Role of diacylglycerol O-acyltransferase 1 (DGAT1) in lipolysis and autophagy of adipose tissue from ketotic dairy cows

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ABSTRACT

High-yielding dairy cows in early lactation often encounter difficulties in meeting the energy requirements essential for maintaining milk production. This is primarily attributed to insufficient dry matter intake, which consequently leads to sustained lipolysis of adipose tissue. Fatty acids released by lipolysis can disrupt metabolic homeostasis. Autophagy, an adaptive response to intracellular environmental changes, is considered a crucial mechanism for regulating lipid metabolism and maintaining a proper cellular energy status. Despite its close relationship with aberrant lipid metabolism and cyto-lipotoxicity in animal models of metabolic disorders, the precise function of diacylglycerol O-acyltransferase 1 (DGAT1) in bovine adipose tissue during periods of negative energy balance (NEB) is not fully understood. Particularly regarding its involvement in lipolysis and autophagy. The objective of the present study was to assess the impact of DGAT1 on both lipolysis and autophagy in bovine adipose tissue and isolated adipocytes. Adipose tissue and blood samples were collected from cows diagnosed as clinically ketotic (n = 15) or healthy (n = 15) following a veterinary evaluation based on clinical symptoms and serum concentrations of BHB, which were 3.19 mM (interquartile range = 0.20) and 0.50 mM (interquartile range = 0.06), respectively. Protein abundance of DGAT1 and phosphorylation levels of unc-51-like kinase 1 (ULK1), were greater in adipose tissue from cows with ketosis, whereas phosphorylation levels of phosphoinositide 3-kinase (PI3K), protein kinase B (AKT), and mammalian target of rapamycin (mTOR) were lower. Furthermore, when adipocytes isolated from the harvested adipose tissue of 15 healthy cows were transfected with DGAT1 overexpression adenovirus or DGAT1 small interfering RNA followed by exposure to epinephrine (EPI), it led to greater ratios and protein abundance of phosphorylated hormone-sensitive triglyceride lipase (LIPE) to total LIPE and adipose triglyceride lipase (ATGL), while inhibiting the protein phosphorylation levels of ULK1, PI3K, AKT, and mTOR. Overexpression of DGAT1 in EPI-treated adipocytes reduced lipolysis and autophagy, whereas silencing DGAT1 further exacerbated EPI-induced lipolysis and autophagy. Taken together, these findings indicate that upregulation of DGAT1 may function as an adaptive response to suppress adipocytes lipolysis, highlighting the significance of maintaining metabolic homeostasis in dairy cows during periods of NEB.

Keywords: DGAT1, bovine adipocytes, lipolysis, autophagy

INTRODUCTION

Dairy cows frequently encounter a state of negative energy balance (NEB) during the early postpartal period, which arises when the energy demands for milk production surpass the energy consumed largely due to sluggish DMI (de Vries and Veerkamp, 2000). Adipose tissue plays a crucial role in regulating energy metabolism during NEB by releasing free fatty acids (FFA) through lipolysis (Gross et al., 2013). Lipolysis is the process of enzymatically breaking down stored triacylglycerol (TAG) in adipose tissue, resulting in the generation of FFA and glycerol (Birsoy et al., 2013, Huang et al., 2021). Excessive lipolysis leads to accumulation of FFA, often surpassing the liver's capacity for oxidation and metabolism, which ultimately causes cellular lipotoxicity (Zechner et al., 2017). While recent studies have established a correlation between peripartal
metabolic disorders in dairy cows and disturbances in lipid metabolism associated with lipolysis (McFadden, 2020), our understanding of the specific therapeutic targets and cellular mechanisms governing lipolysis remains limited.

Autophagy has recently emerged as a critical mechanism for regulating lipid metabolism and maintaining energy homeostasis (Singh et al., 2009), it is a highly conserved cellular process that involves the degradation of cytoplasmic components through lysosomal mechanisms, serving to meet the cell's metabolic requirements and facilitate organelle renewal (He and Klionsky, 2009). In the context of adipose tissue, at least in non-ruminants, autophagy is essential for preserving mitochondrial functionality, facilitating the turnover of lipid droplets, and enhancing the differentiation process of adipocytes (Baerga et al., 2009, Zhang et al., 2012). Studies have demonstrated that autophagy is activated during nutrient deprivation in the peripartal period, including during hyperketonemia and fatty liver in dairy cows (Du et al., 2017, Li et al., 2020b). Recent research has also highlighted the involvement of autophagy in regulating FFA during lipolysis, thereby preventing the lipotoxic effects associated with excessive FFA accumulation (Cingolani and Czaja, 2016, Nguyen et al., 2017). Whether lipolysis and autophagy within adipose tissue during NEB are functionally related is unknown.

Diacylglycerol O-acyltransferase 1 (DGAT1) is a crucial enzyme responsible for TAG biosynthesis and storage in adipose tissue (Chen and Farese, 2005). Recent research has revealed that DGAT1 not only has significant implications for lipid synthesis, but also has a regulatory role in autophagy (Nguyen et al., 2017). However, the precise mechanisms by which DGAT1 affects these processes remain unclear, particularly during NEB. Our hypothesis was that DGAT1 participates in the regulation of adipose tissue lipolysis during the development of ketosis, while disturbances in autophagy contribute to the pathogenesis of ketosis.

**MATERIALS AND METHODS**

**Animals**

The animal experiments were performed in accordance with the guidelines outlined for the ethical treatment and utilization of experimental animals at Heilongjiang Bayi Agricultural University (DWKJXY2023019). The experiment involved selecting Holstein dairy cows from a commercial 6,000 cow dairy farm (Nenjiang, Heilongjiang, China). A total of 150 lactating Holstein cows (number of lactations: median = 3, range = 2 to 4; DIM: median = 17 d, range = 12 to 21 d) were tested between September and October 2022. The goal was to identify ketotic and healthy cows without other complications such as hypocalcemia and mastitis. The veterinarians evaluated the body condition of cows using a 5-point scale (Ferguson et al., 1994). Cows testing positive for sodium nitroprusside ketone bodies in milk were classified by veterinarians as suspected clinically ketotic. Based on the serum concentration of BHB and consideration of clinical symptoms, we randomly selected 15 cows with serum BHB concentrations above 3 mM for the clinical ketosis group, and 15 cows with serum BHB concentrations below 1.2 mM for the healthy control group. (Vanholder et al., 2015). Blood samples were collected by jugular venipuncture using anticoagulant-free blood collection tubes before feeding. After allowing samples to clot at room temperature for 2 h, they were centrifuged at 1900 × g for 15 min at 4°C to separate the serum. Serum glucose, fatty acid, and BHB concentrations were measured using a Hitachi 7170 automatic analyzer (Hitachi laboratory measurement, Tokyo, Japan) and commercial kits from Randox Laboratories (Crumlin, Northern Ireland) (glucose: Cat. No. GL3815; fatty acids: Cat. No. FA115; BHB: Cat. No. RB1008). Physiological parameters of ketotic and healthy cows are presented in Table 1. All cows involved in this study were fed according to National Research Council recommendations during the dry and lactation periods (NRC, 2001).

**Adipose Tissue Collection and Processing.** Subcutaneous adipose tissue samples were obtained from the tail-head region of cows at 17 (±4) days postpartum following previously described methods (Xu et al., 2019b). Briefly, the tail-head and its surrounding fur were thoroughly cleaned with surgical soap. Local anesthesia was administered in the area between the ischium and tailbone. A scalpel incision, approximately 5 cm long, was made, and the skin was pulled using sterile hemostats to facilitate tissue collection. Adipose tissue samples weighing 1 to 2 g were obtained through blunt dissection using sterile forceps and surgical scissors. Sterile gauze was applied for compression hemostasis to prevent external bleeding. The incision made for sampling was closed using 6 to 8 surgical staples (Henry Schein). The collected adipose tissue samples were rinsed with sterile PBS buffer solution. Samples intended for total protein extraction were weighed and stored in cryovials under liquid nitrogen. Adipose samples for preadipocyte isolation were stored in culture media bottles containing sterile PBS buffer supplemented with 2,500 U/mL penicillin and 2,500 mg/mL streptomycin.

To isolate primary preadipocytes from healthy cow adipose tissue, we followed our previously established method (Xu et al., 2019a, Xu et al., 2021). In brief, the collected adipose tissue samples from 15 cows were...
digested with using a collagenase type I digestion solution (1 mg/mL; Sigma-Aldrich) to obtain vascular stromal cells. Ammonium perchloride-potassium lysate buffer (Solarbio, Beijing, China) was added to eliminate the residual red blood cells in cell pellet, and then repeat the previous centrifugation step. After discarding supernatant, the resulting pellet was resuspended in a basic culture medium (BCM) containing 10% fetal bovine serum (Catalog No.10091148; Gibco), DMEM/F12 (HyClone, Logan, Utah), and 1% streptomycin and penicillin mixture. The cells were subsequently incubated in a cell culture incubator with 5% CO2 at a temperature of 37°C for a duration of 24 h.

**Cell Differentiation and Treatment**

To initiate the differentiation of preadipocytes into mature adipocytes, preadipocytes in a 6-well plate were cultured in differentiation culture medium 1, containing 1 μM dexamethasone (Sigma-Aldrich), 0.5 mM 3-Isobutyl-1-methylxanthin (Sigma-Aldrich), 1 μg/mL insulin (Sigma-Aldrich) and in BCM. After 4 d, cells were cultured in differentiation culture medium 2 containing 1 μg/mL insulin. After 2 d, cells were cultured with BCM for another 2 d (Xu et al., 2019a). After differentiation, mature adipocytes were treated with epinephrine (EPI; Sigma-Aldrich) at concentrations of 1 μM for 2 h (Xu et al., 2021b). To investigate the role of DGAT1 in lipolysis, we regulated the expression of DGAT1 in adipocytes and following specific steps as described previously (Xu et al., 2022). In brief, overexpression was induced by transfection of DGAT1 adenovirus (adDGAT1) with a titer of 50 MOI and empty vector (EV) adenovirus for 48 h. Silencing of DGAT1 was achieved by transfecting 100 pmol of DGAT1 small interfering RNA (siDGAT1) and a negative control vector (NC) for 6 h. Both DGAT1 overexpression adenovirus and the empty adenovirus vector were designed and constructed by Hanbio Biotechnology Co. Ltd. The siDGAT1 was synthesized by Hanbio Biotechnology. The siDGAT1 sequences were as follows: sense 5′ACUUGAGAGGUUCUCUAGTT3′ and antisense 5′CUAGAGAACCUACAUAGUATT3′. The siRNA negative control vector was synthesized by Hanbio Biotechnology, and its sequences were as follows: sense 5′UUUCUCGGAACGUACGUTT3′ and antisense 5′ACGUGACCUUCCGAGATT3′.

**Western Blotting.** Total protein from both adipose tissue of ketotic and healthy cows (n = 15 cows per group) and isolated adipocytes (n = 6 replicates per group) was extracted using a Total Protein Extraction Kit (C510003–0050, Sangon Biotech, Shanghai, China) (Xu et al., 2021). Briefly, 200 mg adipose tissue dissolved in 200 μL pre-cooled (4°C) lysis buffer containing phosphatase inhibitor, protease inhibitor and phenylmethylsulfonyl fluoride was homogenized by cryo-milling using a tissue mixer mill at 30 Hz for 2 min (MM400; Retsch, Haan, Germany). Adipose tissue homogenates were then centrifuged at 4°C, 15,000 × g for 10 min, and the resulting supernatant was collected as the total protein extract from the adipose tissue.

For adipocytes, the culture medium was discarded, the remaining culture medium washed with 4°C pre-cooled PBS buffer, then 200 μL of the above-mentioned pre-cooled lysis buffer were added, and a cell scraper used to collect the cells. Cells were mixed with a vortex mixer for 15 s 3 times every 10 min to fully lyse adipocytes.

Total protein concentration of adipose tissue and adipocytes was measured by the bicinchoninic acid method (C503051–0500, Sangon Biotech). Proteins of different molecular weights were separated by SDS-PAGE on a 12% polyacrylamide gel, and a sample containing 30 μg of protein was added to each lane. Subsequently, proteins were transferred to 0.45 μm polyvinylidene difluoride membranes (IPFL00005, Millipore, Bedford, MA) by wet electroblotting with constant voltage (80 V) for 45 min in Western blotting transfer buffer (25 mM Tris, 192 mM glycine with 20% methanol). The polyvinylidene difluoride membrane was blocked in 5% BSA for 3 h, and then incubated with unc-51-like kinase 1 (ULK1; 1:1,000; #8054; Cell Signaling Technology), phosphorylated (Ser555)-ULK1 (p-ULK1; 1:2,000; #5869; Cell Signaling Technology), phosphorylated (Ser473) AKT (p-AKT; 1:2,000; #4060S, Cell Signaling Technology), phosphorylated (Ser2448) mTOR (p-mTOR; 1:1,000, ab99532, Abcam), adipose triglyceride lipase (ATGL; 1:1,000, ab99532, Abcam),

### Table 1. Baseline characteristics of the dairy cows and samples used in this study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 15)</th>
<th>Ketosis (n = 15)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parities</td>
<td>Third</td>
<td>Third</td>
<td></td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>620.60; 4.04</td>
<td>636.20; 8.30</td>
<td>0.066</td>
</tr>
<tr>
<td>Body condition scores</td>
<td>2.73; 0.25</td>
<td>3.25; 0.25</td>
<td>0.001</td>
</tr>
<tr>
<td>DMI (kg/d)</td>
<td>21.36; 1.28</td>
<td>19.85; 1.17</td>
<td>0.013</td>
</tr>
<tr>
<td>Milk production, kg/d</td>
<td>30.81; 1.58</td>
<td>26.99; 1.15</td>
<td>0.001</td>
</tr>
<tr>
<td>Serum BHB (mM)</td>
<td>0.50; 0.06</td>
<td>3.19; 0.20</td>
<td>0.001</td>
</tr>
<tr>
<td>Serum fatty acids (mM)</td>
<td>0.32; 0.06</td>
<td>1.06; 0.11</td>
<td>0.001</td>
</tr>
<tr>
<td>Serum glucose (mM)</td>
<td>3.81; 0.24</td>
<td>2.39; 0.43</td>
<td>0.001</td>
</tr>
</tbody>
</table>

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hormone-sensitive triglyceride lipase (LIVE; 1:1,000, #4107; Cell Signaling Technology), phosphorylated LIPE (p-LIVE; 1:1000, #4139; Cell Signaling Technology), DGAT1 (1:2,000; Ab189994, Abcam), and β-actin (ACTB; 1:2,000; Ab8226, Abcam) antibody diluent at 4°C overnight. The membrane was then washed with Tris-buffered saline-Tween 5 times for 10 min each time and incubated with the following secondary antibodies: horseradish peroxidase (HRP)-conjugated AffiniPure rabbit anti-goat IgG (H+L) (SA00001–4, 1:10,000, Proteintech) or HRP-conjugated AffiniPure goat anti-mouse IgG (H+L) (SA00001–1, 1:10,000; Proteintech) at room temperature for 1 h. The membrane was then washed with Tris-buffered saline-Tween. Immunoreactive bands were visualized with enhanced chemiluminescence solution (Millipore). The Image J software is used for quantifying the pixel density. To standardize the expression levels of the target proteins, the pixel density of phosphorylated proteins such as ULK1, PI3K, AKT, and mTOR was divided by the pixel density of their respective total proteins. For the other target proteins, they were divided by the pixel density of ACTB from the same PVDF membrane. This division allowed us to obtain normalized pixel density values for subsequent statistical analysis.

Statistical Analysis.

Statistical analysis was conducted using SPSS software version 25.0 (IBM Corp.) and GraphPad Prism program (Prism 8.3.0, GraphPad Software). Before analysis, all data underwent tests for normality and homogeneity of variance and residuals using the Shapiro-Wilk and Levene tests, respectively. Basic characteristics of the cows with a skewed distribution underwent a nonparametric statistical analysis using the Wilcoxon test (Zhu et al., 2019). For other data with a Gaussian distribution, parametric statistical analysis was conducted using the independent-sample t-test to compare between 2 groups. Basic characteristics of dairy cows are presented as the median and interquartile range, while other data with normally distributed data are expressed as least squares means ± SEM. Multiple comparisons were evaluated using one-way ANOVA with Bonferroni correction. A P-value less than 0.05 was deemed statistically significant, and a P-value below 0.01 was considered highly significant.

RESULTS

DGAT1 Abundance, Lipolysis, and Autophagy Status in Adipose Tissue of Ketotic Dairy Cows

The protein expression level of DGAT1 in adipose tissue of ketosis cows was greater than that of healthy cows (P < 0.01, Figure 1A, B). In addition, phosphorylation of LIPE (led to greater p-LIVE/LIVE) and protein abundance of ATGL (P < 0.01, Figure 1A-D) were greater in adipose tissue of ketotic cows.

Compared with the control cows, the data revealed that protein abundance ratio of p-ULK1 to ULK1 was upregulated in ketotic cows (P < 0.01, Figure 2A, B), while the protein abundance of phosphorylated PI3K, AKT, and mTOR (P < 0.01, Figure 2A-E) was lower in adipose tissue of ketotic.

Effects of EPI on DGAT1 Abundance, Lipolysis, and Autophagy in Bovine Adipocytes

The level of DGAT1 protein expression in adipocytes treated with 1 μM EPI was greater compared with the control group (P < 0.01, Figure 3A, B). A similar effect was observed for the p-LIVE/LIVE ratio and protein abundance of ATGL (P < 0.01, Figure 3A-D).

Treatment of EPI in bovine adipocytes resulted in greater protein abundance ratio of p-ULK1 to ULK1 (P < 0.01, Figure 4A, B), while the protein abundances of PI3K, AKT, and mTOR phosphorylation were lower compared with the control group (P < 0.01, Figure 4A-E).

Effects of DGAT1 Overexpression on Lipolysis and Autophagy during Lipolysis induced by EPI

Compared with the empty vector group, DGAT1 overexpression upregulated protein abundance of DGAT1 (P < 0.05, Figure 5A, B). Compared with the EPI group, overexpression of DGAT1 attenuated the increase in LIPE phosphorylation induced by EPI (P < 0.05, Figure 5A, C). The protein abundance of ATGL also had a similar trend (P < 0.05, Figure 5A, D). Overexpression of DGAT1 reversed the upregulation in protein abundance of LIPE phosphorylation and ATGL induced by EPI.

Compared with the EPI group, when DGAT1 was overexpressed, it led to reversing the greater phospho-ULK1 induced by EPI (P < 0.05, Figure 6A, B). In contrast, when compared with the EPI group, overexpression of DGAT1 led to a greater abundance of phospho-PI3K, phospho-AKT and phospho-mTOR in response to EPI (P < 0.05, Figure 6A-E).
Effects of DGAT1 Silencing on Lipolysis and Autophagy in response to EPI

Compared with the negative control group, DGAT1 silencing downregulated protein abundance of DGAT1 (P < 0.05, Figure 7A, B). Compared with the EPI group, DGAT1 silencing aggravated the upregulation of p-LIPE/LIPE ratio and ATGL in EPI-treated adipocytes (P < 0.05, Figure 7A-D).

Furthermore, the silencing of DGAT1 further increased the protein abundance of phosphorylate ULK1 in EPI-treated adipocytes when compared with the EPI group (P < 0.05, Figure 8A, B). In addition, protein expression level of phosphorylation of P3K, AKT and mTOR also decreased (P < 0.05, Figure 8A-E).

DISCUSSION

Sustained adipocyte lipolysis in transition dairy cows is generally linked to local lipotoxicity, systemic inflammation, and the onset of ketosis (Schmitt et al., 2011, Dong et al., 2022). Studies with nonruminants have demonstrated that activation of autophagy is key mechanism for mobilizing FFA to avoid cellular lipo-toxicity. Our study provided evidence that increased abundance of DGAT1 in adipocytes is associated with pronounced autophagy in response to lipolytic signals. In fact, a persistent state of lipolysis promotes autophagy, at least partly, through the inhibition of DGAT1. Thus, activity of this enzyme plays a vital role in the metabolic regulation within adipose tissue of ketotic dairy cows.

Hormone-sensitive lipase plays a pivotal role as the rate-limiting enzyme in the initial steps of adipose catabolism, namely the hydrolysis of TAG and diacylglycerol. Moreover, studies have confirmed that, in addition to LIPE, ATGL on lipid droplets triggers the conversion of TAG into diglycerides and FFA (Zechner et al., 2005). In nonruminants, LIPE and ATGL account for over 95% of TAG hydrolysis activity in white adipose tissue (Schweiger et al., 2006). Thus, the increased phosphorylation level of LIPE and the enhanced protein expression level of ATGL in adipose tissue of ketotic cows confirmed a state of active lipolysis in ketotic cows.

Cows with ketosis exhibit altered autophagic activity in liver and adipose tissue (Shen et al., 2021, Xu et al., 2021), suggesting an inherent adaptive mechanism of
Autophagy is an adaptive response in mammalian cells to nutrient stress, primarily regulated by energy stress sensors such as mTOR and AMPK (Ferhat et al., 2019). mTORC1 phosphorylates ULK1, which prevents the phosphorylation and interaction of ULK1 by AMPK. This interaction is crucial for ULK1 in autophagy induction (Kim et al., 2011). In our previous study, we observed increased AMPK phosphorylation in adipose tissue of ketotic dairy cows, highlighting its regulatory role in autophagic activity in bovine adipocytes under oxidative stress (Xu et al., 2021). The results of the present study further demonstrate that in addition to the activation of AMPK, the activation of ULK1 is involved in the induction of autophagy in adipose tissue of ketotic dairy cows. The PI3K/AKT signaling pathway is a well-known contributor to mTOR activation (Guo et al., 2016), and the present study provides further evidence that inhibition of PI3K/AKT/mTOR signaling and enhanced ULK1 phosphorylation are associated with autophagy in adipose tissue of cows experiencing ketosis.

The link between lipolysis and autophagy was investigated using bovine adipocytes, after stimulating lipolysis with EPI. These results align with in vivo findings from ketotic cows demonstrating that the inhibition of PI3K/AKT/mTOR and upregulation of ULK1 phosphorylation were enhanced by EPI. In a previous study with bovine adipocytes, we demonstrated that autophagy is activated during oxidative stress (Xu et al., 2021). A similar study also suggested that the activation of the autophagy-lysosomal pathway is connected to enhanced lipolysis in adipose tissue of ketotic cows and bovine adipocytes (Yu et al., 2022). Thus, the present data further confirmed these previous findings and underscored the link between lipolysis and autophagy. The potential for therapeutic interventions targeting lipolytic control becomes evident when considering how sustained lipolysis can trigger autophagy and contribute to ketosis development.

Figure 2. Abundance of autophagy proteins in adipose tissue of healthy (n = 15) and clinically ketotic (n = 15) cows. (A) Representative Western blots of phosphorylated unc-51-like kinase 1 (p-ULK1), ULK1, phosphorylated phosphoinositide 3-kinase (p-PI3K), PI3K, phosphorylated mammalian target of rapamycin (p-mTOR) and mTOR. (B-E) Quantification of diacylglycerol O-acyltransferase 1 (DGAT1), phosphorylated hormone-sensitive triglyceride lipase (p-LIPE)/LIPE, and adipose triglyceride lipase (ATGL) in adipose tissue. For all bar plots shown, data are expressed as means ± SEM ** P < 0.01; statistical differences assessed by t-test.
Studies with murine adipocytes reported that inhibition of DGAT1 during lipolysis promoted FFA release into the medium (Chitraju et al., 2017). Such effect was demonstrated in the present study when DGAT1 inhibition led to upregulation of LIPE and ATGL during lipolysis. The key role of DGAT1 was further demonstrated when its overexpression reversed the EPI-induced over-induction of these lipolytic enzymes. Because the inhibition of DGAT1 increases lipolysis and production of ROS (Mitra et al., 2017), at least in rodents, we speculate that the marked upregulation of DGAT1 in adipose tissue of ketotic cows may be a metabolic adaptation to control the release of FFA into the circulation. Thus, in the context of ketosis, a sustained lipolytic response could be associated with changes in DGAT1 activity or effects on proteins involved in this process.

Autophagy is a crucial cellular process responsible for recycling damaged or surplus organelles and proteins, thereby maintaining cellular equilibrium and preventing the accumulation of harmful molecules (Glick et al., 2010). Studies suggest that autophagy can regulate lipid metabolism by removing excessive lipid droplets from adipocytes (Dong and Czaja, 2011, Nguyen et al., 2017, Tsai et al., 2017). Thus, the upregulation of autophagy observed upon DGAT1 silencing might indicate a compensatory mechanism to reduce lipotoxicity resulting from increased lipolysis.

In murine adipocytes, the inhibition of DGAT1 exacerbates lipolysis and nearly abolishes diacylglycerol

![Figure 3](image-url)
erol production (Chitraju et al., 2017), with studies reporting that elevated diacylglycerol levels inhibit autophagy in yeast cells (Li et al., 2020a). These previous studies indicated that DGAT1 may play a regulatory role in lipolysis and autophagy. Furthermore, inhibition of DGAT1 can activate AMPK in murine adipocytes (Sharma et al., 2023). Considering our previous studies showing that AMPK overexpression induces autophagy in oxidative stress (Xu et al., 2021), we speculate that suppression of autophagy via the activity of DGAT1 might be associated with its negative regulatory relationship with AMPK and lipolysis. Further investigation into the mechanisms underlying this potential crosstalk is required.

Overall, the present studies provide evidence underscoring a pivotal role for DGAT1 in maintaining adipocyte metabolic homeostasis in ketogenic dairy cows. It highlights the interplay between lipolysis and autophagy and suggests that DGAT1 may act as a regulator of this balance. Further research into how DGAT1 regulates adipose tissue metabolism could identify novel therapeutic targets aimed at enhancing the well-being and productivity of transition dairy cows.

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CONFLICT OF INTEREST There are no conflicts of interest.

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Figure 5. Effects of diacylglycerol O-acyltransferase 1 (DGAT1) overexpression on lipolysis during epinephrine (EPI) induced lipolysis. Bovine adipocytes were treated with overexpression adenovirus of DGAT1 (adDGAT1) for 48 h and further incubated in the absence or presence of 1 μM EPI for 2 h. (A) Representative Western blots of DGAT1, phosphorylated hormone-sensitive triglyceride lipase (p-LIPE), LIPE, and adipose triglyceride lipase (ATGL). (B-D) Quantification of DGAT1, p-LIPE/LIPE, and ATGL in bovine adipocytes. For all bar plots shown, data are expressed as mean ± SEM. The same lowercase letters indicate no significant difference (P > 0.05), whereas different lowercase letters indicate a significant difference (P < 0.05). All experiments were repeated 3 times in duplicate (6 wells/group), n = 6.


**Figure 6.** Effects of diacylglycerol O-acyltransferase 1 (DGAT1) overexpression on autophagy during epinephrine (EPI) induced lipolysis. Bovine adipocytes were treated with overexpression adeno virus of DGAT1 (adDGAT1) for 48 h and further incubated in the absence or presence of 1 μM EPI for 2 h. (A) Representative Western blots of phosphorylated unc-51-like kinase 1 (p-ULK1), phosphorylated phosphoinositide 3-kinase (p-PI3K), PI3K, phosphorylated protein kinase B (p-AKT), AKT, phosphorylated mammalian target of rapamycin (p-mTOR), and mTOR. (B–E) Quantification of p-ULK1/ULK1, p-PI3K/PI3K, p-AKT/AKT, p-mTOR/mTOR in bovine adipocytes. For all bar plots shown, data are expressed as mean ± SEM. The same lowercase letters indicates no significant difference (P > 0.05), whereas different lowercase letters indicate a significant difference (P < 0.05). All experiments were repeated 3 times in duplicate (6 wells/group), n = 6.


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Figure 7. Effects of diacylglycerol O-acyltransferase 1 (DGAT1) silencing on lipolysis during epinephrine (EPI) induced lipolysis. Bovine adipocytes were treated with DGAT1 small interfering RNA (siDGAT1) for 48 h and further incubated in the absence or presence of 1 μM EPI for 2 h. (A) Representative Western blots of DGAT1, phosphorylated hormone-sensitive triglyceride lipase (p-LIPE), LIPE, and adipose triglyceride lipase (ATGL). (B–D) Quantification of DGAT1, p-LIPE/LIPE, and ATGL in bovine adipocytes. For all bar plots shown, data are expressed as mean ± SEM. Different lowercase letters indicate significant differences (P < 0.05). All experiments were repeated 3 times in duplicate (6 wells/group), n = 6.
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Figure 8. Effects of diacylglycerol O-acyltransferase 1 (DGAT1) silencing on autophagy during epinephrine (EPI) induced lipolysis. Bovine adipocytes were treated with DGAT1 small interfering RNA (siDGAT1) for 48 h and further incubated in the absence or presence of 1 μM EPI for 2 h. (A) Representative Western blots of phosphorylated unc-51-like kinase 1 (p-ULK1),ULK1, phosphorylated phosphoinositide 3-kinase (p-PI3K), PI3K, phosphorylated protein kinase B (p-AKT), AKT, phosphorylated mammalian target of rapamycin (p-mTOR), and mTOR. (B–E) Quantification of p-ULK1/ULK1, p-PI3K/PI3K, p-AKT/AKT, and p-mTOR/mTOR in bovine adipocytes. For all bar plots shown, data are expressed as mean ± SEM. Different lowercase letters indicate significant differences (P < 0.05). All experiments were repeated 3 times in duplicate (6 wells/group), n = 6.


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