Meat and Muscle Biology™

All-Trans Retinoic Acid Drives Development Phase-Specific Response to Adipogenic and Myogenic Processes in Bovine Skeletal Muscle-Derived Cells

Xue Cheng Jin[1,#], Dong Qiao Peng[2,#], Won Seob Kim[3], Jin Soo Park[1], Jun Hee Lee[1], Yuan H. Brad Kim[4], Stephen B. Smith[5], and Hong Gu Lee[1]*

[1]Department of Animal Science and Technology, Sanghuh College of Life Science, Konkuk University, Seoul 05029, Korea
[2]College of Animal Sciences, Jilin University, Jilin Provincial Key Laboratory of Livestock and Poultry Feed and Feeding in Northeastern Frigid Area, Changchun 130062, China
[3]Department of Animal Science, Michigan State University, East Lansing, MI 48824, USA
[4]Department of Animal Sciences, Purdue University, West Lafayette, IN 47907, USA
[5]Department of Animal Science, Texas A&M University, College Station, TX 77843, USA
*Corresponding author. Email: hglee66@konkuk.ac.kr (Hong Gu Lee)
[#]These authors contributed equally to this work.

Abstract: Optimal muscle and intramuscular fat development are foundational to enhanced high-quality meat production in beef cattle, involving the proliferation and differentiation of key cellular populations, such as myoblasts and preadipocytes. Vitamin A is an essential nutrient for beef cattle and plays an important role in the formation and maintenance of numerous tissue types such as adipose tissue and skeletal muscle. The objective of this study was to investigate the effect of all-trans retinoic acid (ATRA), the active form of vitamin A, on adipogenic and myogenic processes in bovine skeletal muscle-derived cells (BSMC). As a result, ATRA treatment significantly upregulated *ZFP423* and *MYF5* during the growth phase ($P < 0.05$). Moreover, ATRA treatment in the growth phase significantly elevated mRNA expression markers, including *FABP4* and *PPARγ* in adipogenesis ($P < 0.05$), as well as *MYOD*, *MYOG*, and *MYF6* in myogenesis ($P < 0.05$). In addition, when used only in the differentiation phase, ATRA treatment significantly increased the mRNA expression of myogenesis markers (*MYOD*, *MYOG*, and *MYF6*) ($P < 0.05$) but decreased the mRNA expression of adipogenesis markers (*FABP4* and *PPARγ*) compared with the control group ($P < 0.05$). In conclusion, the ATRA treatment in the growth phase of BSMC indirectly promoted hyperplasia of myoblasts and preadipocytes, and the effect continued into subsequent differentiations. However, ATRA treatment plays a different role in adipogenic and myogenic differentiation by regulating related mRNA. These results will provide a reference for the application of vitamin A from the fetal to the postnatal period in beef cattle production.

Key words: adipogenic process, all-trans retinoic acid, bovine skeletal muscle-derived cells, myogenic process, zinc finger protein 423
Meat and Muscle Biology 8(1): 16978, 1–13 (2024) **doi:10.22175/mmb.16978**
Submitted 14 September 2023 Accepted 7 December 2023

Introduction

As global demand for high-quality protein sources intensifies, the beef industry faces a pressing need to augment both meat quality and yield (Cho et al., 2010). Marbling (intramuscular fat) and skeletal muscle development are critical to this endeavor, which is founded on cellular processes such as hyperplasia (increase in cell number) and hypertrophy (increase in cell size) (Russell and Oteruelo, 1981; Jo et al., 2009; Peng et al., 2021). In beef cattle, a previous report hypothesized that intramuscular fat development in the fetal stage and the first 250 d post-birth is marked by preadipocyte hyperplasia, followed by a shift toward adipocyte hypertrophy that

continues until slaughter (Wang et al., 2016). In addition, the development of skeletal muscle in antenatal bovines mainly includes the hyperplasia of mononuclear myoblasts during the early fetal stage and the hypertrophy of myofibers after the middle fetal stage; then, the newly formed myocytes from satellite cells fuse with myofibers to continue the development of postnatal muscle tissue (Bonnet et al., 2010; Yin et al., 2013). Therefore, researching how nutrient treatments affect the development of intramuscular fat and skeletal muscle at the cellular level is pivotal for practical applications in beef production.

Skeletal muscle is a complex tissue composed of a diverse array of heterogeneous cell populations. A recent study characterized bovine skeletal muscle-derived cells (BSMC), identifying 11 distinct populations of mononuclear cells, including fibro-adipogenic progenitors (FAP), satellite cells, endothelial cells (EC), and smooth muscle and mesenchymal cells (SMMC) (Messmer et al., 2023). The cellular diversity within primary BSMC supports the induction of multipotent differentiation pathways under specific *in vitro* culture conditions, encompassing adipogenic and myogenic differentiation (Li et al., 2017; Messmer et al., 2023). The BSMC can be obtained from the skeletal muscle tissue of live cattle through biopsy, or alternatively, they can be acquired at the time of slaughter to avoid additional harm to the animals. Given the ease of obtaining material from the animals and the potential for various forms of differentiation, primary BSMC culture provides a reliable model for *in vitro* studies of the mechanisms underlying intramuscular fat and skeletal muscle development (El-Habta et al., 2018; Baatar and Hwang, 2020).

Vitamin A and its derivatives (metabolic forms of vitamin A: retinal, retinoic acid) act as essential factors in maintaining normal physiological function in cattle (Chen and Chen, 2014). For beef cattle, supplementing or restricting vitamin A during specific stages is closely related to the development of intramuscular fat and skeletal muscle. The negative relationship between serum vitamin A and marbling was first noted in Black Japanese Steers, in which vitamin A restriction during the fattening period contributed to increased marbling in beef (Oka et al., 1998). Subsequently, studies of the effect of vitamin A and adipocyte hypertrophy on marbling score were carried out in various species of cattle (Peng et al., 2021). Also, numerous studies were done on the hypertrophy of adipocytes to raise the total amount of marbling in cattle, using an *in vitro* model of the 3T3-L1 cell line from mice, but few studies focused on the hyperplasia of preadipocytes (Green and Meuth, 1974; Park et al., 2018). On the other hand, retinoic acid has previously been shown to impact myogenic differentiation in various species, including mice, zebrafish, chickens, and sheep; however, its promotive effects remain controversial (Hamade et al., 2006; Reijntjes et al., 2010; Liu et al., 2016; Li et al., 2020). Our recent study showed that vitamin A supplementation in late pregnancy has the potential to increase birth weight, muscle development, as well as preadipocyte development in Korean native cattle (Jo et al., 2020). Meanwhile, we also found that vitamin A supplementation promoted preadipocyte and muscle development in Korean native calves (Peng et al., 2020a, 2020b; Jin et al., 2022). In addition, vitamin A injection in Angus calves also showed a positive effect on muscle development as well as final marbling score in beef cattle (Harris et al., 2018; Wang et al., 2018). Previous studies suggest that supplementing vitamin A from prenatal to calf stages and restricting it during the fattening stage seems to be an effective measure to enhance intramuscular fat and skeletal muscle development. However, the impact of vitamin A *in vitro* on intramuscular fat and muscle, particularly its effects on different developmental phases of cells, remains unclear.

Therefore, the objective of the present study was to investigate the impact of all-trans retinoic acid (ATRA; the active form of vitamin A) on BSMC of Korean native cattle at different developmental phases of adipogenesis and myogenesis.

Materials and Methods

Isolation of bovine skeletal muscle-derived cells

All animal experiments were performed in accordance with the "Guidelines for Care and Use of Experimental Animals," under approval number KU21206 from Konkuk University. The method used to isolate BSMC from muscle tissue in this study was previously described (Ortiz-Colón et al., 2009; Lavasani et al., 2013; Lee et al., 2023). *Longissimus thoracis* muscle samples from 4 female Korean native cattle (29.5 ± 0.6 mo of age; carcass weight: 415.8 ± 57.4 kg) were obtained from a slaughterhouse in Namyangju (Gyeonggi, Republic of Korea). The muscle sample was first rinsed 3 times with sterile cold 1 × PBS supplemented with 0.2% gentamicin and amphotericin B solution (G/A, 500X, Sigma-Aldrich, NY, USA). After that, it was placed on ice in the same supplemented PBS solution for transportation and arrived

at the laboratory within 1 h of collection. The sample was then minced and enzymatically digested in a shaking water bath (JSSB-30T, JSR, Gongju, Republic of Korea) at 37°C and 80 rpm for 45 min using 0.25% collagenase type II (Gibco, NY, USA) and 0.1% dispase II (Sigma-Aldrich, MO, USA). Following this, Dulbecco's Modified Eagle Medium (DMEM; Hyclone, UT, USA), supplemented with 10% fetal bovine serum (FBS; Biowest, FL, USA) and 1% penicillin/streptomycin (P/S; Gibco), was added to terminate the digestive reaction. The mixture was then filtered through 100-μm and 70-μm cell filters, followed by the removal of red blood cells (RBC) using an RBC lysis buffer. The cells were seeded into 10% collagen-coated (Collagen Solutions, Glasgow, UK) culture plates containing a growth medium composed of DMEM, 10% FBS, 0.2% G/A, and 1% P/S under a 5% CO_2 atmosphere at 37°C. The medium was changed every 3 d, and the BSMC were passaged at 70% confluence by trypsinization (Trypsin-EDTA solution, Gibco). The BSMC at passage 3 were used for the subsequent ATRA treatment experiments.

Induction of adipogenic differentiation

For the adipogenic differentiation, the BSMC were seeded in a 6-well plate containing the growth medium at a density of 5.0×10^4 cells/well. After 48 h of culture, the BSMC reached approximately 90% confluence (considered D0), and the medium was changed to induce adipogenic differentiation. The adipogenic differentiation was induced under the condition of DMEM, 10% FBS, 10 μg/mL insulin (Sigma-Aldrich), 0.5 mM isobutylmethylxanthine (IBMX; Sigma-Aldrich), 1 μM dexamethasone (Sigma-Aldrich), 1 μg/mL rosiglitazone (Sigma-Aldrich), and 1% P/S. After 3 d, the medium was changed to a medium comprising DMEM, 10% FBS, 10 μg/mL insulin, 1 μg/mL rosiglitazone, and 1% P/S to maintain the differentiation, and the medium was changed every 2 d. On day 14 (D14), lipid droplets within the adipocytes were visualized by Oil Red O staining under a microscope.

Induction of myogenic differentiation

For the myogenic differentiation, the BSMC were seeded in a 6-well plate containing the growth medium at the density of 5.0×10^4 cells/well. After 48 h of culture, the BSMC reached approximately 90% confluence (considered D0), and the medium was changed to induce myogenic differentiation. The myogenic differentiation was induced under the condition of DMEM, 2% horse serum (Gibco), 10 μg/mL insulin, 1 μg/mL doxycycline (Sigma-Aldrich), and 1% P/S. The medium was changed every 2 d. Myotubes were observed under the microscope on day 6 (D6) to confirm the myogenic differentiation.

All-trans retinoic acid treatment

Increasing concentrations of ATRA (0.01 μM, 0.1 μM, and 1 μM, Sigma-Aldrich) were added to the medium with the solvent (dimethyl sulfoxide [DMSO], Sigma-Aldrich). Treatment with ATRA was performed independently across 3 phases: the growth phase, the adipogenic differentiation phase, and the myogenic differentiation phase (Figure 1). The growth phase was considered as the 48 h period after seeding. The adipogenic differentiation phase lasted 14 d, and the myogenic differentiation phase lasted 6 d. Each treatment group consisted of 3 wells in a 6-well plate. To prevent degradation of ATRA, experiments were conducted in a dark room under red light.

Oil Red O staining

Oil Red O staining was performed on D14 of the adipogenic differentiation. The BSMC were washed twice with 1 × PBS and then fixed with 10% formalin at room temperature overnight. After washing twice with distilled water (DW), the cells were incubated for 5 min with 60% isopropanol. Then, the cells were stained with an Oil Red O solution comprising 0.3% Oil Red O (Sigma-Aldrich) and 60% isopropanol for 30 min. Then the stained cells were washed 4 times with DW and dissolved in 100% isopropanol for optical density (OD) value detection at 500 nm.

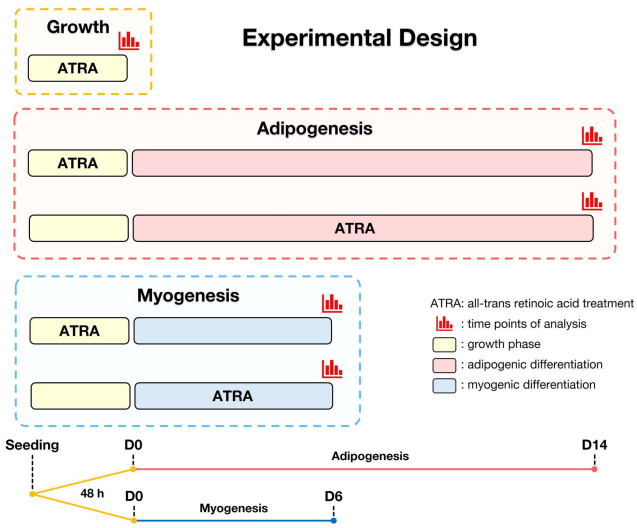

Figure 1. Experimental design for all-trans retinoic acid (ATRA) treatment during different developmental phases of bovine skeletal muscle-derived cells (BSMC).

Reverse transcription-qPCR

Total RNA was extracted from the BSMC at 3 different time points: the end of the growth phase (D0), myogenic differentiation phase (D6), and adipogenic differentiation phase (D14). The extraction was performed using TRIzol Reagent (Molecular Research Center, OH, USA), following the protocol provided by the manufacturer. RNA integrity was evaluated using the RNA Nano 6000 assay kit (Agilent Technologies, CA, USA) on an Agilent Bioanalyzer 2100 system (Agilent Technologies). The RNA integrity number (RIN) of samples used in this study was 8.5 ± 0.5. The cDNA was synthesized from 300 ng of RNA using the iScript cDNA synthesis kit (Bio-Rad, CA, USA). The qPCR reactions were performed using a SYBR green kit (AccuPower 2X GreenStarTM qPCR MasterMix, Bioneer, Seoul, Republic of Korea) on a CFX Connect Real-Time PCR Detection System (Bio-Rad). Specific primers for qPCR were designed using Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/), as shown in Table 1. The amplification efficiencies of all genes were calculated from the standard curve at 10-fold serial dilution and were above 95%. *18S*, *RPLP0*, and *GAPDH* were used as reference genes (Wang et al., 2022), and the data of relative expression were analyzed using the $2^{-\Delta\Delta CT}$ method.

Statistical analysis

The Oil Red O staining and mRNA expression data were analyzed using the GLM procedure in SAS (v. 9.4, SAS Institute, NC, USA), and ATRA treatment was considered as a fixed effect. Tukey's HSD test was performed for multiple comparisons. The BSMC isolated from muscle samples of each individual beef cattle were considered as biological replicates and served as experimental units (n). Differences were considered statistically significant if the P value was less than 0.05.

Results

Induction of adipogenic and myogenic differentiation in bovine skeletal muscle-derived cells

After isolation, BSMC were cultured with the growth medium, and adipogenic or myogenic differentiation was conducted at 90% confluence of BSMC, which is also considered D0 for the following differentiation (Figure 2a and 2b). After culturing the BSMC under adipogenic conditions for 14 d, lipid droplets were observed in the differentiated BSMC using Oil Red O staining (Figure 2c). Similarly, numerous mature myotubes were observed after culturing under myogenic conditions for 6 d (Figure 2d). These results indicated that isolated BSMC from slaughtered beef cattle were capable of differentiating into adipocytes and myotubes.

Effects of all-trans retinoic acid treatment during growth phase on preadipocyte and myoblast-related mRNA expressions in bovine skeletal muscle-derived cells

Following ATRA treatment during the growth phase of BSMC, *ZFP423* mRNA expression was

Table 1. Primer sequences designed for qPCR

Gene name	Forward primer (5'→ 3')	Reverse primer (5'→ 3')	Accession number
ZFP423	AGGGCTGGTCCAAGGTGGTGTA	TGTGGCTCTGCTGCGGCTTATC	NM_001101893
PREF1	CCTCTTGCTCCTGCTGGCTTTC	AAGGTCACGCACTGGTCACAC	NM_174037.2
KLF2	ATTAAGCGTCGTCTTCCCCC	ACCAGGTAGTCAAAATGCCCA	XM_024994816.1
WNT10B	TTGATACTCACAACCGCAACTCCG	CTCGCTCACAGAAGTCAGGAGAGT	XM_010805029.3
PPARγ	TGGAGACCGCCCAGGTTTGC	AGCTGGGAGGACTCGGGGTG	NM_181024.2
FABP4	CGTGGGCTTTGCTACCAG	TGGTTGATTTTCCATCCCAG	NM_174314.2
MYF5	TCCTGATGTACCAAATGTATATGCC	ATCCAGGTTGCTCTGAGTTGG	NM_174116.1
MYOD	AGGCGAAGGAACTGTTGTGT	TCAGGGAAGAGCGCTGAGTA	NM_001040478.2
MYOG	TACAGACGCCCACAATCTGC	GGTTTCATCTGGGAAGGCCG	NM_001111325.1
MYF6	GAAGGAGGGACAAGCATTGA	GAGGAAATGCTGTCCACGAT	NM_181811.2
18S	ACCCATTCGAACGTCTGCCCTATT	TCCTTGGATTGTGGTAGCCGTTTCT	NR_036642
RPLP0	CAACCCGGCTCTGGAGAAACTG	ACTTCACACGGCGCTATGG	NM_001012682.1
GAPDH	CGTGGAGGGACTTATGACCAC	CGCCAGTAGAAGCAGGGATG	NM_001034034.2

ZFP423 = zinc finger protein 423; *PREF1* = preadipocyte factor-1; *KLF2* = Kruppel like factor 2; *WNT10B* = Wnt family member 10B; *PPARγ* = peroxisome proliferator activated receptor gamma; *FABP4* = fatty acid binding protein 4; *MYF5* = myogenic factor 5; *MYOD* = myogenic differentiation 1; *MYOG* = myogenin; *MYF6* = myogenic factor 6; *18S* = 18S ribosomal RNA; *RPLP0* = ribosomal protein lateral stalk subunit P0; *GAPDH* = glyceraldehyde-3-phosphate dehydrogenase.

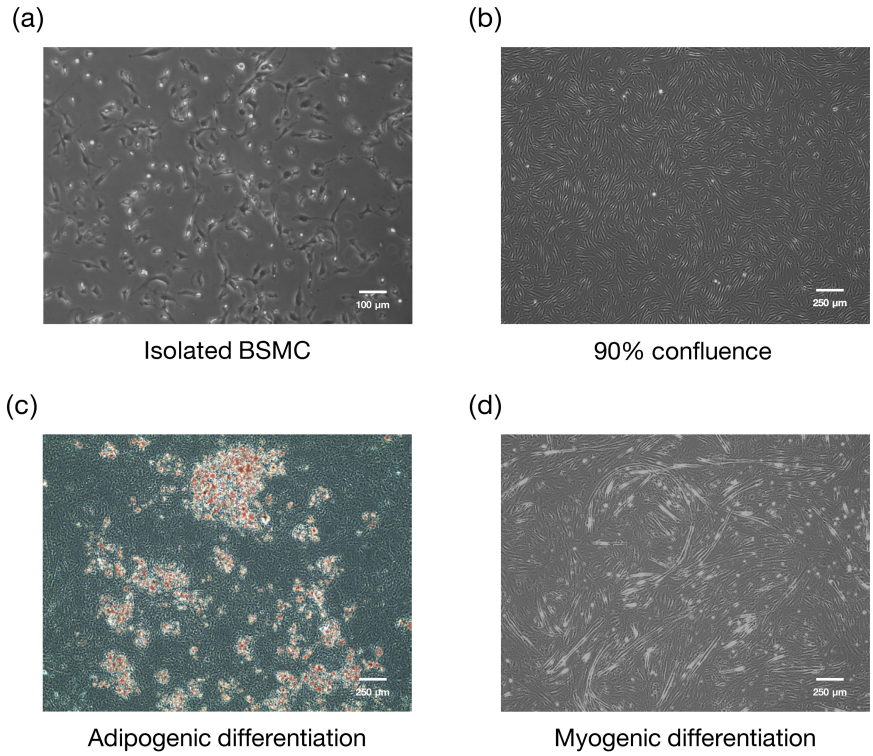

Figure 2. Representative images of bovine skeletal muscle-derived cells (BSMC) from isolation to adipogenic differentiation and myogenic differentiation. (a) The BSMC were isolated by the collagenase digestion method (original magnification × 100). (b) Isolated BSMC were cultured for 48 h until they reached approximately 90% confluence before the induction of adipogenic or myogenic differentiation (original magnification × 40). (c) Adipocytes were stained by the Oil Red O method 14 d after adipogenic differentiation, and the lipid droplets were shown in red (original magnification × 40). (d) Myotubes were observed 6 d after myogenic differentiation (original magnification × 40).

significantly upregulated at D0 ($P < 0.05$, Figure 3a). Similarly, a significant increase in the expression of *KLF2* mRNA was observed after treatment with 0.1 μM ATRA compared with that in control cells ($P < 0.05$, Figure 3b). In addition, *MYF5* mRNA expression increased with both 0.1 μM and 1 μM concentrations ($P < 0.05$, Figure 3c). However, no significant difference was observed in *PREF1* and *WNT10B* mRNA expression ($P > 0.05$, Figure 3d and 3e).

Effects of all-trans retinoic acid treatment during growth phase on subsequent adipogenic and myogenic differentiation in bovine skeletal muscle-derived cells

To further confirm the impact of ATRA treatment during the growth phase on subsequent adipogenic differentiation, Oil Red O staining was performed on D14 of differentiation. Our results revealed that the adipogenesis levels were significantly enhanced at both 0.1 μM and 1 μM concentrations of ATRA compared to control cells ($P < 0.05$, Figure 4a). The significant upregulation of *FABP4* and *PPARγ* mRNA expression at both 0.1 μM and 1 μM concentration supported this observation ($P < 0.05$, Figure 4b and 4c). No significant difference in *PREF1* mRNA expression was found compared to the control group ($P > 0.05$, Figure 4d). A slight decrease was observed in *ZFP423* mRNA after 0.01 μM ATRA treatment compared to the control group, but no significant differences were found in other groups ($P < 0.05$, Figure 4e).

In addition, we evaluated the effect of ATRA treatment during the growth phase on subsequent myogenic differentiation in BSMC. After treatment with ATRA at the growth phase, both 0.1 μM and 1 μM concentrations showed a significant increase in the mRNA expression of myogenic markers including *MYF5*, *MYOD*, *MYOG*, and *MYF6* at D6 of myogenic differentiation ($P < 0.05$, Figure 5a to 5d). Moreover, upregulated expression of *ZFP423* mRNA was detected when cells were subjected to both 0.1 μM and 1 μM ATRA ($P < 0.05$, Figure 5e).

Effects of all-trans retinoic acid treatment during differentiation phase on adipogenic and myogenic differentiation in bovine skeletal muscle-derived cells

We also examined the effect of ATRA treatment during the differentiation phase on adipogenic

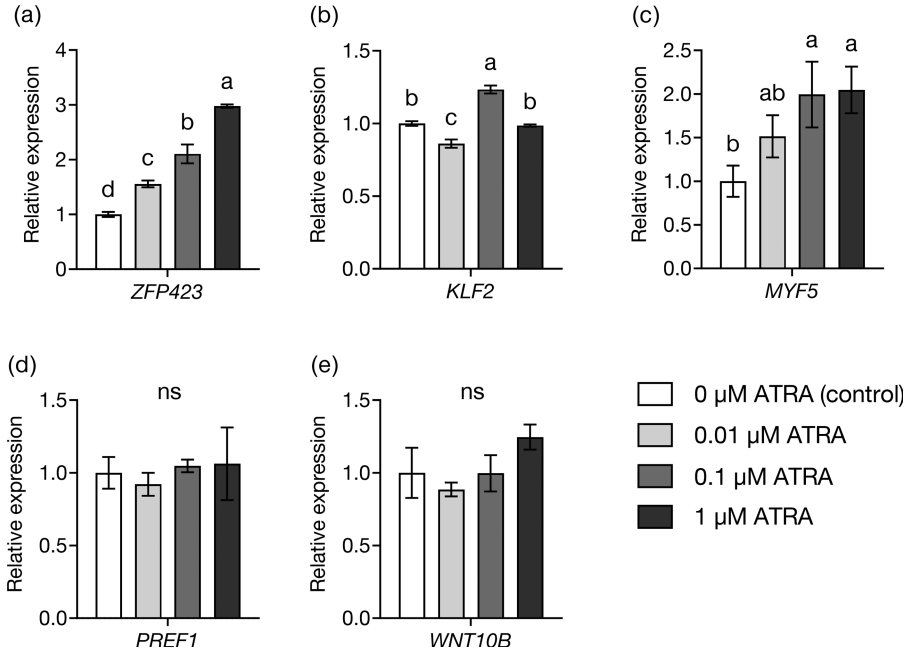

Figure 3. The relative fold changes in mRNA expression of preadipocytes and myoblast-related markers in BSMC treated with all-trans retinoic acid (ATRA) for 48 h during the growth phase (D0). (a) *ZFP423* = zinc finger protein 423. (b) *KLF2* = Kruppel like factor 2. (c) *MYF5* = myogenic factor 5. (d) *PREF1* = preadipocyte factor 1. (e) *WNT10B* = Wnt family member 10B. Relative mRNA expressions (fold changes) were normalized against reference genes (*18S*, *RPLP0*, and *GAPDH*). Error bars represent SEM for each group ($n = 3$). [a–d]Values with different letters differ significantly ($P < 0.05$). ns = not significant difference.

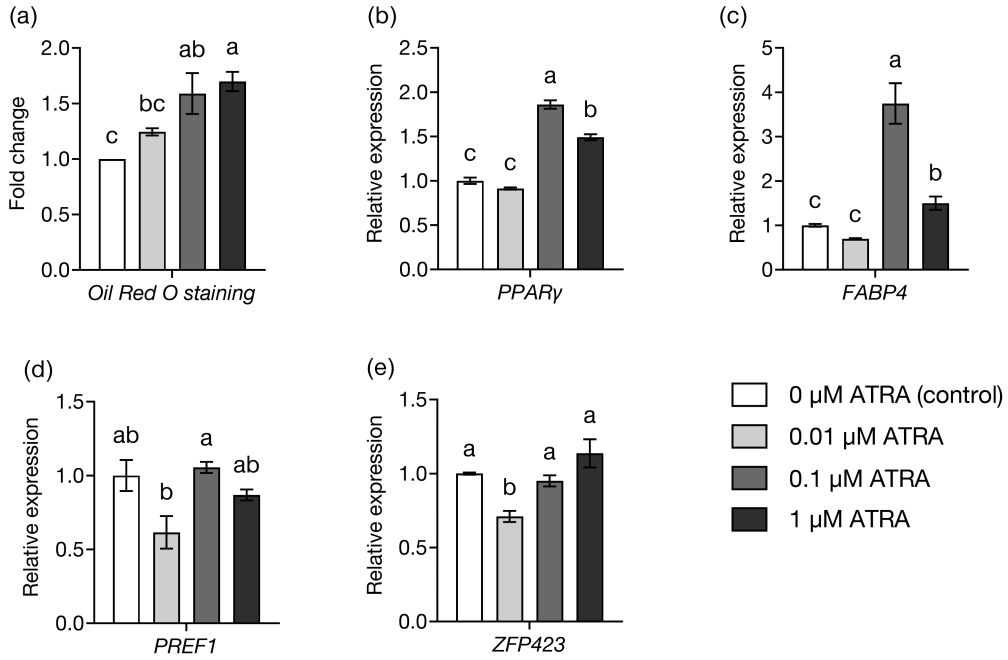

Figure 4. The relative fold changes in mRNA expression of adipogenesis-related marker genes and intensity of Oil Red O staining in bovine skeletal muscle-derived cells (BSMC) with all-trans retinoic acid (ATRA) treatment for 48 h during the growth phase, assessed on day 14 after adipogenic differentiation (D14). (a) Oil Red O staining. (b) *PPARγ* = peroxisome proliferator activated receptor gamma. (c) *FABP4* = fatty acid binding protein 4. (d) *PREF1* = preadipocyte factor 1. (e) *ZFP423* = zinc finger protein 423. Relative mRNA expressions (fold changes) were normalized against reference genes (*18S*, *RPLP0*, and *GAPDH*). Error bars represent SEM for each group ($n = 3$). [a–c]Values with different letters differ significantly ($P < 0.05$).

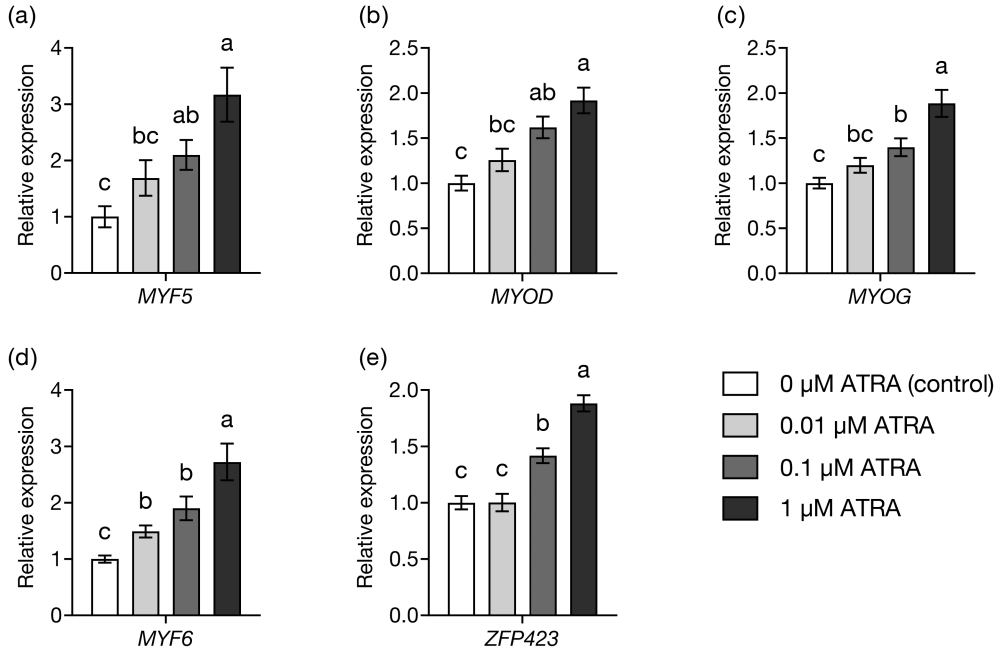

Figure 5. The relative fold changes in mRNA expression of myogenesis-related marker genes in bovine skeletal muscle-derived cells (BSMC) with all-trans retinoic acid (ATRA) treatment for 48 h during the growth phase, assessed on day 6 after myogenic differentiation (D6). (a) *MYF5* = myogenic factor 5. (b) *MYOD* = myogenic differentiation 1. (c) *MYOG* = myogenin. (d) *MYF6* = myogenic factor 6. (e) *ZFP423* = zinc finger protein 423. Relative mRNA expressions (fold changes) were normalized against reference genes (*18S*, *RPLP0*, and *GAPDH*). Error bars represent SEM for each group ($n = 3$). [a–c]Values with different letters differ significantly ($P < 0.05$).

differentiation. The result of Oil Red O staining showed that ATRA treatment inhibited adipogenic differentiation in all concentrations ($P < 0.05$, Figure 6a). Similarly, the adipogenic differentiation-related gene *PPARγ* was significantly downregulated with 1 μM ATRA treatment ($P < 0.05$, Figure 6b). The expression of mRNA for *FABP4* also decreased at both 0.1 μM and 1 μM concentrations ($P < 0.05$, Figure 6c). In contrast, the preadipocyte-related gene *ZFP423* was significantly increased at both the 0.1 μM and 1 μM concentrations ($P < 0.05$, Figure 6d).

Similarly, during the myogenic differentiation phase, treatment with 0.01 μM, 0.1 μM, and 1 μM ATRA only in this phase also increased the mRNA expression of *MYOD*, *MYOG*, *MYF6*, and *ZFP423* ($P < 0.05$, Figure 7a to 7d). Meanwhile, the mRNA expression of *MYF5* was greater in the 1 μM concentration compared with that in the control cells ($P < 0.05$, Figure 7e).

Discussion

To investigate the effect of ATRA on preadipocyte development, BSMC were supplemented with increasing concentrations of ATRA (0 μM, 0.01 μM, 0.1 μM, and 1 μM) during the growth phase. At the end of the growth phase (reached approximately 90% confluence), we found that *ZFP423* and *KLF2* expressed a higher level than the control group. The *ZFP423* has been recognized as a key indicator of adipogenic commitment. A higher-level presence of *ZFP423* suggests an increased number of preadipocytes within the cellular pool prior to adipogenic differentiation, signaling a more advanced adipogenesis activity during the ensuing differentiation phase (Huang et al., 2012). However, we found that the mRNA expression level of *ZFP423* did not change on D14 of adipogenic differentiation. According to a previous study, *ZFP423* was reported to be highly enriched in adipogenic fibroblasts rather than in uncommitted fibroblasts with adipogenic commitment (Gupta et al., 2010). Also, the level of *ZFP423* seemed to experience no change during the adipogenic differentiation phase (Gupta et al., 2012). A low level of *ZFP423* mRNA expression after adipogenic differentiation might be caused by methylation at the *ZFP423* regulatory region (Longo et al., 2018). Also, *ZFP423* mRNA expression negatively correlates with the size of the mature adipocytes (Longo et al., 2018), which indicates that the level of *ZFP423* mRNA expression would be reduced by hypertrophy of the adipocytes. In addition, Banerjee et al. (2003) demonstrated that the Kruppel-like transcription factor 2 (*KLF2*) was highly expressed in preadipocytes but not in mature adipocytes. In our study,

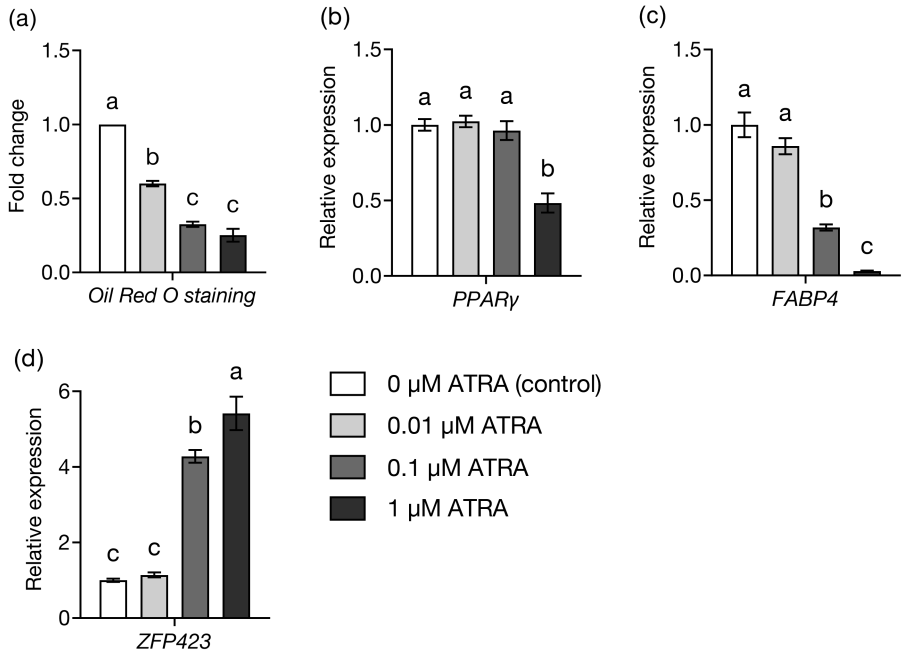

Figure 6. The relative fold changes in mRNA expression of adipogenesis-related marker genes and intensity of Oil Red O saining in bovine skeletal muscle-derived cells (BSMC) after all-trans retinoic acid (ATRA) treatment for 14 d during adipogenic differentiation (D14). (a) Oil Red O staining. (b) *PPARγ* = peroxisome proliferator activated receptor gamma. (c) *FABP4* = fatty acid binding protein 4. (d) *ZFP423* = zinc finger protein 423. Relative mRNA expressions (fold changes) were normalized against reference genes (*18S*, *RPLP0*, and *GAPDH*). Error bars represent SEM for each group ($n = 4$). [a–c]Values with different letters differ significantly ($P < 0.05$).

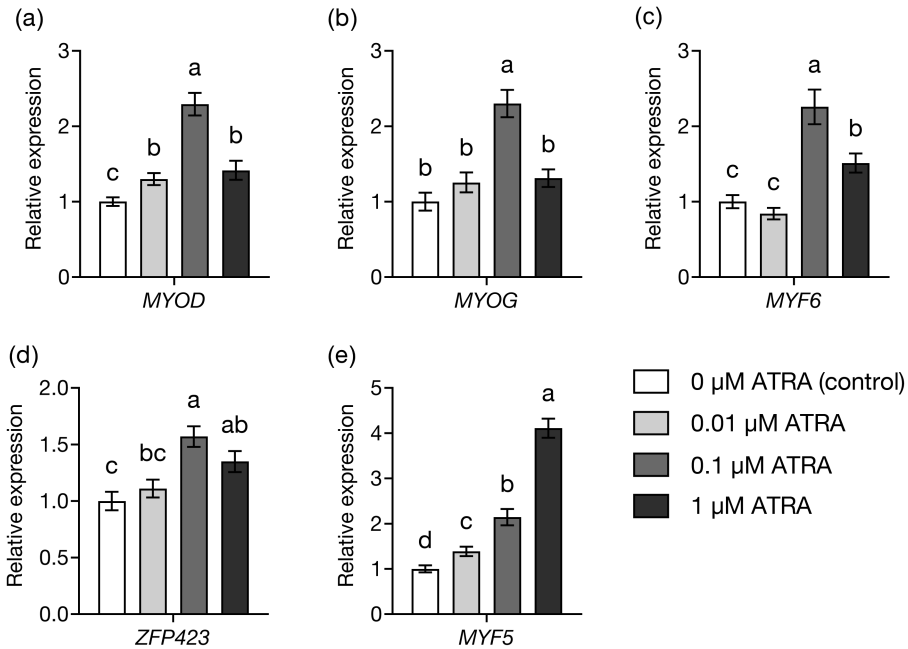

Figure 7. The relative fold changes in mRNA expression of myogenesis-related marker genes in bovine skeletal muscle-derived cells (BSMC) after all-trans retinoic acid (ATRA) treatment for 6 d during myogenic differentiation (D6). (a) *MYOD* = myogenic differentiation 1. (b) *MYOG* = myogenin. (c) *MYF6* = myogenic factor 6. (d) *ZFP423* = zinc finger protein 423. (e) *MYF5* = myogenic factor 5. Relative mRNA expressions (fold changes) were normalized against reference genes (*18S*, *RPLP0*, and *GAPDH*). Error bars represent SEM for each group ($n = 4$). [a–d]Values with different letters differ significantly ($P < 0.05$).

0.1 μM ATRA elevated the mRNA expression level of *KLF2*, which was consistent with previous research. Moreover, a subsequent article implied that *KLF2* did not induce adipogenic commitment from multipotent stem cells, but only maintained the status of preadipocytes before adipogenesis (Wu et al., 2005). Moreover, both *PREF1* and *WNT10B* exhibited high mRNA expression levels in preadipocytes rather than in mature adipocytes (Christodoulides et al., 2009; Wang et al., 2010). However, we did not find a significant difference in our study, which suggests that these genes may not play a crucial role in the development of preadipocytes in BSMC. We also examined the impact of ATRA treatment only during the growth phase of BSMC on subsequent adipogenic differentiation. Our interpretation of the Oil Red O staining indicates that the adipogenesis levels were elevated in the BSMC treated with ATRA during the growth phase. Meanwhile, for adipogenesis marker *FABP4* and *PPARγ* (Moseti et al., 2016), the mRNA expression level was much higher in the ATRA treatment group (0.1 μM and 1 μM ATRA) than in the control group on D14 of adipogenic differentiation. This suggests that supplementing ATRA during the growth phase of BSMC may lead to an increase in preadipocyte development, as indicated by markers *ZFP423* and *KLF2*, thereby resulting in a greater number of mature adipocytes in the terminal adipogenic differentiation.

Meanwhile, ATRA treatment during the adipogenic differentiation phase significantly decreased the adipogenesis and fat deposition level in correlation with the *FABP4*, *PPARγ*, and Oil Red O staining result in this study. This result was consistent with a previous study in which retinoic acid inhibited adipogenesis by marker genes and related pathways in mouse 3T3-L1 and C3H10T1/2 cell lines (Kim et al., 2013; Wang et al., 2017). Retinoic acid also had negative effects on adipocyte differentiation in BSMC isolated from the subcutaneous fat from peripartal Holstein cows (Xu et al., 2021). Additionally, the elevated levels of *ZFP423* mRNA following ATRA supplementation during the adipogenic differentiation phase appear to indicate that more preadipocytes failed to enter the adipogenic differentiation process. Therefore, the aforementioned results imply that supplementing ATRA only during the growth phase of BSMC might maintain some preadipocytes in an undifferentiated state via related genes and pathways, thereby indirectly promoting adipogenesis during the terminal differentiation stage, while during the adipogenic differentiation phase, ATRA supplementation would inhibit the adipogenesis progress.

As precursors of myofibers, myoblasts participate in myogenesis under the regulation of myogenic regulatory factors (MRF), including *MYF5*, *MYOD*, *MYOG*, and *MYF6* (Pownall et al., 2002). *MYF5* and *MYOD* are involved in the determination of entering the myogenic lineage, whereas *MYOG* and *MYF6* play a vital role in the differentiation and maintenance of the terminally differentiated state (Hawke and Garry, 2001). Data from several studies suggest that retinoic acid plays an antiproliferative role in various cells (Cui et al., 2016; Gao et al., 2021; Yoshioka et al., 2021). A recent study of sheep primary myoblasts demonstrated that 0.1 μM ATRA inhibited proliferation through downregulation of cyclin D1, CDK4, and PCNA proteins (Li et al., 2020). Similarly, we observed that the proliferation of BSMC was inhibited at concentrations of 0.1 μM and 1 μM (data not shown). *MYF5* is known to be one of the markers of myogenic commitment and is expressed in most quiescent satellite cells (Beauchamp et al., 2000; Gayraud-Morel et al., 2012). The normal expression of *MYF5* indicates the presence of satellite cells in the BSMC isolated in the present study. Interestingly, treatment with ATRA during the growth phase resulted in a significant increase in *MYF5* mRNA expression in BSMC at D0. Our results appear to contradict the observed reduction in proliferation. This discrepancy might be attributed to the primary isolated BSMC being distinct from established cell lines, encompassing a variety of single-cell populations (Carnac et al., 1993; Messmer et al., 2023). Importantly, satellite cells are multipotent mesenchymal stem cells that, in the absence of *MYF5*, can differentiate into other cell types, including osteoblasts and adipocytes (Tajbakhsh et al., 1996; Asakura et al., 2001). Therefore, we hypothesized that ATRA supplementation during the growth phase prevented cells from entering other lineages by upregulating the expression of the *MYF5* mRNA. Furthermore, the mRNA expression of *MYF5*, *MYOD*, *MYOG*, and *MYF6* was significantly increased on D6 of myogenic differentiation. These results imply that after ATRA treatment during the growth phase, a greater number of myoblasts are involved in subsequent differentiation, thereby enhancing the final level of myogenic differentiation.

Several studies have suggested that retinoic acid is highly correlated with myogenic differentiation in myoblasts, and it has been demonstrated that retinoic acid reverses the antimyogenic effect of TGF-β1 in C2C12 cells (Krueger and Hoffmann, 2010; Lamarche et al., 2015). Liu et al. (2016) reported that treatment with

1 μM retinoic acid during the differentiation phase significantly increased the mRNA expression of *MYOD*, *MYOG*, and *MYF6* in C2C12 cells on D6 of differentiation. Likewise, treatment with 0.01 μM and 0.1 μM ATRA significantly upregulated the protein levels of MYOG and MYHC in sheep primary myoblasts after 48 h of myogenic differentiation (Xu et al., 2021). In addition, we observed that *MYOD*, *MYOG*, and *MYF6* mRNA expression was significantly upregulated by ATRA treatment during the myogenic differentiation phase. This result was likely to be related to the intracellular activation of RAR and RXR signaling (Alric et al., 1998; Zhu et al., 2009). The mRNA expression of *MYF5* should be reduced immediately after the induction of myogenic differentiation (Ott et al., 1991). However, we found that the mRNA expression of *MYF5* was significantly increased on D6 of differentiation, whether treated with ATRA during the growth phase or the differentiation phase. Moreover, we found that *ZFP423* mRNA expression was elevated by ATRA treatment during both the growth phase and differentiation in the myogenic differentiation process. Besides the role played in adipogenic differentiation, a recent study in mice revealed that the expression of *ZFP423* altered the myoblast cell fate and was considered a crucial factor to regulate the progression of the proliferation and differentiation of muscle (Addison et al., 2019). However, whether the increased mRNA expression of *ZFP423* with ATRA treatment and in myoblasts played a similar role in maintaining the myoblast status is still unclear. Further research is needed on the relationship between ATRA and *ZFP423* in the myogenesis process. Hence, it could conceivably be hypothesized that ATRA treatment activates or maintains undifferentiated "reserve cells" by some means (Yoshida et al., 1998; Zammit, 2017).

Conclusions

In this study, ATRA treatment in the growth phase of BSMC increased preadipocyte development and indirectly promoted adipogenesis to mature adipocytes. In the differentiation phase, ATRA treatment decreased adipogenesis. Moreover, more myoblasts were involved in myogenic differentiation after ATRA treatment in the growth phase, as well as in increasing the subsequent myogenesis level. Meanwhile, myogenic differentiation of BSMC was also promoted by the treatment of ATRA in the differentiation phase. These results imply that vitamin A plays differential roles across distinct developmental phases of adipogenic and myogenic processes in BSMC, which provides a reference value for marbling and muscle development in beef production. Future research could involve other developmental models, such as fetuses or calves, to reinforce these findings and broaden our understanding of ATRA effects.

Acknowledgments

This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (2020R1A2B5B02001843), and also by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (NRF-2022M3A9I3082366).

Literature Cited

Addison, W. N., K. C. Hall, S. Kokabu, T. Matsubara, M. M. Fu, F. Gori, and R. Baron. 2019. Zfp423 regulates skeletal muscle regeneration and proliferation. Mol. Cell. Biol. 39:e00447–18. https://doi.org/10.1128/MCB.00447-18

Alric, S., A. Froeschlé, D. Piquemal, G. Carnac, and A. Bonnieu. 1998. Functional specificity of the two retinoic acid receptor RAR and RXR families in myogenesis. Oncogene 16: 273–282. https://doi.org/10.1038/sj.onc.1201484

Asakura, A., M. Komaki, and M. Rudnicki. 2001. Muscle satellite cells are multipotential stem cells that exhibit myogenic, osteogenic, and adipogenic differentiation. Differentiation 68: 245–253. https://doi.org/10.1046/j.1432-0436.2001.680412.x

Baatar, D., and S. G. Hwang. 2020. Effect of testosterone on the differentiation control of stromal vascular cells isolated from *longissimus* muscle of Hanwoo beef cattle. Meat Sci. 159:107916. https://doi.org/10.1016/j.meatsci.2019.107916

Banerjee, S. S., M. W. Feinberg, M. Watanabe, S. Gray, R. L. Haspel, D. J. Denkinger, R. Kawahara, H. Hauner, and M. K. Jain. 2003. The Krüppel-like factor KLF2 inhibits peroxisome proliferator-activated receptor-γ expression and adipogenesis. J. Biol. Chem. 278:2581–2584. https://doi.org/10.1074/jbc.M210859200

Beauchamp, J. R., L. Heslop, D. S. W. Yu, S. Tajbakhsh, R. G. Kelly, A. Wernig, M. E. Buckingham, T. A. Partridge, and P. S. Zammit. 2000. Expression of CD34 and Myf5 defines the majority of quiescent adult skeletal muscle satellite cells. J. Cell Biol. 151:1221–1234. https://doi.org/10.1083/jcb.151.6.1221

Bonnet, M., I. Cassar-Malek, Y. Chilliard, and B. Picard. 2010. Ontogenesis of muscle and adipose tissues and their interactions in ruminants and other species. Animal 4:1093–1109. https://doi.org/10.1017/S1751731110000601

Carnac, G., O. Albagli-Curiel, A. Levin, and A. Bonnieu. 1993. 9-cis-retinoic acid regulates the expression of the muscle determination gene Myf5. Endocrinology 133:2171–2176. https://doi.org/10.1210/endo.133.5.8404668

Chen, W., and G. Chen. 2014. The roles of vitamin A in the regulation of carbohydrate, lipid, and protein metabolism. Journal

of Clinical Medicine 3:453–479. https://doi.org/10.3390/jcm3020453

Cho, S. H., J. Kim, B. Y. Park, P. N. Seong, G. H. Kang, J. H. Kim, S. G. Jung, S. K. Im, and D. H. Kim. 2010. Assessment of meat quality properties and development of a palatability prediction model for Korean Hanwoo steer beef. Meat Sci. 86:236–242. https://doi.org/10.1016/j.meatsci.2010.05.011

Christodoulides, C., C. Lagathu, J. K. Sethi, and A. Vidal-Puig. 2009. Adipogenesis and WNT signalling. Trends Endocrin. Met. 20:16–24. https://doi.org/10.1016/j.tem.2008.09.002

Cui, J., M. Gong, Y. He, Q. Li, T. He, and Y. Bi. 2016. All-trans retinoic acid inhibits proliferation, migration, invasion and induces differentiation of hepa1-6 cells through reversing EMT in vitro. Int. J. Oncol. 48:349–357. https://doi.org/10.3892/ijo.2015.3235

El-Habta, R., M. Sloniecka, P. J. Kingham, and L. J. Backman. 2018. The adipose tissue stromal vascular fraction secretome enhances the proliferation but inhibits the differentiation of myoblasts. Stem Cell Res. Ther. 9:352. https://doi.org/10.1186/s13287-018-1096-6

Gao, L., Y. He, K. Wang, C. Wang, H. Wu, A. Hu, L. Ruan, Q. Bo, W. Chen, C. Hu, L. Li, D. Zhang, T. Xia, S. Qian, Z. Liu, Y. Yang, W. Yang, H. Wang, and Q. Zhao. 2021. All-trans retinoic acid suppressed GES-1 cell proliferation induced by exosomes from patients with precancerous lesions by arresting the cell cycle in S-phase. Eur. J. Cancer Prev. 30:113–119. https://doi.org/10.1097/CEJ.0000000000000571

Gayraud-Morel, B., F. Chrétien, A. Jory, R. Sambasivan, E. Negroni, P. Flamant, G. Soubigou, J.-Y. Coppée, J. Di Santo, A. Cumano, V. Mouly, and S. Tajbakhsh. 2012. Myf5 haploinsufficiency reveals distinct cell fate potentials for adult skeletal muscle stem cells. J. Cell Sci. 125:6198. https://doi.org/10.1242/jcs.128678

Green, H., and M. Meuth. 1974. An established pre-adipose cell line and its differentiation in culture. Cell 3:127–133. https://doi.org/10.1016/0092-8674(74)90116-0

Gupta, R. K., Z. Arany, P. Seale, R. J. Mepani, L. Ye, H. M. Conroe, Y. A. Roby, H. Kulaga, R. R. Reed, and B. M. Spiegelman. 2010. Transcriptional control of preadipocyte determination by Zfp423. Nature 464:619–623. https://doi.org/10.1038/nature08816

Gupta, R. K., R. J. Mepani, S. Kleiner, J. C. Lo, M. J. Khandekar, P. Cohen, A. Frontini, D. C. Bhowmick, L. Ye, S. Cinti, and B. M. Spiegelman. 2012. Zfp423 expression identifies committed preadipocytes and localizes to adipose endothelial and perivascular cells. Cell Metab. 15:230–239. https://doi.org/10.1016/j.cmet.2012.01.010

Hamade, A., M. Deries, G. Begemann, L. Bally-Cuif, C. Genêt, F. Sabatier, A. Bonnieu, and X. Cousin. 2006. Retinoic acid activates myogenesis in vivo through Fgf8 signalling. Dev. Biol. 289:127–140. https://doi.org/10.1016/j.ydbio.2005.10.019

Harris, C. L., B. Wang, J. M. Deavila, J. R. Busboom, M. Maquivar, S. M. Parish, B. McCann, M. L. Nelson, and M. Du. 2018. Vitamin A administration at birth promotes calf growth and intramuscular fat development in Angus beef cattle. J. Anim. Sci. Biotechno. 9:55. https://doi.org/10.1186/s40104-018-0268-7

Hawke, T. J., and D. J. Garry. 2001. Myogenic satellite cells: physiology to molecular biology. J. Appl. Physiol. 91:534–551. https://doi.org/10.1152/jappl.2001.91.2.534

Huang, Y., A. K. Das, Q. Y. Yang, M. J. Zhu, and M. Du. 2012. Zfp423 promotes adipogenic differentiation of bovine stromal vascular cells. PLoS One 7:e47496. https://doi.org/10.1371/journal.pone.0047496

Jin, X. C., D. Q. Peng, S. J. Kim, N. Y. Kim, J. G. Nejad, D. Kim, S. B. Smith, and H. G. Lee. 2022. Vitamin A supplementation downregulates *ADH1C* and *ALDH1A1* mRNA expression in weaned beef calves. Animal Nutrition 10:372–381. https://doi.org/10.1016/j.aninu.2022.06.007

Jo, J., O. Gavrilova, S. Pack, W. Jou, S. Mullen, A. E. Sumner, S. W. Cushman, and V. Periwal. 2009. Hypertrophy and/or hyperplasia: dynamics of adipose tissue growth. PLoS Comput. Biol. 5:e1000324. https://doi.org/10.1371/journal.pcbi.1000324

Jo, Y. H., D. Q. Peng, W. S. Kim, S. J. Kim, N. Y. Kim, S. H. Kim, J. Ghassemi Nejad, J. S. Lee, and H. G. Lee. 2020. The effects of vitamin A supplementation during late-stage pregnancy on longissimus dorsi muscle tissue development, birth traits, and growth performance in postnatal Korean native calves. Asian Austral. J. Anim. 33:742–752. https://doi.org/10.5713/ajas.19.0413

Kim, D. M., H. R. Choi, A. Park, S. M. Shin, K. H. Bae, S. C. Lee, I. C. Kim, and W. K. Kim. 2013. Retinoic acid inhibits adipogenesis via activation of Wnt signaling pathway in 3T3-L1 preadipocytes. Biochem. Bioph. Res. Co. 434:455–459. https://doi.org/10.1016/j.bbrc.2013.03.095

Krueger, C., and F. M. Hoffmann. 2010. Identification of retinoic acid in a high content screen for agents that overcome the anti-myogenic effect of TGF-beta-1. PLoS One 5:e15511. https://doi.org/10.1371/journal.pone.0015511

Lamarche, E., N. Lala-Tabbert, A. Gunanayagam, C. St-Louis, and N. Wiper-Bergeron. 2015. Retinoic acid promotes myogenesis in myoblasts by antagonizing transforming growth factor-beta signaling via C/EBPbeta. Skelet. Muscle 5:8. https://doi.org/10.1186/s13395-015-0032-z

Lavasani, M., A. Lu, S. D. Thompson, P. D. Robbins, J. Huard, and L. J. Niedernhofer. 2013. Isolation of muscle-derived stem/progenitor cells based on adhesion characteristics to collagen-coated surfaces. In: K. Turksen, editor, Stem cells and aging: methods and protocols. Humana Press, Totowa, NJ. p. 53–65. https://doi.org/10.1007/978-1-62703-317-6_5

Lee, J. H., D. Q. Peng, X. C. Jin, S. B. Smith, and H. G. Lee. 2023. Vitamin D3 decreases myoblast fusion during the growth and increases myogenic gene expression during the differentiation in muscle satellite cells from Korean native beef cattle. J. Anim. Sci. 101:skad192. https://doi.org/10.1093/jas/skad192

Li, F., Y. Li, Y. Duan, C.-A. A. Hu, Y. Tang, and Y. Yin. 2017. Myokines and adipokines: Involvement in the crosstalk between skeletal muscle and adipose tissue. Cytokine Growth F. R. 33:73–82. https://doi.org/10.1016/j.cytogfr.2016.10.003

Li, Q., T. Zhang, R. Zhang, X. Qin, and J. Zhao. 2020. All-trans retinoic acid regulates sheep primary myoblast proliferation and differentiation in vitro. Domest. Anim. Endocrinol. 71:106394. https://doi.org/10.1016/j.domaniend.2019.106394

Liu, B., N. Li, Y. Jiang, C. Liu, L. Ma, W. Cong, and J. Xiao. 2016. Effects of excessive retinoic acid on C2C12 myogenesis.

J. Hard Tissue Biol. 25:97–103. https://doi.org/10.2485/jhtb.25.97

Longo, M., G. A. Raciti, F. Zatterale, L. Parrillo, A. Desiderio, R. Spinelli, A. Hammarstedt, S. Hedjazifar, J. M. Hoffmann, C. Nigro, P. Mirra, F. Fiory, P. Formisano, C. Miele, U. Smith, and F. Beguinot. 2018. Epigenetic modifications of the Zfp/ZNF423 gene control murine adipogenic commitment and are dysregulated in human hypertrophic obesity. Diabetologia 61:369–380. https://doi.org/10.1007/s00125-017-4471-4

Messmer, T., R. G. J. Dohmen, L. Schaeken, L. Melzener, R. Hueber, M. Godec, C. Didoss, M. J. Post, and J. E. Flack. 2023. Single-cell analysis of bovine muscle-derived cell types for cultured meat production. Frontiers in Nutrition 10. https://doi.org/10.3389/fnut.2023.1212196

Moseti, D., A. Regassa, and W. K. Kim. 2016. Molecular regulation of adipogenesis and potential anti-adipogenic bioactive molecules. Int. J. Mol. Sci. 17:124. https://doi.org/10.3390/ijms17010124

Oka, A., Y. Maruo, T. Miki, T. Yamasaki, and T. Saito. 1998. Influence of vitamin A on the quality of beef from the Tajima strain of Japanese Black cattle. Meat Sci. 48:159–167. https://doi.org/10.1016/s0309-1740(97)00086-7

Ortiz-Colón, G., A. C. Grant, M. E. Doumit, and D. D. Buskirk. 2009. Bovine intramuscular, subcutaneous, and perirenal stromal-vascular cells express similar glucocorticoid receptor isoforms, but exhibit different adipogenic capacity. J. Anim. Sci. 87:1913–1920. https://doi.org/10.2527/jas.2008-1350

Ott, M.-O., E. Bober, G. Lyons, H. Arnold, and M. Buckingham. 1991. Early expression of the myogenic regulatory gene, *myf-5*, in precursor cells of skeletal muscle in the mouse embryo. Development 111:1097–1107. https://doi.org/10.1242/dev.111.4.1097

Park, S. J., S. H. Beak, D. J. S. Jung, S. Y. Kim, I. H. Jeong, M. Y. Piao, H. J. Kang, D. M. Fassah, S. W. Na, S. P. Yoo, and M. Baik. 2018. Genetic, management, and nutritional factors affecting intramuscular fat deposition in beef cattle – A review. Asian Austral. J. Anim. 31:1043–1061. https://doi.org/10.5713/ajas.18.0310

Peng, D. Q., Y. H. Jo, S. J. Kim, N. Y. Kim, J. G. Nejad, and H. G. Lee. 2020a. Oral vitamin A supplementation during neonatal stage enhances growth, pre-adipocyte and muscle development in Korean native calves. Anim. Feed Sci. Tech. 268:114609. https://doi.org/10.1016/j.anifeedsci.2020.114609

Peng, D. Q., S. J. Kim, and H. G. Lee. 2020b. Metabolomics analyses to characterize metabolic alterations in Korean native calves by oral vitamin A supplementation. Sci. Rep.-UK 10:8092. https://doi.org/10.1038/s41598-020-65023-y

Peng, D. Q., S. B. Smith, and H. G. Lee. 2021. Vitamin A regulates intramuscular adipose tissue and muscle development: Promoting high-quality beef production. J. Anim. Sci. Biotechno. 12:34. https://doi.org/10.1186/s40104-021-00558-2

Pownall, M. E., M. K. Gustafsson, and C. P. Emerson, Jr. 2002. Myogenic regulatory factors and the specification of muscle progenitors in vertebrate embryos. Annu. Rev. Cell Dev. Bi. 18:747–783. https://doi.org/10.1146/annurev.cellbio.18.012502.105758

Reijntjes, S., P. Francis-West, and B. S. Mankoo. 2010. Retinoic acid is both necessary for and inhibits myogenic commitment and differentiation in the chick limb. Int. J. Dev. Biol. 54:125–134. https://doi.org/10.1387/ijdb.082783sr

Russell, R. G., and F. T. Oteruelo. 1981. An ultrastructural study of the differentiation of skeletal muscle in the bovine fetus. Anat. Embryol. 162:403–417. https://doi.org/10.1007/BF00301866

Tajbakhsh, S., D. Rocancourt, and M. Buckingham. 1996. Muscle progenitor cells failing to respond to positional cues adopt non-myogenic fates in myf-5 null mice. Nature 384:266–270. https://doi.org/10.1038/384266a0

Wang, B., X. Fu, M. J. Zhu, and M. Du. 2017. Retinoic acid inhibits white adipogenesis by disrupting GADD45A-mediated Zfp423 DNA demethylation. J. Mol. Cell Biol. 9:338–349. https://doi.org/10.1093/jmcb/mjx026

Wang, Y., C. Hudak, and H. S. Sul. 2010. Role of preadipocyte factor 1 in adipocyte differentiation. Clin. Lipidol. 5:109–115. https://doi.org/10.2217/clp.09.80

Wang, G. H., C. C. Liang, B. Z. Li, X. Z. Du, W. Z. Zhang, G. Cheng, and L. S. Zan. 2022. Screening and validation of reference genes for qRT-PCR of bovine skeletal muscle-derived satellite cells. Sci. Rep.-UK 12:1–11. https://doi.org/10.1038/s41598-022-09476-3

Wang, B., W. Nie, X. Fu, J. M. de Avila, Y. Ma, M. J. Zhu, M. Maquivar, S. M. Parish, J. R. Busboom, M. L. Nelson, and M. Du. 2018. Neonatal vitamin A injection promotes cattle muscle growth and increases oxidative muscle fibers. J. Anim. Sci. Biotechno. 9:82. https://doi.org/10.1186/s40104-018-0296-3

Wang, B., Q. Yang, C. L. Harris, M. L. Nelson, J. R. Busboom, M. J. Zhu, and M. Du. 2016. Nutrigenomic regulation of adipose tissue development - Role of retinoic acid: A review. Meat Sci. 120:100–106. https://doi.org/10.1016/j.meatsci.2016.04.003

Wu, J., S. V. Srinivasan, J. C. Neumann, and J. B. Lingrel. 2005. The KLF2 transcription factor does not affect the formation of preadipocytes but inhibits their differentiation into adipocytes. Biochemistry-US 44:11098–11105. https://doi.org/10.1021/bi050166i

Xu, Q., Y. Fan, J. J. Loor, Y. Liang, X. Sun, H. Jia, C. Zhao, and C. Xu. 2021. All-trans retinoic acid controls differentiation, proliferation, and lipolysis in isolated subcutaneous adipocytes from peripartal Holstein cows. J. Dairy Sci. 104:4999–5008. https://doi.org/10.3168/jds.2020-19408

Yin, H., F. Price, and M. A. Rudnicki. 2013. Satellite cells and the muscle stem cell niche. Physiol. Rev. 93:23–67. https://doi.org/10.1152/physrev.00043.2011

Yoshida, N., S. Yoshida, K. Koishi, K. Masuda, and Y. Nabeshima. 1998. Cell heterogeneity upon myogenic differentiation: Down-regulation of MyoD and Myf-5 generates 'reserve cells.' J. Cell Sci. 111:769–779. https://doi.org/10.1242/jcs.111.6.769

Yoshioka, H., S. S. Ramakrishnan, J. Shim, A. Suzuki, and J. Iwata. 2021. Excessive all-trans retinoic acid inhibits cell proliferation through upregulated microRNA-4680-3p in cultured human palate cells. Frontiers in Cell and Developmental Biology 9. https://doi.org/10.3389/fcell.2021.618876

Zammit, P. S. 2017. Function of the myogenic regulatory factors Myf5, MyoD, myogenin and MRF4 in skeletal muscle, satellite

cells and regenerative myogenesis. Semin. Cell Dev. Biol. 72:19–32. https://doi.org/10.1016/j.semcdb.2017.11.011

Zhu, G. H., J. Huang, Y. Bi, Y. Su, Y. Tang, B. C. He, Y. He, J. Luo, Y. Wang, L. Chen, G. W. Zuo, W. Jiang, Q. Luo, J. Shen, B. Liu, W. L. Zhang, Q. Shi, B. Q. Zhang, Q. Kang, J. Zhu, J. Tian, H. H. Luu, R. C. Haydon, Y. Chen, and T. C. He. 2009. Activation of RXR and RAR signaling promotes myogenic differentiation of myoblastic C2C12 cells. Differentiation 78:195–204. https://doi.org/10.1016/j.diff.2009.06.001