J. Dairy Sci. 100:1–9
https://doi.org/10.3168/jds.2016-12159

Fatty acid elongase 6 plays a role in the synthesis of long-chain fatty acids in goat mammary epithelial cells

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ABSTRACT

In nonruminants, it is well established that elongation of very long-chain fatty acid-like fatty acid elongase 6 (ELOVL6) catalyzes the synthesis of C18:0 from C16:0 in lipogenic tissues like adipose and liver. However, the role of ELOVL6 in regulating lipid metabolism in ruminant mammary gland remains unknown. In the present study, *ELOVL6* was overexpressed or knocked down via adenoviral transfection to assess its role in goat mammary epithelial cells. Results revealed that *ELOVL6* overexpression had a weak effect on the expression of genes related to triacylglycerol (TAG) synthesis and desaturation. Overexpression of *ELOVL6* increased the content of C18:0 at the expense of C16:0, and increased the elongation index of C16:0. Overexpression of *ELOVL6* had no significant effect on the elongation index of C16:1n-7 and the desaturation indices of C16:0 and C18:0. Knockdown of *ELOVL6* had a negative effect on mRNA expression of the esterification genes *GPAM* and diacylglycerolacyltransferase 2 (*DGAT2*) and TAG concentration; however, it increased the concentration of C16:0 and decreased C18:1n-7 and C18:n-9 in goat mammary epithelial cells. Accordingly, downregulation of *ELOVL6* significantly decreased the elongation indices of C16:0 and C16:1n-7. The lack of change in the desaturation indices of C16:0 and C18:0 upon knockdown of *ELOVL6* was consistent with the minor change in *SCD1* expression. In conclusion, these are the first results highlighting an important role of ELOVL6 in long-chain fatty elongation and TAG synthesis in ruminant mammary cells.

Key words: elongase, lactation, fatty acid composition, milk fat

INTRODUCTION

Fat composition is one of the most valuable components in terms of dairy production, in which the high proportion of PUFA contributes a special function for human health (Haenlein, 2004; Shingfield et al., 2008). In nonruminants, the fatty acid elongases are rate-limiting enzymes controlling the synthesis of long-chain fatty acids [**LCFA**; e.g., 16–18 carbons (Green and Olson, 2011)], some of which are substrates for the biosynthesis of PUFA. In the ruminant mammary gland, de novo fatty acid synthesis results in the production of short- and medium-chain fatty acids and a portion of 16:0 (Harvatine et al., 2009). Although the ruminant mammary gland secretes PUFA in milk, it is unknown if elongases play a role in this process.

In rodents, the elongation of very long-chain fatty acid-like fatty acid elongase family (**Elovl**) includes 7 isotypes, Elovl1 to Elovl7. Each elongase exhibits different fatty acid substrate preferences. The enzymes Elovl1, Elovl3, and Elovl4 catalyze a broad array of saturated and monounsaturated fatty acids containing up to 24 carbons (Matsuzaka and Shimano, 2009). Substrates of Elovl2 include PUFA with 20 and 22 carbons (Tvrdik et al., 2000). The Elovl5 is involved in the elongation of various PUFA containing 18 and 20 carbons (Green et al., 2010). The Elovl6 catalyzes the chain elongation of palmitate (C16:0) to stearate (C18:0) and the elongation of palmitoleate (C16:1n-7) to vaccinate (C18:1n-7); hence, its activity is closely associated with de novo fatty acid synthesis.

In mice, Elovl6 is highly expressed in lipogenic organs and is sensitive to dietary, hormonal, and developmental control (Matsuzaka and Shimano, 2009). The mRNA abundance of Elovl6 in liver and adipose tissue was markedly elevated upon refeeding fasting animals; however, dietary PUFA decreased Elovl6 profoundly (Matsuzaka et al., 2002). Loss of Elovl6 function in mice fed a high-sucrose diet increased the content of

Received October 15, 2016.
Accepted February 14, 2017.
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C16:0 and C16:1n-7, but reduced C18:0 and C18:1n-9 in the liver (Matsuzaka et al., 2007). Overexpression of Elovl6 increased synthesis of 18:0 and 18:1n-9 (Green et al., 2010). Collectively, the data from rodents underscore an important role for Elovl6 in the overall process of LCFA synthesis.

The recent demonstration that ELOVL6 is expressed in goat and bovine mammary tissue (Bionaz et al., 2012a,b; Shi et al., 2015) and its expression is altered by dietary lipid supplementation in cow mammary tissue (Leroux et al., 2016) suggested that ELOVL6 may play a role in fatty acid elongation. Compared with rodents and humans, the role of ELOVL6 in the ruminant mammary gland is not known. Whether ELOVL6 is essential for the synthesis and alteration of LCFA composition in ruminant mammary cells remains to be determined. We hypothesized that ELOVL6 has a role in the overall process of LCFA synthesis in goat mammary tissue. To assess the role of ELOVL6, both adenoviral-mediated RNA interference and overexpression were performed in goat mammary epithelial cells (**GMEC**).

MATERIALS AND METHODS

Adenovirus Generation

The whole process for generation and proliferation of recombinant adenovirus expressing ELOVL6 (Ad-ELOVL6) was carried out as previously described (Shi et al., 2013a). Briefly, the *ELOVL6* cDNA (GenBank no. KF667508) was subcloned into the adenoviral plasmic (**pAd**) Track-CMV plasmid vector to generate pAdTrack-CMV-ELOVL6 vectors. The vector was inserted into an adenoviral vector (pAdEasy-1) to generate adenoviral plasmids in BJ5183 cells. The adenoviral plasmids linearized by *Pac*I (New England Biolabs, Ipswich, MA) were transfected into 293A cells to generate the adenovirus pAd-ELOVL6.

To knock down *ELOVL6*, a short hairpin RNA sequence (**shELOVL6**; described in Supplemental Table S1; https://doi.org/10.3168/jds.2016-12159) was designed and synthesized with the *Xho*I and *Kpn*I restriction sites (Invitrogen Biotechnology Co. Ltd., Shanghai, China). The sequence was constructed into the pENTR/CMV-GFP/U6 vector and then switched into an adenoviral vector (pAd/PL-DEST) using the Gateway technique (Invitrogen, Carlsbad, CA) to generate the pAd-shELOVL6 vector. The P*ac* I linearized adenoviral plasmids were transfected into 293A cells to generate the adenovirus (Ad-shELOVL6). The process of Ad-shELOVL6 generation was described previously (Shi et al., 2013b).

Cell Culture

The GMEC were isolated from peak lactation Xinong Saanen goats as described previously (Hu et al., 2009; Wang et al., 2010; Shi et al., 2014). Details of cell culture were described recently (Lin et al., 2013; Shi et al., 2013b). Briefly, cells were incubated at 37°C in 5% CO_2 and air. Culture medium was composed of Dulbecco's modified Eagle medium/F12 (Hyclone, Beijing, China) containing insulin (5 mg/L, Sigma-Aldrich, St. Louis, MO), hydrocortisone (5 mg/L, Sigma), penicillin/streptomycin (10k unit/L, Harbin Pharmaceutical Group, China), epidermal growth factor (1 mg/L, Sigma-Aldrich), and fetal bovine serum (10%, Gibco, Waltham, MA). To promote lactogenesis, GMEC were cultured in medium described above plus prolactin (2 μg/mL, Sigma-Aldrich) for 24 h before initial experiments. The 293A cells for adenovirus generation were cultured in basal Dulbecco's modified Eagle medium (Gibco) containing 10% fetal bovine serum.

The GMEC at about 80% confluence were transfected with adenovirus supernatant (Ad-ELOVL6, Ad-shELOVL6, Ad-GFP, or Ad-shNC). The transfected GMEC were collected after 48 h of culture for lipid extraction, total RNA extraction, and triacylglycerol (**TAG**) analysis.

Total RNA Extraction and Quantitative Real-Time PCR

Total RNA from GMEC was extracted using the RNA Prep pure cell Kit (Tiangen Biotech Co. Ltd., Beijing, China) according to the manufacturer's protocol (http://www.tiangen.com/asset/imsupload/up0203129001467351047.pdf). Genomic DNA contamination was removed using DNase provided with the kit. Synthesis of cDNA was conducted using the PrimeScript RT kit (Takara Bio Inc., Otsu, Japan) according to the manufacturer's instructions (http://www.clontech.com/CN/Products/Real-Time_qPCR_and_Reverse_Transcription/Reagents_and_Standards/Two-Step_RT-qPCR/PrimeScript_RT_with_gDNA_Eraser?sitex=10022:22372:US). The quantitative real-time PCR (**qPCR**) was performed according to the manufacturer's instructions using SYBR Green (SYBR Premix Ex Taq II, Perfect Real Time, Takara Bio Inc.).

The synthesis of long-chain fatty acids is associated with desaturation and the resulting fatty acids are incorporated into TAG (Harvatine et al., 2009; Green et al., 2010). Thus, several genes related to TAG synthesis [diacylglycerolacyltransferase 1 (*DGAT1*), diacylglycerolacyltransferase 2 (*DGAT2*), glycerol-3-phosphate acyltransferase (*GPAM*), perilipin2 (*PLIN2*)] and fatty