ABSTRACT: Our objective was to determine if palmitoleic (C16:1 cis-9) acid supplementation to primary bovine adipocytes regulates lipogenic gene expression and rates of lipogenesis. Stromal vascular cells were isolated from subcutaneous and intermuscular fat, propagated, and frozen for use in this study. Cells were passaged 4 times, allowed to reach confluence, held for 2 d, and then differentiated with a standard hormone cocktail (d 0). At d 2, secondary differentiation media containing 1 of 4 concentrations of palmitoleic acid (0, 50, 150, or 300 μM) were added for 10 d. Cells were harvested on d 6 and 12 to assess fatty acid concentrations and gene expression. In addition, 13C2 and 13C18:0 stable isotopes were added on d 6 to measure lipogenesis and desaturase activity, respectively. Concentrations of C16:1 and total fatty acids increased (P < 0.05) linearly in response to palmitoleic acid supplementation. Concentrations of C18:1 cis-11 and C20:1 cis-13 also increased (P < 0.01) in response to supplementation, suggesting elongation of palmitoleic acid in vitro. Concentrations of C16:1, C18:1 cis-11, and total fatty acids were also greater (P < 0.05) at d 12 compared with d 6. In contrast, C16:0, C18:0, and C18:1 cis-9 concentrations decreased (P < 0.05) in response to palmitoleic acid supplementation and were not affected (P > 0.05) by harvest day. The ratio of C18:1 cis-9/C18:0 and fractional synthetic rate (FSR) of desaturation decreased (P < 0.05) in response to increasing palmitoleic acid supplementation. In addition, FSR of lipogenesis was reduced (P < 0.05) in palmitoleic acid-treated cells. Messenger RNA abundance as determined by real-time quantitative PCR for stearoyl-CoA desaturase 1 (SCD1), fatty acid synthase (FASN), and elongase protein 6 (ELOVL6) genes were reduced (P < 0.05) by palmitoleic acid supplementation. Expression of a β-oxidation gene, carnitine palmitoyltransferase 1A (CPT1A), was upregulated (P < 0.05) with palmitoleic acid supplementation in a dose-responsive manner. Supplementation of palmitoleic acid to bovine adipocytes results in increased incorporation of this fatty acid and its elongation products into the adipocyte, which downregulates SCD1, FASN, and ELOVL6 to decrease lipogenesis and upregulates CPT1A, potentially increasing β-oxidation. These results suggest that palmitoleic acid, an end product of desaturation, can act as a regulator of lipogenesis, desaturation, and β-oxidation in bovine adipocytes.

Key words: adipocyte, bovine, desaturase, lipogenesis, palmitoleic acid

INTRODUCTION

Over the past decade, researchers have begun to view white adipose tissue as a complex, metabolically active tissue (McGillis, 2005). Excess adipose tissue is not advantageous to human health, with regard to common medical disorders associated with obesity, or to livestock producers, who strive for a balance between maximal intramuscular fat and minimal subcutaneous fat, which influence beef quality and yield grades, respectively. Interest in fatty acid composition of meat and milk products has increased with concerns of SFA in the human diet. Research shows that adipocytes produce and secrete leptin (Flier, 1998) and adipokines (MacDougald and Burant, 2007) to coordinate systemic metabolism.
Cao et al. (2008) identified palmitoleic (C16:1 cis-9) acid as a lipokine that increases insulin sensitivity in skeletal muscle and decreases lipogenesis in liver of mice. In the study, administration of palmitoleic acid to mice reduced lipogenic gene expression in the liver and increased insulin signaling in skeletal muscle. In humans, circulating palmitoleic acid concentration is strongly associated with abdominal obesity in children (Okada et al., 2005) and adults (Gong et al., 2010), and is a predictor of insulin sensitivity in adults (Stefan et al., 2010). In finishing beef cattle, the correlations between tissue palmitoleic and oleic (C18:1 cis-9) acid concentrations, and carcass fat content are large (r = 0.68 and 0.81, respectively; S. Duckett, unpublished data). Palmitoleic acid in circulation and adipose tissues originate predominately from de novo fatty acid synthesis, as dietary sources of C16:1 are minor. Therefore, we expect palmitoleic acid to function similarly at the primary site of lipogenesis, the liver, for most monogastrics and adipose tissue in ruminants (Vernon, 1980; Smith and Crouse, 1984), across species. We hypothesize that palmitoleic acid supplementation to bovine adipocytes will decrease lipogenesis and lipogenic gene expression.

MATERIALS AND METHODS

The experimental procedures were reviewed and approved by Clemson University Animal Care and Use Committee.

Cell Culture

Primary bovine stromal vascular (SV) cells were harvested from adipose tissue from 3 beef carcasses using slight modifications of methods described by Hirai et al. (2007), as described by Pratt et al. (2010). Cells were plated at 1 x 10⁴ cells/cm² and passaged every 2 to 4 d when 60% confluent. Cells were incubated at 37°C under 5% CO₂ humidified atmosphere in media [Dulbecco’s modified eagles medium (DMEM), 10% fetal calf serum (FCS), and 2 x antibiotic/antimycotic (AB/AM; containing 10,000 U/mL penicillin G, 10,000 μg/mL streptomycin, and 25 μg/mL amphotericin B)], with replacement every 2 d. After 4 passages, cell lines were stored in liquid nitrogen at 1 x 10⁶ cells/mL in freezing media (DMEM, 20% FCS, and 10% dimethyl sulfoxide) for later use. Cell culture hormones and DMEM were purchased from Sigma-Aldrich (St. Louis, MO) and AB/AM and serum were purchased from Thermo Scientific Hyclone (Logan, UT).

Treatments

Individual cultures from 3 carcasses were used in duplicate for this study. Cells were thawed, cultured in growth media for 3 passages, and seeded in 6-well plates (1.6 cm²/well) at 1 x 10⁵ cells/cm². Cells were allowed to reach confluence, held for 2 d, and differentiated on d 0 with DMEM containing 5% FCS, 2 x AB/AM, 2.5 μg/mL insulin, 0.5 mM 2-isobutyl-1-methylxanthine, 0.25 μM dexamethasone, 5 μM troglitazone (TRO), and 10 mM acetate (Hirai et al., 2007; Pratt et al., 2010). Secondary differentiation media (DMEM, 5% FCS, 2 x AB/AM, 2.5 μg/mL insulin, 5 μM TRO, and 10 mM acetate) were applied for 4 (d 6) or 10 d (d 12), along with palmitoleic acid at varying concentrations (0, 50, 150, or 300 μM, final concentration). Palmitoleic acid was purchased from Acros Organics (Geel, Belgium). Palmitoleic acid was bound to BSA (2:1, wt/wt), according to Duckett et al. (2002). Individual fatty acids were identified by comparison of retention times with standards (Sigma; Matreya, Pleasant Gap, PA). Fatty acids were quantified by incorporating an internal standard, methyl tricosanoic (C23:0) acid, into each sample during methylation and expressed as a weight percentage of total fatty acids per well.

Fatty Acids

 Cellular fatty acids were extracted and transmethylated, as described by Folch et al. (1957) and Park and Goins (1994), respectively. Fatty acid methyl esters were analyzed using an Agilent 6850 gas chromatograph (GC) equipped with an Agilent 7673A automatic sampler (Agilent Technologies, Inc., Santa Clara, CA). Separations were accomplished using a 100-m Supelco SP-2560 (Supelco, Inc., Bellefonte, PA) capillary column (0.25-mm i.d. and 0.20-μm film thickness), according to Duckett et al. (2002). Individual fatty acids were identified by comparison of retention times with standards (Sigma; Matreya, Pleasant Gap, PA). Fatty acids were quantified by incorporating an internal standard, methyl tricosanoic (C23:0) acid, into each sample during methylation and expressed as a weight percentage of total fatty acids per well.

Lipogenesis

To measure lipogenesis in vitro, we replaced a portion of the unlabeled acetate in the secondary differentiation media with isotopically labeled acetate (2.5 mM 1-13C2) and incubated cultures treated with 0, 150, and 300 μM C16:1 on d 6 for 0, 6, 12, and 24 h. At harvest, cells were placed directly into 2:1 chloroform:methanol (vol/vol) to terminate all enzymatic activity. Fatty acid methyl esters were prepared as stated above for analysis with an Agilent 6890N GC equipped with an Agilent 5973 mass spectrometer (MS), using a 100-m Varian CP7489 (Varian Instruments Inc., Walnut Creek, CA) capillary
The relative abundance (RA), where, p is the number of precursor monomers present in the polymer, p = 8. Finally, our estimate of lipogenesis could be derived by calculating FSR (Wolfe and Chinkes, 2005).

\[
\text{FSR}_{\text{lipogenesis}} = \frac{(p \times MPE_{\text{precursor}})}{(MPE_{\text{C18:0}} \times (1 - MPE_{\text{C18:0}})^p - 1)^x (\text{time} 1 - \text{time} 0)}
\]

Desaturation

Stearoyl-CoA desaturase 1 (SCD1) is the enzyme responsible for creating a double bond at the Δ9 position of several fatty-acyl CoA and its preferred substrates are C16:0 and C18:0 (Enoch et al., 1976). Stearoyl-CoA desaturase transcription and activity are regulated by certain fatty acids (Ntambi, 1995), but the effect of palmitoleic acid supplementation on SCD1 activity has not been reported to date. In the absence of a suitable SCD1 antibody for Western blotting, SCD1 activity was measured enzymatically. Initially, 2 SV cell lines were treated with 20 μM 1-13C18:0 to cultures on d 6, as described above. Enrichment of C18:0 was detected by 6 h post inclusion of tracer; however, TTR of C18:1 cis-9 was not evident in cultures (data not shown). Therefore, we repeated the experiment with 2 cell lines treated with 0 or 150 μM C16:1 and increased the amount of tracer to 100 μM 1-13C18:0 on d 6. Samples were prepared and analyzed, as described above, for analysis with GC-MS. Ions of mass-to-charge ratio (m/z) 298 (m) and 299 (m + 1) were selectively measured to calculate the isotopic enrichments of C18:0. Similarly, isotope abundance of 296 (m) and 297 (m + 1) was measured for C18:1 cis-9. Because tracer enrichment can be measured in this case and the product of the SCD reaction is not a polymer of the tracer, it was not necessary to use MIDA. The FSR was calculated for rate of desaturation, using the precursor enrichment, MPE_{C18:0}.

\[
\text{FSR}_{\text{desaturation}} = \frac{TTR_{C18:1 \text{ cis-9}, \text{time} 1} - TTR_{C18:1 \text{ cis-9}, \text{time} 0}}{MPE_{C18:0} \times (\text{time} 1 - \text{time} 0)}
\]

Gene Expression

Total cellular RNA (tcRNA) was isolated from cells using the mirVana microRNA Isolation kit (Ambion, Austin, TX), according to manufacturer instructions, and RNA quality was assessed as described by DUCKETT et al. (2009). RNA quality was assessed using Nanodrop 2000 Spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA) and Agilent Bioanalyzer 2100. All tcRNA samples used in real-time quantitative PCR (qPCR) had a 260:280 absorbance ratio >1.8 on the Nanodrop and RNA integrity number >8.0 (1.0 to 10.0 scale), using Agilent RNA 6000 Nano kit. Real-time qPCR was conducted using an Eppendorf MasterCycler ep realplex (Westbury, NY), with the QuantiTect SYBR Green RT-PCR One Step Kit (Qiagen, Valencia, CA), according to manufacturer directions. Two genes, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and β-actin, were evaluated for data normalization (DUCKETT et al., 2009). To determine the appropriate normalization gene to be used, the cycle threshold values for GAPDH, β-actin and all target genes per sample were entered into the BESTKEEPER program (http://www.genequantification.info). The program determines the most stable gene to be used for normalization by repeated pair-wise correlation and regression analysis (PFAFF et al., 2002). Both GAPDH and β-actin exhibited a correlation coefficient of 0.99 (P < 0.001) in the analysis and were suitable for data normalization. Primers for bovine mRNA were designed using Primer 3 software (http://frodo.wi.mit.edu/primer3) and parameters defined by DUCKETT et al. (2009). Primer sets were first
evaluated according to Duckett et al. (2009) to verify identity. Genes of interest for this study were those involved in fatty acid biosynthesis, including acetyl-CoA carboxylase (ACC), fatty acid synthase (FASN), fatty acid elongase (ELOVL) -5 and -6, SCD1, sterol regulatory element binding protein-1c (SREBP), and carnitine palmitoyl-transferase 1A (CPT1A).

**Statistical Analysis**

The data were analyzed using Proc GLM procedure (SAS Inst. Inc., Cary, NC) for treatment comparisons over time. A 2-way ANOVA was performed as a completely randomized design, with factors fatty acid concentration (0, 50, 150, and 300 μM C16:1), day (d 6 and 12), and 2-way interaction in the model. Least squares means were computed and separated statistically using Fisher’s Protected LSD test. Linear regression was performed using the bivariate fit model of JMP Version 7 (SAS Inst. Inc.). Relative gene expression data were analyzed using Pair-wise Fixed Reallocation Randomization Test (Pfaffl et al., 2002) with preplanned treatment comparisons.

**RESULTS AND DISCUSSION**

The differentiation hormones and media used in our experiment have been tested for their efficacy in inducing differentiation in bovine SV cultures (Aso et al., 1995; Grant et al., 2008a,b; Lengi and Corl, 2010). Classically, preadipocytes change morphologically from fibroblastic to round and accumulate lipid (Napolitano, 1963), in addition to changes in gene expression (Taniguchi et al., 2008). Previously, our SV cultures displayed characteristic changes in fatty acid composition and adipogenic gene expression upon hormonal treatment, consistent with adipocyte differentiation (Pratt et al., 2010). In the current study, cells changed shape from fibroblastic to round after differentiation and became lipid filled with fatty acid supplementation (Figure 1).

**Fatty Acids**

We successfully incorporated our fatty acid supplement into bovine adipocyte cultures, based on detection of increasing amounts of C16:1 in palmitoleic acid-treated cells after all media were removed (Figure 2). Rates of palmitoleic acid incorporation into the adipocytes were 0.24 and 0.61 μg/well per μM supplemented palmitoleic acid for d 6 and 12, respectively. After completion of the fatty acid analysis, we discovered high concentrations of fatty acids believed to be C18:1 cis-11 and C20:1 cis-13. To confirm identity, ion masses were checked with GC-MS and samples were spiked with standards of known identity. The identities of these peaks were confirmed as being C18:1 cis-11 and C20:1 cis-13. A significant interaction (P < 0.001) between palmitoleic acid supplementation and day of harvest was present for total fatty acids, C16:1, C18:1 cis-11, and C20:1 cis-13 (Figure 2). Each of these fatty acids gravimetrically increased (P < 0.01) in response to greater palmitoleic acid supplementation and the magnitude of response was greater (P < 0.01) on d 12 than d 6. No other fatty acid peaks detected showed a similar pattern of response (P > 0.05). As C18:1 cis-11 and C20:1 cis-13 were not present in increased concentrations in control cells and they increased dramatically in cultures supplemented with palmitoleic acid, we suspect they were elongated from palmitoleic acid.

After accounting for any endogenous concentrations of C16:1, C18:1 cis-11, and C20:1 cis-13 in our cultures, we estimated the percentage of supplemental palmitoleic acid that was elongated. In the 50-μM palmitoleic acid-treated cells on d 6, 66 ± 2% of palmitoleic acid was elongated to C18:1 cis-11 and C20:1 cis-13. With greater palmitoleic acid supplementation, the percentage of palmitoleic acid converted to C18:1 cis-11 and C20:1 cis-13 decreased (P < 0.01) to 55 ± 2% and 49 ± 2% for 150 and 300 μM treatments, respectively. Results were similar for d-12 cultures in that the percentage of palmitoleic acid elongated was 69 ± 3%, 63 ± 2%, and 54 ± 2% for the 50-, 150-, and 300-μM palmitoleic acid treatments, respectively. Despite the linear incorporation of C16:1 into the adipocytes, the percentage elongated into C18:1 cis-11 and C20:1 cis-13 in bovine adipocytes was curvilinear, suggesting that the elongase reaction...
Palmitoleic acid reduces lipogenesis in vitro was limited by another factor other than substrate availability.

There was an interaction \((P < 0.05)\) between palmitoleic acid supplementation and day of harvest for linoleic (C18:2 n-6) acid (Figure 3). On d 12, linoleic acid content did not differ \((P > 0.05)\) with palmitoleic acid supplementation (Figure 3). On d 6, linoleic acid content was less \((P < 0.05)\) for 50- and 300-μM palmitoleic acid supplementation compared with controls. Stearic (C18:0) acid content was less \((P < 0.05)\) for 50 and 300 μM compared with controls (0 μM; Table 1). Oleic (C18:1 cis-9) and arachidonic (C20:4 n-6) acids were reduced \((P < 0.05)\) in palmitoleic acid-supplemented cells, regardless of supplementation. Palmitic (C16:0) acid content did not differ \((P > 0.05)\) with palmitoleic acid supplementation.

Lipogenesis

Enrichment for C16:0, as calculated by TTR, was detected in cells treated with 1-13C2 in the media on d 6. There was a significant interaction \((P < 0.01)\) of TTRC16:0 between palmitoleic acid supplementation and time of labeled acetate incubation. All palmitoleic acid treatments had TTRC16:0 >0 \((P < 0.01)\) at 12 h, but cells treated with 150 μM palmitoleic acid had reduced \((P < 0.05)\) TTRC16:0 compared with 0-μM palmitoleic acid-treated cells and numerically less TTR C16:0 than 300-μM palmitoleic acid-treated cells (Figure 4). At 24 h

Table 1. Main effect of palmitoleic acid supplementation on palmitic (C16:0), stearic (C18:0), and oleic (C18:1 cis-9) acids, and desaturation index (C18:1 cis-9/C18:0) of bovine adipocytes

<table>
<thead>
<tr>
<th>Fatty acid, μg/well</th>
<th>Palmitoleic acid supplementation</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 μM</td>
<td>50 μM</td>
<td>150 μM</td>
</tr>
<tr>
<td>C16:0</td>
<td>14.83</td>
<td>12.79</td>
<td>16.13</td>
</tr>
<tr>
<td>C18:0</td>
<td>15.41a</td>
<td>11.20b</td>
<td>13.45ab</td>
</tr>
<tr>
<td>C18:1 cis-9</td>
<td>15.35a</td>
<td>9.42b</td>
<td>11.02b</td>
</tr>
<tr>
<td>C20:4</td>
<td>6.57a</td>
<td>5.27b</td>
<td>5.10b</td>
</tr>
<tr>
<td>C18:1 cis-9/C18:0</td>
<td>1.01a</td>
<td>0.84b</td>
<td>0.78bc</td>
</tr>
</tbody>
</table>

\(a-c\)Within a row, means without a common superscript differ \((P < 0.05)\).
after 1-13C2 treatment, TTR_{C16:0} of 0-, 150-, and 300-μM cells differed (P < 0.001) from each other. Similarly, FSR_{C16:0} from 0 to 24 h after labeled acetate treatment was reduced (P < 0.05) ~45% and 30% by 150-μM and 300-μM palmitoleic acid supplementation, respectively. Therefore, lipogenesis, as measured by the rate of labeled acetate incorporation into the primary product of de novo fatty acid synthesis, C16:0, was decreased by 150-μM palmitoleic acid supplementation. In addition, doubling the amount of exogenous palmitoleic acid to 300 μM did not further reduce lipogenesis.

The concentration of C16:0 present in our cultures did not differ due to palmitoleic acid treatment, but synthesis of 13C16:0 from labeled 13C acetate was reduced in palmitoleic acid-treated cells. Unchanged content of C16:0 could indicate that C16:0 was not desaturated or elongated but was conserved as a SFA in palmitoleic acid-treated cells. The ratio of MUFA:SFA is a critical factor affecting membrane fluidity and survivability of a cell (Ntambi and Miyazaki, 2004).

**Desaturation**

The ratios of MUFA to SFA are often calculated as indicators of desaturation and have been associated with SCD1 activity (Baumgard et al., 2002; Mosley and McGuire, 2007). Because our fatty acid treatment confounded the C16:1/C16:0 ratio and peaks for C14:1 and C14:0 were not detectable, we used C18:1 cis-9/C18:0 ratio as our desaturation index. There was no main effect of harvest day on C18:1 cis-9/C18:0 ratio, but palmitoleic acid supplementation reduced (P < 0.001) the C18:1 cis-9/C18:0 ratio (Table 1). The 50-, 150-, and 300-μM palmitoleic acid treatment groups had a smaller (P < 0.05) C18:1 cis-9/C18:0 ratio compared with 0-μM palmitoleic acid-supplemented cells. In addition, cells treated with 300 μM palmitoleic acid had a reduced (P < 0.05) desaturation index compared with cells treated with 50-μM palmitoleic acid. These results suggest that desaturation was decreased by palmitoleic acid supplementation; however, desaturation ratios are not always reflective of SCD1 activity (Archibeque et al., 2005).

To get a more accurate measurement of desaturation activity than simply using a desaturation index, we measured desaturation of stable isotope tracer in our cultures. We were able to detect significant enrichment of C18:0 in our cells 6 h after 1-13C18:0 addition. There was no effect (P > 0.05) of palmitoleic acid supplementation on TTR_{C18:0} of 0-, 150-, and 300-μM cells differed (P < 0.001) from each other. For TTR_{C18:1 cis-9}, there was significant interaction (P < 0.01) between palmitoleic acid supplementation and time of incubation with 1-13C18:0. Enrichment of C18:1 cis-9 was first detected at 12 h for 0-μM palmitoleic acid-treated cells and at 24 h for 150-μM palmitoleic acid-treated cells (Figure 4). At 24 h when enrichment was above background for both amounts of palmitoleic acid, TTR_{C18:1 cis-9} was greater (P < 0.001) in 0-μM palmitoleic acid-treated cells than in 150-μM palmitoleic acid-treated cells. Similarly, the

![Figure 4. Tracer-to-tracee ratio (TTR) and fractional synthetic rate for the production of 13C16:0 from 1-13C2 (lipogenesis) and 1-13C18:1 cis-9 from 1-13C18:0 (desaturation) in bovine adipocyte cultures treated with palmitoleic acid, respectively. Molar percent excess (MPE) per hour was calculated over a 24-h period after stable-isotope addition to the media. *Within a time point, palmitoleic acid-treated cells differed from unsupplemented cells (P < 0.05). a-bBars of the same color without a common letter differ (P < 0.05). †Analysis of the 300-μM concentration was not performed for desaturation activity.](image-url)
desaturation rate, calculated as FSR<sub>C18:1 cis-9</sub> from 0 to 24 h after the addition of 1-<sup>13</sup>C18:0, was reduced by >70% in cells supplemented with palmitoleic acid.

**Gene Expression**

Fatty acid synthase mRNA was downregulated (P < 0.01) by 35% and 25% in 50-μM and 150-μM palmitoleic acid-treated cells, respectively (Figure 5). At 300 μM of palmitoleic acid, FASN mRNA expression was not different (P > 0.05) from control (0 μM). Gene expression of ACC was not different (P < 0.05) in palmitoleic acid-supplemented cells compared with controls. Both ACC and FASN are key enzymes in de novo fatty acid synthesis. Fatty acid synthesis begins with ACC catalyzed addition of a carboxyl group to acetyl-CoA to form malonyl-CoA. Fatty acid synthase is a large, multimeric protein that sequentially adds acetyl-CoA to malonyl-CoA or a fatty acyl-CoA until the fatty acid reaches a length of 16 carbons, C16:0. Changes in FASN gene expression with palmitoleic acid supplementation followed a similar pattern as rate of lipogenesis using <sup>13</sup>C2; however, gene expression was unchanged at 300 μM, but lipogenic rate was still reduced compared with controls.

Expression of SCD1 mRNA was downregulated (P<0.05) by 60% or more at all 3 amounts of palmitoleic acid supplementation compared with control (0 μM). Stearoyl-CoA desaturase is the enzyme responsible for the desaturation of SFA to MUFA by inserting a double bond in the Δ<sub>9</sub> position. As reviewed by Ntambi and Miyazaki (2004), SCD1 has been extensively studied especially in the Murine Model. Transcription of SCD1 mRNA is controlled primarily by SREBP, which binds to sterol response element in the promoter region of the SCD1 gene (Lay et al., 2002; Ebrelé et al., 2004). The promoter region of the bovine SCD1 gene contains a fat-specific element, PUFA response element, and SREBP-response region (Keating et al., 2006). Expression of SCD1 is also regulated by its products and not by the availability of its substrates (Keating et al., 2006). Our study confirmed that palmitoleic acid, an end product of SCD1, also downregulates SCD1 mRNA expression in bovine cells. In addition, the MUFA:SFA and FSR<sub>desaturase</sub> show that palmitoleic acid impacts actual activity of the desaturase, as well. Due to the similarities between the percentage decrease in desaturase activity and gene expression, the regulation of palmitoleic acid on SCD1 most likely occurs at the level of transcription; although, Cao et al. (2008) report SCD1 protein stability may also be affected by palmitoleic acid. The PUFA response element in the bovine SCD1 promoter region is the most likely target of transcriptional repression by palmitoleic acid (Keating et al., 2006). In a mouse reporter assay, promoter activity of SCD1 was suppressed by palmitoleic acid supplementation (Cao et al., 2008). When the PUFA response element of the SCD1 promoter region was mutated, palmitoleic acid no longer suppressed SCD1 promoter activity (Cao et al., 2008).

Several elongase enzymes are present in mammalian cells and are responsible for adding acetyl-CoA to the carboxylic acid end of fatty acyl-CoA molecule. However, each elongase isoform has preferred fatty-acyl substrates of particular C length (Guillou et al., 2010). In humans, most ELOVL proteins identified elongate PUFA greater than or equal to 18C; however, ELOVL6 is attributed to the conversion of 12-C to 16-C SFA and MUFA, including C16:1 to C18:1 cis-11 (Matsuzaka et al., 2002; Leonard et al., 2004). It has been proposed that palmitoleic acid can be elongated into C18:1 cis-11 and potentially elongated further into a 20-C fatty acid, C20:1 cis-13 (Matsuzaka and Shimano, 2009). In addition, ELOVL5 is reported to have some activity for this reaction (Wang et al., 2006), especially in absence of ELOVL6 (Matsuzaka et al.,

![Figure 5. Relative expression of lipogenic genes in cells supplemented with 0, 50, 150, and 300 μM C16:1 to d 6 post differentiation. Acetyl-CoA carboxylase (ACC), fatty acid synthase (FASN), stearoyl-CoA desaturase 1 (SCD1), fatty acid elongase (ELOVL)-5 and -6, and carnitine palmitoyl-transferase 1A (CPT1A). Cycle threshold (C<sub>T</sub>) values were normalized to glyceraldehyde-3-phosphate dehydrogenase. *P ≤ 0.05. Gene expression was upregulated or downregulated compared with 0-μM palmitoleic acid-treated cells.](image-url)
2007). Primarily regulated by lipogenic transcription factors (Leonard et al., 2004; Wang et al., 2006), these 2 ELOVL isoforms are the only bovine elongase enzymes published on the National Center for Biotechnology Information (NCBI) to date (Zimin et al., 2009). In this study, mRNA expression of ELOVL5 was not altered (P > 0.05) with palmitoleic acid supplementation. Expression of ELOVL6 mRNA was downregulated (P < 0.05) with 50-μM and 150-μM palmitoleic acid supplementation compared with controls. As FASN is not capable of synthesizing fatty acids >16 C in length, acetate molecules were most likely added to palmitoleic acid by ELOVL6 in our study. Elongation with acetate increased the fatty acid chain length to 18 and 20 C, subsequently changing the double bond to Δ11 and Δ13 positions, respectively, with each 2-C addition.

In our adipocyte cultures, CPT1A mRNA was upregulated (P < 0.05) by palmitoleic acid supplementation. Carnitine palmitoyl-transferase 1A is responsible for transporting long-chain fatty acids through the outer mitochondrial membrane for the purpose of β-oxidation in the mitochondrial matrix. In ruminants, CPT1A is expressed in numerous tissues, including adipose tissue in contrast to a more restricted tissue abundance in monogastrics (Price et al., 2003). Price et al. (2003) found N-terminal sequence differences in ovine CPT1 that alter enzyme kinetics for certain substrates, which differ from that of the rat. Inhibition of CPT1A is primarily attributed to malonyl-CoA, the product of the ACC reaction, in monogastric species (McGarry and Brown, 1997). Overexpression of CPT1A increases long-chain fatty acid oxidation in human embryonic kidney cells (Jambor de Sousa et al., 2005). Palmitoleic acid supplementation stimulates CPT1A transcription, which may also lead to increased β-oxidation and ATP production.

Fatty acid synthase, ELOVL6, and SCD1 genes are all transcriptionally controlled by SREBP (Eberlé et al., 2004), suggesting coordinated regulation. There was no effect (P > 0.05) of palmitoleic acid supplementation on SREBP gene expression (data not shown). Because post-translational modification is a major point of regulation for SREBP activity (Brown and Goldstein, 1997), a lack of change in SREBP gene expression may not be indicative of SREBP activity. The SREBP promoter region contains a sterol response element to which proteolytically mature SREBP can bind to activate its own transcription (Shimano, 2000). In addition, liver X receptor and insulin can stimulate SREBP transcription (Eberlé et al., 2004), but those activators were not explored in this study, as SREBP mRNA abundance were not altered.

In summary, supplementation of palmitoleic acid to bovine adipocytes results in increased incorporation of this fatty acid and its elongation products into the adipocyte, which downregulates SCD1, FASN, and ELOVL6 to decrease lipogenesis and desaturation, and upregulates CPT1A to increase β-oxidation. These results suggest that exogenous palmitoleic acid, an end product of desaturation, acts as a regulator of lipogenesis, desaturation, and β-oxidation in bovine adipocytes. However, with a large percentage of palmitoleic acid presumably elongated into other fatty acids, it remains to be determined whether effects of palmitoleic acid supplementation are due to palmitoleic acid directly or its elongation products. More research is needed to ascertain the mode of action and efficacy in vivo of palmitoleic acid administration to modulate lipogenesis in beef cattle production.

LITERATURE CITED


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