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# Comparisons of extracellular matrix-related gene expression levels in different adipose tissues from Korean cattle

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ABSTRACT

Adipose tissues are related to the meat quality and economic value of cattle. In particular, the extracellular matrix (ECM) is an important factor that influences adipose tissue remodeling. The objective of this study was to measure the expression levels of ECM-related genes in different adipose tissues from bulls, cows, and steers of Korean cattle (Hanwoo). The sample tissues were separated from omental (OM), subcutaneous (SC), and intramuscular (IM) adipose tissues. The expression levels of 24 ECM-related genes and proteins were determined using real-time PCR and Western blot analysis, respectively. We also observed that castration resulted in increased ($P < 0.05$) expression of 17 genes, whereas it caused decreased ($P < 0.05$) expression of *COL1A2*, *MMP1*, *MMP3*, *MMP7*, and *VCAM1* in the OM adipose tissue of cows and steers compared to bulls. The mRNA levels of 24 ECM-related genes were similar between IM and SC adipose tissues. Furthermore, except for *ITGB1* (IM), *ICAM1* (IM), and *COL1A1* (OM), the expression levels were generally similar between cows and steers compared to bulls. The *FN1*, *MMP7*, and *LOX* mRNA levels exhibited significantly strong ($P < 0.001$) positive correlations (r=0.74, r=0.73, and r=0.74, respectively) with the marbling score. The protein levels can reflect the mRNA levels. Therefore, our findings suggest that castration affects the expression levels of ECM-related genes, which is in accordance with different fat depots.

## 1. Introduction

Excessive adiposity causes metabolic syndromes at different fat depots or regional adipose tissue (Bjørndal et al., 2011; Tchkonia et al., 2013). Most information available regarding regulation factors and mechanisms relates to their influence on the development and growth of different adipose tissues. The region-specific factors could contribute to differential adipose tissue growth (Dijan et al., 1983) and metabolic stimuli (Yang and Smith, 2007). For example, the subcutaneous and internal adipose tissue depots have different metabolic properties (Saremi et al., 2014). The different expression patterns could have influenced adipocyte development and proliferation, triggering adipocyte proliferation (Soret et al., 2016). The proteins expressed in adipose tissue were observed in different regional tissue from cows, steers, and bulls using proteomic analysis (Cho et al., 2016).

The location and distribution of multi-site tissues are important for improving the meat quality in cattle industry because internal and subcutaneous fat are not useful, while intramuscular fats have high value. The significant regional differences in lipid metabolism and adipocyte morphology have led to the identification of regulatory genes. The Korean beef industry has been attempting to drop the level of inedible fat without impacting the beef quality. Therefore, the beef industry needs technology that enables it to selectively manipulate fat deposition.

The development and function of adipose tissue may be regulated through interactions between cells and their surrounding environment. The extracellular matrix (ECM) signaling mechanisms involve a complex functional system, including transmembrane molecules and components that play a role in the maintenance of tissue morphogenesis. Therefore, ECM interactions directly and/or indirectly regulate differentiation, proliferation, growth factors, cell behaviors, and apoptosis (Gumbiner, 1996; Gattazzo et al., 2014). Adipocytes also contribute to the synthesis and remodeling of ECM with several enzymes involved in each of these processes (Mariman and Wang, 2010; Lu et al., 2012). Cell adhesion and migration in the ECM are crucial components of biological processes, such as adipogenesis (Lu et al.,

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2012). The interactions between adipose tissue and the ECM are mediated by transmembrane molecules. Overall, ECM proteins modulated in regional adipose tissues differentiate tissues in terms of lipolysis and adipokine secretion (Choe et al., 2016). Morphogenesis depends on the ECM, ECM-receptors (i.e., integrins and other ECM receptors), ECM-degrading enzymes [i.e., matrix metalloproteinases (MMPs)] and their inhibitors, and tissue inhibitors of metalloproteinases (TIMPs) (Lu et al., 2011). Notably, the ECM signaling mechanisms provide structural anchorage for cells, remodeling adipocytes (Berryman et al., 2011; Pope et al., 2016).

Body fat deposition is caused by several lipid metabolism processes, such as adipogenesis, lipogenesis, fatty-acid β-oxidation, and ECM pathways (Lee et al., 2013; Jeong et al., 2012, 2013; Hausman et al., 2009). Castration is the most common method performed on cattle to stop the production of male hormones. The sex of the cattle greatly affects the texture of beef due to the presence of intramuscular fat content (Venkata Reddy et al., 2015). Steers (known as a castrated bull) markedly increase their intramuscular fat (IMF) deposition, improving the beef quality in Korean cattle (Bong et al., 2012; Jeong et al., 2013). The castrated-bull (i.e., steer) appears feminine compared to the bull, but it is typically not referred to as a cow. However, little is known about the effects of different fat depots on the differential expression of genes associated with ECM signaling in different sex types. Therefore, we investigated the expression levels of ECM-related genes and proteins in different adipose tissues of Korean bulls, cows, and steers.

## 2. Material and methods

### 2.1. Animals and sample preparation

All experimental procedures were approved and performed by the National Institute of Animal Science Institutional Animal Use and Care Committee in Korea. We assumed that bulls have the lowest marbling score (MS) with relatively uniform values (1 on a scale of 1–9; 1 representing the lowest MS and 9 representing the highest MS) according to the Korean Beef Marbling Standard, while steers have higher MS values, ranging between 7 and 9, and cows have MS values, ranging between 4 and 7. Bulls were weaned at a mean age of 3 months and fed with 30% concentrates and 70% roughage until 6 months. Following castration, all animals (i.e., bulls, cows, and steers) were fed as described in a previous study (Jeong et al., 2012). Roughage was offered *ad libitum*, and the animals had free access to fresh water throughout. We used adipose tissue samples of nine bulls, 10 cows, and 10 steers. All tissues were collected immediately after slaughter. Subcutaneous (SC) adipose tissues were taken from the dorsal area of the 13th rib. Omental (OM) adipose tissues were dissected out from the omentum. A *longissimus dorsi* muscle (LM) tissue from the left side of the 13th rib was collected from carcasses. Intramuscular (IM) adipose tissues were separated the LM tissues. After slaughter and a 24-h chill period, cold carcass weights were measured, and the left side of each carcass was cut between the last rib and first lumbar vertebra to determine the MS. The frozen LM tissue covered with foil was placed on dry ice to maintain the frozen state of the muscle tissue for IM adipose tissue collection. The collection time for 1 g of IMF was approximately 30 min. The slaughter age was approximately 31 months. Carcass characteristics are listed for bulls (491 ± 14 kg), cows (406 ± 13 kg), and steers (452 ± 12 kg) in Table 1.

### 2.2. RNA extraction and quantitative real-time PCR

Total RNAs were extracted using TRIzol reagent (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions. Total RNAs were quantified by absorbance at 260 nm using a Nanodrop ND-1000 (NanoDrop, Wilmington, USA) and the integrity of total RNAs was checked with 1.2% (w/v) agarose gel. Total RNAs (diluted to 1-μg amounts) was reverse transcribed into cDNA using a QuantiTect reverse transcription kit (Qiagen, Valencia, USA) according to the manufacturer's instructions. Real-time PCR was performed using QuantiTect SYBR Green Master Mix (Qiagen, Valencia, USA) and a 7500 real-time PCR System (Applied Biosystems, Foster City, USA). Real-time PCR analysis was performed as previously described (Jeong et al., 2012). All data were normalized to the expression level of the endogenous genes *Ribosomal protein S9* (*RPS9*) and *Ribosomal protein, large, P0* (*RPLP0*). Relative quantification analysis was performed using the comparative Ct (ΔΔCT) method (Livak and Schmittgen, 2001). Primers are listed in Table 2.

**Table 1**
Carcass characteristics of Korean bulls, cows, and steers.

| Variables | Bulls (n=9) | Steers (n=10) | Cows (n=10) |
|---|---|---|---|
| Age, months | 31.6 ± 0.24 | 31.7 ± 0.16 | 31.4 ± 0.30 |
| Carcass weight, kg | 490.9 ± 13.59[a] | 452.6 ± 12.28[a] | 406.1 ± 13.37[b] |
| Backfat thickness, mm | 7.7 ± 1.48[b] | 16.7 ± 1.51[a] | 19.1 ± 1.90[a] |
| Rib-eye area, $cm^2$ | 100.9 ± 3.46[a] | 91.2 ± 1.72[b] | 90.6 ± 2.45[b] |
| Yield index | 68.0 ± 0.97[a] | 62.0 ± 1.18[b] | 61.5 ± 1.47[b] |
| Yield grade[1] | 277.8 ± 14.70[a] | 155.6 ± 16.67[b] | 142.9 ± 20.20[b] |
| Marbling score[2] | 1.0 ± 0.00[c] | 6.9 ± 0.37[a] | 4.7 ± 0.47[b] |
| Quality grade[3] | 10.0 ± 0.00[c] | 38.9 ± 1.05[a] | 32.9 ± 1.84[b] |

Mean ± SEM, [a–c]Means in row with different superscripts differ ($P < 0.05$).
[1] Yield grade: 300=A, 200=B, 100=C.
[2] Marbling score: 1=trace, 9= very abundant.
[3] Quality grade: 40=1++ or 1+, 30=1, 20=2, 10=3.

### 2.3. Protein extraction and Western analysis

Samples were homogenized using a Polytron homogenizer for 10 s in cold Pro-PREP protein extraction solution (Intron, Seongnam, Korea). The lysate samples were incubated at −20 °C for 1 h. After centrifugation at 10,000*g* for 10 min, the protein concentrations were determined by the Bradford method according to the manufacturer's instructions. Total proteins were prepared for Western blot analysis with boiling in 5× sample buffer (250 mM Tris-HCl (pH 6.8), 0.5 M DTT, 10% SDS, 0.5% bromophenol blue, and 50% glycerol). The 20-μg proteins (n=4) were separated using sodium dodecyl sulfate–polyacrylamide gel electrophoresis by molecular mass on 8–15% collagen type I, alpha (COL1A; 139 kDa; Santa Cruz Biotechnology, Santa Cruz, USA); collagen type III, alpha (COL3A; 138 kDa, Abcam, Cambridge, UK); integrin, alpha 5 (ITGA5; 145 kDa, Abcam, Cambridge, UK); integrin, beta 1 (ITGB1; 138 kDa, Santa Cruz Biotechnology, Santa Cruz, USA); matrix metallopeptidase 1 (MMP1; 54 kDa, Abcam, Cambridge, UK); and matrix metallopeptidase (MMP2; 74 kDa, Abcam, Cambridge, UK) separating gels. The separated proteins were transferred onto polyvinylidene fluoride membranes (Millipore, Billerica, USA). COL1A, COL3A, ITGA5, and ITGB1 proteins on the 8% gels and MMP1 and MMP2 proteins on 5% gels were used. The transferred membranes were blocked with 5% skim milk in Tris-buffered saline containing 0.1% Tween 20 for 1 h. The membrane was incubated with commercial primary antibodies (1:500 dilution of antibodies against COL1A and MMP1, 1:1000 dilution of antibodies against COL3A, ITGA5, ITGB1, and MMP2). Western blot analysis was performed as previously described (Jeong et al., 2013). The blots were treated with secondary horseradish peroxidase conjugated with anti-goat or anti-rabbit antibodies (1:5000 dilution; Santa Cruz Biotechnology, Santa Cruz, USA) and developed using an enhanced chemiluminescence detection kit (Millipore, Billerica, USA). The signal intensity values were quantified using an EZ-Capture II chemiluminescence imaging system with a cooled camera (Atto, Tokyo, Japan) and measurements captured using system analyzer version 2.0 software. The relative protein levels were expressed as the intensity of each protein/intensity of β-tubulin.