ABSTRACT

Fatty liver is a major metabolic disorder of high-producing dairy cows during the transition period. In nonruminants, it is well established that insulin-induced gene 1 (INSIG1) plays a crucial role in regulating hepatic lipogenesis by controlling the anchoring of sterol regulatory element-binding protein 1 (SREBP-1) on the endoplasmic reticulum along with SREBP cleavage-activating protein (SCAP). Whether the INSIG1-SCAP-SREBP-1c transport axis is affected in cows experiencing fatty liver is unknown. Thus, the aim of this study was to investigate the potential role of INSIG1-SCAP-SREBP-1c axis in the progression of fatty liver in dairy cows. For in vivo experiments, 24 dairy cows at the start of their fourth lactation (median; range 3–5) and 8 d in milk (median; range 4–12 d) were selected into a healthy group [n = 12; triglyceride (TG) content <1%] and a severe fatty liver group (n = 12; TG content >10%) according to their hepatic TG content. Blood samples were collected for detecting serum concentrations of free fatty acids, β-hydroxybutyrate, and glucose. Compared with healthy cows, cows with severe fatty liver had higher serum concentrations of β-hydroxybutyrate and free fatty acids and lower concentration of glucose. Liver biopsies were used to detect the status of INSIG1-SCAP-SREBP-1c axis, and the mRNA expression of SREBP-1c-target lipogenic genes ACACA, FASN, and DGAT1 was greater in the liver of dairy cows with severe fatty liver. In vitro experiments were conducted on hepatocytes isolated from 5 healthy 1-d-old female Holstein calves, and hepatocytes from each calf were run independently. First, hepatocytes were treated with 0, 200, or 400 μM palmitic acid (PA) for 12 h. Exogenous PA treatment decreased INSIG1 protein abundance, enhanced the endoplasmic reticulum to Golgi export of SCAP–precursor SREBP-1c complex and the nuclear translocation of mature SREBP-1c, all of which was associated with increased transcriptional activation of lipogenic genes and TG synthesis. Second, hepatocytes were transfected with INSIG1-overexpressing adenovirus for 48 h and treated with 400 μM PA 12 h before the end of transfection. Overexpressing INSIG1 inhibited PA-induced SREBP-1c processing, upregulation of lipogenic genes, and TG synthesis in hepatocytes. Overall, the present in vivo and in vitro results indicated that the low abundance of INSIG1 contributes to SREBP-1c processing and hepatic steatosis in dairy cows. Thus, the INSIG1-SCAP-SREBP-1c axis may be a novel target for treatment of fatty liver in dairy cows.

Key words: fatty liver, insulin-induced gene 1, sterol regulatory element-binding protein 1c, SREBP cleavage-activating protein

INTRODUCTION

The liver plays a central and integrative role in the maintenance of lipid homeostasis. Proper coordination of synthesis, storage, secretion, and oxidation of fatty acids is essential to prevent abnormal lipid accumulation that can result in fatty liver disease (Su et al., 2019; Stefan and Cusi, 2022). In the first month postpartum, 30% to 40% of dairy cows have moderate fatty liver, and about 10% have severe fatty liver, underscoring the high incidence of this metabolic disorder during early lactation (Bobe et al., 2004; Gross et al., 2013). Dairy
cows with moderate or severe fatty liver generally have decreased health status, milk production, and reproductive performance, causing huge economic losses (Fry et al., 2018). Thus, a better understanding of the molecular mechanisms controlling fatty liver in dairy cows and identification of feasible therapeutic targets are important to ensure profitability in the dairy industry.

Insulin-induced genes (INSIG1 and INSIG2) are anchor proteins of the endoplasmic reticulum (ER) with central roles in regulating lipogenesis (Dong and Tang, 2010). Studies in humans on the expression and distribution of INSIG revealed that INSIG1 is highly expressed in the liver, accounting for more than 75% of the total INSIG (Dong and Tang, 2010; Smith et al., 2010). In nonruminants, INSIG1 is intricately involved in lipid disorders and the progression of fatty liver (Ouyang et al., 2020). In ruminants, nearly all detailed studies on INSIG function were carried out in mammary tissue (Bionaz and Loor, 2008; Li et al., 2019), with data indicating that INSIG1 and INSIG2 isoforms synergistically regulate lipogenesis in mammary epithelial cells (Li et al., 2019). Although studies several decades ago reported a low capacity of the ruminant liver for lipogenesis (Hanson and Ballard, 1967), studies in the last 2 decades (van Dorland et al., 2009; Khan et al., 2014) have demonstrated alterations in the hepatic mRNA abundance for several lipogenic genes during the transition into lactation. However, it remains to be determined whether INSIG1 is involved in regulating this process in the ruminant liver.

Studies in nonruminants indicated that INSIG1 regulated lipogenesis by repressing the cleavage and activation of sterol regulatory element-binding protein-1c (SREBP-1c), a master lipogenic transcription factor (Engelking et al., 2004; Dong and Tang, 2010). SREBP-1c is synthesized as an inactive precursor SREBP-1c (pSREBP-1c) in the ER (Ferré and Foufelle, 2007, 2010). Newly synthesized pSREBP-1c forms a complex with SREBP cleavage-activating protein (SCAP), an ER-to-Golgi transport protein required for SREBP-1c processing (Radhakrishnan et al., 2007). After export from the ER to Golgi, pSREBP-1c is processed sequentially, and the transcriptionally active fragments of SREBP-1c are released. The released mature SREBP-1c (mSREBP-1c) fragments enter the nucleus where they activate transcription of genes necessary for lipogenesis (Wang et al., 2015). Transcriptional and cleavage activation of SREBP-1c are proven triggers of hepatic steatosis in dairy cows with fatty liver (Li et al., 2014; Zhu et al., 2019). However, the mechanistic details of the cleavage activation of SREBP-1c by SREBP-1c processing in cows with fatty liver and the potential role of INSIG1 in this process is still unknown. We speculate that INSIG1 may contribute to hepatic steatosis by regulating SREBP-1c processing in dairy cows with severe fatty liver.

**MATERIALS AND METHODS**

**Animals and Sampling**

Experimental animal protocols were approved by the Ethics Committee for Animal Care and Use of Jilin University (permit number: SY202209007; Changchun, Jilin, China). The animals received humane care according to Laboratory animal—The guidelines for ethical review of animal welfare (GB/T 35892–2018, China).

Dairy cows used in the current study were selected from a dairy farm located in Bayan Nur, Inner Mongolia, China. All cows were housed in a climate-controlled barn with individual tie stalls and received a total mixed ration for ad libitum access. Cows eligible for selection (n = 76) were free of comorbidities, at the start of their fourth lactation (median; range 3–5), and 8 DIM (median; range 4–12 DIM). Liver samples were obtained via liver biopsy, and the hepatic triglyceride (TG) contents were measured. Cows with hepatic TG content higher than 10% were classified as having severe fatty liver, whereas those below 1% were classified as healthy. After screening, 12 cows with severe fatty liver and 12 healthy cows were randomly selected. Dry matter intake was calculated as the difference between daily feed offered and feed refused for the last 3 d before biopsy. Body condition score was assigned based on a 1- to 5-point scale as described in a previous study (Edmonson et al., 1989). The basic physiological parameters of cows are provided in Table 1.

Blood samples were collected via coccygeal venipuncture (without anticoagulant) between 0730 and 0830 before the morning feeding on 3 consecutive days. Serum was separated within an hour by centrifugation at 3,500 × g for 15 min at 4°C. Concentrations of free fatty acids (FFA), BHB, and glucose were determined using a Hitachi 7170 autoanalyzer (Hitachi) with commercially available kits (BHB: RB1008; FFA: FA115; glucose: GL3815; Randox Laboratories). Liver samples were obtained via liver biopsy by an experienced veterinarian and then fixed with 4% paraformaldehyde or immediately frozen in liquid nitrogen.

**Isolation and Culture of Calf Primary Hepatocytes**

A total of 5 calves were used to isolate hepatocytes. Primary hepatocytes were isolated from 1-d-old female Holstein calves (healthy, 30–40 kg, fasting) as previously described (Zhu et al., 2019), with modifications.
Immediately after the calves were anesthetized, the caudate liver lobe was aseptically removed through surgical hepatectomy. The excised caudate lobe was used for subsequent hepatocyte isolation under sterile conditions. Perfusion solution A (140 mM NaCl, 6.7 mM KCl, 10 mM HEPES, 2.5 mM glucose, and 0.5 mM EDTA, pH 7.4; 37°C, 50 mL/min for 10–15 min) and perfusion solution B (140 mM NaCl, 6.7 mM KCl, 30 mM HEPES, 2.5 mM glucose, and 5 mM CaCl₂, pH 7.4; 37°C, 50 mL/min for 3 min) were used to perfuse the liver until the effluent became clear. Subsequently, the liver was digested with collagenase IV solution (0.1 g of collagenase IV solution (17104019; Gibco) dissolved in 0.5 L of perfusion solution B, pH 7.2–7.4) at a flow rate of 20 mL/min for 15 to 20 min. The liver was then moved to a sterile flat plate with fetal bovine serum (FBS; FB15015; Clark Bioscience) to terminate the digestion. After that, the liver was cut open to release hepatocytes, and the hepatic capsule, connective tissue, blood vessels, and incompletely digested liver were removed. The suspension was filtered sequentially with 100-μm (CLS431752; Corning) and 70-μm (CLS431751; Corning) cell strainers. The obtained hepatocyte suspension was washed twice with cold RPMI-1640 medium (SH30027.02; Hyclone Laboratories) and centrifuged at 500 × g for 5 min at 4°C. The cell pellets were resuspended using adherent medium (RPMI-1640 medium containing 10% FBS, 10⁻⁶ M insulin, 10⁻⁶ M dexamethasone, 10 μg/mL vitamin C). Cells were then seeded into a 6-well culture plate (1 × 10⁶ cells per well) and cultured in a 37°C incubator with 5% CO₂ and humidified atmosphere. After 4 h, the adherent medium was replaced with growth medium (RPMI-1640 medium containing 10% FBS). All cells were harvested 72 h after isolation.

**PA Preparation and Cell Treatment**

The 100 mM stock solution of palmitic acid (PA) was prepared by dissolving the PA (P0500; Sigma-Aldrich) in 0.1 M NaOH at 70°C until clear and then complexed to 10% fatty acid–free BSA (V900933; Sigma-Aldrich) at 55°C for 10 min. The working solution was prepared by diluting stock solution with RPMI-1640 medium containing 2% BSA. An equivalent amount of BSA was administered to the control group.

Hepatocytes were isolated from each calf independently and were not cocultured. The concentration of PA used in this study was based on hematology standards of dairy cows with fatty liver (Rukkwamsuk et al., 1999, 2000). To detect the concentration-dependent effect of PA on INSIG1 expression, SREBP-1c processing and lipogenesis, and the optimal concentration for in vitro culture, hepatocytes were treated with 0, 200, or 400 μM PA for 12 h in RPMI-1640 medium containing 2% BSA. To gain better insight into whether and how INSIG1 participates in fatty liver disease in dairy cows in vitro, hepatocytes were transfected with Ad-INSIG1 at a multiplicity of infection of 50 for 48 h and treated with 400 μM PA for 12 h before the end of transfection.

**Measurement of TG Content**

The TG content was detected using the TG content assay kit (E1013; Applygen Technologies Inc.) according to the manufacturer’s instructions (https://www.applygen.com/upload/download/20210713/20210713024705477.pdf). For liver samples, approximately 50 mg of liver tissue was chopped and homogenized in 1 mL of lysis buffer. For cell samples, every 1 × 10⁷ hepatocytes were lysed with 1 mL of lysis buffer. After lysis, a total of 20-μL supernatant was pipetted for detecting the protein concentration using the bicinchoninic acid assay (BCA) regent (P1511; Applygen Technologies Inc.). The remaining lysate was heated at 70°C for 10 min and then centrifuged at 800 × g for 5 min at 4°C. Then 10 μL of supernatant was incubated with 190 μL of chromogenic liquid at 37°C for 15 min. The absorbance was detected at 550 nm using a Microplate Reader (Thermo Scientific). The TG values of liver samples were expressed as a percentage of wet.

### Table 1. The basic physiological parameters of the healthy and severe fatty liver cows

<table>
<thead>
<tr>
<th>Item</th>
<th>Healthy (n = 12)</th>
<th>Severe fatty liver (n = 12)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG content (% wet weight)</td>
<td>0.85 ± 0.03</td>
<td>11.85 ± 0.42</td>
<td>0.000</td>
</tr>
<tr>
<td>BW (kg)</td>
<td>634.00 ± 6.83</td>
<td>655.93 ± 9.03</td>
<td>0.066</td>
</tr>
<tr>
<td>BCS</td>
<td>3.63 ± 0.04</td>
<td>3.79 ± 0.07</td>
<td>0.061</td>
</tr>
<tr>
<td>DMI (kg/d)</td>
<td>20.93 ± 0.45</td>
<td>18.94 ± 0.57</td>
<td>0.013</td>
</tr>
<tr>
<td>BHB (mM)</td>
<td>0.67 ± 0.05</td>
<td>2.08 ± 0.08</td>
<td>0.000</td>
</tr>
<tr>
<td>FFA (mM)</td>
<td>0.36 ± 0.04</td>
<td>0.89 ± 0.04</td>
<td>0.000</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td>4.11 ± 0.08</td>
<td>2.85 ± 0.11</td>
<td>0.000</td>
</tr>
</tbody>
</table>

FFA = free fatty acids; TG = triglyceride. The data were analyzed using the 2-tailed unpaired Student t-test. The data are presented as means ± SEM.
weight. The TG values of cell samples were normalized to protein concentrations and expressed as micromoles per milligram of protein.

**Protein Extraction and Western Blotting**

Protein abundance of INSIG1 in the ER fraction, SCAP and pSREBP-1c in the Golgi fraction, and mSREBP-1c in the nuclear fraction were determined by western blotting. Total proteins were extracted from liver tissue or cultured primary hepatocytes using a commercial lysis buffer (P0013J; Beyotime Institute of Biotechnology) according to the manufacturer's instructions (https://www.beyotime.com/product/P0013J.htm). The ER, Golgi, and nuclear fractions used for western blotting were isolated from fresh liver tissue or hepatocytes using an ER isolation kit (ER-036; Invent Biotechnologies), a Golgi isolation kit (GO-037; Invent Biotechnologies), or a nuclear isolation kit (SC-003 or NT-032; Invent Biotechnologies) according to the manufacturer's instructions (ER-036: https://www.inventchina.cn/pro_view.asp?id=122&class=2; GO-037: https://www.inventchina.cn/pro_view.asp?id=123&class=2; SC-003: https://www.inventchina.cn/pro_view.asp?id=87&class=2; NT-032: https://www.inventchina.cn/pro_view.asp?id=110&class=2). The BCA reagent (P1511; Applygen Technologies Inc.) was used to determine protein concentrations for standardizing the quantity of protein loaded onto each lane. Protein from each sample was individually mixed with loading buffer and denatured at 95°C for 8 min. Then proteins were separated in SDS-polyacrylamide gels and transferred onto polyvinylidene fluoride membranes. Membranes were then blocked in 5% BSA/Tris-buffered saline-Tween at room temperature for 1 h. Blots were incubated with anti-INSIG1 antibody (1:500; ab70784; Abcam), anti-Calnexin antibody (1:500; sc-23954; Santa Cruz Biotechnology), anti-SCAP antibody (1:1,000; ab190103; Abcam), anti-GM130 antibody (1:1,000; ab52649; Abcam), anti-SREBP-1c antibody (1:500; NB600–582 or NB100–2215; Novus Biologicals), anti-β-actin antibody (1:2,000; ab8226; Abcam), or anti-Lamin B1 antibody (1:1,000; ab16048; Abcam) overnight at 4°C, followed by incubation with the corresponding secondary antibody at room temperature for 45 min. Immunoreactive bands were visualized by enhanced chemiluminescence solution (WBULS0500; Millipore) and imaged using an automatic chemiluminescence image analysis system (Tanon). All bands were quantified using Image-Pro Plus 6.0 (Media Cybernetics) and normalized to the corresponding loading control (total protein: β-actin; ER fraction: Calnexin; Golgi fraction: GM130; nuclear fraction: Lamin B1).

**RNA Extraction and Quantitative Real-Time PCR Assay**

Total RNA was extracted from liver samples or cultured primary hepatocytes using RNAiso Plus (D9108; TaKaRa Biotechnology) according to the manufacturer's instructions (https://www.taakarabiomed.com.cn/Download/9108-9109.pdf). The purity of RNA was assessed by A260/A280 and A260/A230 ratios, and the results indicated that all total RNA used in the present study was pure and free from protein and organic pollutants. The RNA integrity was assessed by gel electrophoresis, and the results showed clear 18S and 28S ribosomal RNA bands, with the 28S rRNA band approximately twice as intense as the 18S rRNA band, indicating intact RNA. The concentration of purified total RNA was determined by UV spectrometer at 260 nm. cDNA was reverse-transcribed from 1 μg purified total RNA using a reverse transcription kit (RR047A; TaKaRa Biotechnology). The relative mRNA expression was determined via quantitative real-time PCR technology using the TB Green reagent kit (RR82LR; TaKaRa Biotechnology) on the 7500 Real-Time PCR System (Applied Biosystems Inc.). The primer sequences are listed in Supplemental Table S1 (https://doi.org/10.6084/m9.figshare.22106147.v1; Zhu et al., 2023). The reaction conditions were as follows: 95°C for 3 min; 95°C for 15 s, 35 cycles; 60°C for 1 min. The relative expression of target genes was normalized to reference genes (ACTB and GAPDH) using the 2^−ΔΔCq method.

**Oil Red O Staining**

Hepatocytes were washed 3 times in PBS and then fixed with 4% paraformaldehyde for 15 min. Subsequently, hepatocytes were incubated with 60% isopropanol for 10 s, dried, and stained with Oil Red O working solution for 10 to 15 min. Hepatocytes were washed with 60% isopropanol once for 3 s and PBS twice for 5 min. Then hepatocytes were counterstained with hematoxylin for 6 to 8 min. The features were observed and captured under a light microscope (Olympus). Oil Red O stock solution was prepared by dissolving 0.35 g of Oil Red O powder in 100 mL of isopropanol. Oil Red O working solution was prepared by diluting Oil Red O stock solution with distilled water at a ratio of 3:2.

**Statistical Analysis**

Data from multiple repeats (at least 3 times) were analyzed using the appropriate statistical analysis methods with the Statistical Package for the Social Sciences 19.0 software (SPSS Inc.). All data were test-
ed for normality and homogeneity of variance using the Shapiro-Wilk and Levene tests, respectively. Statistical significance was calculated using the 2-tailed unpaired Student t-test or ANOVA with Bonferroni correction. Data are presented as the means ± standard error of the mean. A $P$-value < 0.05 was considered significant.

**RESULTS**

**Cows with Severe Fatty Liver Had Lower Protein Abundance of Hepatic INSIG1 and Increased Processing of SREBP-1c**

Compared with healthy cows, hepatic TG content ($P < 0.01$) was higher and DMI ($P < 0.05$) was lower in dairy cows with severe fatty liver (Table 1). Serum concentrations of BHB ($P < 0.01$) and FFA ($P < 0.01$) were higher and glucose ($P < 0.01$) was lower in cows with severe fatty liver than in healthy cows (Table 1).

Western blotting results showed that the protein abundance of INSIG1 in the ER fraction was lower in the livers of cows with severe fatty liver than in those of healthy cows (Figure 1A; $P < 0.05$). In contrast, the protein abundance of SCAP ($P < 0.01$) and pSREBP-1c ($P < 0.01$) in the Golgi fraction and mSREBP-1c ($P < 0.01$) in the nuclear fraction were greater in the livers of cows with severe fatty liver relative to those of healthy cows (Figure 1A). Furthermore, the mRNA expression of SREBP-1c-target lipogenic genes *ACACA* ($P < 0.01$), *FASN* ($P < 0.01$), and *DGAT1* ($P < 0.05$) was greater in the livers of cows with severe fatty liver than in those of healthy cows (Figure 1B).
PA Reduced INSIG1 Protein Abundance and Induced SREBP-1c Processing and TG Synthesis in Calf Hepatocytes

Calf hepatocytes were treated with different concentrations of PA (0, 200, or 400 μM) for 12 h. In the ER fraction, the protein abundance of INSIG1 was decreased by PA treatment (Figure 2A; *P < 0.05 or **P < 0.01). In addition, Golgi-localized SCAP (*P < 0.01) and pSREBP-1c (*P < 0.01) and nuclear-localized mSREBP-1c (*P < 0.01) were increased upon PA treatment (Figure 2A). Following treatment with PA, the mRNA expression of SREBP-1c-target lipogenic genes ACACA (*P < 0.01), FASN (*P < 0.05 or **P < 0.01), and DGAT1 (*P < 0.01) was increased (Figure 2B). Furthermore, TG content was also increased by PA treatment (Figure 2C; *P < 0.05 or **P < 0.01).

Overexpressing INSIG1 Altered Lipid Metabolism in Calf Hepatocytes

To gain better insight into whether and how INSIG1 participates in fatty liver disease in dairy cows, calf hepatocytes were transfected with Ad-INSIG1 for 48 h and treated with 400 μM PA for 12 h before the end of transfection. Ad-INSIG1 transfection significantly increased the protein abundance of INSIG1 in ER fraction either with or without PA treatment (Figure 3A; *P < 0.01). In comparison with the PA plus Ad-Control group, the protein abundance of SCAP (*P < 0.01) and
pSREBP-1c (P < 0.01) in the Golgi fraction and mSREBP-1c (P < 0.01) in the nuclear fraction was lower in the PA plus Ad-INSIG1 group (Figure 3A). The mRNA expression of SREBP-1c-target lipogenic genes ACACA (P < 0.05), FASN (P < 0.05), and DGAT1 (P < 0.01) was lower in the PA plus Ad-INSIG1 group than in the PA plus Ad-Control group (Figure 3B). Similarly, the TG content was significantly lower in the PA plus Ad-INSIG1 group (Figure 4A; P < 0.01). The decrease in lipid accumulation was also confirmed by Oil Red O staining where INSIG1 overexpression reduced PA-induced lipid droplet formation (Figure 4B).

DISCUSSION

Fatty liver is a major metabolic disorder during the transition period, especially in overconditioned cows that experience greater decreases in DMI and more pronounced negative energy balance (NEB; Herdt, 1988). Fat mobilization due to NEB causes high blood
concentrations of FFA, which are the pathological basis of fatty liver in dairy cows owing to their re-esterification into TG within hepatocytes (Bobe et al., 2004). Palmitic acid is a representative saturated fatty acid released during lipolysis (Rukkwamsuk et al., 2000) and accounts for around 30% of all FFA (Contreras et al., 2010). In this study, the amount of TG revealed a clear state of hepatic steatosis in cows with severe fatty liver. The decreased DMI, lower serum glucose concentration, and higher serum FFA concentrations in cows with severe fatty liver compared with healthy cows indicated they suffered a more pronounced NEB.

In nonruminants, SREBP-1c is a central transcription factor that regulates lipogenesis and is synthesized as an inactive pSREBP-1c in the ER. It requires transport to the Golgi with the escort protein SCAP and then cleavage to mSREBP-1c before import into the nucleus to activate the transcription of lipogenic genes (Brown and Goldstein, 1997; McPherson and Gauthier, 2004). It is well established that activated SREBP-1c aggravated lipogenesis and contributed to the development of fatty liver (Li et al., 2014; Zhu et al., 2019). In the present study, we also observed that the cleavage activation of SREBP-1c and subsequent transcription of its downstream lipogenic target genes were enhanced in cows with severe fatty liver.

SREBP-1c activation requires the removal of INSIG1, which serves as a retention protein of the SCAP-pSREBP complex in the ER (Yang et al., 2002; Dong and Tang, 2010). A substantial and growing body of evidence from humans and mice demonstrated that low expression of INSIG1 was associated with hepatic steatosis (Azzu et al., 2021; Zhao et al., 2021). Specifically, mice subjected to a high-fat diet had lower hepatic INSIG1 expression in parallel with hepatic steatosis (Zhao et al., 2021). Investigations in transgenic mice (Engelking et al., 2004; McPherson and Gauthier, 2004) and Zucker diabetic rats (Takaishi et al., 2004) demonstrated that overexpression of hepatic INSIG1 inhibits SREBP-1c processing and reduces hepatic lipogenesis. Additional support for a biologic role of INSIG1 at the onset of fatty liver can be discerned through the cleavage activation of SREBP-1c and accumulation of TG in hepatocytes upon deficiency of INSIG1 mRNA, as achieved with RNA interference (Adams et al., 2003, 2004; Sever et al., 2003) or by mutational inactivation in cultured hamster cells (Lee et al., 2005). In addition, studies in ruminant mammary tissue underscored the potential importance of INSIG1 in aspects related to milk fat synthesis (Li et al., 2019; Chen et al., 2021). In the present study, we provide evidence for the low abundance of INSIG1 in the liver of dairy cows with severe fatty liver. These results underscored a possible role of INSIG1 in regulating SREBP-1c processing and hepatic steatosis in the liver of dairy cows.

A previous study showed that an overload of PA decreases the protein abundance of INSIG1 in bovine mammary epithelial cells (Chen et al., 2021). Here,
we demonstrated that PA had a dose-dependent effect and decreased INSIG1 protein abundance in hepatocytes. Furthermore, PA treatment enhanced SREBP-1c processing and subsequent transcription of its downstream lipogenic genes, ultimately increasing TG synthesis. Based on these results, we speculate that PA promoted lipid accumulation via mediating the INSIG1-SCAP-SREBP-1c transport axis in calf hepatocytes. To further investigate this, INSIG1 was overexpressed in hepatocytes, and then cells were challenged with PA. In line with previous studies in mice (Engelking et al., 2004; McPherson and Gauthier, 2004; Takeishi et al., 2004), our results showed that overexpression of INSIG1 inhibited PA-induced SREBP-1c processing and upregulation of lipogenic genes. Simultaneously, in response to INSIG1 overexpression, the PA-induced lipid accumulation was mitigated.

Taken together, the present study indicates that PA-induced low abundance of INSIG1 favors SREBP-1c processing and the transcription of lipogenic genes, thereby exacerbating lipid accumulation in hepatocytes. Given that hepatic steatosis in cows with severe fatty liver is accompanied by high concentrations of PA, low abundance of INSIG1, and increased SREBP-1c processing, we speculate that low abundance of INSIG1 contributes to the occurrence and development of fatty liver in dairy cows by driving SREBP-1c processing. Further research on the exact regulatory mechanisms linking INSIG1 and SREBP-1c processing is required. The present study extends the function of INSIG1 as a suppressor of SREBP-1c processing in response to PA lipotoxicity in dairy cows and highlights the INSIG1-SCAP-SREBP-1c axis as a promising target for fatty liver therapy.

CONCLUSIONS

In the present study, severe fatty liver was associated with low abundance of hepatic INSIG1 and enhanced SREBP-1c processing. Palmitic acid decreased INSIG1 protein abundance and enhanced the ER-to-Golgi export of SCAP-pSREBP-1c complex, the nuclear translocation of mSREBP-1c, and the transcription of its downstream lipogenic genes, all of which led to lipid accumulation in hepatocytes. More importantly, overexpression of INSIG1 inhibited PA-induced SREBP-1c processing, upregulation of lipogenic genes, and lipid accumulation. Overall, our data indicated that the low abundance of INSIG1 contributes to SREBP-1c processing and leads to hepatic steatosis in dairy cows. Thus, the INSIG1-SCAP-SREBP-1c axis may be a novel target for the prevention and treatment of fatty liver in dairy cows.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (Beijing, China; grant nos. 32022084 and 32172927), the Science Foundation of Education Department of Jilin Province (Changchun, China; grant no. JJKH2022104IKJ), and the Fundamental Research Funds for the Central Universities (Jilin, China). The authors have not stated any conflicts of interest.

REFERENCES

Zhu et al.: INSIG1 AND HEPATIC LIPIDOSIS


ORCIDS

Yiwei Zhu https://orcid.org/0000-0001-9789-7229
Lin Lei https://orcid.org/0000-0001-5740-1508
Xinghui Wang https://orcid.org/0000-0001-9269-6161
Qiamming Jiang https://orcid.org/0000-0001-9522-1856
Jian J. Loor https://orcid.org/0000-0003-1586-4365
Fanrong Kong https://orcid.org/0000-0003-0777-3324
Guowen Liu https://orcid.org/0000-0002-2672-6104
Xinwei Li https://orcid.org/0000-0002-0765-677X