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AMPKα, *C/EBPβ*, *CPT1β*, *GPR43*, *PPARγ*, and *SCD* Gene Expression in Single- and Co-cultured Bovine Satellite Cells and Intramuscular Preadipocytes Treated with Palmitic, Stearic, Oleic, and Linoleic Acid

S. H. Choi[1,2,a], S. K. Park[3,a], B. J. Johnson[4], K. Y. Chung[5], C. W. Choi[6], K. H. Kim[7,8], W. Y. Kim[2], and S. B. Smith[1,*]
[1] Department of Animal Science, Texas Agricultural Experiment Station,
Texas A&M University, College Station, TX 77843, USA

ABSTRACT: We previously demonstrated that bovine subcutaneous preadipocytes promote adipogenic gene expression in muscle satellite cells in a co-culture system. Herein we hypothesize that saturated fatty acids would promote adipogenic/lipogenic gene expression, whereas mono- and polyunsaturated fatty acids would have the opposite effect. Bovine semimembranosus satellite cells (BSC) and intramuscular preadipocytes (IPA) were isolated from crossbred steers and cultured with 10% fetal bovine serum (FBS)/ Dulbecco's Modified Eagle Medium (DMEM) and 1% antibiotics during the 3-d proliferation period. After proliferation, cells were treated for 3 d with 3% horse serum/DMEM (BSC) or 5% FBS/DMEM (IPA) with antibiotics. Media also contained 10 μg/mL insulin and 10 μg/mL pioglitazone. Subsequently, differentiating BSC and IPA were cultured in their respective media with 40 μM palmitic, stearic, oleic, or linoleic acid for 4 d. Finally, BSC and IPA were single- or co-cultured for an additional 2 h. All fatty acid treatments increased (p = 0.001) carnitine palmitoyltransferase-1 beta (*CPT1β*) gene expression, but the increase in *CPT1β* gene expression was especially pronounced in IPA incubated with palmitic and stearic acid (6- to 17- fold increases). Oleic and linoleic acid decreased (p = 0.001) stearoyl-CoA desaturase (*SCD*) gene expression over 80% in both BSC and IPA. Conversely, palmitic and stearic acid increased *SCD* gene expression three fold in co-cultured in IPA, and stearic acid increased *AMPKα* gene expression in single- and co-cultured BSC and IPA. Consistent with our hypothesis, saturated fatty acids, especially stearic acid, promoted adipogenic and lipogenic gene expression, whereas unsaturated fatty acids decreased expression of those genes associated with fatty acid metabolism. (**Key Words:** Bovine, Co-culture, Fatty Acids, Gene Expression, Preadipocytes, Satellite Cells)

INTRODUCTION

The primary objective of this study was to demonstrate the effects of saturated fatty acids (palmitic and stearic acid) and unsaturated fatty acids (oleic and linoleic acid) on specific gene expression in bovine satellite cells (BSC) and intramuscular preadipocytes (IPA). The secondary objective was to document the effects of brief co-culture of BSC and IPA on adipogenic and lipogenic gene expression. We hypothesized that palmitic and stearic acid would increase expression of genes associated with lipid accumulation in BSC and IPA during differentiation. We also tested the effects of oleic acid and linoleic acid, as we predicted that they would depress adipogenic and/or lipogenic gene expression. Stearic acid is the most abundant fatty acid to pass from the rumen to the small intestine in cattle, although palmitic acid also is abundant in the small intestine of cattle (Ekeren et al., 1992; Chung et al., 2006b). Oleic acid is absorbed from the gastrointestinal tract of cattle (Ekeren et al., 1992) and is produced endogenously

* Corresponding Author: S. B. Smith. Tel: +1-979-845-3939, Fax: +1-979-458-2702, E-mail: sbsmith@tamu.edu
[2] Department of Animal Science, Chungbuk National University, Cheongju 361-763, Korea.
[3] Department of Food Science and Technology, Sejong University, Seoul, 143-747, Korea.
[4] Department of Animal and Food Science, Texas Tech University, Lubbock, TX 79409, USA.
[5] Hanwoo Research Institute, National Institute of Animal Science, RDA, 232-950, Korea.
[6] Department of Animal Resources, Daegu University, Gyeongsan, 712-749, Korea.
[7] Graduate School of International Agricultural Technology, Seoul National University, Pyeongchang 232-916, Korea.
[8] Institute of Green Bio Science & Technology, Seoul National University, Pyeongchang, 232-916, Korea.
[a] These authors contributed equally to this work and co-first authors.
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(St. John et al., 1991; Chang et al., 1992; Archibeque et al., 2005; Kadegowda et al., 2013), and linoleic acid is the most abundant polyunsaturated fatty acid in the diet and tissues of beef cattle (Chung et al., 2006b).

To accomplish these objectives, we documented the effects of exposure to fatty acids during culture on the expression of genes associated with adipocyte differentiation (CCAAT/enhancer-binding protein beta [*C/EBPβ*] and peroxisome proliferator-activated receptor gamma [*PPARγ*]), lipid metabolism (carnitine palmitoyltransferase-1 beta [*CPT1β*] and stearoyl-CoA desaturase [*SCD*]), and the regulation of triacylglycerol turnover (G protein-coupled protein receptor 43 [*GPR43*] and AMP-activated protein kinase alpha-1 [*AMPKα*]). In the present study, we hypothesized that co-culture of preadipocytes with myoblasts would modify the effects of saturated and unsaturated fatty acids on adipogenic and lipogenic gene expression.

MATERIALS AND METHODS

Chemicals

Fatty acids were purchased from Nu-Chek prep, Inc (Elysian, MN, USA). Other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA), Invitrogen (Carlsbad, CA, USA), Calbiocem (La Jolla, CA, USA), Gibco (Grand Island, NY, USA) or Applied Biosystems (Foster City, CA, USA).

Bovine satellite cell and preadipocyte isolation

Muscle-derived BSC and IPA were isolated from semimembranosus muscle of 14-mo-old crossbred steers raised at Texas Tech University. Steers were killed with a captive bolt stunner followed by exsanguination. Using sterile techniques, approximately 500 g of the semimembranosus muscle and IPA within the muscle were dissected and transported to the laboratory. Subsequent procedures were conducted in a sterile field in a tissue culture hood. After removal of connective tissue and IPA, the muscle was passed through a sterile meat grinder. The ground muscle was incubated with 0.1% pronase (Calbiochem) in Earl's Balanced Salt Solution (Sigma-Aldrich, USA) for 1 h at 37℃ with frequent mixing. The dissected IPA was incubated with 0.1% type IV collagenase (Sigma-Aldrich, USA) in Earl's Balanced Salt Solution for 1 h at 37℃ with frequent mixing (Suryawan and Hu, 1997; Ohyama et al., 1998). After incubation, both mixtures were centrifuged at 1,500×*g* for 4 min, the pellets were suspended in phosphate buffered saline (Gibco) (140 mM NaCl, 1 mM KH_2PO_4, 3 mM KCl, 8 mM Na_2HPO_4), and the suspensions were centrifuged at 500×*g* for 10 min. The supernatants were centrifuged at 1,500×*g* for 10 min to pellet the mononucleated cells. The PBS wash and differential centrifugation were repeated twice. The resulting cells preparations were suspended in cold (4℃) Dulbecco's Modified Eagle Medium (DMEM; Gibco) containing 10% fetal bovine serum (FBS) (Gibco) and 10% (vol/vol) dimethylsulfoxide (Sigma-Aldrich, USA) and frozen. Cells were stored frozen in liquid nitrogen.

Cell culture

The BSC and IPA were obtained from a single batch and experiments were replicated in three independent incubations (Choi et al., 2013a). Cells were plated at a density of 1×10^4 cells per well and grown at 37℃ under a humidified atmosphere of 5% CO_2. Upon reaching confluence after 3 d, the growth medium was replaced with 3% horse serum/DMEM plus antibiotics (BSC) or 5% FBS/DMEM plus antibiotics (IPA). Differentiation medium also contained 10 μg/mL insulin and 10 μM pioglitazone (Kang et al., 2003; Chung and Johnson, 2009), and BSC and IPA were allowed to differentiate for 3 d. Subsequently, no fatty acids (control cells) or 40 μM fatty acids (palmitic, stearic, oleic, or linoleic acid) were added to the media, and the differentiating IPA and BSC were incubated for an additional 4 d. Fatty acids were dissolved in ethanol prior to addition to the cell culture (Satory and Smith, 1999; Chung et al., 2006a). Finally, differentiated IPA and BSC were single-cultured and co-cultured for an additional 2 h in DMEM plus antibiotics in the presence of the fatty acids.

Real-time polymerase chain reaction

RNA was extracted with Tri Reagent (Sigma Chemicals, St. Louis, MO, USA) as reported previously (Smith et al., 2012). The concentration of RNA was quantified with a NanoDrop ND-100 Spectrophotometer (Thermo Scientific, Washington, DE, USA). The 260:280 ratio for all samples was greater than 1.85. Quantitative real time polymerase chain reaction (qRT-PCR) was used to analyze the expression of *C/EBPβ*, *CPT1β*, *GPR43*, *PPARγ*, *AMPK-α*, and *SCD* (primers listed in Table 1). Commercially available eukaryotic 40S ribosomal protein S9 (*RPS9*) RNA (Applied Biosystems; GeneBank Accession #X03205) was used as the endogenous control. Other studies in bovine adipose tissue explants (Hosseini et al., 2010) and bovine liver (Baxa et al., 2010) demonstrated that *RPS9* mRNA expression was stable and suitable as a housekeeping gene under their conditions. Additionally, *RPS9* was used as a housekeeping gene for the expression genes in bovine muscle (Chung and Johnson, 2009; Baxa et al., 2010).

Complementary DNA was produced from 1 μg RNA using Taq- Man Reverse Transcriptase Reagents (Applied Biosystems, USA) by the protocol recommended by the manufacturer. Random hexamers were used as primers in cDNA synthesis. Measurement of the relative quantity of the cDNA of interest was carried out using TAMRA PCR

Table 1. Forward and reverse primers and probes for real-time PCR for specific gene mRNA

Maker gene	Gene No.		Sequence (5' to 3')
RPS9	DT860044	Forward	GAGCTGGGTTTGTCGCAAAA
		Reverse	GGTCGAGGCGGGACTTCT
		Taqman probe	6FAM-ATGTGACCCCGCGGAGACCCTTC-TAMRA
AMPK-α	NM_001109802	Forward	ACCATTCTTGGTTGCTGAAACTC
		Reverse	CACCTTGGTGTTTGGATTTCTG
		Taqman probe	6FAM-CAGGGCGCGCCATACCCTTG-TAMRA
C/EBPβ	NM_176788	Forward	CCAGAAGAAGGTGGAGCAACTG
		Reverse	TCGGGCAGCGTCTTGAAC
		Taqman probe	6FAM-CGCGAGGTCAGCACCCTGC-TAMRA
CPT1β	NM_001034349	Forward	ACACATCTACCTGTCCGTGATCA
		Reverse	CCCCTGAGGATGCCATTCT
		Taqman probe	6FAM-TCCTGGAAGAAACGCCTGATTCGC-TAMRA
GPR43	FJ_562212	Forward	GGCTTTCCCCGTGCAGTA
		Reverse	ATCAGAGCAGCGATCACTCCAT
		Taqman probe	6FAM-AAGCTGTCCCGCCGGCCC-TAMRA
PPARγ	NM_181024	Forward	ATCTGCTGCAAGCCTTGGA
		Reverse	TGGAGCAGCTTGGCAAAGA
		Taqman probe	6FAM-CGCGAGGTCAGCACCCTGC-TAMRA
SCD	AB075020	Forward	TGCCCACCACAAGTTTTCAG
		Reverse	GCCAACCCACGTGAGAGAAG
		Taqman probe	6FAM-CCGACCCCCACAATTCCCG-TAMRA

PCR, polymerase chain reaction; *RPS9*, ribosomal protein S9; *AMPK-α*, AMP-activated protein kinase alpha; *C/EBPβ*, CCAAT/enhancer-binding protein beta; *CPT1β*, carnitine palmitoyltransferase-1 beta; *GPR43*, G protein-coupled protein receptor 43; *PPARγ*, peroxisome proliferator-activated receptor gamma; *SCD*, stearoyl-CoA desaturase.

Master Mix (Applied Biosystems, USA), appropriate forward and reverse primers, and 1 μL of the cDNA mixture. Assays were performed in duplicate in the GeneAmp 5700 Sequence Detection System (Applied Biosystems, USA) using thermal cycling parameters recommended by the manufacturer (40 cycles of 15 s at 95°C and 1 min at 60°C). Cycle threshold (CT) values were means of duplicate measurements. The comparative CT values were employed to determine expression levels for target genes; fold change was determined as $2^{\Delta\Delta CT}$ with *RPS9* as the endogenous control. Titration of the target mRNA primers against increasing amounts of cDNA gave linear responses with slopes between –2.8 and –3.0. In order to reduce the effect of assay-to-assay variation in the PCR assay, all values were calculated relative to a calibration standard run on every qRT-PCR assay. The ABI Prism 7000 detection system (Applied Biosystems, USA) was used to perform the assay utilizing the thermal cycling variables recommended by the manufacturer (50 cycles of 15 s at 95°C and 1 min at 60°C).

Statistical analysis

Data were analyzed by analysis of variance as a three-factor design with cell type (adipocytes, myoblasts), culture method (single-culture, co-culture), and treatment (fatty acids) as the main effects (SuperAnova, Abacus Concepts, Berkley, CA, USA). The model also tested all possible two- and three-way interactions among main effects. Means were separated by Fisher's Protected LSD (SuperAnova, USA) if their respective *F*-test indicated significant differences ($p<0.05$).

RESULTS

Cell type, culture method, and fatty acid treatment main effects

There were highly significant differences ($p<0.001$) between IPA and BSC for *AMPKα*, *C/EBPβ*, *CPT1β*, *PPARγ*, and *SCD* gene expression (Table 2). The mRNA levels for *AMPKα*, *C/EBPβ*, and *PPARγ* were higher in IPA than in BSC after differentiation, whereas *CPT1β* and *SCD* gene expression was higher in BSC than in IPA after differentiation. The mRNA levels for *GPR43* did not differ between cell types ($p = 0.303$).

The culture method main effect (single or co-culture) was significant ($p<0.001$) for *AMPKα*, *C/EBPβ*, *GPR43*, *PPARγ*, and *SCD* gene expression (Table 2). The mRNA levels for *AMPKα*, *C/EBPβ*, *GPR43*, *PPARγ*, and *SCD* were greater in co-cultured cells than in single-cultured cells. Culture method had no effect on *CPT1β* gene expression ($p = 0.197$).

Fatty acid treatments affected the expression of all genes ($p<0.001$) except *GPR43* and *PPARγ* ($p = 0.136$ and 0.165, respectively). Expression of *AMPKα*, *C/EBPβ*, and *SCD* was highest in cells incubated with stearic acid, whereas oleic and linoleic acid depressed *SCD* gene expression relative to control samples (Table 2). All fatty

Table 2. Main effects for cell type, culture method, and fatty acid for gene expression in single- and co-cultured bovine satellite cells and intramuscular preadipocytes incubated in the absence and presence of 40 μM palmitic, stearic, oleic, or linoleic acid

Gene[a]	Main effects									SEM	Treatment p-values		
	Cell type[b]		Culture method[c]		Fatty acid treatment[d]								
	BSC	IPA	Single	Co-culture	Control	Palmitic	Stearic	Oleic	Linoleic		CT	CM	FA
AMPKα	0.88	1.41	0.97	1.32	1.11^{h}	1.13^{h}	1.43^{g}	0.99^{h}	1.05^{h}	0.05	<0.001	<0.001	<0.001
C/EBPβ	0.01^{e}	5.02	2.06	2.95	0.83^{i}	4.39^{h}	5.43^{g}	0.60^{i}	1.30^{i}	0.51	<0.001	<0.001	<0.001
CPT1β	1,823	17	953	886	198^{d}	1,096^{h}	931^{i}	1,450^{g}	923^{i}	141	<0.001	0.197	<0.001
GPR43	0.92	1.09	0.53	1.47	0.89	0.67	1.37	1.03	1.05	0.11	0.303	<0.001	0.136
PPARγ	1.71	2.48	1.30	2.89	1.83	1.70	2.52	2.04	2.38	0.15	<0.001	<0.001	0.165
SCD	1.56	0.98	1.08	1.48	1.80^{h}	1.45^{i}	2.68^{g}	0.29^{j}	0.16^{j}	0.15	<0.001	<0.001	<0.001

SEM, standard error of the mean; CT, cell type; CM, culture method; FA, fatty acid treatment; BSC, bovine satellite cells; IPA, intramuscular preadipocytes; *AMPK-α*, AMP-activated protein kinase alpha; *C/EBPβ*, CCAAT/enhancer-binding protein beta; *CPT1β*, carnitine palmitoyltransferase-1 beta; *GPR43*, G protein-coupled protein receptor 43; *PPARγ*, peroxisome proliferator-activated receptor gamma; *SCD*, stearoyl-CoA desaturase.

[a] Relative *AMPKα*, *C/EBPβ*, *GPR43*, *PPARγ*, *CPT1β*, and *SCD* mRNA levels in total RNA isolated from BSC, IPA single- or co-cultured with insulin (10 μM), and pioglitizone (10 μM). Data are for three culture preparations.

[b] Data are means for 30 observations, pooled across culture method and fatty acid treatment.

[c] Data are means for 30 observations, pooled across cell type and fatty acid treatment.

[d] Data are means for 15 observations, pooled across cell type and culture method.

[e] *C/EBPβ* was detectable only in control BSC and BSC incubated with 40 μM palmitic acid.

[ghij] Means within a gene for fatty acid treatments with common superscripts are not different (p>0.05).

acids strongly increased *CPT1β* gene expression, and the greatest increase was caused by oleic acid.

Cell type×culture method×fatty acid three-way interactions

There were significant cell type×culture method×fatty acid interactions for *AMPKα*, *C/EBPβ*, *PPARγ*, and *SCD* gene expression (p≤0.052) (Figures 1 and 2). Relative to the control cells, palmitic acid increased *AMPKα* gene expression in single-cultured IPA and both palmitic and stearic acid increased *AMPKα* gene expression in co-cultured IPA (Figure 1A). Similarly, palmitic and stearic acid increased *C/EBPβ* gene expression in single cultured IPA and stearic acid increased *C/EBPβ* gene expression more than palmitic acid in co-cultured IPA (Figure 1B). Linoleic acid increased *PPARγ* gene expression in single cultured BSC and stearic, oleic, and linoleic acid increased *PPARγ* gene expression in single cultured IPA (Figure 2A). None of the fatty acids treatments affected *PPARγ* gene expression in co-cultured IPA. Relative to the control cells, palmitic acid decreased *SCD* gene expression in single- and co-cultured BSC but increased *SCD* gene expression in co-cultured IPA (Figure 2B).

Culture method×fatty acid, cell type×fatty acid, and culture method×cell type two-way interactions for *CPT1β* and *GPR43*

The cell type×culture method×fatty acid interactions for *CPT1β* and *GPR43* gene expression were not significant (p = 0.312 and 0.113, respectively) (Table 3). Nor was the culture method×fatty acid interaction significant for *CPT1β* and *GPR43* gene expression (p = 0.341 and 0.125, respectively). The cell type×fatty acid interaction was significant for *CPT1β* (p<0.001) but not for *GPR43* gene expression (p = 0.141). Also, the culture method x cell type was not significant for *CPT1β* (p = 0.141) but tended to be significant for *GPR43* gene expression (p = 0.073) (Table 3).

DISCUSSION

Most studies of the regulation of adipogenesis have been conducted in cultured primary preadipocyte cultures or 3T3-L1 secondary preadipocyte cultures. A few studies have investigated the differentiation of bovine adipocytes and myoblasts in single cultures (Chung et al., 2006a; Ouellette et al., 2009), and we are aware of three studies describing the interaction between adipocytes and myoblastic cells in a co-culture system (Hausman et al., 2005; Choi et al., 2013a; Park et al., 2013), two of which originated from our laboratory (Choi et al., 2013a; Park et al., 2013). We have used this set of genetic markers of adipose tissue differentiation (*C/EBPβ*, *PPARγ*) and lipid metabolism (*AMPKα*, *CPT1β*, *GPR43*, *SCD*) in studies of the effects of fatty acids on bovine preadipocytes (Chung et al., 2006a) and mature adipose tissues (Smith et al., 2012; Choi et al., 2013a). The expression of *PPARγ* is promoted strongly by *C/EBPβ*, which initiates preadipocyte differentiation (Wu et al., 1996; Saladin et al., 1999). Recent studies demonstrated that inhibition of SCD catalytic activity reduced the activities of genes associated with *de novo* fatty acid synthesis, such as fatty acid synthase while increasing expression of genes associated with fatty acid oxidation, such as *CPT1β* (Kim et al., 2011; Kadegowda et al., 2013). Acetate and propionate activate the GPR43 receptor (Brown et al., 2003); this leads to a reduction in lipolysis (Ge et al., 2008), which in turn

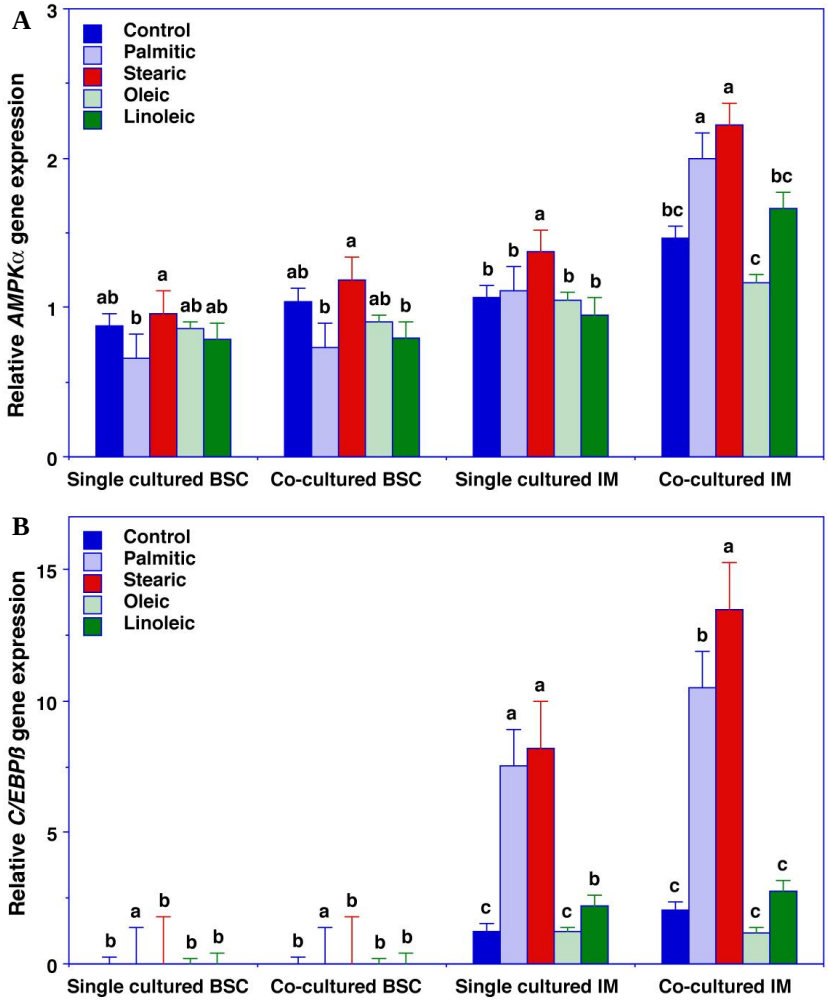

Figure 1. *AMPKα* gene expression (A) and *C/EBPβ* gene expression (B) in bovine satellite cells (BSC) and intramuscular preadipocytes (IPA). Cells were plated at a density of 1×10^4 cells per well and grown at 37℃ under a humidified atmosphere of 5% CO_2. Upon reaching confluence after 3 d, the growth medium was replaced with 3% horse serum/DMEM plus antibiotics (BSC) or 5% FBS/DMEM plus antibiotics (IPA). Differentiation medium also contained 10 μg/mL insulin and 10 μM pioglitazone, and BSC and IPA were allowed to differentiate for 3 d. Subsequently,no fatty acids (control cells) or 40 μM fatty acids (palmitic, stearic, oleic, or linoleic acid) were added to the media, and the differentiating IPA and BSC were incubated for anadditional 4 d. Differentiated IPA and BSC were single-cultured and co-cultured for an additional 2 h in DMEM plus antibiotics in the presence of the 40 μM fatty acids. A. *AMPKα*: The cell type×culture method×fatty acid three-way interaction was significant (p = 0.029). Relative to the control cells, palmitic acid increased *AMPKα* gene expression in single cultured IM (intramuscular) preadipocytes and both palmitic and stearic acid increased *AMPKα* gene expression in co-cultured IPA. B. *C/EBPβ*: The cell type×culture method×fatty acid three-way interaction was significant (p = 0.003). Relative to the control cells, palmitic and stearic acid increased *C/EBPβ* gene expression in single cultured IPA and stearic acid increased *C/EBPβ* gene expression more than palmitic acid in co-cultured IPA. All data are the means±SEM for 3 independent incubations. [abc] Means within a cell type and culture method with common superscripts are not different (p>0.05). *AMPK-α*, AMP-activated protein kinase alpha; CCAAT/enhancer-binding protein beta; DMEM, Dulbecco's Modified Eagle Medium; FBS, fetal bovine serum; SEM, standard error of the mean.

increases lipid accumulation in adipocytes and promotes metabolism of fatty acids and glucose in other tissues (Kimura et al., 2013). These effects may be attenuated by *AMPKα* (Yin et al., 2003). Expression of *AMPKα* promotes fatty acid oxidation by upregulating *PPAR* gamma coactivator-1alpha (*PGC-1α*) gene expression (Wan et al., 2013). In our previous study (Choi et al., 2013a), co-culture of subcutaneous (SC) preadipocytes with BSC increased *GPR43* gene expression nearly fivefold, which would antagonize the actions of *AMPKα* if activated by appropriate ligands (i.e., volatile fatty acids). In this study, co-culture increased *GPR43* gene expression two- to five fold. Although culture method had no effect on *CPT1β* gene expression, the elevated *GPR43* gene expression induced by co-culture suggests that the presence of IPA may increase lipid accumulation in BSC.

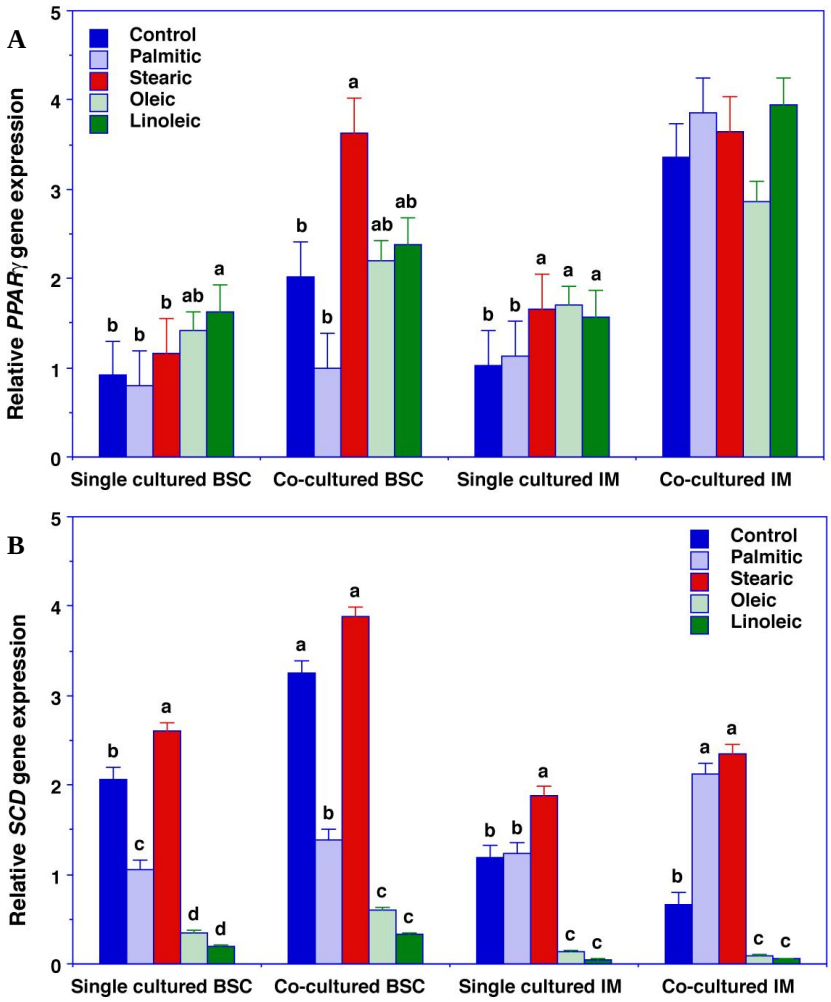

Figure 2. *PPARγ* gene expression (A) and *SCD* gene expression (B) in bovine satellite cells (BSC) and intramuscular preadipocytes (IPA). Cells were plated at a density of 1×10^4 cells per well and grown at 37℃ under a humidified atmosphere of 5% CO_2. Upon reaching confluence after 3 d, the growth medium was replaced with 3% horse serum/DMEM plus antibiotics (BSC) or 5% FBS/DMEM plus antibiotics (IM preadipocytes). Differentiation medium also contained 10 μg/mL insulin and 10 μM pioglitazone, and BSC and IPA were allowed to differentiate for 3 d. Subsequently,no fatty acids (control cells) or 40 μM fatty acids (palmitic, stearic, oleic, or linoleic acid) were added to the media, and the differentiating IPA and BSC were incubated for an additional 4 d. Differentiated IPA and BSC were single-cultured and co-cultured for an additional 2 h in DMEM plus antibiotics in the presence of the 40 μM fatty acids. A. *PPARγ*: The cell type×culture method×fatty acid three-way interaction was significant (p = 0.053). Relative to the control cells, linoleic acid increased *PPARγ* gene expression in single cultured BSC and stearic, oleic, and linoleic acid increased *PPARγ* gene expression in single cultured IPA. None of the fatty acids treatments affected *PPARγ* gene expression in co-cultured IPA. B. *SCD*: The cell type×culture method×fatty acid three-way interaction was significant (p = 0.001). Relative to the control cells, palmitic acid decreased *SCD* gene expression in single- and co-cultured cultured BSC but increased *SCD* gene expression in co-cultured IPA. All data are the means±SEM for 3 independent incubations. [abc] Means within a cell type and culture method with common superscripts are not different (p>0.05). *PPARγ*, peroxisome proliferator-activated receptor gamma; *SCD*, stearoyl-CoA desaturase; DMEM, Dulbecco's Modified Eagle Medium; FBS, fetal bovine serum; IM, intramuscular adipocyte; SEM, standard error of the mean.

Co-culture generally increased *PPARγ* gene expression in BSC and IPA, which we did not observe previously in SC preadipocytes co-cultured with BSC (Choi et al., 2013a). However, *PPARγ* gene expression was profoundly higher is SC preadipocytes in our previous study than in the IPA of the current study. Conversely, *C/EBPβ* gene expression was not detectable in SC preadipocytes (Choi et al., 2013a) but readily measurable in IPA in the current study. The expression of *C/EBPβ* occurs soon after preadipocytes are induced to differentiation (Darlington et al., 1998). These data suggest that IPA still were in early stages of differentiation even after 7 d of differentiation.

In an early study, we demonstrated high levels of *SCD* gene expression in bovine *longissimus dorsi* and SC adipose tissue (Cameron et al., 1994). In a recent study (Choi et al., 2014), intramuscular (IM) adipose tissue

Table 3. Culture method×fatty acid and cell type×fatty acid interaction means for gene expression in single- and co-cultured bovine satellite cells and intramuscular preadipocytes incubated in the absence and presence of 40 μM palmitic, stearic, oleic, or linoleic acid

Gene/culture method[a]		Treatment					SEM	p-values
		Control	Palmitic	Stearic	Oleic	Linoleic		
Culture method×fatty acid[b]								
CPT1β	Single culture	178	1,069	980	1,582	959	141	0.341
	Co-culture	217	1,123	882	1,318	887		
GPR43	Single culture	0.58	0.34	0.50	0.78	0.46	0.11	0.125
	Co-culture	1.19	1.01	2.24	1.29	1.64		
Cell type×fatty acid[c]								
CPT1β	BSC	392^{g}	2,165^{ef}	1,835^{f}	2,889^{e}	1,832^{f}	141	<0.001
	IPA	3.32^{j}	27.91^{h}	26.79^{h}	11.93^{i}	14.13^{i}		
GPR43	BSC	0.69	0.46	1.72	0.93	0.78	0.11	0.141
	IPA	1.08	0.89	1.02	1.14			
Culture method×cell type[d]		Single culture		Co-culture				
CPT1β	BSC	1,883		1,738			141	0.141
	IPA	12		22				
GPR43	BSC	0.29		1.54			0.11	0.073
	IPA	0.77		1.41				

SEM, standard error of the mean; *CPT1β*, carnitine palmitoyltransferase-1 beta; *GPR43*, G protein-coupled protein receptor 43; BSC, bovine satellite cells; IPA, intramuscular preadipocytes.

[a] Relative *CPT1β* and *GPR43*mRNA levels in total RNA isolated from BSC, IPA single- or co-cultured with insulin (10 μM), and pioglitizone (10 μM).

[b] Data are means for 6 observations, pooled over cell type.

[c] Data are means for 6 observations, pooled over culture method.

[d] Data are means for 30 observations, pooled over fatty acid treatment.

[efghij] Means within a gene with common superscripts are not different (p>0.05).

explants were removed from cattle at 12, 14, or 16 mo of age and cultured for 48 h in the absence and presence of 40 μM α-linolenic acid, *trans*-10, *cis*-12 conjugated linoleic acid (CLA), oleic acid, stearic acid, and *trans*-vaccenic acid. The mRNA levels for *SCD* increased over threefold between 12 and 16 mo of age in IM adipose tissue, similar to the pattern of *SCD* gene expression in bovine SC adipose tissue (Martin et al., 1999). However, whereas unsaturated fatty acids strongly depressed *SCD* gene expression in both BSC and IPA in this study, they had no effect on *SCD* gene expression in IM adipose tissue explants (Choi et al., 2014). We previously demonstrated that *trans*-10, *cis*-12 CLA strongly depressed *SCD* expression in SC preadipocytes (Chung et al., 2006b; Choi et al., 2013a), but CLA only slightly depressed *SCD* gene expression in intact IM adipose tissue in our recent study (Choi et al., 2014). Conversely, whereas stearic acid strongly stimulated *C/EBPβ* gene expression in BSC and IPA, stearic acid had no effect on *C/EBPβ* gene expression in IM adipose tissue explants (Choi et al., 2014). Based on this and previous studies (Chung et al., 2006b; Choi et al., 2013a), we conclude that fatty acids can exert effects on adipogenic or lipogenic gene expression only during the early phases of preadipocyte differentiation.

The saturated fatty acids, palmitic and stearic acid are noteworthy for their strong effects on gene expression. Palmitic acid increased *C/EBPβ* gene expression in both BSC and IPA (although *C/EBPβ* gene expression remained barely detectable in BSC). Palmitic acid also increased *AMPKα* and *SCD* gene expression in co-cultured IPA. Dietary palmitate, fed in the form of palm oil, increased *de novo* fatty acid synthesis, enzymes associated with fatty acid synthesis, and adipocyte volume in bovine SC adipose tissue (Choi et al., 2013b). Elevated palmitic acid in plasma or muscle of young cattle may promote *trans*-differentiation of satellite cells, leading to muscle steatosis. This observation certainly warrants further investigation into the effects of palmitic acid on myogenic gene expression.

Of the fatty acids tested in this study, stearic acid elicited the most consistent increases in adipogenic and lipogenic gene expression. The pronounced effects of stearic acid on *PPARγ*, *C/EBPβ*, and *SCD* gene expression in IPA suggests that stearic acid may promote adipogenesis and fatty acid desaturation during growth in cattle. Stearic acid is the most abundant fatty acid in the digesta of cattle, and is the most abundant saturated fatty acid in bovine plasma (Ekeren et al., 1992; Chung et al., 2006b; Brooks et al., 2010), and thus is readily available to promote adipogenesis. Circulating oleic and linoleic acid may antagonize the effects of saturated fatty acids in promoting *SCD* gene expression in cattle, consistent with an early study with 3T3-L1 preadipocytes (Sessler et al., 1996) but not *PPARγ*, *C/EBPβ*, or *AMPKα* gene expression, as these unsaturated fatty acids had no effect on the expression of

these genes in IPA.

Finally, the results of this study confirm our earlier report (Choi et al., 2013a) that co-culture of BSC and IPA alters the expression of genes associated with adipogenesis and lipogenesis. These data suggest that the coexistence of BSC and IPA within intact muscle would promote IPA differentiation and may even cause *trans*-differentiation of BSC to IM adipocytes.

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