Conjugated linoleic acid synthesis-related protein proteasome subunit α 5 (PSMA5) is increased by vaccenic acid treatment in goat mammary tissue

Y. C. Jin,*† Z. H. Li,‡ Z. S. Hong,§ C. X. Xu,# J. A. Han,‡ S. H. Choi,|| J. L. Yin,‡ Q. K. Zhang,‡ K. B. Lee,¶ S. K. Kang,‡ M. K. Song,** Y. J. Kim,†† H. S. Kang,† Y. J. Choi,‡¹ and H. G. Lee†¹

*Department of Animal Science, College of Animal Science and Veterinary Medicine, Jilin University, Changchun 130062, People's Republic of China

†Department of Animal Science/Bio-Resources and Development Institute, Pusan National University, Miryang, Gyeongnam 627-706, Republic of Korea

‡Department of Agricultural Biotechnology, Seoul National University, Seoul 151-742, Republic of Korea

§Department of Animal Science and Technology, Tianjin Agricultural University, Tianjin 300384, People's Republic of China

#Department of Molecular Oncology, H. Lee Moffitt Cancer Center, Tampa, FL 33612

||Department of Animal Science, Texas A&M University, College Station 77843-2471

¶Department of Biotechnology, SongDo Techno Park, Incheon 406-840, Republic of Korea

**Department of Animal Science, Chungbuk National University, Cheong-Ju, Chungbuk 361-763, Republic of Korea

††Department of Food Science and Biotechnology, Korea University, Chochiwon, Chungnam 339-700, Republic of Korea

ABSTRACT

This study was conducted to identify proteins associated with the endogenous synthesis of conjugated linoleic acid (CLA) from *trans*-vaccenic acid (TVA; *trans*-11 C18:1, a precursor for CLA endogenous synthesis) in mammary tissues. Six lactating goats were divided into 2 groups. One group was given an intravenous bolus injection of TVA (150 mg) twice daily over 4 d; the other group received saline injections. Treatment with TVA increased the concentration of *cis*-9,*trans*-11 CLA and TVA in goat milk. Additionally, TVA treatment increased the expression of stearoyl-CoA desaturase (*SCD*) in mammary tissue. Using 2-dimensional gel electrophoresis and electrospray ionization quadrupole time-of-flight mass spectrometry, 3 proteins affected by infusions of TVA were identified. Proteasome (prosome, macropain) subunit α type 5 (PSMA5) was upregulated, whereas peroxiredoxin-1 and translationally controlled tumor protein 1 were downregulated in TVA-treated animals compared with the vehicle-injected controls. Only the effect of TVA on PSMA5 could be confirmed by Western blot analysis. To further explore the regulation of PSMA5 in mammary epithelial cells when TVA is converted into CLA, we used a differentiated bovine mammary epithelial cell line treated with TVA for 6 h. Changes in *cis*-9,*trans*-11 CLA concentrations and mRNA expression patterns of both *SCD* and PSMA5 were monitored. The concentration of *cis*-9,*trans*-11

CLA increased after TVA treatment. The mRNA expression level of PSMA5 was significantly elevated to 6 h, but *SCD* mRNA expression only increased in 2 h after TVA treatment. These results indicate that PSMA5 is highly expressed in goat mammary tissue and bovine mammary epithelial cells when TVA is converted into CLA. Our data suggest that PSMA5 protein is associated with CLA biosynthesis in mammary tissue.

Key words: two-dimensional gel electrophoresis, conjugated linoleic acid biosynthesis, mammary tissue, proteasome (prosome, macropain) subunit, α type 5 (PSMA5)

INTRODUCTION

Conjugated linoleic acid (CLA) is a collective term that refers to the positional and geometric isomers of linoleic acid (*cis*-9,*cis*-12-octadecadienoic acid) with conjugated double bonds. Conjugated linoleic acid is a minor lipid fraction constituent of dairy and meat products derived from ruminant animals. Recently, increased attention has been given to the beneficial health properties of CLA, which include anticancer (Aro et al., 2000) and antidiabetic activities (Houseknecht et al., 1998), as well as the ability to enhance immune responses (Hayek et al., 1999), reduce atherosclerosis (Lee et al., 1994), and reduce fat mass (Blankson et al., 2000).

The major isomer of CLA in food is *cis*-9,*trans*-11 CLA; thus, most CLA studies have focused on *cis*-9,*trans*-11 CLA, including mechanisms governing the endogenous synthesis of CLA in ruminants. Previous studies have indicated that endogenous *cis*-9,*trans*-11 CLA is synthesized from *trans*-vaccenic acid (TVA; *trans*-11 C18:1) via the major CLA synthesis pathway

Received February 17, 2011.

Accepted March 7, 2012.

¹Corresponding authors: cycow@snu.ac.kr and hglee66@pusan.ac.kr

Table 1. Chemical composition and nutritional value of forage, concentrates, and consumed dairy goat diets (DM basis)

Item	Hay	Alfalfa hay	Concentrates	Total diet
DM (% as fed)	90.6	88.0	88.3	88.8
CP (%)	5.8	13.0	19.6	13.5
Crude fat (%)	0.67	1.1	4.7	2.2
Crude fiber (%)	29.8	32.73	7.7	23.3
NDF (%)	68.6	43.9	29.7	45.2
Ca (%)	0.1	0.6	0.8	0.5
P (%)	0.1	0.2	0.5	0.3
TDN (%)	61.5	62.4	71.5	65.3
NE _L ¹ (Mcal/kg of DM)	1.4	1.4	1.6	1.5

¹Estimated from NRC (2007). NE_L(Mcal/kg) = 0.0245 × TDN (%) – 0.12.

in ruminants (Griinari et al., 2000; Bauman et al., 2001; Corl et al., 2001). *Trans*-vaccenic acid is the predominant precursor of *cis*-9,*trans*-11 CLA in milk fat and is formed during the biohydrogenation of linoleic acid and linolenic acid into stearic acid in the rumen (Griinari et al., 2000). In nonruminant animals, the intake of TVA may contribute to the supply of CLA because nonruminant animals can also synthesize CLA from TVA (Salminen et al., 1998; Adlof et al., 2000; Santora et al., 2000; Banni et al., 2001; Loor et al., 2002; Corl et al., 2003; Jin et al., 2010). Previous reports have indicated that stearyl-CoA desaturase (**SCD**, also known as Δ^9 -desaturase) plays a key role in the synthesis of *cis*-9,*trans*-11 CLA (Pollard et al., 1980) by participating in the final step of endogenous conversion of CLA from TVA in the mammary gland (Griinari et al., 2000; Bauman et al., 2001; Corl et al., 2001).

A study conducted by Ntambi (1995) revealed some of the mechanisms involved in Δ^9 -desaturation. Specifically, it is known that electrons flow sequentially from NADPH through NADH-cytochrome b5 reductase to cytochrome b5, to the terminal Δ^9 -desaturase, and finally to an active oxygen, which is reduced to H₂O (Ntambi, 1995). However, the mechanisms underlying CLA synthesis in the mammary gland in response to dietary intake of TVA are still unclear. Aside from SCD, we do not know the identity of other proteins involved in the endogenous synthesis of CLA from TVA in the mammary gland.

In addition, even though proteomic research has focused on changes in the expression of some proteins in transgenic mice that arise in response to CLA (de Roos et al., 2005), we provide the first proteomic profiles for the biosynthesis of CLA in rat mammary glands and mouse mammary gland epithelial cells (HC11) in response to treatment with TVA (Jin et al., 2010). However, other proteins involved in the endogenous synthesis of CLA might be important and thus we aimed to identify new proteins that are associated with

CLA synthesis in goat mammary tissue and confirm their response to TVA treatment in bovine mammary epithelial cells.

MATERIALS AND METHODS

Experimental Design, Animals, and Diets

All animal experiments were approved by the Animal Care and Use Committee of Seoul National University. Six multiparous lactating Saanen goats (240 ± 10 DIM) were divided into 2 groups (3 goats/group) with an average daily milk yield of 1.17 ± 0.60 kg. Every day, each goat received 580 g of hay, 920 g of alfalfa hay, and 800 g of fodder concentrates. Experiment feeds were analyzed for DM, CP, crude fat, crude fiber, NDF, Ca, and P according to the methods of Association of Official Analytical Chemists (AOAC, 1995). The ingredients and chemical composition of the diets are shown in Table 1. The goats were milked twice daily (at 0700 and 1900 h).

Trans-vaccenic acid (Matreya LLC, Pleasant Gap, PA) was dissolved in 0.9% saline containing 10% intralipid (Fresenius Kabi, Seoul, Republic of Korea) to yield a 30 mg/mL concentration. After a 7-d adaptation period, one group was treated with 5 mL of TVA solution and the other group was given 5 mL of saline (10% intralipid in 0.9% saline) via a jugular vein injection as previously described (Viswanadha et al., 2003; Jin et al., 2010). Goats were given the intravenous injections twice daily 30 min after each milking. This treatment was continued for 4 d.

On the morning of d 5, milk and mammary tissue were sampled. The milk was collected from both gland halves and a representative aliquot was used for milk FA analysis. After milking, biopsies were taken from lactating goats anesthetized with xylazine hydrochloride (0.05 mg/kg of BW, intravenous; Rompun, Bayer Korea, Seoul, Republic of Korea). Goats were in a lat-

eral recumbent position so that the entire right lateral side of the udder was exposed. Skin at the surgical site was aseptically prepared using alternating applications of a povidone iodine solution and 70% alcohol scrubs. Mammary tissue was excised from an area 2 to 3 cm below the skin via a 5- to 6-cm incision made in the right udder 8 to 10 cm dorsal to the base of teat. Approximately 5 g of mammary tissue was excised from right side of the udder while carefully avoiding the gland cistern and major blood vessels. Mammary tissue was rapidly flash-frozen in liquid nitrogen and stored at -80°C until subsequent 2 dimensional gel electrophoresis (**2-DE**) and Western blot analysis.

Protein Sample Preparation for 2-DE

Protein samples were prepared from mammary tissues using a previously described method (Aksu et al., 2002; Jin et al., 2010). Briefly, mammary tissue was homogenized in modified lysis buffer containing 9 M urea, 50 mM KCl, 25 mM Tris-HCl, pH 7.1, 70 mM dithiothreitol (**DTT**), 2% immobilized pH gradient (**IPG**) buffer (pH 3–10 nonlinear), 1× nuclease mix, and 1× protease inhibitor (GE Healthcare, Piscataway, NJ). The samples were incubated at room temperature (22°C) for 30 min, stirred every 5 min, and then centrifuged at $19,000 \times g$ for 30 min at 20°C . The supernatant was collected and stored in -80°C until analysis.

2-DE

The 2-DE was conducted as previously described (Desrivieres et al., 2003; Jin et al., 2010). Briefly, 100 μg of pooled, solubilized mammary gland proteins per group of goats was mixed with a rehydration solution containing 9 M urea, 4% CHAPS {3[(3-cholamidopropyl)dimethylammonio]propanesulfonic acid}, 0.5% (vol/vol) IPG buffer (pH 3–10 nonlinear), 80 mM DTT, and 0.002% (wt/vol) bromophenol blue, to a total volume of 350 μL . The samples were then applied onto 18-cm IPG DryStrips (pH 3–10 nonlinear; GE Healthcare) for simultaneous rehydration. Electrophoresis in the first dimension was performed using an isoelectric focusing (**IEF**) electrophoresis unit (GE Healthcare); the temperature maintained at 20°C for all IEF steps. The IEF was performed with the following voltage-time program: 30 V for 12 h, 500 V for 1 h, 1,000 V for 1 h, and 8,000 V for 6 h to deliver a total of 48,000 V·h. After IEF, the focused IPG strips were incubated for 15 min in equilibration buffer [50 mM Tris-HCl, pH 8.8; 6 M urea, 2% SDS, 30% glycerol, and 0.002% (wt/vol) bromophenol blue] containing 1% DTT, followed by equilibration buffer containing 135 mM iodoacet-

amide instead of DTT for 15 min. Next, the equilibrated strips (18 cm) were inserted onto SDS-PAGE gels (12.5%) and SDS-PAGE was performed using an Ettan DALT 2-D gel system (GE Healthcare). Upon completion of the 2-dimensional SDS-PAGE, the gels were stained using a PlusOne Silver Staining Kit (GE Healthcare) according to the manufacturer's instructions. The silver-stained gels were scanned using a UMAX Power Look 2100XL scanner (software: Magic Scan, version V5.2; UMAX Technologies Inc., Dallas, TX). Spot detection, pair matching, and normalization were performed using ProteomWeaver software (Definiens, Munich, Germany).

In-Gel Digestion

For protein identification, spots were excised from the gels and then subjected to in situ digestion with trypsin, as previously described (Shevchenko et al., 1996). Briefly, the excised gel spots were destained by shaking with 100 μL of destaining solution (30 mM potassium ferricyanide and 100 mM sodium thiosulfate) for 5 min. After removing the solution, the gel spots were incubated with 200 mM ammonium bicarbonate for 20 min. The gel pieces were then dehydrated with 100 μL of acetonitrile and dried in a vacuum centrifuge. The dried gel pieces were rehydrated with 20 μL of 50 mM ammonium bicarbonate containing 0.2 μg of modified trypsin (Promega, Madison, WI) for 45 min on ice. After removing the solution, 30 μL of 50 mM ammonium bicarbonate was added and the gel pieces were digested overnight at 37°C . The peptide solution was then desalted using a C18 nanocolumn constructed in our laboratory as described below.

Desalting and Concentration of Peptide

Custom-made chromatographic columns were used to desalt and concentrate the peptide mixture before mass spectrometric analysis. To accomplish this, 100 to 300 nL of Poros reverse phase R2 material (bead size, 20–30 μm ; PerSeptive Biosystems, Framingham, MA) was packed into a constricted GELoader tip (Eppendorf, Hamburg, Germany). A 10- μL syringe was used to force liquid through the column by applying gentle air pressure. Next, 30 μL of the peptide mixture from the digestion supernatant was diluted in 30 μL of 5% formic acid, loaded onto the column, and washed with 30 μL of 5% formic acid. For MS/MS analysis, the peptides were eluted with 1.5 μL of 50% methanol:49% H_2O :1% formic acid directly into a precoated borosilicate nanoelectrospray needle (Micromass, Manchester, UK).

Protein Identification by Electrospray Ionization Quadrupole Time-of-Flight Mass Spectrometry Analysis

Tandem MS (MS/MS) analysis of the peptides recovered from in-gel digestion was performed with nano-electrospray ionization (ESI) using a quadrupole-time-of-flight (Q-TOF)2 mass spectrometer (Micromass). The source temperature was 80°C and a potential of 1 kV was applied to the precoated borosilicate nanoelectrospray needles (EconoTip, New Objective, Woburn, MA) in the ion source in combination with a nitrogen back-pressure of 0 to 3.4×10^4 Pa5 psi to produce a stable flow rate (10–30 nL/min). The cone voltage was 40 V. A quadrupole analyzer was used to select precursor ions for fragmentation in the hexapole collision cell. The collision gas was air applied at a pressure of 6 to 7×10^7 Pa to induce a collision energy of 20 to 30 V. Product ions were analyzed using an orthogonal TOF analyzer fitted with a reflector, a microchannel plate detector, and a time-to-digital converter. All data were processed using a Mass Lynx Windows NT PC system. For protein identification, the peptide masses determined by Q-TOF MS were matched with the theoretical peptides from proteins in the NCBI (<http://www.ncbi.nlm.nih.gov/>) database using MASCOT (<http://www.matrixscience.com>) and Profound software (<http://www.matrixscience.com>). In addition, all MS/MS spectra recorded on tryptic peptides derived from each spot were searched against protein sequences present in the NCBI-nr and EST databases (<http://www.ncbi.nlm.nih.gov/nucest>) using MASCOT search program.

Cell Culture

Bovine MAC-T mammary epithelial cells were kindly provided by F. Q. Zhao (University of Vermont, Burlington). The cells were grown in Dulbecco's modified Eagle's/high glucose medium (Hyclone, Logan, UT) supplemented with 10% fetal bovine serum (Hyclone), 5 µg/mL insulin (Sigma, St. Louis, MO), 1 µg/mL hydrocortisone (Sigma), 50 µg/mL gentamicin (Sigma), 100 IU/mL penicillin (Sigma), and 100 µg/mL streptomycin (Sigma) at 37°C in 5% CO₂ and 95% air. After the cells reached confluence in 150-mm dishes or 6-well plates, differentiation was induced by incubation in medium containing 5% fetal bovine serum, 5 µg/mL insulin, 1 µg/mL hydrocortisone, 5 µg/mL prolactin (Sigma), 50 µg/mL gentamicin, 100 IU/mL penicillin, and 100 µg/mL streptomycin for 4 d as previously described (Huynh et al., 1991). After 90 h of differentiation induction, 50 µM TVA was added to the culture medium and the cells were incubated for an additional 6 h. The TVA was dissolved in essentially fatty acid-

free BSA complexes at a molar ratio of 4:1 as previously described (Kim et al., 2000). During differentiation induction, the medium was replaced every day. Among 5 replicate trials, 2 replicates (150-mm dishes) were used for FA extraction, and total RNA was extracted from 3 replicates (6-well plates). The RNA was extracted by washing the cells with PBS and then adding 1 mL of TRIzol reagent (Invitrogen, Carlsbad, CA) to the culture dish and scraping with a cell scraper. The cell lysate were passed several times through a pipette, transferred to 1.5 mL tubes, and stored at –80°C until analysis.

Analysis of Milk and Cellular Fatty Acid Methyl Esters

Lipids for FA analysis were extracted from milk samples and MAC-T cells using the Folch method (Folch et al., 1957). Briefly, lipids were extracted using a chloroform/methanol solution (2/1; vol/vol) from the milk samples or MAC-T cell pellets. Next, methyl esters of the fatty acids were prepared by transesterification with a 14% (wt/vol) boron trifluoride/methanol solution at 90°C for 60 min as previously described (Morrison and Smith, 1964). The fatty acid methyl esters were quantified by Agilent 7890A gas chromatograph (Agilent Technologies Inc., Alpharetta, GA) using an SP-2560 fused silica capillary column (100 m × 0.25 mm i.d. and film thickness of 0.2 µm; Supelco Inc., Bellefonte, PA). The analysis involved a programmed run with temperature ramps as follows: the oven temperature was initially 70°C for 5 min after which it was increased to 175°C at a rate of 10°C/min and held for 20 min. Next, the temperature was increased to 240°C at a rate of 4°C/min and held for 20 min. The injector and detector temperatures were maintained at 250°C, and the flow rate of the hydrogen carrier gas was 1 mL/min. The hydrogen flow rate to the detector was 40 mL/min, the airflow rate was 400 mL/min, and the nitrogen-gas take-up flow rate was 45 mL/min.

RNA Isolation and Quantitative Real-Time PCR

For transcriptional analysis, total RNA was extracted from goat mammary tissue and MAC-T cells using TRIzol reagent according to the manufacturer's instructions. The integrity of RNA was measured using an Agilent 2100 Bioanalyzer (Agilent Technologies Inc.). The concentration of RNA was measured using a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE). Additionally, the optical density (OD) A260/A280 and OD A260/A230 ratios were determined to verify RNA purity. For this, the OD A260/A280 ratio should be 1.8 to 2.0 and

the OD A260/A230 should be 1.8 or greater. Reverse transcription was performed using an Oligo(dT)₁₂₋₁₈ primer and Moloney murine leukemia virus reverse transcriptase. First, a mixture containing 3 µg of RNA, 1 µL of Oligo (dT)₁₂₋₁₈ (Invitrogen), and diethyl pyrocarbonate-treated water in a total volume of 12 µL was prepared. The mixture was incubated at 65°C for 5 min in a C1000 thermal cycler (Bio-Rad, Laboratories Inc., Hercules, CA) and then kept on ice for 3 min. A master mix composed of 5 µL of 5× First-Strand buffer (Invitrogen), 1 µL of 0.1 M DTT (Invitrogen), 1 µL of 10 mM dNTP (Invitrogen), and 1 µL of Moloney murine leukemia virus reverse transcriptase (Invitrogen) was added to the RNA samples. The PCR was performed using the following temperature program: 37°C for 50 min and 70°C for 15 min. The genes examined and their specific PCR primers are listed in Table 2; β-actin was used as a control.

Real-time (RT)-PCR was performed in 20-µL reaction mixtures in 96-well plates using the iQ5 Real-Time PCR Detection System (Bio-Rad Laboratories Inc.). The optimal reaction conditions were attained using 10 µL of iQ SYBRgreen Supermix Kit (Bio-Rad Laboratories Inc.), 2 µg of cDNA, and 0.3 µL of each 10 µM forward and reverse primers at an annealing temperature of 55°C or 60°C. The following PCR program was used: initial denaturation at 95°C for 3 min followed by 40 cycles of amplification at 94°C for 15 s, annealing at 55°C or 60°C for 30 s, and extension at 72°C for 30 s. The samples were then heated to 95°C for 1 min, followed by 55°C for 1 min, and held at 4°C. The relative quantification of gene expression from different samples was analyzed using the 2^{-ΔΔCT} method (Livak and Schmittgen, 2001). All quantitative RT-PCR analyses were repeated at least 3 times.

Western Blotting

Mammary tissue was homogenized in lysis buffer containing 150 mM NaCl, 50 mM Tris-HCl, pH 8.0, 1% IGEPAL CA-630 (chemically equivalent to Nonidet P-40, nonionic, nondenaturing detergent; Sigma), 0.1% SDS, 0.25% sodium deoxycholate (Sigma), 1 mM EDTA, and 1× protease inhibitor (GE Healthcare). The mixtures were incubated for 30 min on ice and stirred every 5 min. After incubating, the samples were centrifuged at 19,000 × g for 30 min at 4°C, and the supernatant was collected for Western blotting. Protein concentrations were measured using a bicinchoninic acid (BCA) protein assay (Pierce, Rockford, IL). The proteins (30 µg) were separated on 10% (wt/vol) SDS-PAGE and transferred to nitrocellulose membranes (Millipore, Billerica, MA). The membranes were blocked overnight with 5% skim milk in Tris-buffered

Table 2. Gene information and PCR primer sequences

Gene ¹	Forward primer	Reverse primer	GenBank accession no.	Product size (bp)	Species/reference
LPL	5'-tgaccagctagtgaagtc-3'	5'-cggtagccttacttgatt-3'	DQ997818	91	Capra hircus
FAS	5'-gacagagcagccttcataa-3'	5'-ggaggttgaggagagcataa-3'	DQ223929	140	Capra hircus
ACC	5'-gatacgcatacacaattggc-3'	5'-ttaacttccagcagaggt-3'	Z117803	90	Capra hircus
β-Actin ²	5'-aaagcaacacgtgagaagat-3'	5'-cctggatggccacatacat-3'	AF481159	76	Capra hircus
β-Casein	5'-gtgaggaacacagcaaca-3'	5'-ttttgtgggagctgttagg-3'	NM_181008	115	Bos taurus (Zhou et al., 2008)
α _{S2} -Casein	5'-agctctccaccagfaggaa-3'	5'-gcaagcggaatttctgtaa-3'	NM_174528.2	150	Bos taurus (Zhou et al., 2008)
PSMA5	5'-catgagtggttaattgtg-3'	5'-agcctgagtcacactctca-3'	NM_001015566	115	Bos taurus
SCD	5'-cccttctctgagctgtctg-3'	5'-atgtgactcttccctctga-3'	NM_173959	181	Bos taurus (Zhou et al., 2008)
β-Actin ²	5'-ctcttcacgctcttctct-3'	5'-ggcagctgctcttcttgc-3'	BC142413	178	Bos taurus (Lengi and Corl, 2007)

¹LPL = lipoprotein lipase; FAS = fatty acid synthase; ACC = acetyl-CoA carboxylase; PSMA5 = proteasome (prosome, macropain) subunit α type 5; SCD = stearoyl-CoA desaturase.

²One primer sequence for β-actin specifically targeted goat mammary tissue and the other specifically targeted bovine mammary epithelial cells.

saline-Tween (TBST) buffer (20 mM Tris-HCl, pH 7.6; 137 mM NaCl, and 0.01% Tween 20) at 4°C. The membranes were then incubated for 3 h at 4°C with primary antibodies: β -actin (ab6276, 1:5,000, 42 kDa; Abcam, Cambridge, UK), mouse monoclonal antibody (CD.E10) against human SCD [ab19862, 1:2,000, 41.5 kDa; Abcam), rabbit polyclonal antibody against human peroxiredoxin-1 (**PRDX1**; ab41906, 1:1,000, 22 kDa; Abcam), rabbit polyclonal antibody specific for human translationally controlled tumor protein (**TCTP**; ab37506, 1:250, 22 kDa; Abcam), and rabbit anti-Proteasome (prosome, macropain) subunit α type 5 (**PSMA5**) (K231) antibody (#2457, 1:2,000, 27 kDa; Cell Signaling Technology Inc., Danvers, MA). The membranes were then washed 3 times with 1 $\times$ Tris-buffered saline-Tween, after which they were incubated with the appropriate secondary antibodies: horseradish peroxidase-conjugated rabbit polyclonal antibody specific for mouse IgG-H&L (ab6728, 1:2,000; Abcam) or horseradish peroxidase-conjugated goat polyclonal antibody against rabbit IgG-H&L (ab6741, 1:2,000; Abcam) for 3 h at 4°C. Antibody binding was visualized using an enhanced chemiluminescence system and detection kit (GE Healthcare) after which gels were exposed to x-ray film (Fujifilm Corp., Tokyo, Japan) for 1 to 3 min. The films were then scanned and the bands were quantified using ImageJ 1.43 software (<http://rsb.info.nih.gov/ij/download.html>). Protein levels were normalized by comparison with β -actin signals on the same membrane.

Statistical Analysis

The experimental data are expressed as the mean $\pm$ standard error of the mean (SEM). Data were analyzed using SPSS 14.0 for Windows (SPSS Inc., Chicago, IL). Two-treatment effects were evaluated using an independent sample *t*-test or one-way ANOVA followed by the Duncan multiple range test. A *P*-value <0.05 was considered statistically significant.

RESULTS

Effects of TVA on the Endogenous Synthesis of CLA

Figures 1a and b show the changes in TVA and *cis*-9,*trans*-11 CLA concentrations in the goat milk and the desaturase index of TVA [$\text{desaturase index} = \text{cis-9,trans-11 CLA}/(\text{TVA} + \text{cis-9,trans-11 CLA})$] in mammary tissue in response to TVA administration. The TVA treatment significantly increased *cis*-9,*trans*-11 CLA concentrations in the milk. The TVA concentration was also increased 2.5-fold in the milk, and the

desaturation index was increased 1.5-fold in the goat mammary tissue, respectively, by TVA treatment.

We used RT-PCR to evaluate the expression of genes involved in FA synthesis. The data show that TVA treatment significantly decreased the mRNA abundance of fatty acid synthase (**FAS**) and acetyl-CoA carboxylase (**ACC**), whereas lipoprotein lipase (**LPL**) mRNA levels were not increased (Figure 2a). Moreover, the protein level of SCD was significantly upregulated by TVA supplementation (Figures 2b and c).

Figure 1. The effects of *trans*-vaccenic acid (TVA) treatment on goat milk TVA and conjugated linoleic acid (CLA) levels, and the desaturase index in goat mammary tissue. (a) Concentrations (mg/mL of milk) of TVA and *cis*-9,*trans*-11 CLA in goat milk; (b) desaturase index in goat mammary tissue. We used a desaturase index as a proxy for Δ^9 -desaturase activity in the mammary gland. Desaturase index = *cis*-9,*trans*-11 CLA/(TVA + *cis*-9,*trans*-11 CLA).

Figure 2. Effect of *trans*-vaccenic acid (TVA) treatment on the expression of conjugated linoleic acid (CLA) synthesis-associated enzymes in goat mammary tissue. (a) Quantitative analyses showed whether transcription of enzymes was up- or downregulated in the TVA-treatment group compared with the control group (vehicle-injected goats). Data are presented as the mean $\pm$ SEM ($n = 3$); (b) Western blotting analysis showed that stearoyl-CoA desaturase (SCD) expression was upregulated in the TVA-treated group compared with the control group; (c) data analysis of protein SCD expression compared with β -actin. * $P < 0.05$.

Identification of Proteins Affected by TVA Treatment in Goat Mammary Tissues Using ESI-Q-TOF MS Analysis

The 2-DE protein profile results showed that the presence of TVA induced changes in the expression of several proteins in goat mammary tissues (data not shown). Based on this protein profile, we selected 1 up-regulated and 2 downregulated proteins that responded to TVA treatment. These 3 proteins showed at least a 2-fold increase or decrease in staining density relative to the control. This was followed by performing ESI-Q-TOF MS to identify the 3 representative proteins (Table 3). Results of this analysis indicated that the upregulated protein was PSMA5 and the downregulated proteins were PRDX1 and TCTP1.

Protein Expression Levels Determined by Western Blot Analysis

Among the 3 representative proteins, only the results of PSMA5 expression were consistent as measured by both the proteomic analysis and Western blotting (Figure 3 and Table 3). The Western blot results showed that PSMA5 expression was significantly increased in mammary tissue from TVA-treated goats compared with control goats ($P < 0.05$; Figure 3).

RT-PCR Analysis of SCD and PSMA5 mRNA Expression in MAC-T cells

To examine the association between SCD and PSMA5 in CLA synthesis, differentiated bovine mammary epithelial MAC-T cells were treated with TVA. Changes in CLA concentration along with mRNA abundance of SCD and PSMA5 were then recorded. β -Casein and α_{S2} -casein are gene markers of MAC-T cell differentiation, so we monitored the mRNA expression of these genes during the 4 d of differentiation. The mRNA expression of β -casein and α_{S2} -casein on d 1, 2, and 4 rapidly increased by 3.1-, 3.3-, and 15.1-fold ($P < 0.01$) and 2.3-, 3.2-, and 9.4-fold ($P < 0.01$), respectively, compared with the levels measured before differentiation induction (data not shown).

After 90 h of differentiation induction in the MAC-T cells, 50 μ M TVA was added to the medium and the cells were incubated for an additional 6 h. During this period, we monitored changes in *cis*-9,*trans*-11 CLA concentration and mRNA expression patterns of SCD and PSMA5. The results show that the concentration of *cis*-9,*trans*-11 CLA increased ($P < 0.05$) until 4 h and then decreased slightly (Figure 4a). The mRNA expression levels of both SCD and PSMA5 were significantly elevated at 2 h. After 2 h of TVA treatment, the

Table 3. Differentially expressed proteins in goat mammary tissue identified by electrospray ionization quadrupole time-of-flight mass spectrometry (ESI-Q-TOF/MS)

Spot no.	NCBI accession no.	Protein	MS/MS sequence ¹	Protein score	Mass (bp)/pI ²	Sequence coverage (%)	Expressional fold change ³ (TVA vs. control)
534	NP_001015566	Proteasome (prosome, macropain) subunit, α type 5 (PSMA5)	LFQVEYAIEAIK, ITSPLMEPSSIEK	50	26,579/4.74	10	$\uparrow$ 2.34
142	NP_776856	Peroxisome oxidin-1 (PRDX1)	QITINDLPVGR	53	22,379/8.81	5	$\downarrow$ 0.44
57	BAC56463	Similar to tumor protein, translationally controlled 1 (TCTP1)	EVADGLCLEVEGK	43	12,928/4.84	11	$\downarrow$ 0.20

¹PSMA5 was matched to 2 candidate peptides in the database search, and both peptide sequences are shown.²Isotopic point.³*Trans*-vaccenic acid (TVA)-induced changes in the expression of identified proteins are indicated by up (increase) or down (decrease) arrows **Figure 3.** Western blots analysis of proteasome (prosome, macropain) subunit α type 5 (PSMA5), peroxiredoxin-1 (PRDX1), and translationally controlled tumor protein 1 (TCTP1) in mammary tissues from *trans*-vaccenic acid (TVA)-treated and control (vehicle-treated) goats. (a) Western blot results showed that PSMA5 was increased in the TVA-treated group compared with the control; (b) data analysis of each protein expression compared with β -actin. The data are expressed as the mean $\pm$ SEM of 3 goats per treatment group. * $P < 0.05$.

concentration of SCD mRNA declined to basal levels ($P < 0.05$). On the other hand, PSMA5 mRNA expression was upregulated at 6 h of TVA treatment ($P < 0.05$; Figure 4b).

DISCUSSION

The health benefits of CLA are documented but systematic exploration of CLA synthesis from TVA in the mammary gland has only recently been initiated (Jin et al., 2010). Corl et al. (2003) demonstrated that endogenous synthesis of *cis*-9,*trans*-11 CLA occurs in the mammary gland. They also found that the enzyme SCD (also known as Δ^9 -desaturase) is involved in CLA synthesis. Using a *trans*-11 vaccenic-1-¹³C acid complex

Figure 4. (a) Changes in the concentration of *cis*-9,*trans*-11 conjugated linoleic acid (CLA) in response to *trans*-vaccenic acid (TVA). After 90 h of differentiation induction in MAC-T cells, 50 μ M TVA was added to the differentiation induction medium and the cells were incubated for an additional 6 h. (b) Examination of temporal stearoyl-CoA desaturase (SCD) and proteasome (prosome, macropain) subunit α type 5 (PSMA5) mRNA expression patterns in MAC-T cells (gene expression relative to 0 h) in response to treatment with 50 μ M TVA over a 6-h incubation. Data were collected during TVA treatment at each time point. Letters (a, b, c, d, and e) denote significant differences at specific time points ($P < 0.05$).

(13 C-TVA), Mosley et al. (2006 a,b) demonstrated that *cis*-9,*trans*-11 CLA is synthesized from TVA by the SCD enzyme in the mammary glands of dairy cattle

and lactating women. Our results were consistent with those previously reported. Other studies (Griinari et al., 2000; Santora et al., 2000; Corl et al., 2001) have suggested that the synthesis of CLA in goat mammary glands and MAC-T cells represents a response to increased TVA levels.

Whereas ACC and FAS are involved in de novo FA synthesis in the mammary gland, LPL is concerned with the uptake of plasma FA to promote milk fat synthesis during lactation. These FA can be desaturated by SCD, thereby converting them into *cis*-9 unsaturated FA (Bernard et al., 2008). In lactating dairy cows, mammary epithelial cells have high levels of SCD activity (Bickerstaffe and Annison, 1970; Kinsella, 1970; McDonald and Kinsella, 1973; Ward et al., 1998), and SCD plays a key role in the endogenous synthesis of *cis*-9,*trans*-11 CLA in mammary glands (Griinari et al., 2000; Corl et al., 2001; Loores et al., 2005). Moreover, rats, mice, and humans can also endogenously synthesize CLA because they express the SCD enzyme (Salminen et al., 1998; Adlof et al., 2000; Santora et al., 2000; Banni et al., 2001; Turpeinen et al., 2002; Corl et al., 2003; Lock et al., 2004; Kraft et al., 2006). Furthermore, compared with oleic acid, TVA supplementation leads to increased SCD transcription in bovine mammary epithelial cells (Matitashvili and Bauman, 2000). Increases in SCD activity reduce the activities of FAS and ACC in bovine MAC-T cells (Jayan and Herbein, 2000). Our findings are consistent with these previous findings and suggest that the expression of SCD protein in the mammary gland is upregulated in response to TVA treatment.

Besides SCD, other proteins might be involved in the endogenous synthesis of CLA from TVA in the mammary gland. We therefore used a proteomic analysis to find novel proteins associated with CLA synthesis. We only found that PSMA5 was increased in mammary tissue when TVA was converted into CLA. Based on this finding, we concluded that PSMA5 might play an important role in endogenous CLA synthesis in mammary tissue.

Proteasome (prosome, macropain) subunit α type 5 is an α -type subunit of the 20S proteasome core complex. Assembly of the eukaryotic 20S proteasome is believed to begin with the formation of an α -ring and requires the participation of PSMA5 (Hirano et al., 2005, 2006; Le Tallec et al., 2007; Kusmierczyk et al., 2008; Yashiroda et al., 2008; Murata et al., 2009). This produces a 20S proteasome core and two 19S regulatory subunits that form the 26S proteasome. This proteasome is a large multi-subunit complex involved in proteolytic degradation via the ubiquitin-proteasome pathway, which is involved in various biological processes such as the cell cycle, apoptosis, angiogenesis, cell adhesion,

and transcription (Read et al., 1995; King et al., 1996; Oikawa et al., 1998; Orlowski, 1999; Desterro et al., 2000). A recent study found higher PSMA5 proteasome activity in colon tumor tissue compared with the surrounding normal tissue (Arlt et al., 2009). Furthermore, a proteomics approach showed that expression of PSMA5 is significantly lower in males with a high body mass index compared with those with a low body mass index (Sakamoto et al., 2009). Moreover, PSMA5 was found to have high level of endo-RNase activity that is a part of the 26S complex specifically cleaved cellular to high-molecular-weight cellular RNA as well as adenylate-uridylylate (AU)-rich elements (Alu/ARE)-containing mRNA, including c-myc and c-fos (Kulichkova et al., 2010). All of these findings demonstrate that PSMA5 is a part of the 26S complex that may play important roles in various biological processes involved in RNA metabolism and protein degradation.

Stearoyl-CoA desaturase 1 is a short-lived integral membrane protein of the endoplasmic reticulum (ER; Oshino and Sato, 1972). Previous studies hypothesized that short-lived cytosolic proteins and some ER proteins are degraded by the ubiquitin-proteasome pathway (Hilt and Wolf, 1996; Hochstrasser, 1996; Matlack et al., 1998). Man et al. (2006) proposed an integral mouse SCD-1 model of membrane topology. This model shows that SCD-1 has 4 transmembrane domains in the ER membrane and that both the NH₂ and COOH termini are oriented toward the cytosol. The single cytoplasmic loop and COOH terminus contain 8 histidine (His) residues (known as the His box), and the 2 ER luminal loops contain 2 of the 3 conserved His motifs that bind iron within the catalytic center of the desaturase (Paton and Ntambi, 2009). Mziaut et al. (2000) reported that the N-terminal segment of SCD contains a sequence responsible for the rapid degradation of the protein. Stearoyl-CoA desaturase in liver microsomes undergoes highly selective degradation. This protein has a half-life of 3 to 4 h and generates a 20-kDa C-terminal SCD fragment (Ozols, 1997; Heinemann and Ozols, 1998). Kato et al. (2006) also showed that SCD1 is naturally short lived and constitutively degraded within the ER by the ubiquitin-proteasome system. Therefore, we presumed that the PSMA5 protein is involved in SCD degradation.

In the present study, we investigated PSMA5 protein expression to determine whether it was similar to SCD expression. The results suggest that PSMA5 was upregulated in goat mammary tissue in response to TVA treatment ($P < 0.05$). We first detected increased PSMA5 expression in mammary tissues following TVA treatment. The increased protein expression of SCD and PSMA5 in the mammary tissue by TVA suggests that these 2 proteins are closely related to endogenous

CLA synthesis in mammary epithelial cells. Therefore, we used MAC-T cells to investigate the relationship between SCD and PSMA5 in the process of endogenous CLA synthesis.

We found that transcripts of SCD in differentiated MAC-T cells increased and were then degraded rapidly with a half-life of approximately 2 h after TVA treatment. Meanwhile, PSMA5 transcription also increased after the addition of TVA and continued until 6 h of TVA treatment. Thus, mRNA expression of PSMA5 and SCD showed an overall inverse relationship after 2 h. In our previous study (Jin et al., 2010), we found that protein disulfide isomerase is related to CLA biosynthesis and its major function is to regulate protein unfolding in the ER. Proteasome (prosome, macropain) subunit α type 5, located on the outer ER membrane surface, eliminates misfolded or unassembled proteins via endoplasmic reticulum-associated protein degradation. Therefore, PSMA5 is a part of the ubiquitin-proteasome system that may be involved in constitutive SCD degradation when TVA is converted into CLA in differentiated MAC-T cells.

The data generated in this study improve our understanding of the molecular mechanisms through which TVA exerts its specific effects on endogenous CLA synthesis in ruminant mammary tissues. To the best of our knowledge, this is the first proteomic study of CLA biosynthesis in response to TVA treatment in goat mammary glands. We found that PSMA5 protein expression increased during CLA biosynthesis following TVA administration. Our results indicated that PSMA5 is a candidate marker for assessing CLA biosynthesis induced by TVA in the mammary tissues of lactating goats.

ACKNOWLEDGMENTS

We gratefully acknowledge Feng-Qi Zhao (Department of Animal Science, College of Agriculture and Life Sciences, University of Vermont, Burlington) for the generous gift of MAC-T cells for the cell culture study. This work was supported by a Bio-Scientific Research Grant (PNU-2008-101-2009009100) funded by Pusan National University (Gyeongnam, Republic of Korea).

REFERENCES

- Adlof, R. O., S. Duval, and E. A. Emken. 2000. Biosynthesis of conjugated linoleic acid in humans. *Lipids* 35:131–135.
- Aksu, S., C. Scheler, N. Focks, F. Leenders, F. Theuring, J. Salnikow, and P. R. Jungblut. 2002. An iterative calibration method with prediction of post-translational modifications for the construction of a two-dimensional electrophoresis database of mouse mammary gland proteins. *Proteomics* 2:1452–1463.
- AOAC. 1995. Official Methods of Analysis. 16th ed. Association of Official Analytical Chemists, Washington, DC.

- Arlt, A., I. Bauer, C. Schafmayer, J. Tepel, S. S. Mürköster, M. Brosch, C. Röder, H. Kalthoff, J. Hampe, M. P. Moyer, U. R. Fölsch, and H. Schäfer. 2009. Increased proteasome subunit protein expression and proteasome activity in colon cancer relate to an enhanced activation of nuclear factor E2-related factor 2 (Nrf2). *Oncogene* 28:3983–3996.
- Aro, A., S. Männistö, I. Salminen, M. L. Ovaskainen, V. Kataja, and M. Uusitupa. 2000. Inverse association between dietary and serum conjugated linoleic acid and risk of breast cancer in postmenopausal women. *Nutr. Cancer* 38:151–157.
- Banni, S., E. Angioni, E. Murru, G. Carta, M. P. Melis, D. Bauman, Y. Dong, and C. Ip. 2001. Vaccenic acid feeding increases tissue levels of conjugated linoleic acid and suppresses development of premalignant lesions in rat mammary gland. *Nutr. Cancer* 41:91–97.
- Bauman, D. E., B. A. Corl, L. H. Baumgard, and J. M. Griinari. 2001. Conjugated linoleic acid (CLA) and the dairy cow. Pages 237–266 in *Recent Advances in Animal Nutrition*. P. C. Garnsworthy and D. J. Cole, ed. Nottingham University Press, Nottingham, UK.
- Bernard, L., C. Leroux, and Y. Chilliard. 2008. Expression and nutritional regulation of lipogenic genes in the ruminant lactating mammary gland. *Adv. Exp. Med. Biol.* 606:67–108.
- Bickerstaffe, R., and E. F. Annison. 1970. Lipid metabolism in the perfused chicken liver. The uptake and metabolism of oleic acid, elaidic acid, *cis*-vaccenic acid, *trans*-vaccenic acid and stearic acid. *Biochem. J.* 118:433–442.
- Blankson, H., J. A. Stakkestad, H. Fagertun, E. Thom, J. Wadstein, and O. Gudmundsen. 2000. Conjugated linoleic acid reduces body fat mass in overweight and obese humans. *J. Nutr.* 130:2943–2948.
- Corl, B. A., D. M. Barbano, D. E. Bauman, and C. Ip. 2003. *cis*-9,*trans*-11 CLA derived endogenously from *trans*-11 18:1 reduces cancer risk in rats. *J. Nutr.* 133:2893–2900.
- Corl, B. A., L. H. Baumgard, D. A. Dwyer, J. M. Griinari, B. S. Phillips, and D. E. Bauman. 2001. The role of delta(9)-desaturase in the production of *cis*-9,*trans*-11 CLA. *J. Nutr. Biochem.* 12:622–630.
- de Roos, B., G. Rucklidge, M. Reid, K. Ross, G. Duncan, M. A. Navarro, J. M. Arbones-Mainar, M. A. Guzman-Garcia, J. Osada, J. Browne, C. E. Loscher, and H. M. Roche. 2005. Divergent mechanisms of *cis*9, *trans*11-and *trans*10, *cis*12-conjugated linoleic acid affecting insulin resistance and inflammation in apolipoprotein E knockout mice: A proteomics approach. *FASEB J.* 19:1746–1748.
- Desrivieres, S., T. Prinz, L. N. Castro-Palomino, M. Meyer, G. Boehm, U. Bauer, J. Schäfer, T. Neumann, C. Shemanko, and B. Groner. 2003. Comparative proteomic analysis of proliferating and functionally differentiated mammary epithelial cells. *Mol. Cell. Proteomics* 2:1039–1054.
- Desterro, J. M., M. S. Rodriguez, and R. T. Hay. 2000. Regulation of transcription factors by protein degradation. *Cell. Mol. Life Sci.* 57:1207–1219.
- Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226:497–509.
- Griinari, J. M., B. A. Corl, S. H. Lacy, P. Y. Chouinard, K. V. Nurmela, and D. E. Bauman. 2000. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by delta(9)-desaturase. *J. Nutr.* 130:2285–2291.
- Hayek, M. G., S. N. Han, D. Wu, B. A. Watkins, M. Meydani, J. L. Dorsey, D. E. Smith, and S. N. Meydani. 1999. Dietary conjugated linoleic acid influences the immune response of young and old C57BL/6NCrIBR mice. *J. Nutr.* 129:32–38.
- Heinemann, F. S., and J. Ozols. 1998. Degradation of stearoyl-coenzyme A desaturase: Endoproteolytic cleavage by an integral membrane protease. *Mol. Biol. Cell* 9:3445–3453.
- Hilt, W., and D. H. Wolf. 1996. Proteasomes: Destruction as a programme. *Trends Biochem. Sci.* 21:96–102.
- Hirano, Y., H. Hayashi, S. Iemura, K. B. Hendil, S. Niwa, T. Kishimoto, M. Kasahara, T. Natsume, K. Tanaka, and S. Murata. 2006. Cooperation of multiple chaperones required for the assembly of mammalian 20S proteasomes. *Mol. Cell* 24:977–984.
- Hirano, Y., K. B. Hendil, H. Yashiroda, S. Iemura, R. Nagane, Y. Hioki, T. Natsume, K. Tanaka, and S. Murata. 2005. A heterodimeric complex that promotes the assembly of mammalian 20S proteasomes. *Nature* 437:1381–1385.
- Hochstrasser, M. 1996. Protein degradation or regulation: Ub the judge. *Cell* 84:813–815.
- Houseknecht, K. L., J. P. Vanden Heuvel, S. Y. Moya-Camarena, C. P. Portocarrero, L. W. Peck, K. P. Nickel, and M. A. Belury. 1998. Dietary conjugated linoleic acid normalizes impaired glucose tolerance in the Zucker diabetic fatty fa/fa rat. *Biochem. Biophys. Res. Commun.* 244:678–682.
- Huynh, H. T., G. Robitaille, and J. D. Turner. 1991. Establishment of bovine mammary epithelial cells (MAC-T): An in vitro model for bovine lactation. *Exp. Cell Res.* 197:191–199.
- Jayan, G. C., and J. H. Herbein. 2000. “Healthier” dairy fat using *trans*-vaccenic acid. *Nutr. Food Sci.* 30:304–309.
- Jin, Y. C., H. G. Lee, C. X. Xu, J. A. Han, S. H. Choi, M. K. Song, Y. J. Kim, K. B. Lee, S. K. Kim, H. S. Kang, B. W. Cho, T. S. Shin, and Y. J. Choi. 2010. Proteomic analysis of endogenous conjugated linoleic acid biosynthesis in lactating rats and mouse mammary gland epithelial cells (HC11). *Biochim. Biophys. Acta* 1804:745–751.
- Kato, H., K. Sakaki, and K. Mihara. 2006. Ubiquitin-proteasome-dependent degradation of mammalian ER stearoyl-CoA desaturase. *J. Cell Sci.* 119:2342–2353.
- Kim, E. J., W. Y. Kim, Y. H. Kang, Y. L. Ha, L. A. Bach, and J. H. Y. Park. 2000. Inhibition of Caco-2 cell proliferation by (n-3) fatty acids: Possible mediation by increased secretion of insulin-like growth factor binding protein-6. *Nutr. Res.* 20:1409–1421.
- King, R. W., R. J. Deshaies, J. M. Peters, and M. W. Kirschner. 1996. How proteolysis drives the cell cycle. *Science* 274:1652–1659.
- Kinsella, J. E. 1970. Stearic acid metabolism by mammary cells. *J. Dairy Sci.* 53:1757–1765.
- Kraft, J., L. Hanske, P. Mockel, S. Zimmermann, A. Hartl, J. K. G. Kramer, and G. Jahreis. 2006. The conversion efficiency of *trans*-11 and *trans*-12 18:1 by delta 9-desaturase differs in rats. *J. Nutr.* 136:1209–1214.
- Kulichkova, V. A., A. S. Tsimokha, O. A. Fedorova, T. N. Moiseeva, A. Botril, L. Lezina, L. N. Gauze, I. M. Konstantinova, A. G. Mittenberg, and N. A. Barlev. 2010. 26S proteasome exhibits endoribonuclease activity controlled by extra-cellular stimuli. *Cell Cycle* 9:840–849.
- Kusmierczyk, A. R., M. J. Kunjappu, M. Funakoshi, and M. Hochstrasser. 2008. A multimeric assembly factor controls the formation of alternative 20S proteasomes. *Nat. Struct. Mol. Biol.* 15:237–244.
- Le Tallec, B., M. B. Barrault, R. Courbeyrette, R. Guérois, M. C. Marsolier-Kergoat, and A. Peyroche. 2007. 20S proteasome assembly is orchestrated by two distinct pairs of chaperones in yeast and in mammals. *Mol. Cell* 27:660–674.
- Lee, K. N., D. Kritchevsky, and M. W. Pariza. 1994. Conjugated linoleic acid and atherosclerosis in rabbits. *Atherosclerosis* 108:19–25.
- Lengi, A. J., and B. A. Corl. 2007. Identification and characterization of a novel bovine stearoyl-CoA desaturase isoform with homology to human SCD5. *Lipids* 42:499–508.
- Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method. *Methods* 25:402–408.
- Lock, A. L., B. A. Corl, D. M. Barbano, D. E. Bauman, and C. Ip. 2004. The anticarcinogenic effect of *trans*-11 18:1 is dependent on its conversion to *cis*-9, *trans*-11 CLA by Δ 9-desaturase in rats. *J. Nutr.* 134:2698–2704.
- Loor, J. J., A. Ferlay, A. Ollier, K. Ueda, M. Doreau, and Y. Chilliard. 2005. High-concentrate diets and polyunsaturated oils alter *trans* and conjugated isomers in bovine rumen, blood, and milk. *J. Dairy Sci.* 88:3986–3999.
- Loor, J. J., X. Lin, and J. H. Herbein. 2002. Dietary *trans*-vaccenic acid (*trans*11–18:1) increases concentration of *cis*9, *trans*11-conjugated linoleic acid (rumenic acid) in tissues of lactating mice and suckling pups. *Reprod. Nutr. Dev.* 42:85–99.
- Man, W. C., M. Miyazaki, K. Chu, and J. M. Ntambi. 2006. Membrane topology of mouse stearoyl-CoA desaturase 1. *J. Biol. Chem.* 281:1251–1260.

- Matitashvili, E., and D. E. Bauman. 2000. Effect of different isomers of C18:1 and C18:2 fatty acids on lipogenesis in bovine mammary epithelial cells. *J. Anim. Sci.* 83(Suppl. 1):165. (Abstr.)
- Matlack, K. E. S., W. Mothes, and T. A. Rapoport. 1998. Protein translocation: Tunnel vision. *Cell* 92:381–390.
- McDonald, T. M., and J. E. Kinsella. 1973. Stearyl-CoA desaturase of bovine mammary microsomes. *Arch. Biochem. Biophys.* 156:223–231.
- Morrison, W. R., and L. M. Smith. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. *J. Lipid Res.* 5:600–608.
- Mosley, E. E., M. K. McGuire, J. E. Williams, and M. A. McGuire. 2006a. *cis*-9, *trans*-11 conjugated linoleic acid is synthesized from vaccenic acid in lactating women. *J. Nutr.* 136:2297–2301.
- Mosley, E. E., B. Shafii Dagger, P. J. Moate, and M. A. McGuire. 2006b. *cis*-9, *trans*-11 conjugated linoleic acid is synthesized directly from vaccenic acid in lactating dairy cattle. *J. Nutr.* 136:570–575.
- Murata, S., H. Yashiroda, and K. Tanaka. 2009. Molecular mechanisms of proteasome assembly. *Nat. Rev. Mol. Cell Biol.* 10:104–115.
- Mziaut, H., G. Korza, and J. Ozols. 2000. The N-terminus of microsomal delta 9 stearoyl-CoA desaturase contains the sequence determinant for its rapid degradation. *Proc. Natl. Acad. Sci. USA* 97:8883–8888.
- NRC. 2007. Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids. National Academies Press, Washington, DC.
- Ntambi, J. M. 1995. The regulation of stearoyl-CoA desaturase (SCD). *Prog. Lipid Res.* 34:139–150.
- Oikawa, T., T. Sasaki, M. Nakamura, M. Shimamura, N. Tanahashi, S. Omura, and K. Tanaka. 1998. The proteasome is involved in angiogenesis. *Biochem. Biophys. Res. Commun.* 246:243–248.
- Orlowski, R. Z. 1999. The role of the ubiquitin-proteasome pathway in apoptosis. *Cell Death Differ.* 6:303–313.
- Oshino, N., and R. Sato. 1972. The dietary control of the microsomal stearyl CoA-desaturation enzyme system in rat liver. *Arch. Biochem. Biophys.* 149:369–377.
- Ozols, J. 1997. Degradation of hepatic stearyl CoA delta 9-desaturase. *Mol. Biol. Cell* 8:2281–2290.
- Paton, C. M., and J. M. Ntambi. 2009. Biochemical and physiological function of stearoyl-CoA desaturase. *Am. J. Physiol. Endocrinol. Metab.* 297:E28–E37.
- Pollard, M. R., F. D. Gunstone, A. T. James, and L. J. Morris. 1980. Desaturation of positional and geometric isomers of monoenoic fatty acids by microsomal preparations from rat liver. *Lipids* 15:306–314.
- Read, M. A., A. S. Neish, F. W. Lusinskas, V. J. Palombella, T. Maniatis, and T. Collins. 1995. The proteasome pathway is required for cytokine-induced endothelial-leukocyte adhesion molecule expression. *Immunity* 2:493–506.
- Sakamoto, K., Y. Sato, T. Shinka, M. Sei, I. Nomura, M. Umeno, A. A. Ewis, and Y. Nakahori. 2009. Proteasome subunits mRNA expressions correlate with male BMI: Implications for a role in obesity. *Obesity (Silver Spring)* 17:1044–1049.
- Salminen, I., M. Mutanen, M. Jauhiainen, and A. Aro. 1998. Dietary *trans* fatty acids increase conjugated linoleic acid levels in human serum. *J. Nutr. Biochem.* 9:93–98.
- Santora, J. E., D. L. Palmquist, and K. L. Roehrig. 2000. *Trans*-vaccenic acid is desaturated to conjugated linoleic acid in mice. *J. Nutr.* 130:208–215.
- Shevchenko, A., O. N. Jensen, A. V. Podtelevnikov, F. Sagliocco, M. Wilm, O. Vorm, P. Mortensen, A. Shevchenko, H. Boucherie, and M. Mann. 1996. Linking genome and proteome by mass spectrometry: Large-scale identification of yeast proteins from two-dimensional gels. *Proc. Natl. Acad. Sci. USA* 93:14440–14445.
- Turpeinen, A. M., M. Mutanen, A. Aro, I. Salminen, S. Basu, D. L. Palmquist, and J. M. Griinari. 2002. Bioconversion of vaccenic acid to conjugated linoleic acid in humans. *Am. J. Clin. Nutr.* 76:504–510.
- Viswanadha, S., J. G. Giesy, T. W. Hanson, and M. A. McGuire. 2003. Dose response of milk fat to intravenous administration of the *trans*-10, *cis*-12 isomer of conjugated linoleic acid. *J. Dairy Sci.* 86:3229–3236.
- Ward, R. J., M. T. Travers, S. E. Richards, R. G. Vernon, A. M. Salter, P. J. Buttery, and M. C. Barber. 1998. Stearoyl-CoA desaturase mRNA is transcribed from a single gene in the ovine genome. *Biochem. Biophys. Acta* 1391:145–156.
- Yashiroda, H., T. Mizushima, K. Okamoto, T. Kameyama, H. Hayashi, T. Kishimoto, S. Niwa, M. Kasahara, E. Kurimoto, E. Sakata, K. Takagi, A. Suzuki, Y. Hirano, S. Murata, K. Kato, T. Yamane, and K. Tanaka. 2008. Crystal structure of a chaperone complex that contributes to the assembly of yeast 20S proteasomes. *Nat. Struct. Biol. Mol.* 15:228–236.
- Zhou, Y., R. M. Akers, and H. Jiang. 2008. Growth hormone can induce expression of four major milk protein genes in transfected MAC-T cells. *J. Dairy Sci.* 91:100–108.