

OPEN

Up-regulation of bone morphogenetic protein and its signaling molecules following castration of bulls and their association with intramuscular fat content in Korean cattle

Da Jin Sol Jung¹ & Myunggi Baik^{1,2*}

We evaluated whether castration affects bone morphogenetic protein 2 (BMP2) level and the expression of its signaling molecules in Korean cattle bulls. We also checked whether castration affects the expression of muscle fiber type and oxidative and glycolytic enzyme genes. Enzyme-linked immunosorbent assays revealed that steers had higher plasma BMP2 and leptin concentrations than bulls. Quantitative real-time PCR showed that steers had higher mRNA levels of the *lysyl oxidase* gene, a downstream target of the BMP signaling pathway, in the *longissimus thoracis* (LT) muscle. Steers had higher adipogenic *peroxisome proliferator-activated receptor gamma* and lipogenic *fatty acid binding protein 4* mRNA levels in the LT than bulls. Steers had lower mRNA levels for several muscle fiber type 1 genes and fiber type 2A myosin heavy chain 2 gene than bulls. Steers had higher mRNA levels of the glycolytic enzyme *phosphoglycerate kinase 1* gene than bulls. Transcript levels of oxidative enzyme genes did not differ between bulls and steers. Regression analysis revealed a positive association between plasma BMP2 levels and intramuscular fat (IMF) content in the steer group. These findings suggest that upregulation of the BMP signaling pathway in response to castration induces increased adipogenic gene expression, contributing to the increased IMF deposition observed in castrated animals.

Marbling refers to the dispersion or scattering of fat inside lean meat, and the degree of marbling is the major determinant of the quality grade of Korean cattle beef¹. Castration significantly increases the marbling score (MS) and intramuscular fat (IMF) accumulation, improving beef quality in Korean cattle^{1,2}. Previously, we showed that the activation of adipogenesis and lipogenesis is involved in increased IMF deposition post-castration²⁻⁴. Both hyperplasia (adipocyte number) and hypertrophy (adipocyte size) contribute to IMF deposition^{5,6}. Adipocyte hyperplasia may result from new preadipocyte recruitment and commitment by mesenchymal stem cells (MSCs) from the vascular stroma of adipose tissue⁷, adipocyte proliferation via mitotic clonal expansion of the committed preadipocytes during differentiation⁸, and new adipocyte differentiation from preadipocytes⁹.

The bone morphogenetic protein (BMP) signaling pathway is thought to be required for the differentiation of MSCs into the adipocyte lineage based on cell culture studies¹⁰⁻¹². *Lysyl oxidase* (LOX) is a downstream target gene of the BMP signaling pathway, and is implicated as an early marker of adipogenic commitment in cell culture¹⁰. Little is known about the involvement of BMP signaling molecules in marbling formation in bovine species. It is known that leptin can induce adipocyte differentiation in preadipocytes^{13,14}. Limited information is available regarding whether castration affects plasma leptin levels.

¹Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Science, College of Agriculture and Life Sciences, Seoul National University, Gwanak-gu, Seoul, 08826, Republic of Korea. ²Institutes of Green Bio Science Technology, Seoul National University, Pyeongchang-gun, Gangwon, 25354, Republic of Korea. *email: mgbaik@snu.ac.kr

Variables	Bulls (n = 10)	Steers (n = 10)	P-value
Carcass weight, kg	445 ± 9.37	434 ± 9.47	0.42
Back fat thickness, mm	4.7 ± 0.63	11.7 ± 0.80	<0.001
Rib eye area, cm	86.4 ± 2.97	95.9 ± 6.89	0.23
Yield index ¹	69.0 ± 0.36	66.6 ± 0.97	0.04
Yield grade ²	30.0 ± 0.00	22.0 ± 2.00	0.003
Marbling score ³	1.1 ± 0.10	7.1 ± 0.35	<0.001
Quality grade ⁴	11.0 ± 1.00	44.0 ± 1.63	<0.001
IMF content, %	4.18 ± 1.69	14.5 ± 5.48	<0.001

Table 1. Carcass characteristics of the Korean cattle bulls and steers. Values are mean ± standard deviation. ¹Yield index = $68.184 - 0.625 \times \text{back fat thickness} + 0.13 \times \text{rib eye area} - 0.024 \times \text{carcass weight} + 3.23$. ²Yield grade: 30 = A; 20 = B; 10 = C. ³Marbling score: 1 = min; 9 = max. ⁴Quality grade: 50 = 1++; 40 = 1+; 30 = 1; 20 = 2; 10 = 3.

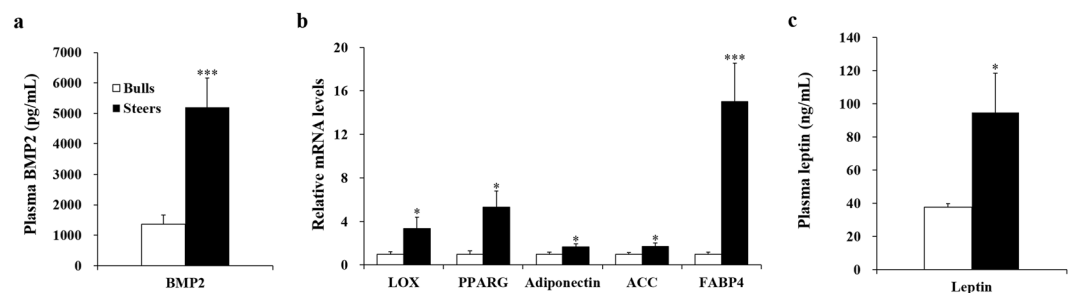


Figure 1. Plasma BMP2 and leptin levels and expression levels of LOX and adipogenic genes in Korean cattle bulls and steers. (a,c) The plasma BMP2 and leptin levels were measured using ELISA (n = 10/group). (b) mRNA levels in the *longissimus thoracis* were determined using quantitative PCR and normalized to β -actin (n = 10/group). mRNA levels in bulls were normalized to 1.0. Values are shown as the mean + standard error. *p < 0.05; ***p < 0.001. BMP2, bone morphogenetic protein 2; LOX, lysyl oxidase; PPARG, peroxisome proliferator-activated receptor gamma; ACC, acetyl-CoA carboxylase; FABP4, fatty acid binding protein.

Castration reduces circulating testosterone² and affects muscle fiber types in cattle¹⁵. Thus, castration may affect the expression of genes involved in myofiber type composition and muscle metabolism through this endocrine change.

In this study, we aimed to understand changes in the expression of BMP2-adipogenesis signaling molecules caused by castration in cattle. We also evaluated whether castration of bulls affects the expression levels of muscle fiber type and metabolism-related genes.

Results and Discussion

Carcass characteristics of Korean cattle bulls and steers. The carcass weights of bulls and steers averaged 445 ± 9.37 and 434.3 ± 9.47 kg, respectively (Table 1). Steers had greater (p < 0.001) back fat thickness and greater (p < 0.001) MS and quality grade than bulls. Steer LT had a 4.0-fold greater (p < 0.001) IMF content ($14.5 \pm 5.48\%$) than bull LT ($4.18 \pm 1.69\%$). Bulls and steers were slaughtered at a similar weight, so steers were about 6 months older at slaughter. Age affects marbling development;¹ therefore, in addition to castration itself, age differences may affect differences in MS and IMF content between bulls and steers.

Comparison of plasma BMP2 levels and expression of BMP2-adipogenesis signaling molecules in the LT between bulls and steers.

Hyperplasia requires the proliferation and differentiation of preadipocytes into new adipocytes, a process known as adipogenesis^{8,9,16}. BMPs are a group of growth factors in the transforming growth factor-beta superfamily¹⁷. BMP2 induces adipocyte development, including adipogenesis and preadipocyte commitment¹⁰. In this study, steers had higher (p < 0.001) plasma BMP2 concentrations than bulls (Fig. 1a). Consistent with our results, a study on humans found that circulating serum BMP2 levels were higher in a moderate obesity group than in lean healthy controls¹⁸. After castration of bulls, their circulating serum testosterone levels decreased². A previously published mouse experiment showed that BMP signaling was blocked after testosterone injection¹⁹. Strong negative correlations have been observed between testosterone and body fat²⁰. In this study, the decreased testosterone levels following castration may have triggered the higher circulating plasma BMP2 levels in steers, contributing to increased IMF deposition in castrated animals. Regression analysis revealed a positive association (p < 0.05) between plasma BMP2 levels and IMF content in the steer group, but not in the bull group (Table 2). The explanation for the absence of a significant association in the bull group may be that IMF content was relatively homogenous among animals in the bull group, as the standard deviation of IMF content in the bull group was much lower than that in the steer group (Table 1).

Variables	Bull (n = 10)			Steer (n = 10)		
	Coefficient	P value	R ²	Coefficient	P value	R ²
Plasma leptin	-0.186	0.607	0.035	0.477	0.164	0.227
Plasma BMP2	-0.028	0.940	0.001	0.636	0.048	0.404
LOX mRNA level	0.209	0.563	0.044	-0.199	0.582	0.040
PPARG mRNA level	0.444	0.199	0.197	0.465	0.176	0.216
Adiponectin mRNA level	-0.160	0.659	0.026	-0.505	0.137	0.255
ACC mRNA level	0.593	0.071	0.352	-0.331	0.351	0.109
FABP4 mRNA level	-0.120	0.741	0.014	-0.364	0.301	0.133

Table 2. Regression analysis of levels of plasma bone morphogenetic protein 2 (BMP2) and its signaling molecules in the *longissimus thoracis* with intramuscular fat content (IMF%) in Korean cattle. BMP2, bone morphogenetic protein 2; LOX, lysyl oxidase; PPARG, peroxisome proliferator activated receptor gamma; ACC, acetyl-CoA carboxylase alpha; FABP4, fatty acid binding protein 4.

The LOX gene is a downstream target of the BMP signaling pathway¹⁰. Steers had higher ($p < 0.05$) LOX mRNA levels in the LT than bulls (Fig. 1b). In mesenchymal stem cells derived from human adipose tissue, LOX expression was upregulated by BMP2 induction²¹. BMP2/4 induces the expression of LOX, which contributes to preadipocyte commitment by murine mesenchymal progenitor C3H10T1/2 cells¹¹. Steers had higher ($p < 0.05$) PPARG mRNA levels in the LT than bulls. Steers also had higher ($p < 0.05$) adiponectin mRNA levels in the LT than bulls. In murine mesenchymal progenitor C3H10T1/2 cells, the induction of adipogenesis by BMP2 occurs through the activation of PPARG²². PPARG regulates the expression of several adipocyte-secreted proteins, including adiponectin²³. In human mesenchymal cells (hMSCs), a combination of BMP2 and 3-isomethyl-1-methylxanthine induces adiponectin expression²⁴. In our study, the up-regulation of BMP2 and LOX following castration may have led to the activation of preadipocyte commitment and subsequent adipocyte hyperplasia by adipogenesis through activation of PPAR and adiponectin in castrated animals. Steers had higher acetyl CoA carboxylase ($p < 0.05$) and fatty acid binding protein 4 ($p < 0.001$) mRNA levels than bulls. Thus, de novo fatty acid synthesis and fatty acid transport may be subsequently activated through BMP signaling, resulting in increased IMF deposition in castrated animals.

Comparison of expression levels of muscle fiber type and metabolism-related genes in the LT between bulls and steers.

In our previous study, castration profoundly reduced circulating testosterone in cattle². This endocrine change may affect muscle fiber type composition and muscle metabolism. In the present study, we checked whether castration affects the expression levels of genes related to muscle fiber type and muscle metabolism. Steers had lower mRNA levels of muscle fiber type 1 (slow twitch, red muscle, oxidative) genes, including *troponin C1* ($p < 0.05$), *troponin T1* ($p < 0.001$), and *myoglobin* ($p < 0.05$), than bulls (Fig. 2a). Previously, a lower proportion of type 1 fiber was observed in the *longissimus dorsi* muscle of steers compared to that of bulls in French Montbe'liard cattle at 16 months of age¹⁵. In our study, steers had lower ($P < 0.01$) mRNA levels of the fiber type 2A (fast twitch, oxidative, glycolytic) myosin heavy chain (MYH) isoform *MYH2* gene than bulls. mRNA levels of the fast type 2B (fast-twitch, glycolytic) *MYH4* gene did not differ ($p > 0.05$) between bulls and steers. Collectively, our results reveal that castration decreases transcription of muscle fiber types 1 and 2A genes in the LT.

These changes in fiber type gene expression may affect muscle metabolism. Thus, we compared the transcript levels of oxidative and glycolytic enzyme genes in the LT between bulls and steers. mRNA levels of oxidative enzyme genes, including *citrate synthase* and *isocitrate dehydrogenase 1*, did not differ between bulls and steers (Fig. 2b). Steers had higher ($p < 0.05$) mRNA levels of the glycolytic enzyme *phosphoglycerate kinase 1* gene than bulls (Fig. 2b). Steers also tended to have higher ($p = 0.10$) mRNA levels of the glycolytic enzyme *hexokinase 2* gene than bulls. mRNA levels of *muscle type phosphofructokinase 1* and *lactate dehydrogenase A* genes did not differ between bulls and steers. Consistent with our results, a previous study reported that steers exhibited higher glycolytic enzyme activities than bulls; however, the same study found that steers had lower oxidative enzyme activities than bulls, which contrasted with the results of the present study¹⁵. This inconsistency may be due to a difference in cattle breed (Korean cattle vs. French Montbe'liard cattle). In skeletal muscle forkhead box O1 transgenic mice, expression of muscle fiber type I genes, including slow isoforms of troponins and myoglobin, was down-regulated, but expression of mitochondrial oxidative enzyme genes involved in electron transport system (*cytochrome c oxidase II and IV*) did not differ, between forkhead box O1 mice and the control²⁵. Collectively, our results indicate that castration moderately affects gene expression levels of glycolytic enzymes but does not affect gene expression of oxidative enzymes.

Comparison of plasma leptin levels between bulls and steers.

In this study, steers had higher ($p < 0.05$) plasma leptin concentrations than bulls (Fig. 1c). White adipose tissue is the main leptin-producing tissue²⁶. Rodent primary culture experiments have shown that leptin is secreted after the differentiation of preadipocytes into adipocytes²⁷. Consistent with our study, higher leptin concentrations were indicative of greater adiposity in beef cattle²⁸. Therefore, higher body fat content in steers may be one explanation for the higher plasma leptin concentrations observed in our study and other studies. Positive correlations between adipocyte size and leptin expression have been found in human^{29,30} and rodent studies³¹. Similarly, plasma leptin concentrations

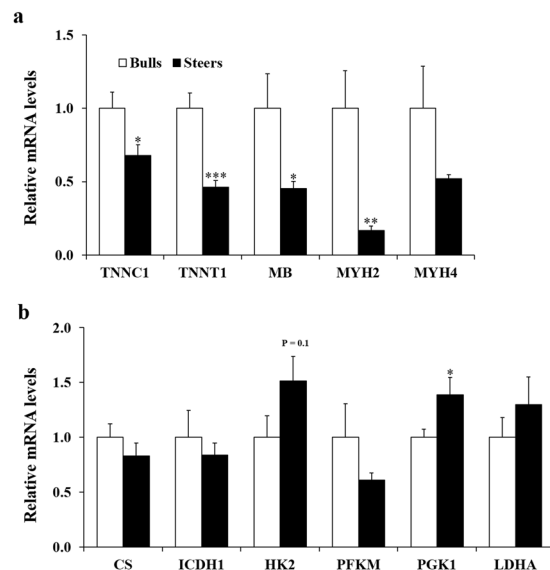


Figure 2. Expression levels of muscle fiber type and oxidative and glycolytic enzyme genes in the longissimus thoracis of Korean cattle bulls and steers. mRNA levels of muscle fiber type (a) and oxidative and glycolytic enzyme (b) genes were determined using quantitative PCR and normalized to β -actin ($n = 10/\text{group}$). mRNA levels in bulls were normalized to 1.0. Values are shown as the mean + standard error. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. *TNNC1*, troponin C1; *TNNT1*, troponin T1; *MB*, myoglobin; *MYH2*, myosin heavy chain 2; *MYH4*, myosin heavy chain 4; *CS*, citrate synthase; *ICDH1*, isocitrate dehydrogenase 1; *HK2*, hexokinase 2; *PFKM*, phosphofructokinase 1, muscle type; *PGK1*, phosphoglycerate kinase 1; *LDHA*, lactate dehydrogenase A.

are strongly related to adipose cell size in cattle³². Because larger adipocytes accommodate more leptin mRNA, adipocyte size may influence leptin synthesis and secretion³³. Several studies have reported that hypertrophy is actively involved in marbling and IMF deposition^{34,35}. Our previous study also revealed that castration induces hypertrophy of subcutaneous and abdominal fat in Korean cattle³⁶. Therefore, we speculate that increased adipocyte hypertrophy may have contributed to the increased plasma leptin concentrations in castrated animals, although we did not measure the size of intramuscular adipocytes in this study.

Conclusions

Our findings suggest that upregulation of the BMP signaling pathway in response to castration induces increased adipogenic gene expression in bulls, contributing to the increased IMF content observed in castrated animals. Our findings also indicate that castration affects the expression of some of muscle fiber type genes and has moderate effects on the expression of glycolytic pathway genes, but not oxidative enzymes. Our results reveal a new adipogenesis pathway for bovine IMF deposition.

Materials and Methods

Animals and tissue samples. All experimental procedures involving animals were approved by the Seoul National University Institutional Animal Care and Use Committee (SNU-IACUC: SNU-161117-3) and conducted in accordance with the Animal Experimental Guidelines of the SNU-IACUC. This study examined 10 Korean cattle bulls and 10 steers. We used bulls and steers, which have clear differences in IMF content¹, as an experimental model to understand the involvement of BMP2 signaling molecules in IMF deposition and the metabolic differences between bulls and steers. A feeding regime was followed as previously described with modifications². Briefly, 20 bulls were weaned at an average age of 3 mo and fed with 30% concentrates and 70% roughage until 6 mo of age. Ten bulls were castrated at 6 mo, the age at which Korean cattle bulls are routinely castrated. We castrated bulls under the guidance of an expert veterinarian. Bulls and steers were fed with concentrates composed of 15% crude protein (CP) and 71% total digestible nutrients (TDN) from 7 mo to 13 mo of age, 13% CP and 72% TDN until 19 mo of age, and 11% CP and 73% TDN after 20 mo of age. For roughage, timothy (2.5–3.5 kg/d; approximately 1.2% BW/animal) was fed from 7 mo to 13 mo of age, rice straw (2.0–3.0 kg/d; approximately 0.5% BW/animal) until 19 mo of age, and rice straw (1.0–1.5 kg/d; approximately 0.15% BW/animal) after 20 mo of age.

Before slaughter, blood was collected from the jugular vein into EDTA Vacutainer tubes, and plasma was prepared as previously described³⁷. Bulls and steers were slaughtered at 25.7 ± 0.52 and 31.8 ± 0.11 months, respectively. Carcass traits, including MS, beef quality grade, ribeye area, fat thickness, yield index, and yield grade, were determined as previously described². *Longissimus thoracis* (LT) muscles from the left carcass side between the 12th and 13th ribs were collected immediately after slaughter and stored at -70°C until analysis. The IMF content of the LT muscles was measured following the procedure of Folch *et al.*³⁸. Briefly, LT tissues were ground into a fine powder, which was homogenized in a 2:1 chloroform-methanol mixture (vol/vol). The fat content was measured after the fat-containing solvents were evaporated.

Gene name (Symbol)	Gene bank accession no.	Primer	Sequence (5'-3')	T _m , °C	Length (bp)
<i>β-actin (ACTB)</i> *	NM_173979.3	Forward Reverse	AGCAAGCAGGAGTACGATGAGT ATCCAACCGACTGCTGTCA	60.5 58.0	120
<i>Lysyl oxidase (LOX)</i>	NM_173932.4	Forward Reverse	AACAATGTCGTCCGCTGTGA CCTTGGGAGTTTGGCTTGC	60.2 60.5	102
<i>Peroxisome proliferator activated receptor gamma (PPARG)</i>	NM_181024.2	Forward Reverse	AATCCCTGTTCCGTGCTGTG AAAGTTGGTGGCCAAAACG	59.6 58.9	149
<i>Adiponectin</i>	NM_174742.2	Forward Reverse	CCCTGACTGAAGTCTGTGGC TCTTCCATGTTGTCCTCGCC	60.3 60.0	115
<i>Acetyl-CoA carboxylase alpha (ACC)</i>	NM_174224.2	Forward Reverse	AGACGTTGGAAGCAGAGAGG TTCAGCTCCAGAGGTTTGGC	59.4 55.0	142
<i>Fatty acid binding protein 4 (FABP4)</i>	NM_174314.2	Forward Reverse	GCTGCACCTTCTTCTCACCT TTCTGGTAGCAAAGCCAC	58.1 60.3	140
<i>Troponin C1, slow skeletal and cardiac type (TNNC1)</i>	NM_001034351.2	Forward Reverse	GTGAGCCGCCAGTATGGATG CACGAAGATGTCAAAGGCCG	60.9 59.6	97
<i>Troponin T1, slow skeletal type (TNNT1)</i>	XM_010815469.3	Forward Reverse	GCCTCTGAACATCGACCACA AGCTTCGCCATCAGGTCAA	55.0 60.0	111
<i>Myoglobin (MB)</i>	NM_173881.2	Forward Reverse	GAGTCACATGCCAACAAGCAC GAAGTCTGAAGGATGCTTGGC	60.3 59.2	99
<i>Myosin heavy chain 2 (MYH2)</i>	NM_001166227.1	Forward Reverse	AAGAGCCCTTGAATGAGGC ATGGCCATTTCTGGTCCG	60.0 60.1	138
<i>Myosin, heavy chain 4, skeletal muscle (MYH4)</i>	NM_174224.2	Forward Reverse	GGTCCAAGTGTGAAGAGGG GATGCAGCGTACAAAGTGGG	60.3 59.6	150
<i>Citrate synthase (CS)</i>	NM_001044721.1	Forward Reverse	CCATGGCTTACTCACTGCG TTCGTGGAAGAAGCACTGGC	59.3 60.9	97
<i>Isocitrate dehydrogenase (NADP(+)) 1 (IDH1)</i>	NM_181012.3	Forward Reverse	TCCGAAATATCCTGGGTGGC CCCTGGCACAACAAAATCGG	59.5 60.0	149
<i>Hexokinase 2 (HK2)</i>	XM_015473383.2	Forward Reverse	TGCTCGCCTACTTCTTACGG CCATCTCCTTGGGAAAACGC	60.1 60.2	126
<i>Phosphofructokinase, muscle (PFKM)</i>	NM_001075268.1	Forward Reverse	GGACAATCTGCAAAGAAGCCC CCACCAGAGGTTAACACGGC	52.4 60.0	126
<i>Phosphoglycerate kinase 1 (PGK1)</i>	NM_001034299.1	Forward Reverse	GTGGAGGAAGAAGGGAAGGG GAAAGTGAAGCTCGGAAGG	59.4 59.2	89
<i>Lactate dehydrogenase A (LDHA)</i>	NM_174099.2	Forward Reverse	GCTATTAATCGGTGCCCCAGG TTGCCATCTGGACTTAGACCC	60.9 60.0	110

Table 3. Sequences of the primers used in real-time PCR analysis. *ACTB = Internal control gene.

Blood plasma analysis. The plasma BMP2 and leptin concentrations were quantified using enzyme-linked immunosorbent assays (ELISA) according to the manufacturer's instructions. BMP2 was analyzed using a Bovine Bone Morphogenetic Protein 2 ELISA Kit (MyBioSource, San Diego, CA, USA) and leptin was analyzed using a Bovine Leptin ELISA Kit (MyBioSource). The intra- and inter-assay coefficients of variation for the Leptin and BMP2 kits were both less than 15%.

RNA extraction and quantitative real-time polymerase chain reaction. Total RNA was extracted from LT tissue using TRIzol reagent (Molecular Research Center, Cincinnati, OH, USA), based on the manufacturer's instructions. The RNA was quantified using a NanoPhotometer (Implen, Munich, Germany), and the quality was assessed using ethidium bromide staining of the 28S and 18S agarose gel electrophoresis bands and a Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA), as previously described³⁹. cDNA was synthesized from reverse-transcribed total RNA using an iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA, USA), according to the manufacturer's instructions.

Quantitative real-time PCR was performed using QuantiTect SYBR Green RT-PCR Master Mix (QIAGEN, Hilden, Germany), as previously described³⁹. We followed the "Minimum Information for Publication of Quantitative Real-Time PCR Experiments" (MIQE) guidelines for qPCR as closely as possible⁴⁰. All qPCR analyses were conducted in a 25- μ L total reaction volume that contained 20 ng cDNA, 12.5 μ L SYBR Green RT-PCR Master Mix, and 1.25 μ L of 10 μ M primers. The thermal cycling parameters were: 95 °C for 15 min, followed by 40 cycles at 94 °C for 15 s, 55 °C for 30 s, and 72 °C for 30 s. Table 3 lists the primers used. We used two different exons for forward and reverse primers to prevent amplification of the DNA template. We indicated the melting temperatures (T_m) of all primers. The T_ms of all of the primers were 52.4–60.9 °C. An annealing temperature of 55 °C was used for amplification of all genes, resulting in a single major peak in all cases. The $\Delta\Delta$ CT method was used to determine the relative fold change in gene expression⁴¹. We evaluated whether β -actin, ribosomal protein lateral stalk subunit P0, and 18 s RNA were suitable reference genes. β -actin expression was generally uniform in the LT between bulls and steers and was therefore used as the reference gene. We also used β -actin as a reference gene in the LT in two previous studies^{2,42}.

Statistical analyses. Differences between bulls and steers were examined using the general linear model procedure in SAS 9.1 software (SAS Institute, Cary, NC, USA). The IMF contents were not normally distributed

due to marked differences between the bull and steer groups. Thus, we performed a linear regression analysis to separately examine the relationship between gene expression and IMF content (%) within the bull and steer groups using SAS 9.4 software. This resulted in the following equation:

$$\text{IMF}\%_i = \beta_0 + \beta_1 \text{Expression}_i + \epsilon_i,$$

where IMF%_i is the variable of IMF%, Expression_i is the variable expression level, β_0 is the intercept, β_1 is the coefficient of expression level, and ϵ_i is random error.

Received: 29 April 2019; Accepted: 12 December 2019;

Published online: 24 December 2019

References

- Park, S. J. *et al.* Genetic, management, and nutritional factors affecting intramuscular fat deposition in beef cattle - A review. *Asian-Australas J Anim Sci* **31**, 1043–1061 (2018).
- Bong, J. J. *et al.* Differential expression of genes associated with lipid metabolism in longissimus dorsi of Korean bulls and steers. *Meat Sci* **91**, 284–293 (2012).
- Baik, M. *et al.* Triennial Growth and Development Symposium: Molecular mechanisms related to bovine intramuscular fat deposition in the longissimus muscle. *Journal of Animal Science* **95** (2017).
- Jeong, J., Kwon, E. G., Im, S. K., Seo, K. S. & Baik, M. Expression of fat deposition and fat removal genes is associated with intramuscular fat content in longissimus dorsi muscle of Korean cattle steers. *J Animal Sci* **90**, 2044–2053 (2012).
- Hood, R. L. & Allen, C. E. Cellularity of bovine adipose tissue. *J Lipid Res* **14**, 605–610 (1973).
- Owens, F. N., Dubeski, P. & Hanson, C. F. Factors that alter the growth and development of ruminants. *J Anim Sci* **71**, 3138–3150 (1993).
- Yu, Z. K., Wright, J. T. & Hausman, G. J. Preadipocyte recruitment in stromal vascular cultures after depletion of committed preadipocytes by immunocytotoxicity. *Obes Res* **5**, 9–15 (1997).
- Tang, Q. Q. & Lane, M. D. Adipogenesis: from stem cell to adipocyte. *Annu Rev Biochem* **81**, 715–736 (2012).
- Lai, N., Sims, J. K., Jeon, N. L. & Lee, K. Adipocyte induction of preadipocyte differentiation in a gradient chamber. *Tissue Eng Part C Methods* **18**, 958–967 (2012).
- Huang, H. *et al.* BMP signaling pathway is required for commitment of C3H10T1/2 pluripotent stem cells to the adipocyte lineage. *Proc Natl Acad Sci USA* **106**, 12670–12675 (2009).
- Huang, H. Y. *et al.* Involvement of cytoskeleton-associated proteins in the commitment of C3H10T1/2 pluripotent stem cells to adipocyte lineage induced by BMP2/4. *Mol Cell Proteomics* **10**, M110 002691 (2011).
- Tang, Q. Q., Otto, T. C. & Lane, M. D. Commitment of C3H10T1/2 pluripotent stem cells to the adipocyte lineage. *Proc Natl Acad Sci USA* **101**, 9607–9611 (2004).
- Fruhbeck, G. Intracellular signalling pathways activated by leptin. *Biochem J* **393**, 7–20 (2006).
- Bates, S. H. *et al.* STAT3 signalling is required for leptin regulation of energy balance but not reproduction. *Nature* **421**, 856–859 (2003).
- Brandstetter, A. M., Picard, B. & Geay, Y. Muscle fibre characteristics in four muscles of growing male cattle: II. Effect of castration and feeding level. *Livestock Production Science* **53**, 25–36 (1998).
- Esteve Rafols, M. Adipose tissue: cell heterogeneity and functional diversity. *Endocrinología y nutrición: organo de la Sociedad Espanola de Endocrinología y Nutrición* **61**, 100–112 (2014).
- Ozkaynak, E. *et al.* Osteogenic protein-2. A new member of the transforming growth factor-beta superfamily expressed early in embryogenesis. *The Journal of biological chemistry* **267**, 25220–25227 (1992).
- Guiu-Jurado, E. *et al.* Bone morphogenetic protein 2 (BMP2) may contribute to partition of energy storage into visceral and subcutaneous fat depots. *Obesity (Silver Spring, Md.)* **24**, 2092–2100 (2016).
- Guo, W. *et al.* Testosterone administration inhibits hepcidin transcription and is associated with increased iron incorporation into red blood cells. *Aging cell* **12**, 280–291 (2013).
- Tsai, E. C., Matsumoto, A. M., Fujimoto, W. Y. & Boyko, E. J. Association of bioavailable, free, and total testosterone with insulin resistance: influence of sex hormone-binding globulin and body fat. *Diabetes care* **27**, 861–868 (2004).
- Knippenberg, M. *et al.* Differential Effects of Bone Morphogenetic Protein-2 and Transforming Growth Factor- β 1 on Gene Expression of Collagen-Modifying Enzymes in Human Adipose Tissue-Derived Mesenchymal. *Stem Cells* **15**, 2213–2225 (2009).
- Hata, K. *et al.* Differential roles of Smad1 and p38 kinase in regulation of peroxisome proliferator-activating receptor gamma during bone morphogenetic protein 2-induced adipogenesis. *Mol Biol Cell* **14**, 545–555 (2003).
- Berger, J. P. Role of PPARgamma, transcriptional cofactors, and adiponectin in the regulation of nutrient metabolism, adipogenesis and insulin action: view from the chair. *International journal of obesity (2005)* **29**(Suppl 1), S3–4 (2005).
- van Zoelen, E. J., Duarte, I., Hendriks, J. M. & van der Woning, S. P. TGF β -induced switch from adipogenic to osteogenic differentiation of human mesenchymal stem cells: identification of drug targets for prevention of fat cell differentiation. *Stem Cell Res Ther* **7**, 123–123 (2016).
- Kamei, Y. *et al.* Skeletal muscle FOXO1 (FKHR) transgenic mice have less skeletal muscle mass, down-regulated Type I (slow twitch/red muscle) fiber genes, and impaired glycemic control. *The Journal of biological chemistry* **279**, 41114–41123 (2004).
- Zhang, Y. Positional cloning of the mouse obese gene and its human homologue. *Nature* **372**, 425–432 (1994).
- Mitchell, S. E. *et al.* ob gene expression and secretion of leptin following differentiation of rat preadipocytes to adipocytes in primary culture. *Biochemical and biophysical research communications* **230**, 360–364 (1997).
- Geary, T. W. *et al.* Leptin as a predictor of carcass composition in beef cattle. *J Anim Sci* **81**, 1–8 (2003).
- Jernas, M. *et al.* Separation of human adipocytes by size: hypertrophic fat cells display distinct gene expression. *FASEB journal: official publication of the Federation of American Societies for Experimental Biology* **20**, 1540–1542 (2006).
- Skurk, T., Alberti-Huber, C., Herder, C. & Hauner, H. Relationship between adipocyte size and adipokine expression and secretion. *The Journal of clinical endocrinology and metabolism* **92**, 1023–1033 (2007).
- Guo, K. Y., Halo, P., Leibel, R. L. & Zhang, Y. Effects of obesity on the relationship of leptin mRNA expression and adipocyte size in anatomically distinct fat depots in mice. *American journal of physiology. Regulatory, integrative and comparative physiology* **287**, R112–R119 (2004).
- Delavaud, C. *et al.* Plasma leptin concentration in adult cattle: effects of breed, adiposity, feeding level, and meal intake. *J Anim Sci* **80**, 1317–1328 (2002).
- Auwerx, J. & Staels, B. Leptin. *Lancet (London, England)* **351**, 737–742 (1998).
- Du, M. *et al.* Fetal programming of skeletal muscle development in ruminant animals. *J Anim Sci* **88**, E51–60 (2010).
- Du, M. *et al.* Meat Science and Muscle Biology Symposium: manipulating mesenchymal progenitor cell differentiation to optimize performance and carcass value of beef cattle. *J Anim Sci* **91**, 1419–1427 (2013).
- Baik, M., Jeong, J. Y., Vu, T.-T. T., Piao, M. Y. & Kang, H. J. Effects of castration on the adiposity and expression of lipid metabolism genes in various fat depots of Korean cattle. *Livestock Science* **168**, 168–176 (2014).

37. Kang, H. J. *et al.* Effects of ambient temperature and rumen-protected fat supplementation on growth performance, rumen fermentation and blood parameters during cold season in Korean cattle steers. *Asian-Australas J Anim Sci* In press (2018).
38. Folch, J., Lees, M. & Sloane Stanley, G. H. A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry* **226**, 497–509 (1957).
39. Park, S. J., Kang, H. J., Na, S., Lee, S. H. & Baik, M. Differential expression of extracellular matrix and integrin genes in the longissimus thoracis between bulls and steers and their association with intramuscular fat contents. *Meat Sci* **136**, 35–43 (2018).
40. Bustin, S. A. *et al.* The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clinical chemistry* **55**, 611–622 (2009).
41. Livak, K. J. & Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods (San Diego, Calif.)* **25**, 402–408 (2001).
42. Jeong, J. Y., Kim, J. S., Nguyen, T. H., Lee, H. J. & Baik, M. Wnt/beta-catenin signaling and adipogenic genes are associated with intramuscular fat content in the longissimus dorsi muscle of Korean cattle. *Animal genetics* **44**, 627–635 (2013).

Acknowledgements

This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (2017R1A2B4003207).

Author contributions

D.J.S.J. performed most of the experiments, performed the statistical analysis, and wrote the draft of the paper. M.B. guided all experiments and revised the paper. All authors approved the final version of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to M.B.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2019