Bovine Adipose Triglyceride Lipase is Not Altered and Adipocyte Fatty Acid-Binding Protein is Increased by Dietary Flaxseed

Jeffrey Deilulis · Jonghyun Shin · Eric Murphy · Scott L. Kronberg · Maurice L. Eastridge · Yeunsu Suh · Jong-Taek Yoon · Kichoon Lee

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Abstract In this paper, we report the full-length coding sequence of bovine ATGL cDNA and analyze its expression in bovine tissues. Similar to human, mouse, and pig ATGL sequences, bovine ATGL has a highly conserved patatin domain that is necessary for lipolytic function in mice and humans. This suggests that ATGL is functionally intact as a triglyceride lipase in cattle. Tissue distribution of ATGL gene expression was highest in fat and muscle (skeletal and cardiac) tissue, while protein expression was solely detectable in the adipose tissue. The effect of 109 days of flaxseed supplementation on ATGL and adipocyte fatty acid-binding protein (FABP4 or A-FABP, E-FABP or FABP5) expression was examined in Angus steers. Supplemented steers had greater triacylglycerol (TAG) content in the muscle compared with unsupplemented ones. Additionally, supplementation increased A-FABP expression and decreased stearoyl-CoA desaturase 1 (SCD-1) expression in muscle, while total ATGL expression was unaffected. In summary, supplementation of cattle rations with flaxseed increased muscle TAG concentrations attributed in part to increased expression of key enzymes involved in lipid trafficking (A-FABP) and metabolism (SCD-1).

Keywords Lipolysis · Adipose triglyceride lipase · Bovine · Flaxseed

 Abbreviations

AA Amino acid
A-FABP Adipocyte-type fatty acid-binding protein
ATGL Adipose triglyceride lipase
E-FABP Epidermal fatty acid-binding protein
FS Flaxseed supplementation
H-FABP Heart-type fatty acid binding protein
HRP Horse radish peroxidase
HSL Hormone sensitive lipase
LPL Lipoprotein lipase
M-MLV Moloney murine leukemia virus
PKA Protein kinase A
PMSF Phenylmethysulfonyl fluoride
PPARγ Peroxisome proliferator-activated receptor gamma
PVDF Polyvinylidene fluoride
SCD-1 Stearoyl-CoA desaturase 1
TBST Tris-buffered saline with Tween twenty
TAG Triacylglycerol
Introduction

Altering the fatty acid composition of beef products for human consumption adds value, creating a functional food which may enhance the overall health of meat-consuming individuals. In the Western diet, the consumption of n-3 fatty acids via dietary intake is low because of relatively low rates of fish consumption and the industrial production of animal feeds rich in grains containing n-6 fatty acids, leading to production of meat rich in n-6 and low in n-3 fatty acids [1]. In addition, it is important to mention that beef fat has a high degree of saturation due to the biohydrogenation of unsaturated dietary fatty acids by rumen microorganisms, though there is a variation in the n-6/n-3 ratio between breeds and production systems. It has been demonstrated that feeding diets high in flaxseed increases n-3 fatty acid content in beef and poultry [2–4]. We have previously shown that n-3 levels in Angus beef increased from 27 to 45 mg per 100 g [2]. The acceptable macronutrient distribution range set by the National Academy of Sciences, Institute of Medicine, Food and Nutrition Board in 2005 for total n-3 fatty acids is 0.6–1.2% of energy, 1.3–2.6 g for a person consuming 2,000 kilocalories per day. Studies indicate that in humans, a high intake of n-6 fatty acids shifts the physiologic state to one that is prothrombotic and proaggregatory, characterized by increases in blood viscosity and vasoconstriction, and reduced bleeding time, while high intake of n-3 fatty acids have anti-inflammatory, anti-thrombotic, anti-hypertensive, and anti-arrhythmic properties, while also improving arterial compliance, vasodilation, and serum lipid profiles [5–8]. The average Western diet has an n-6 to n-3 fatty acid ratio of approximately 20–30:1 [5], hence large scale production of n-3 enriched meats would provide alternative sources of n-3 fatty acids for consumers.

Supplementation with flaxseed changes not only the fatty acid composition of skeletal muscle of Angus steers [2], but may also affect lipid metabolism within the muscle altering total fat concentration. Because dietary n-3 fatty acids increase the expression of PPARγ in flax-supplemented cattle [2], the impact of this dietary regimen on the expression of adipose triglyceride lipase (ATGL) and on adipocyte fatty acid-binding protein (A-FABP, FABP-4) was examined in this study. ATGL is a newly discovered lipase which hydrolyzes the first ester bond of stored triacylglycerols, releasing nonesterified free fatty acids [9]. It is associated with lipid droplets and is found at high concentrations in adipose tissue, though it is present in tissues with lipid stores [10]. ATGL activity is required for PKA-stimulated fatty acid and glycerol release in murine embryonic fibroblast adipocytes [11] and is the rate-limiting step in hormone-induced lipolysis [9, 12]. The product, diacylglycerol, is then hydrolyzed by activated hormone sensitive lipase (HSL), which has a higher substrate affinity for diacylglycerol than triacylglycerol (TAG) [12]. A direct interaction between A-FABP and HSL promotes the hydrolysis of lipid droplet stored TAG, thus it is thought that A-FABP may be involved in fatty acid trafficking from the lipid droplet to the adipocyte membrane where the fatty acid leaves the cell [13, 14]. Thus, ATGL, HSL, and A-FABP work in concert to mobilize esterified fatty acids stores from lipid storing tissues, although the impact of n-3 fatty acid supplementation on expression of these key proteins in cattle is not fully characterized.

To address this question, we examined the expression of these proteins in longissimus muscle of Angus steers finished on a diet containing flaxseed rations. In addition, while the sequences of ATGL for mouse, swine, human, and avian species are known [9, 15, 16], the sequence of bovine ATGL and its response to dietary manipulation is not known. Herein, we report for the first time the sequence for bovine ATGL and characterize ATGL gene expression in cattle. We also demonstrate that ATGL gene expression was not influenced by dietary n-3 fatty acids in cattle, although the expression of A-FABP was increased, consistent with our previous work demonstrating an increase in PPARγ expression in these cattle [2].

Materials and Methods

Feeding Regimen for Angus Cattle

The experimental methods for the feeding of Angus steers were described in our previous report [2]. Briefly, 20 Angus steers that were 16 months old with a mean body weight of 414 kg (SD = 39 kg) at the start of the study were divided into two groups: flaxseed supplement (907 g/day) and control groups (10 steers per group). The basal diet of the grazing steers was forage during the first 2.5 months of the trial, then a combination of forage and concentrate for the next month and primarily concentrate for the final month [2]. Intake of feedstuffs for the basal diet of grazing steers was ad libitum and the percentage of their diet from flaxseed (907 g/day for final 107 days of supplementation) was therefore not determined. At the end of the flaxseed supplementation period, the steers were slaughtered and cross-sectional pieces of longissimus dorsi muscles (ribeye steaks) between the 12th and 13th ribs were collected from each carcass. Muscle tissues were stored at −80°C for later use.

Four Angus cattle with about 460 kg of body weight (≈550 day old) were euthanized by captive bolt stunning and exsanguination at the Ohio State University’s Meat Science Laboratory located in the Department of Animal Sciences, Columbus, OH, USA. Adipose tissue, heart, muscle, lung, liver, and kidney were collected immediately.
after euthanization, snap-frozen in liquid nitrogen and stored at −80°C before total RNA and protein isolations to examine tissue distribution of ATGL expression. Animal care and procedures were approved by the OSU Institutional Animal Care and Use Committee.

**RNA Isolation**

RNA was isolated from bovine tissue samples using TriZol™ (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions. RNA quality was assessed by agarose gel electrophoresis. Approximately 1 μg of total RNA was reverse transcribed according to the manufacturer’s instructions (Invitrogen Life Technologies—M-MLV reverse transcriptase).

**Cloning of Bovine ATGL**

Subcutaneous dorsal fat adipose (100 mg) was sampled within 3 min of euthanization, snap-frozen in liquid nitrogen, and stored at −80°C before total RNA isolation. Complementary DNA from subcutaneous adipose tissue of Angus cattle was used as a template for PCR. Primers were designed according to several partial bovine DNA sequences that contain conserved sequence homology among human (Genbank accession numbers: AY894804, NM_020376) and mouse (AJ278476, AK031609) sequences. A MJ Research PTC-200 thermal cycler (MJ Research Inc., South San Francisco, CA, USA) and DNA Taq polymerase (Invitrogen) were used for all PCR. The PCR products were separated by electrophoresis on a 1% agarose gel and the appropriate band(s) excised and gel extracted using the Qiagen Gel Extraction kit (Qiagen, Alameda City, CA, USA). The product was then ligated to the pCR 2.1-TOPO vector using the TOPO TA Cloning Kit (Invitrogen). Positive clones were sequenced by The Ohio State University sequencing core facility using an Applied Biosystems 3730 DNA Analyzer (Foster City, CA, USA). The resulting sequences confirmed bovine ATGL by homology to the mouse, human, and pig by identification of characteristic enzyme domains. Real-time primers were then designed to measure ATGL gene expression ("F2" 5'-GTGGACGTGGCATTCAGA-3', "R2" 5'-TA CAGGGATGCGCTCCGCTT-3'). Primers were designed to span genomic introns, thus allowing for detection of genomic DNA contamination. PCR using F2/R2 primers were optimized for use in real-time PCR.

**Quantitative Real-Time PCR Detection of Total Gene Expression**

Real-time PCR was performed using SYBR green I nucleic acid dye (Molecular Probes Invitrogen detection technologies) on an ABI 7300 (Applied Biosystems). AmpliTaq Gold™ (Applied Biosystems) was used in all real-time reactions as was the following thermal profile: 95°C 10 m, 40 cycles of 94°C 30 s, 58°C 60 s, 82°C 30 s. The CT values for the internal control (cyclophilin) and target genes, as determined by the ABI software, were used to calculate gene expression using the 2-ΔΔCT method. All target genes were normalized to cyclophilin and changes were calculated as relative fold-change to cyclophilin. Randomly selected samples from all real-time runs were resolved by agarose gel electrophoresis to ensure the amplification of one product. In addition, dissociation/melting curves yielded single peaks, indicating a single product with lack of primer dimers or genomic DNA contamination. "No template" negative controls were included in all PCR to detect contamination. Primer sequences used for the quantitative real-time PCR are shown in Table 1.

**Protein Isolation and Immunoblotting**

Approximately 80 mg of tissue was homogenized in 800 μl of lysis buffer (1% Triton X-100, 150 mM NaCl, 20 mM HEPES pH 7.5, 10% glycerol, 1 mM EDTA, 100 mM NaF, 100 μM sodium orthovanadate, 1 mM PMSF, and 10 μL/mL protease inhibitor cocktail (Sigma–Aldrich, P8340). The protein content of cell lysate was determined using the bicinchoninic acid (BCA) Protein Assay Kit (Pierce Chemical Co., Rockford, IL, USA). Samples were separated by SDS-PAGE using the mini-Protean system (Bio-RadHer). The protein was wet-transferred to a PVDF membrane (Amersham Biosciences Hybond-P™), blocked in 5% non-fat dry milk in 1x-TBST (0.1% Tween 20) and incubated overnight at 4°C with primary antibody (1:3,000) specific to ATGL (Cell Signaling Technology, Inc., Danvers, MA, USA) in 5% non-fat dry milk. After washing in 1x-TBST, blots were incubated with the appropriate HRP-conjugated secondary antibody for 1 h at room temperature. Blots were washed before addition of ECL plus™ (Amersham Biosciences) and bands were detected with Hyperfilm™ (Amersham Biosciences).

**Analysis of Triacylglycerol Mass**

Samples were taken from visibly lean portions of frozen ribeye steak and immersed in liquid nitrogen. These samples were then pulverized at liquid nitrogen temperatures to a fine homogeneous powder. Pulverized muscle tissue from flaxseed-supplemented and non-supplemented Angus steers was weighed, homogenized in hexane: 2-propanol (3:2 by vol) using a Polytron tissue homogenizer, and the lipids were extracted [17]. The samples were subjected to centrifugation (2,750g, 4°C, 10 min) and the lipid containing solvent removed from the pellet. The samples were
Table 1. Primer sequences for quantitative real-time polymerase chain reaction

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Product size (bp)</th>
<th>GenBank accession #</th>
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<tr>
<td>ATGL-F1</td>
<td>5'-CCGGCGATGTTCCTCCCAAGGAGA-3'</td>
<td>742</td>
<td>FJ897536</td>
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<tr>
<td>ATGL-R1</td>
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<td>983</td>
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<tr>
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<td>5'-GTGACCGGCTGATCTCAAG-3'</td>
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<td></td>
</tr>
<tr>
<td>ATGL-R2</td>
<td>5'-CCCTAGACCAAGGGGCAGC-3'</td>
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<td></td>
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<tr>
<td>ATGL-F3</td>
<td>5'-GTCGACGTTGACATCTCGA-3'</td>
<td>251</td>
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<tr>
<td>ATGL-R1</td>
<td>5'-AACCGGAGGCGCTACCTGTA-3'</td>
<td></td>
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<tr>
<td>LPL-F</td>
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<tr>
<td>HSL-F</td>
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<td>226</td>
<td>NM_001080220</td>
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<tr>
<td>HSL-R</td>
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<tr>
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<td>A-FABP-F</td>
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<tr>
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<td>5'-AGCTTGGTCACTGCGAAGCT-3'</td>
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ATGL adipose triglyceride lipase, LPL lipoprotein lipase, HSL hormone sensitive lipase, CYC cyclolin, A- or E-FABP adipocyte- or epidermal-fatty acid binding protein, SCD-1 stearoyl-CoA desaturase (delta-9-desaturase), F forward, R reverse

Reduced in volume using nitrogen evaporation and the lipid redissolved in a known volume of hexane:2-propanol (3:2 by vol). To analyze TAG mass, the samples were quantitatively applied to a thin layer chromatography plate (silica gel G, Analtech, Newark, DE, USA) and the neutral lipids separated using petroleum ether/diethyl etheracetic acid (75:25:1.3 by vol). This method separates TAG from cholesteryl esters as well as from other neutral lipids, such as cholesterol and unesterified fatty acids [8]. The TAG was removed from the plate by scraping and the sample placed into an acid-washed test tube to which concentrated sulfuric acid was added [18]. The tube was heated at 200°C for 15 min in a heating block and the sample subjected to centrifugation (2,500 g for 10 min) to pellet the silica. The absorbance at 375 nm was measured in a quartz cuvette using a Beckman DU-640 spectrophotometer (Fullerton, CA, USA). Absorbance was converted to mass using triolein glycerol (MW 882, Nuchek Prep, Elysian, MN, USA) and the values are expressed as µg/g wet weight (ww).

Bioinformatics, Sequence Analysis, and Statistical Analysis

Bioinformatics and sequence analysis were performed as previously described [11]. The sequence Scanner (v 1.0) was used to generate the chromatogram of cloned bovine ATGL cDNA by using Applied Biosystems genetic analyzer instruments. ATGL nucleotide and putative protein sequences of pig [15] and avian [16] were already reported and used for sequence analysis and comparison. Mouse and human ATGL nucleotide and putative protein sequences were obtained from NCBI. The alignment and comparison of nucleotide or protein sequences were done using ClustalX and GeneDoc™ software. The phylogenetic tree was constructed by CLUSTAL W (Version 1.83) and MEGA 4 software (neighbor joining method) [19] using the putative ATGL protein sequences of various species. The neighbor joining method is a simplified version of the minimum evolution (ME) method using distance measures to correct for multiple hits at the same sites, and chooses a topology showing the smallest value of the sum of all branches as an estimate of the correct tree [19].

With the exception of the phylogenetic branch lengths, all results are presented as mean ± SEM. Comparison of two means was accomplished by a Student’s t test at P ≤ 0.05 and 0.01. Comparisons among gene expression data were performed using one-way analysis of variance (ANOVA) followed by the Tukey’s test at P ≤ 0.05. Statistical analysis was performed using Minitab software (version 15.0).

Results

We identified several partial bovine DNA sequences in The Institute for Genomic Research (TIGR) database that were homologous to the human ATGL sequence. Primers were designed based on these sequences to clone a full length sequence of bovine ATGL. Soon after we cloned this sequence, another group submitted a bovine ATGL
alignment of cow, human, and mouse ATG, following the guidelines of the Nomenclature Committee. The amino acid positions marked with an asterisk are identical in all three species. The positions marked with an arrow are hypothetical domains.

Sequence alignment of cow (EMBL X05457) and X05457 are identical except for a 1-bp mismatch. At positions 296 and 298, human, mouse, and cow ATG proteins share greater homology to human than bovine ATG. Polyphage analysis revealed that bovine and porcine ATG (AY838486) has a 99% sequence homology to the human sequence (AY838485). The first 180 aa of the putative domain, which is not conserved.
responsible for the lipolytic activity of ATGL (Fig. 1). The hydrophobic domain indicated in open arrows (310–398 aa) is conserved across human, cattle, pig, and mouse sequences with significant interspecies variations only occurring up and downstream of the domain.

ATGL gene expression in analyzed tissues revealed that the cardiac and skeletal muscle exhibited higher expression than the subcutaneous adipose tissue (Fig. 3). This may be unique to cattle, as expression of this gene in mouse and pig is highest in subcutaneous adipose tissue [15]. Expression in the spleen, lung, kidney, and liver were many fold lower than heart, muscle or adipose (Fig. 3).

To compare relative amounts of ATGL protein in different tissues, Western blot analysis for ATGL protein was performed using antibodies that recognize a conserved sequence around Pro186 of human ATGL. The molecular weight of ATGL protein as calculated by the Protein Molecular Weight program in Sequence Manipulation Suite [20] showed that mouse ATGL (486 aa) is 53.66 kDa, cattle (486 aa) is 53.35 kDa, chicken ATGL (483 aa) is 53.59 kDa, and pig ATGL (486 aa) is 53.19 kDa. Western blot analysis showed a single band (53 kDa) of ATGL proteins for the mouse, cattle, chicken, and pig (Fig. 4a), which is consistent with predicted molecular weights of ATGL. Therefore, we concluded that the antibodies were usable for the study of bovine ATGL protein expression. Western blot analysis for tissue distribution of ATGL protein showed that a strong single band of the expected size of the bovine ATGL protein was detected in adipose tissues, but other tissues expressed very low amounts of ATGL protein in cattle (Fig. 4b). When the membrane was probed with adipocyte-fatty acid-binding protein (A-FABP) antibody, A-FABP was detected only in adipose tissue at a short exposure time. Due to variation in amount of beta-actin among different tissues, Coomassie staining was assessed to verify similar amounts of protein loading among samples.

In order to determine if flaxseed supplementation (FS) altered muscle TAG mass, we analyzed the TAG content in the skeletal muscle. We found that the FS group exhibited a
Significant (P < 0.05) 1.5-fold increase in TAG per g of tissue over the control group (Fig. 5). This increase in TAG content was not the result of a net gain in lipid synthesis as the total phospholipid mass was not significantly different between groups (4.1 ± 0.4 nmol/g ww in control vs. 4.7 ± 0.6 nmol/g ww in FS group). Thus, the increased TAG content was due to not only synthesis, but also to accretion of dietary fatty acids.

To better understand how flaxseed supplementation specifically modulates TAG content, we focused on the expression of pertinent genes involved with fatty acid metabolism within the skeletal muscle. We found that flaxseed supplementation did not affect ATGL gene expression and protein content in skeletal muscle of the steers compared to control animals (Fig. 6a, b) when normalized to housekeeping genes/loading controls. Gene expression of HSL and lipoprotein lipase in the muscle were also unaffected by flaxseed supplementation. However, we examined the expression of adipose specific fatty acid binding proteins (A-FABP and E-FABP) as possible molecular indicators for increased muscle TAG accumulation. Both A-FABP and E-FABP were examined as these proteins are found in the adipocyte; in the A-FABP gene-ablated mouse, E-FABP expression demonstrates a compensatory increase in its expression [21]. A-FABP significantly (P < 0.05) increased with flaxseed supplementation (Fig. 6d), while E-FABP expression was unaltered (Fig. 6e). Stearoyl-CoA desaturase-1 (SCD-1) expression, the rate limiting enzyme catalyzing the desaturation of saturated fatty acids, was significantly (P < 0.05) decreased in muscle tissue by flaxseed supplementation (P ≤ 0.05) (Fig. 6g).

**Discussion**

In this study, we cloned the cDNA encoding for bovine ATGL and compared its amino acid sequence with other species to determine interspecies variations and to examine interspecies conservation of lipase enzyme motifs. Comparison analysis of amino acid sequences of putative cattle ATGL protein with mouse, human, and pig revealed that two major domains; a patatin domain (1–180) and a hydrophobic domain (309–396) are conserved in these mammals. In the patatin domains, the active serine hydrolase motif (GXGXXG) was GASAG and the glycine-rich motif (GXGXXG) was GCGFLG in all four species compared. These motives are conserved from yeast to mammals [22], suggesting functional importance for
ATGL activity across species. The hydrophobic domain of ATGL is responsible for the localization of ATGL protein on the surface of lipid droplets called the adiposome [23]. Point mutations and deletion of the hydrophobic domain in the human population cause neutral lipid storage disorder with cardiomyopathy due to inability of mutant ATGL to access the TAG substrate stored in the adiposomes [23]. However, mutations of the ATGL protein at the hydrophobic domain maintained lipase activities [23]. Conservation of two important domains in bovine ATGL suggests the evolutionary importance in maintaining the lipase activities as well as association with the surface of the adiposome.

ATGL is associated with the lipid droplet [10]. Therefore, it is logical to assume that tissues with a greater abundance of accumulated TAG, and thus a larger lipid droplet, will have increased expression of ATGL as seen in tissue distribution (Fig. 3). High relative expression of ATGL in adipose tissue is consistent with expression in mice; however, ATGL gene expression in cattle skeletal muscle seems to be high relative to most tissues tested, a pattern that was also observed in pig [15, 24]. Although ATGL gene expression in muscle was high, ATGL protein was not. The reasons for this observation are unclear; however, it might be explained by differences in translation efficiencies or degradation rates among the tissues. Again, this pattern is also observed in pig [15]. Cattle muscle ATGL expression is probably due to the presence of lipid droplets (adiposomes) in the myocytes within the muscle fibers themselves, as well as the presence of intramuscular adipocytes. Recent studies have shown that the disruption of the ATGL gene in mice and mutations of ATGL gene in the human resulted in excessive lipid accumulation in the muscle and heart, causing myocardial dysfunction [23, 25]. Recent studies showing the negative association of intramuscular fat with ATGL expression strongly suggest the involvement of ATGL in lipid metabolism in muscle [26, 27].

Others have shown that flaxseed supplementation during the finishing stage of beef cattle improved carcass quality with increased marbling scores (amount of intramuscular fat) and percentage of carcasses grading USDA Choice or greater [4, 28]. Increased TAG contents in the muscle of Angus beef cattle in the current study agree with these previous studies. The amount of TAG in the muscle can be largely attributed to intramuscular adipocytes with a minor contribution from lipid droplets within myofibers [29]. Therefore, increased TAG content in the muscle by flaxseed supplementation may be due to hypertrophy and/or hyperplasia of intramuscular adipocytes. A-FABP, an adipogenic marker, is expressed during adipocyte differentiation and maturation [30]. In fact, increased A-FABP expression is associated with increased marbling in cattle and increased TAG content [31], similar to what we have reported herein. In addition, elevated A-FABP expression is associated with increased intramuscular fat content in ducks [32], chickens [33, 34], and hogs [35, 36], suggesting that it is a broad biomarker for increased intramuscular fat deposition. Increased A-FABP expression in the muscle by flaxseed supplementation further supports the supposition that increased TAG is associated with increased adipogenic activities in the muscle.

PPARγ is a major transcription factor, promoting adipogenesis [30]. Our previous study showed that flaxseed supplementation in Angus steers increased PPARγ mRNA expression by 2.7-fold in the muscle compared with the control [2]. Comparative analysis of mammalian A-FABP promoters revealed that two PPAR binding sites are conserved in A-FABP promoters in mammals, including human, mouse, dog, pig, and cattle, and that PPAR agonists are strong transcription activators of A-FABP genes in mammals [37, 38]. Therefore, because A-FABP is a putative PPARγ-regulated gene, the effects of n-3 PUFA, alpha-linolenic acid on A-FABP expression may be attributable to PPAR agonism. This conclusion is supported by the findings that the n-3 long chain fatty acids alpha-linolenic acid and docosahexaenoic acids [39] as well as synthetic PPAR agonists (thiazolidinedione/glitazone class of compounds) acting as PPARγ ligands modulate PPAR binding activity, regulating expression of responsive genes. There is also evidence that A-FABP plays a role in regulating PPARγ activity by binding PPARγ directly and by channeling fatty acid ligands directly to PPARγ [40]. These findings support a role for regulation of a FABP-PPAR axis by dietary fatty acid ligands. Also, it should be noted that PPARγ activation in muscle cells of mouse, pigs, cattle, and humans results in transdifferentiation to adipocytes [41–46]. It is important to note that PPARγ expression is significantly elevated in these same cattle [2], consistent with the elevated expression of A-FABP. Thus, it is possible that enhancement of PPARγ activation/expression in the muscle by flaxseed supplementation may increase adipogenic activities as evidenced by increased TAG concentration and A-FABP expression.

Higher gene expression of ATGL was found in the adipocyte fraction in mice, pigs, and chickens compared to the stromal-vascular fraction which is partially composed of preadipocytes [15, 16, 47]. Localization of ATGL protein on the surface of lipid droplets and induction of ATGL expression during adipocyte differentiation further indicate association of ATGL expression with TAG content [10, 47]. In the current study, TAG content and A-FABP gene expression increased in the muscle with flaxseed supplementation, although ATGL expression was not different between groups. If ATGL expression were normalized to
TAG content, then ATGL expression would be lower in the FS group than the control. Though A-FABP is known to bind to HSL [13, 14, 48], we did not observe a difference in HSL gene expression between groups. A-FABP has an important role in modulating HSL activity by acting as a chaperone protein and directing HSL to the lipid droplet [14] where it may have a role in fatty acid efflux from the lipid droplet and out of the adipocyte. However, it is important to note that elevated A-FABP expression did not result in a change in muscle tissue E-FABP expression (Fig. 6d, e), though in A-FABP gene-ablated mice there was a compensatory increase in E-FABP expression [21]. In addition, muscle tissue H-FABP expression was not enhanced by dietary flaxseed supplementation [2], indicating that the elevation of A-FABP expression following flaxseed supplementation is specific to A-FABP and does not result in the overall increased expression of FABP found in muscle tissue. Hence, A-FABP is an important regulator of adipocyte lipid metabolism and is a consistent marker across species for intramuscular fat deposition, which is consistent with our results.

SCD-1 is a lipogenic enzyme that catalyzes desaturation resulting in the synthesis of monounsaturated fatty acids. SCD-1 gene expression is largely controlled by the transcription factors PPARα and SREBP-1c [49, 50]. Dietary PUFA decreases the expression of SCD-1 in the liver and adipose of diabetic mice [51, 52], which is consistent with our observations. More applicable, supplementation of beef cattle with n-3 PUFA in the form of fish oil also decreases SCD-1 mRNA expression in the muscle [53]. Archibeque et al. [54] reported that flaxseed supplementation at 10% of daily dry matter intake, which amounted to 1,096 g/day compared to 907 g/day for the present study, did not affect SCD-1 enzyme activities in the muscle of Angus steers; however, they did not report fatty acid composition or content of muscle. Herein, we demonstrate reduction of SCD-1 mRNA expression in skeletal muscle by flaxseed supplementation. This reduction is further supported by a 40% reduction in oleic acid content, the end product of SCD-1 enzyme activity, in the muscle of Angus beef with flaxseed supplementation [2], thereby mechanistically accounting for our previous observations in these cattle. Therefore, our data indicate that flaxseed supplementation decreases oleic acid content in the muscle via a reduction in SCD-1 expression.

In summary, bovine ATGL cDNA was cloned and sequenced for the first time. We showed that expression of bovine ATGL protein is adipose tissue specific among various tissues. Comparative analysis revealed that bovine ATGL protein also contained two conserved domains that are important for ATGL functioning. Flaxseed supplementation for approximately 3.5 months before slaughter increased muscle TAG concentration in Angus steers compared to unsupplemented animals. We attribute the increased muscle TAG content in part to the effects of flaxseed supplementation-derived alpha-linolenic acid on A-FABP expression via PPARγ agonism and our previously reported finding of elevated PPARγ expression. Although flaxseed supplementation did not alter expression of HSL or ATGL, it did decrease the expression of SCD-1, consistent with a reduction in oleic acid content in these cattle [2]. Flaxseed supplementation may have beneficial effects on marbling characteristics due to the altered expression of key genes associated with marbling in cattle and TAG content.

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References


