|
| gene | top round ($n = 8$) | loin ($n = 8$) | *t* value | *p* value |
| TM2 | 1.929 ± 0.3208 | 4.646 ± 0.3208 | −5.899 | $5.25 \times 10^{-5}$*** |
| ND2 | 3.010 ± 0.5681 | 6.124 ± 0.5681 | −3.877 | 0.00191** |
| COX3 | 3.980 ± 0.7806 | 7.150 ± 0.7806 | −2.872 | 0.0131* |

We examined their LSM for testing if there was a significant difference between top round and loin muscles using Student's *t* test. These result also showed that TM2, ND2, and COX3 genes had a high expression pattern in the loin muscle type (**Table 5**).

TM2 was related to myofibrils and muscle development. Recently, PPAR$\gamma$, which is predominantly expressed at an early stage of adipocytes as a key transcriptional factor controlling adipocyte differentiation, has been reported to be inhibited by myogenic proteins and the formation of myotubes (*19*, *20*). Other reports demonstrated that myofibril proteins were decreased during the adipogenesis (*21*, *22*) and intramuscular fat development (*23*, *24*). To date, however, the relationship between myofibrils and fat deposition is not clear. As Wang et al. (*23*) proposed, the gene for mRNA expression of myogenesis and muscle development-related genes, which included the TM2 gene, loosely mirrored that of adipogenic-related genes by using microarray analysis. Other reports, which detected DEGs by using the DDRT-PCR, showed that titin and triadin genes, which are involved in muscle contraction, have a tendency to exhibit higher expression levels in low-marbled steer than in high-marbled steer in the late stage of the test period (*24*). Unlike, the TM2 gene expression pattern was shown to be higher in loin muscle, which exhibits more marbling fat content and more oxidative than in the top round muscle in the present study. We therefore suggest that higher expressed TM2 gene in loin muscle is not a result of fat deposition within the muscle types.

Triacylglycerol (TG) is the major component of marbling fat in skeletal muscles and is found mostly within the intramuscular adipocytes. The adipose fatty acid binding protein (A-FABP; FABP4) gene plays a major role in storage or oxidation of the fatty acid. Recently, Jurie et al. (*5*) reported that the TG content also strongly correlated with A-FABP activity and oxidative enzymes activities in a study where the different genotypes of marbling fat levels were three type tissues and three breeds of cattle. Lee et al. (*25*) reported that the FABP4 gene was highly expressed in *longissimus* muscle of highly marbled Hanwoo steers. FABP4 is exclusively expressed within adipocytes. In addition, these authors suggested that higher lipogenesis in *longissimus* at a late fattening stage was due to an increase in the uptake of free fatty acid by FABP4 within a comparative study of mRNA expression of lipogenesis-related genes using the real-time PCR between 12 (early) and 28 (late) month Hanwoo steers (*26*). Moreover, Diamon et al. (*27*) showed that the FABP4 protein content within adipocytes positively correlated with adipocyte number and lipid content in cross-bred pigs. This is in agreement with Cianzio et al. (*28*); these researchers demonstrated that intramuscular adipocyte number was a good indicator of marbling score. Moreover, intracellular fatty acids in intramuscular adipocytes bind the FABP4, which facilitates the transport of fatty acids from plasma membrane to the sites of fatty acid oxidation or esterification into TG. According to these results, we suggested that higher marbled tissue exhibits a higher adipocyte number, FABP4 content, and more utilized fatty acid for TG formation.

In biochemical processes, fatty acids must be activated in the cytoplasm before being utilized in adipocytes. Activation is catalyzed to fatty acyl-CoA, which is an energy-rich molecule, by fatty acid acyl-CoA synthetase and occurs in the mitochondria. Finally, three molecules of fatty acyl-CoA are used as substrates for TG formation. Among the TG formation process, the first activation reaction is the consumption of 2 mol equiv of ATP (*29*). In present study, we found that two genes (ND2 and COX3) were involved in the mitochondrial oxidative phosphorylation pathway; ATP production showed higher levels of gene expression in loin muscle (**Table 5**). To confirm that mRNA levels of these genes were associated with marbling fat content, we performed regression analysis within muscle types. As shown **Figure 2**, the expression of ND2 ($P < 0.01$) and COX3 ($P < 0.001$) significantly increased in loin muscle with more intramuscular fat, while correlation was not detected in top round muscle. Interestingly, chemical inhibition of the mitochondrial respiratory chain (MRC) leads to reduced fat accumulation in the adipocytes of *Caenorhabditis elegans* as well as mammals, indicating that mitochondria play a key role in fat synthesis (*30*). Moreover, we previously reported that ND1 and ATP6 expression is strongly correlated with meat quality as indicated by higher intramuscular fat content in *longissimus* tissue (*31*). Berk et al. (*32*) found that adipocyte fatty acid uptake was increased in obese, diabetic, and fat transgenic mice versus control mice. These results may indicate that oxidative metabolism in mitochondria was positively related with intramuscular fat content and fatty acid utilization for TG formation. Recently, a haplotype of mitochondrial genome polymorphisms, which contained a synonymous transition single nucleotide polymorphism (SNP) (9104 C > T) in the porcine COX3 gene, has been reported to have a significant effect on fat content in an Iberian pig breed (*33*). This result also suggests that the COX3 gene might be a candidate gene, which may explain the intramuscular fat content variation observed in the animal. However, further investigation is needed to confirm the relationship between mitochondrial genes and marbling trait, fatty acid utilization in skeletal muscle, and adipocytes.

In conclusion, we identified 11 DEGs between muscle tissues using DDRT-PCR. These genes were validated by real-time

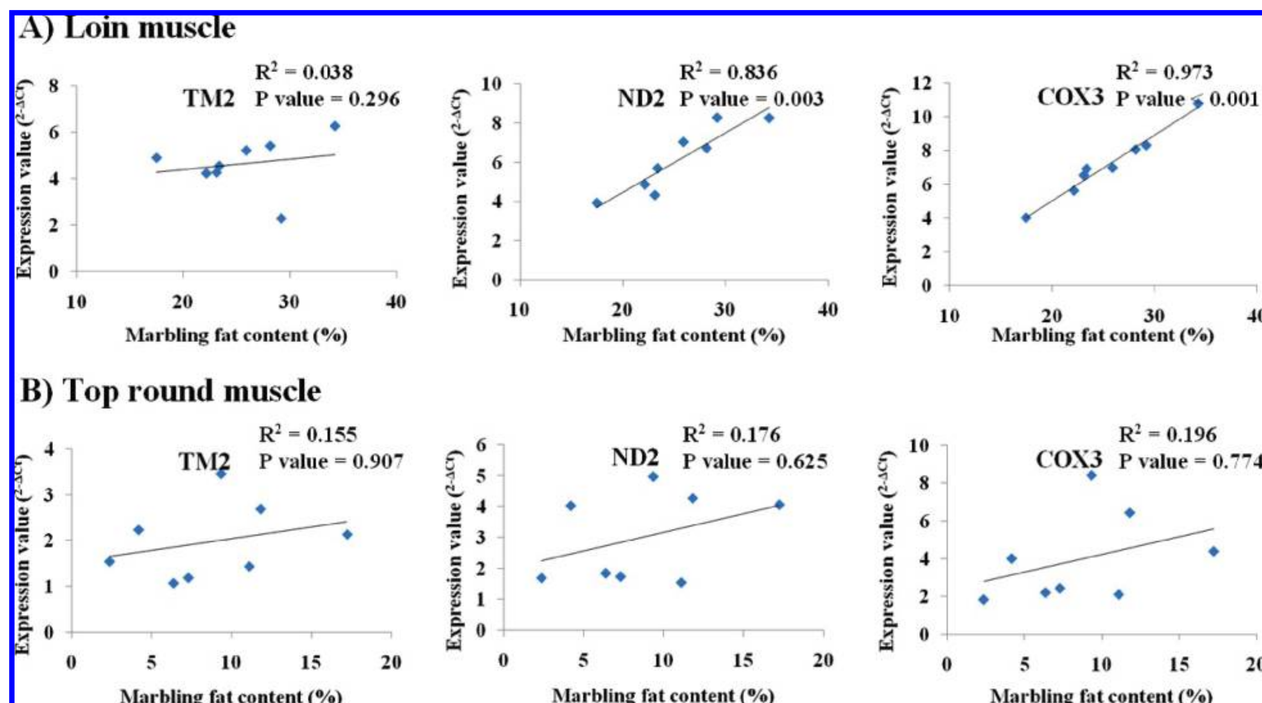


**Figure 2.** Regression analysis between expression levels (*y*-axis) generated by real-time PCR and marbling fat content (%, *x*-axis) within top round and loin muscle types from eight Korean cattle (Hanwoo) steers.

PCR. Of the validated genes, the ND2 and COX3 genes showed higher gene expressions in loin muscle and were correlated with marbling fat content in Korean cattle (Hanwoo) steer. Therefore, these genes could be good indicators for intramuscular fat content within the skeletal muscle of cattle.

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**Received for review July 2, 2009. Revised manuscript received September 25, 2009. Accepted October 1, 2009. This study was supported by 2009 Post Doctoral Course Program and Agenda (20091FHT020710485) of National Institute of Animal Science, Rural Development Administration, Republic of Korea.**