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**Author for correspondence:**
Jianfa Wang, Email: wjflw@sina.com

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# Liver X receptor α participates in LPS-induced reduction of triglyceride synthesis in bovine mammary epithelial cells

Jianfa Wang[1,2], Shuai Lian[1,2], Jun Song[1,2], Hai Wang[1,2], Xu Zhang[1,2], Xianjing He[1], Dandan Hao[1] and Rui Wu[1,2]

[1]College of Animal Science and Veterinary Medicine, Heilongjiang Bayi Agricultural University, No. 2 Xinyang Road, Sartu District, Daqing 163319, P.R. China and [2]Heilongjiang Provincial Key Laboratory of Prevention and Control of Bovine Diseases, No. 2 Xinyang Road, Sartu District, Daqing 163319, P.R. China

**Abstract**

Lipopolysaccharides (LPS) could induce milk fat depression *via* regulating the body and blood fat metabolism. However, it is not completely clear how LPS might regulate triglyceride synthesis in dairy cow mammary epithelial cells (DCMECs). DCMECs were isolated and purified from dairy cow mammary tissue and treated with LPS. The level of triglyceride synthesis, the expression and activity of the liver X receptor α (LXRα), enzymes related to *de novo* fatty acid synthesis, and the expression of the fatty acid transporters were investigated. We found that LPS decreased the level of triglyceride synthesis *via* a down-regulation of the transcription, translation, and nuclear translocation level of the LXRα. The results also indicated that the transcription level of the LXRα target genes, sterol regulatory element binding protein 1 (*SREBP1*), fatty acid synthetase (*FAS*), acetyl-CoA carboxylase-1 (*ACC1*), were significantly down-regulated in DCMECs after LPS treatment. Our data may provide new insight into the mechanisms of milk fat depression caused by LPS.

Milk fat is a globular component of milk and varies with genetics, nutrition, season and health status (Bernard *et al*., 2018, Mccarthy *et al*., 2018, Ventto *et al*., 2017). Milk from Holstein cows averages around 3.6% milk fat. Milk fat depression is indicated by a decrease in milk fat by 0.2% or more (Mccarthy *et al*., 2018). When there is a milk fat depression, the production of milk fat decreases, with possible economic consequences. We are concerned with effects of lipopolysaccharides (LPS) on milk fat content. LPS are the major component of the outer membrane of Gram-negative bacteria, contributing greatly to the structural integrity of the bacteria, and protecting the membrane from certain kinds of chemical attack. The rumen concentration of LPS increases when rumen pH decreases. Previous study showed markedly greater concentrations of rumen LPS with increasing dietary grain level and found a strong negative relationship between rumen LPS and milk fat content (Zebeli and Ametaj, 2009). It is also known that feeding high-concentrate diets can increase the release of LPS in the rumen and pro-inflammatory cytokines in the mammary gland of dairy cows (Zhou *et al*., 2014). In addition, LPS-induced activation of the acute phase response and the inflammatory response may contribute to nutrient partitioning and re-distribution of energy, and ultimately lead to milk fat depression (Dong *et al*., 2013, Stefanska *et al*., 2018). However, it is not completely clear how LPS regulate triglyceride (TG) synthesis in dairy cow mammary epithelial cells (DCMECs).

Elevated interleukin 1 beta (IL-1β) level in mammary gland was observed in LPS-induced mastitis (Miao *et al*., 2012). A new study showed that IL-1β directly inhibits milk fat production in DCMECs concurrently with enlargement of cytoplasmic lipid droplets (Matsunaga *et al*., 2018). This study suggested that LPS-induced IL-1β is a key inhibitor of milk fat production in DCMECs. In addition, the regulation of milk fat synthesis involves multiple transcription factors to control the milk fat enzymatic machinery (Oppi-Williams *et al*., 2013). Liver X receptor α (LXRα) is a nuclear receptor and transcription factor that acts as a key sensor of cholesterol and lipid homeostasis (Oppi-Williams *et al*., 2013, Yao *et al*., 2016). LXRα target genes include *SREBP1* (sterol regulatory element binding protein 1), *FAS* (fatty acid synthetase), *ACC1* (acetyl-CoA carboxylase-1), and *CD36* (cluster of differentiation 36) (Oppi-Williams *et al*., 2013, Yao *et al*., 2016). These genes play vital roles in regulating milk fat synthesis in bovine mammary gland (Bionaz and Loor, 2008). Moreover, LXRα agonist T0901317 and Platycodin D can suppress LPS-induced mastitis in mice and DCMECs (Fu *et al*., 2014, Wang *et al*., 2017). Therefore, we hypothesize that LXRα may be a key factor in LPS-induced inhibition of triglyceride synthesis in DCMECs. In the present study, we

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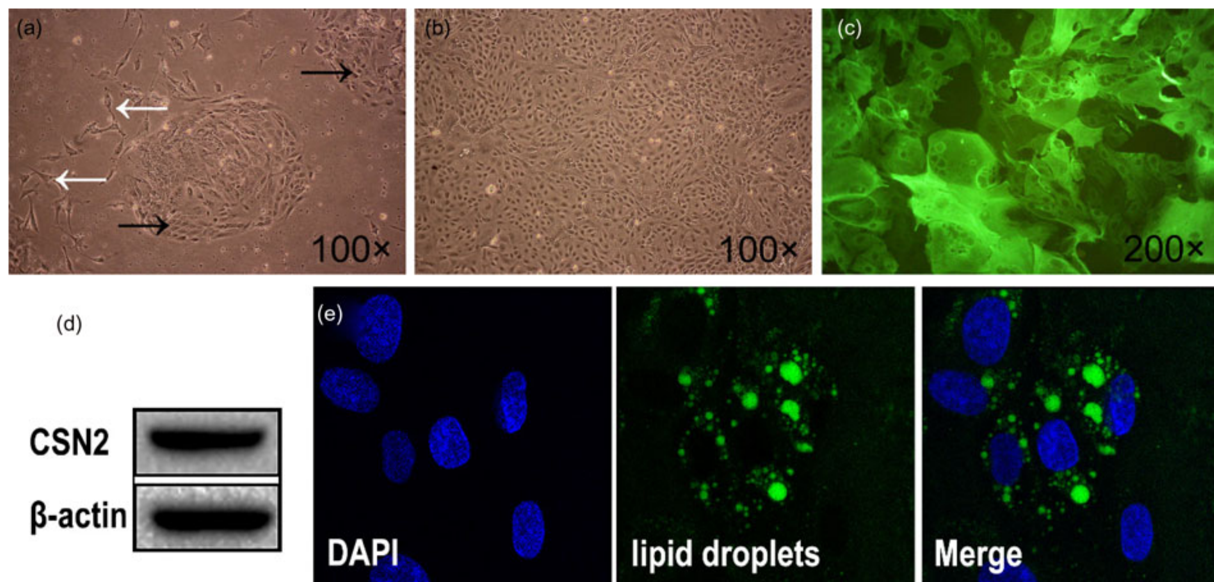


**Fig. 1.** Characterization of the lactating DCMECs culture model. (a) Morphology of the primary cells cultured on the 4th day. Black arrows indicate epithelial-like cells and white arrows indicate fibroblast-like cells. (b) Morphology of the purified DCMECs. (c) Immunofluorescence staining of CK-18 in the purified DCMECs. (d) The expression level of the CSN2 in the purified DCMECs were detected by western blot. (e) The result of BODIPY493/503 staining of lipid droplets (yellow) in the purified DCMECs (400 × ).

investigate the change of LXRα pathway after LPS challenge, so as to elucidate the mechanisms of LPS regulation of triglyceride synthesis in DCMECs.

## Material and methods

### Establishment and characterization of the lactating DCMECs culture model

All experimental protocols involving animals were approved by the Animal Care and Use Committee of Heilongjiang Bayi Agricultural University. Primary cell cultures were established from lactating Holstein mammary parenchymal tissue obtained *post mortem* (cows were slaughtered according to the Humane Methods of Livestock Slaughter Act). Tissue pieces were digested with collagenase I (Invitrogen, Carlsbad, CA, USA). Then, fibroblast-free cultures were obtained by differential trypsinization according to our previous study (Xu *et al*., 2017). Pure DCMECs were used for the following experiments. Immunofluorescence was used to detect expression of cytokeratin-18 (CK-18) to identify purified DCMECs according to our previous studies (Xu *et al*., 2017, Zhao *et al*., 2015). The lactating DCMECs culture model with high lipid production ability was prepared by addition of oleic acid to the culture medium. Then, the expression of the β-casein (CSN2) was analyzed by Western blotting. Primary and secondary antibodies against CK-18, CSN2, and β-actin are listed in online Supplementary Table S1. BODIPY (493/503, Invitrogen, Carlsbad, CA, USA) was used to visualize intramuscular lipid droplets in DCMECs according to Spangenburg *et al*. (2011).

### Measurement of TG

LPS (Sigma-Aldrich, St. Louis, MO, USA) was added to the medium in 6-well culture plates at a final concentration of 10 μg/ml during 0–48 h. Total TG (the intracellular TG and TG secreted into the medium) was measured using the triglyceride assay kit (Applygen Technologies, Beijing, China) according to previous studies (Wang *et al*., 2018, He *et al*., 2019).

### BODIPY493/503 staining of lipid droplets

Staining for lipid droplets in DCMECs was performed as described previously (Spangenburg *et al*., 2011). Briefly, DCMECs grown on coverslips at a density of $1 \times 10^5$ cell/ml, were treated as indicated above. Then, the coverslips were washed with PBS, fixed with 4% paraformaldehyde for 30 min at 4°C, and incubated with BODIPY493/503 for 30 min at room temperature (RT). After further washing, the cell nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich) for 5 min at RT. The coverslips were observed and photographed using a confocal laser-scanning microscope (Leica, Mannheim, Germany). The ratio of area integrated optical density (AIOD) of lipid droplets (yellow) to nuclei (blue) was calculated.

### Immunofluorescence staining

Nuclear translocation for LXRα in DCMECs was performed as described previously (Sticozzi *et al*., 2010). Briefly, coverslips were incubated for 1 h with primary antibody, followed by 1 h with secondary antibodies. Nuclei were stained with DAPI for 1 min after removal of secondary antibodies. Finally, the coverslips were observed and photographed using a confocal laser-scanning microscope (Leica).

### Western blotting

The translation level of LXRα in DCMECs was performed as Western blotting protocol. DCMECs were harvested and protein concentration was determined by the method of Bradford (Biyotime, Shanghai, China). The protein samples were electrophoresed using a 12.5% SDS-polyacrylamide gel and transferred onto polyvinylidene difluoride membranes (Bio-Rad Laboratories, Hercules, CA). Membranes were incubated with

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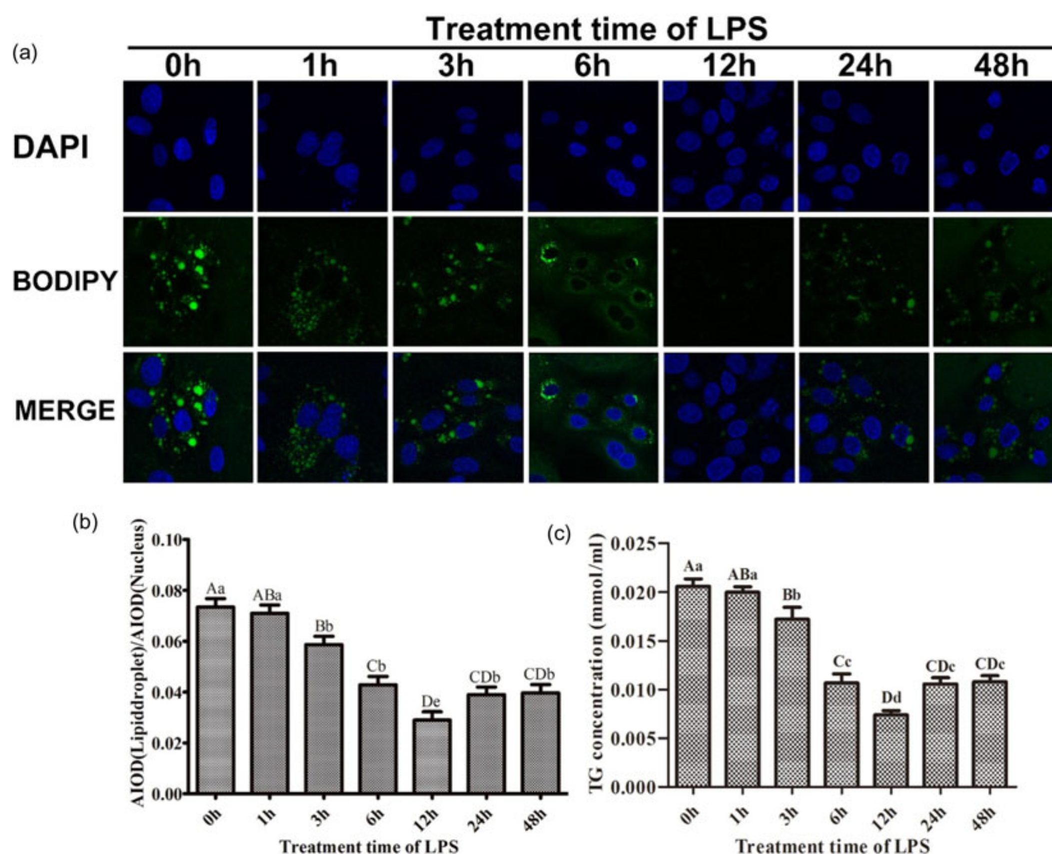


**Fig. 2.** LPS decreases lipid droplets synthesis and secretion in the lactating DCMECs. (a) The typical images of BODIPY493/503 staining for lipid droplets (yellow) in DCMECs (400 ×). (b) The ratio of AIOD of lipid droplets to nuclei. (c) The result of TG content in culture medium. The data with different capital letters between two groups showed very significant differences ($P < 0.01$); data with different lower-case letters between two groups showed significant differences ($P < 0.05$); data with the same letters between two groups showed no significant differences ($P > 0.05$).

the appropriate primary antibodies and secondary antibodies (online Supplementary Table S1). The immunoreactive bands were detected using ECL Western Blotting Substrate (Solarbio, Beijing, China). The images of the protein bands were obtained with a Bio-Rad ChemiDoc$^{TM}$ XRS+ (Bio-Rad, Hercules, CA, USA).

### *Quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR)*

Total RNA from DCMECs was extracted with TRIzol reagent (Sigma-Aldrich). High quality RNA (OD260/280 = 1.8–2.0) was reverse-transcribed to cDNA using PrimeScript RT Reagent Kit with gDNA Eraser (TaKaRa, Dalian, China). The mRNA level of *LXRα*, *SREBP1*, *FAS*, *ACC1*, and *CD36* genes were evaluated by qRT-PCR using a SYBR Green QuantiTect reverse RT-PCR Kit (Roche, Penzberg, Germany). Primer sequences used in the study are listed in online Supplementary Table S2. Quantitative RT-PCR was conducted on the CFX96 Real-Time PCR Detection System (Bio-Rad). The relative expression level of genes was calculated by normalizing to *GAPDH* (glyceraldehyde 3-phosphate dehydrogenase) using the comparative cycle threshold method (Taylor *et al*., 2010, Liu *et al*., 2015).

### *Statistical analysis*

Each sample was assessed in triplicate and the experimental data were expressed as the mean ± SD. Significance values were calculated by one-way analysis of variance (ANOVA) using SPSS version 19.0 software (SPSS, IBM, Armonk, NY, USA). The differences were considered significant at *P*-values of <0.05. All experiments were performed a minimum of three times using different samples.

## Results

### *Characterization of the lactating DCMECs culture model*

After 4 d of primary cell culture, epithelial-like cells (Fig. 1a; black arrow) were observed, which had clear boundaries with areas of fibroblast-like cells (Fig. 1a; white arrow). Mammary epithelial-like cells and fibroblasts were separated according to their differing sensitivity to trypsin. Purified DCMECs with characteristic cobblestone morphology were typically observed (Fig. 1b). Immunofluorescence staining exhibited intensely positive staining for CK-18 in the purified DCMECs' cytoplasm (Fig. 1c). We found that oleic acid could increase the lipid production in DCMECs. CSN2 was detected by western blotting in the purified DCMECs (Fig. 1d). In addition, fluorescence staining result showed that the milk lipid droplets DCMECs stained with BODIPY493/503 (yellow; Figure 1e). These results suggest that the lactating DCMECs culture model is suitable for investigating the milk fat synthesis function.

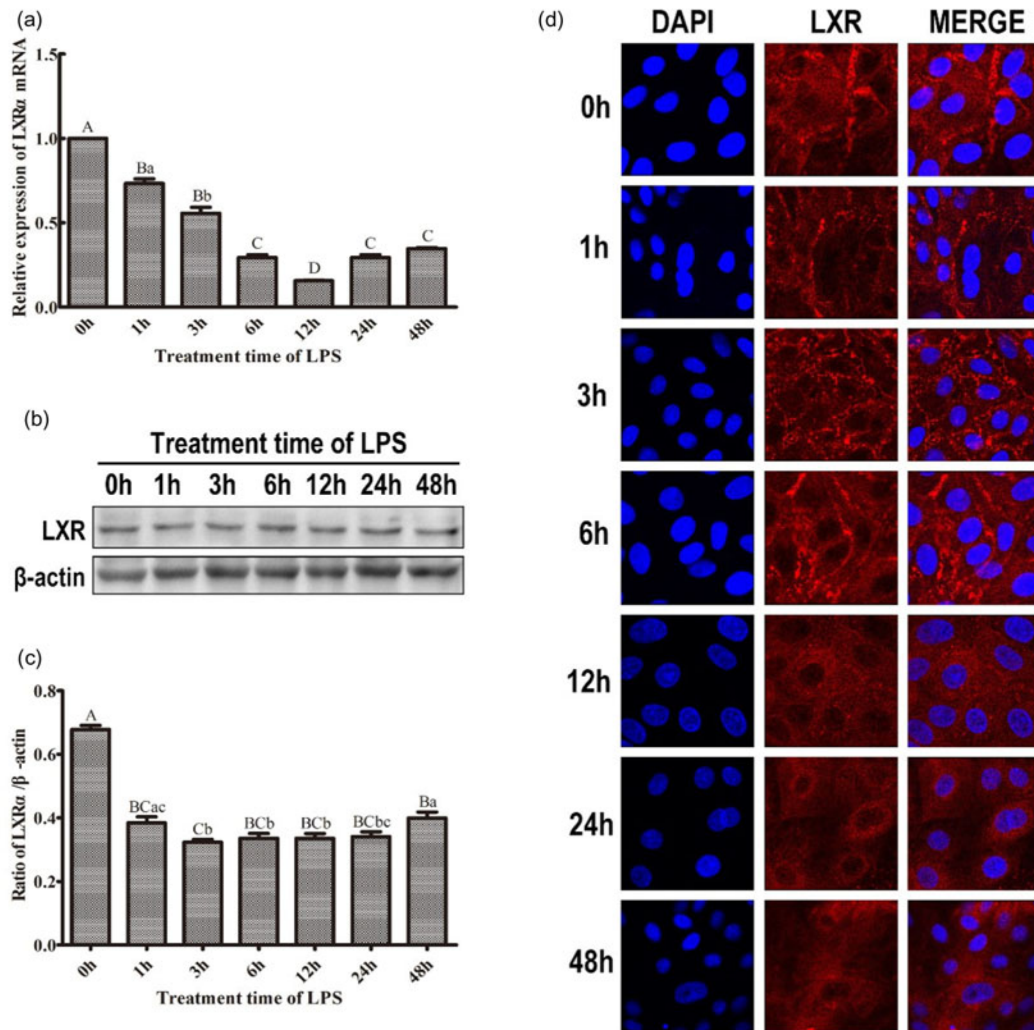


**Fig. 3.** LPS inhibits the transcription, translation and translocation of LXR in the lactating DCMECs. (a) The relative transcription level of LXRα gene. (b) The western blotting result of LXRα. (c) The relative expression level of LXRα. (d) The nuclear translocation of LXRα (red) in DCMECs (400 ×). The data with different capital letters between two groups showed very significant differences ($P < 0.01$); data with different lower-case letters between two groups showed significant differences ($P < 0.05$); data with the same letters between two groups showed no significant differences ($P > 0.05$).

### *LPS decreases lipid droplets synthesis and secretion in the lactating DCMECs*

As shown in Fig. 2, both the number and the size of lipid droplets were decreased after LPS treatment (Fig. 2a). Lipid droplets in DCMECs treated with LPS for 12 h appeared to be lower than at other times (Fig. 2a and b). In addition, the number and the size of lipid droplets in DCMECs treated with LPS for 24 h and 48 h appeared to be larger than those at 12 h after LPS treatment (Fig. 2a). The result of total TG content (Fig. 2c) was consistent with the ratio of AIOD of lipid droplets to nuclei (Fig. 2b). Total TG content at 1, 3, 6, 12, 24, and 48 h after LPS treatment was 0.89-, 0.71-, 0.56-, 0.38-, 0.48-, and 0.51-fold lower than the control, respectively. Based on these data, we found that LPS could decrease lipid droplet synthesis in lactating DCMECs culture model.

### *LPS inhibits the expression of LXRα in the lactating DCMECs*

As shown in Fig. 3, the transcription level (Fig. 3a) and translation level (Fig. 3b and c) of LXRα were down-regulated in DCMECs treated with LPS, and then began to increase gradually at 12 h, and 24 h after LPS treatment respectively. The transcription level of LXRα at 12 h after LPS treatment was 6.4-fold lower than the control ($P < 0.01$). Consistent with the above results, LPS could inhibit the translocation of LXRα from the cytoplasm to the nucleus (Fig. 3d). These results suggest that LPS could inhibit the expression level and activity of LXRα in lactating DCMECs culture model.

### *LPS regulates the transcription of LXRα target genes in the lactating DCMECs*

As shown in Fig. 4, LPS could inhibit the transcription of *SREBP1*, *FAS*, and *ACC1* genes in a time-dependent manner. These results suggest that LPS could inhibit the *de novo* synthesis of fatty acids in DCMECs. However, LPS treatment could increase the transcription level of *CD36* gene (Fig. 4d). The transcription level of *CD36* at 6 h after LPS treatment was 3.4-fold higher than the control ($P < 0.01$). We hypothesize that *CD36* gene may be involved in LPS-induced innate immune responses in DCMECs.

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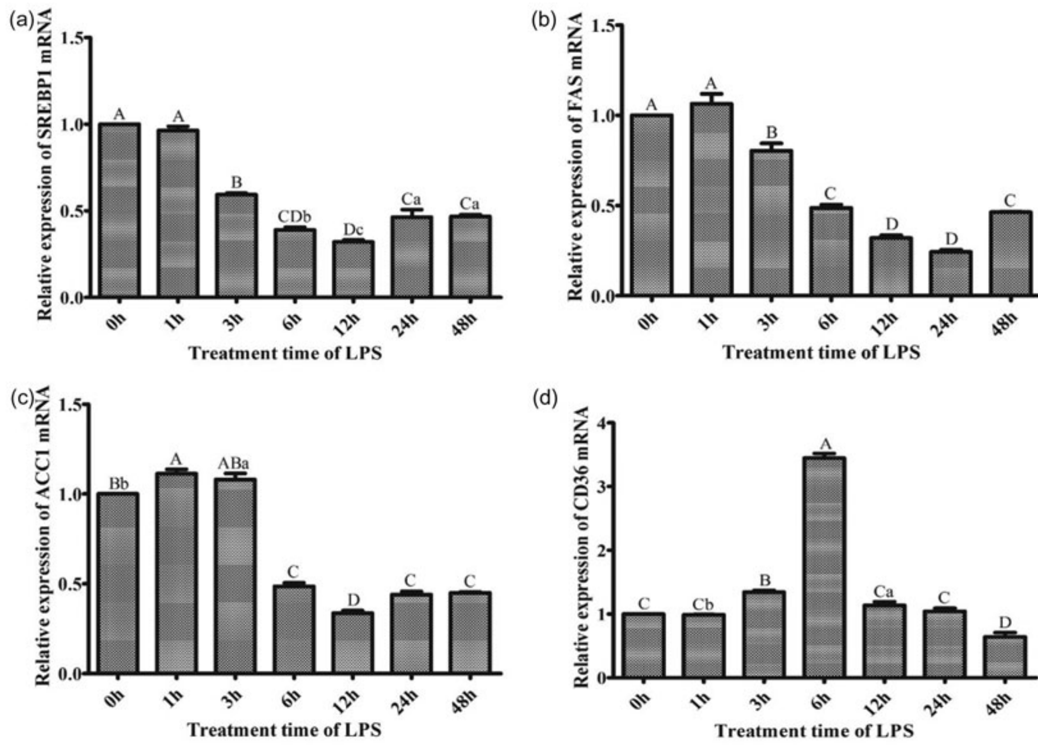


**Fig. 4.** LPS regulates the transcription of LXRα target genes in the lactating DCMECs. (a) The transcription level of *SREBP1* gene. (b) The transcription level of *FAS* gene. (c) The transcription level of *ACC1* gene. (d) The transcription level of *CD36* gene. The data with different capital letters between two groups showed very significant differences ($P < 0.01$); data with different lower-case letters between two groups showed significant differences ($P < 0.05$); data with the same letters between two groups showed no significant differences ($P > 0.05$).

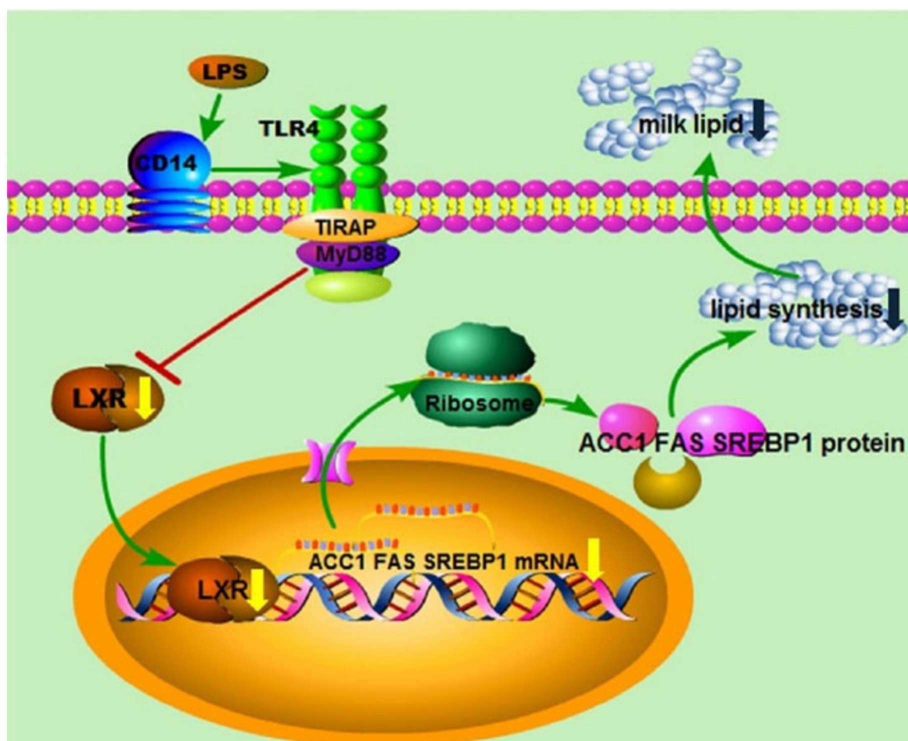


**Fig. 5.** A schematic drawing illustrating the proposed mechanism of LPS-induced reduction of milk lipid.

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## Discussion

LXRs are nuclear receptors that regulate the synthesis of lipid and control cholesterol homeostasis upon activation by oxysterols or synthetic agonists. Farke *et al.* (2008) identified the presence of LXRα in bovine mammary tissue, but did not elucidate its role in lipid metabolism. Mani *et al.* (2009) reported that LXRα should be considered a potentially important regulator of milk fat synthesis, as expression of LXRα is increased during the transition from pregnancy to lactation. McFadden and Corl (2010) found that activation of LXRα could enhance de novo fatty acid synthesis in bovine mammary epithelial cells. In addition, LXR activation increases *SREBP1* and *FAS* mRNA and protein abundance. On the other hand, Oppi-Williams *et al.* (2013) reported that reducing *LXRα* mRNA abundance in mammary alveolar cells (Mac-T) did not alter mRNA abundance of genes involved in de novo lipogenesis or the rate of *de novo* lipogenesis. Li *et al.* (2015) found that LXRα regulated fatty acid synthase promoter activity by directly interacting with LXRα response elements by increasing *SREBP1* abundance in goat mammary epithelial cells (Li *et al.*, 2015). Fu *et al.* (2014) and Wang *et al.* (2017) reported that LXRα agonist T0901317 and Platycodin D could suppress LPS-induced mastitis in mice and DCMECs. Overall, the evidence suggests that LXRα may serve as a new target for mastitis therapy and regulation of milk fat synthesis (Hu *et al.*, 2019). In the current research, our objective was to evaluate the role of LXRα in LPS-induced reduction of triglyceride synthesis in lactating DCMECs.

Cell culture is a convenient tool for studying the mechanisms of milk fat synthesis. Several immortalized bovine mammary epithelial cell lines have been established, such as BMEC + H, HH2A, ET-C, BME-UV, and Mac-T (Hosseini *et al.*, 2013). However, these cell lines are not suited for studying the mechanisms of milk fat synthesis. In the present study, lactating DCMECs culture model with high lipid production ability was prepared by addition of oleic acid to the culture medium. Fluorescent dyes offer an indirect measurement for intracellular lipids in different types of cell. The most common dye for milk lipids is Nile red (Matsunaga *et al.*, 2018). BODIPY staining has been used more recently as a potential alternative to Nile red (Govender *et al.*, 2012). We found that BODIPY staining is an alternative to the Nile Red fluorescence method for the evaluation of intracellular lipids in DCMECs. The results of BODIPY staining and TG detection showed that oleic acid could induce high lipid production ability of DCMECs. The lactating DCMECs culture model is suitable for investigating the milk fat synthesis function.

The intracellular lipids and total TG were detected by BODIPY staining and enzymatic colorimetric assay in the DCMECs layer and culture medium, respectively. We found that LPS could decrease milk lipid droplets synthesis. LPS could decrease the cell viability and induce inflammatory response in DCMECs (Liu *et al.*, 2016). Besides cell viability and inflammatory response, the regulation of milk fat synthesis in DCMECs with LPS treatment also involves multiple transcription factors to control the milk fat enzymatic machinery. LXRα is a nuclear receptor and transcription factor that plays important role in milk fat synthesis and mastitis therapy in dairy cows (Hu *et al.*, 2019). Therefore, the transcription, translation, and translocation of LXR were detected in this study. We found that LPS could inhibit the transcription, translation and nuclear translocation of LXR. The results suggest that LXRα participate in LPS-induced reduction of triglyceride synthesis in DCMECs.

SREBP1 is a membrane-bound transcription factor that directly regulates the synthesis and uptake of cholesterol and fatty acids. The promoter of *SREBP1c* contains a liver X response element (LXRE) (Mcfadden and Corl, 2010). ACC1 and FAS are key enzymes regulating de novo synthesis in dairy cow mammary gland. The promoters of the lipogenic enzymes ACC1, and FAS all contain a sterol response element (SRE) capable of binding SREBP1 (McFadden and Corl, 2010, Oppi-Williams *et al.*, 2013). LXR indirectly promotes the transcription of *ACC1* and *FAS* by increasing the expression of SREBP1c. Furthermore, LXRα can increase the transcription of *ACC1* and *FAS* by binding to LXRE found within the promoter region of these lipogenic genes (Talukdar and Hillgartner, 2006). CD36 is a scavenger receptor that functions in high-affinity tissue uptake of long-chain fatty acids and contributes to milk lipid accumulation (Pepino *et al.*, 2014). Zhou *et al.* (2008) found that *CD36* is a shared target of LXRα, pregnane X receptor (PXR), and peroxisome proliferator-activated receptor (PPAR) gamma. Therefore, *SREBP1*, *FAS*, *ACC1*, and *CD36* are LXRα target genes. Consistent with the LXRα and previous report (Liu *et al.*, 2016), we found that LPS could inhibit the transcription of *SREBP1*, *FAS*, and *ACC1* genes in a time-dependent manner. The results suggest that LPS could inhibit the de novo synthesis of fatty acids in DCMECs *via* inhibiting the expression and activity of the LXRα. Contrary to the LXRα and previous report (Liu *et al.*, 2016), we found that LPS treatment could increase the transcription level of *CD36* gene. Liu *et al.* (2016) reported that LPS significantly decreased the mRNA expression levels of *CD36* in DCMECs compared with the control group. Consistent with our results, Cao *et al.* (2016) reported that *CD36* mRNA levels in mammary gland were significantly increased in *E. coli*-induced dairy goat mastitis compared with healthy goats. They also found that at concentrations of 1–10 μg/ml, LPS did not induce cell apoptosis or necrosis but up-regulated the expression of *CD36* mRNA after LPS treatment for 12 h (Cao *et al.*, 2016). They proved that CD36 regulates LPS-induced signaling pathways and mediates the internalization of E. coli in cooperation with toll-like receptor 4 in goat mammary gland epithelial cells (Cao *et al.*, 2016). We hypothesize that *CD36* gene may be involved in LPS-induced innate immune responses in DCMECs.

In conclusion, LPS can decrease the level of triglyceride synthesis in lactating DCMECs *via* down-regulating the expression and activity of the LXRα (Fig. 5). Our data may provide new insight into the mechanisms of milk fat depression caused by LPS.

**Supplementary material.** The supplementary material for this article can be found at https://doi.org/10.1017/S0022029920000990

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## References

**Bernard L, Bonnet M, Delavaud C, Delosière M, Ferlay A, Fougère H and Graulet B** (2018) Milk fat globule in ruminant: major and minor compounds, nutritional regulation and differences among species. *European Journal of Lipid Science and Technology* **120**, 1–58.

**Bionaz M and Loor JJ** (2008) Gene networks driving bovine milk fat synthesis during the lactation cycle. *BMC Genomics* **9**, 366.

**Cao D, Luo J, Chen D, Xu H, Shi H, Jing X and Zang W** (2016) CD36 regulates lipopolysaccharide-induced signaling pathways and mediates the

internalization of *Escherichia coli* in cooperation with TLR4 in goat mammary gland epithelial cells. *Scientific Reports* **6**, 23132.
**Dong H, Wang S, Jia Y, Ni Y, Zhang Y, Zhuang S, Shen X and Zhao R** (2013) Long-term effects of subacute ruminal acidosis (SARA) on milk quality and hepatic gene expression in lactating goats fed a high-concentrate diet. *PLoS ONE* **8**, e82850.
**Farke C, Meyer HH, Bruckmaier RM and Albrecht C** (2008) Differential expression of ABC transporters and their regulatory genes during lactation and dry period in bovine mammary tissue. *Journal of Dairy Research* **75**, 406–414.
**Fu Y, Tian Y, Wei Z, Liu H, Song X, Liu W, Zhang W, Wang W, Cao Y and Zhang N** (2014) Liver X receptor agonist prevents LPS-induced mastitis in mice. *International Immunopharmacology* **22**, 379–383.
**Govender T, Ramanna L, Rawat I and Bux F** (2012) BODIPY staining, an alternative to the Nile Red fluorescence method for the evaluation of intracellular lipids in microalgae. *Bioresource Technology* **114**, 507–511.
**He XJ, Lian S, Zhang X, Hao DD, Shan XF, Wang D, Sun DB, Wu R and Wang JF** (2019) Contribution of PPARγ in modulation of LPS-induced reduction of milk lipid synthesis in bovine mammary epithelial cells. *International Journal of Agriculture Biology* **22**, 835–839.
**Hosseini A, Sharma R, Bionaz M and Loor JJ** (2013) Transcriptomics comparisons of Mac-T cells *vs.* mammary tissue during late pregnancy and peak lactation. *Advance Dairy Research* **1**, 1–12.
**Hu X, Zhang N and Fu Y** (2019) Role of liver X receptor in mastitis therapy and regulation of milk fat synthesis. *Journal of Mammary Gland Biology and Neoplasia* **24**, 73–83.
**Li J, Luo J, Zhu J, Sun Y, Yao D, Shi H and Wang W** (2015) Regulation of the fatty acid synthase promoter by liver X receptor α through direct and indirect mechanisms in goat mammary epithelial cells. *Comparative Biochemistry and Physiology. B: Biochemistry and Molecular Biology* **184**, 44–51.
**Liu R, Jin C, Wang Z, Wang Z, Wang J and Wang L** (2015) Effects of manganese deficiency on the microstructure of proximal tibia and OPG/RANKL gene expression in chicks. *Veterinary Research Communication* **39**, 31–37.
**Liu L, Zhang L, Lin Y, Bian Y, Gao X, Qu B and Li Q** (2016) 14-3-3γ regulates cell viability and milk fat synthesis in lipopolysaccharide-induced dairy cow mammary epithelial cells. *Experimental and Therapeutic Medicine* **11**, 1279–1287.
**Mani O, Sorensen MT, Sejrsen K, Bruckmaier RM and Albrecht C** (2009) Differential expression and localization of lipid transporters in the bovine mammary gland during the pregnancy-lactation cycle. *Journal of Dairy Science* **92**, 3744–3756.
**Matsunaga K, Tsugami Y, Kumai A, Suzuki T, Nishimura T and Kobayashi K** (2018) IL-1β directly inhibits milk lipid production in lactating mammary epithelial cells concurrently with enlargement of cytoplasmic lipid droplets. *Experimental Cell Research* **370**, 365–372.
**Mccarthy MM, Overton TR, Mechor GD, Bauman DE, Jenkins TC and Nydam DV** (2018) Field study to investigate the associations between herd level risk factors for milk fat depression and bulk tank milk fat percent in dairy herds feeding monensin. *Journal of Dairy Science* **101**, 3118–3125.
**Mcfadden JW and Corl BA** (2010) Activation of liver X receptor (LXR) enhances de novo fatty acid synthesis in bovine mammary epithelial cells. *Journal of Dairy Science* **93**, 4651–4658.
**Miao J, Fa Y, Gu B, Zhu W and Zou S** (2012) Taurine attenuates lipopolysaccharide-induced disfunction in mouse mammary epithelial cells. *Cytokine* **59**, 35–40.
**Oppi-Williams C, Suagee JK and Corl BA** (2013) Regulation of lipid synthesis by liver X receptor α and sterol regulatory element-binding protein 1 in mammary epithelial cells. *Journal of Dairy Science* **96**, 112–121.
**Pepino MY, Kuda O, Samovski D and Abumrad NA** (2014) Structure-function of CD36 and importance of fatty acid signal transduction in fat metabolism. *Annual Review of Nutrition* **34**, 281–303.
**Spangenburg EE, Pratt SJP, Wohlers LM and Lovering RM** (2011) Use of BODIPY (493/503) to visualize intramuscular lipid dropletsin skeletal muscle. *Journal of Biomedicine and Biotechnology* **2011**, 598358.
**Stefanska B, Człapa W, Pruszynska-Oszmałek E, Szczepankiewicz D, Fievez V, Komisarek J, Stajek K and Nowak W** (2018) Subacute ruminal acidosis affects fermentation and endotoxin concentration in the rumen and relative expression of the CD14/TLR4/MD2 genes involved in lipopolysaccharide systemic immune response in dairy cows. *Journal of Dairy Science* **101**, 1297–1310.
**Sticozzi C, Pecorelli A, Belmonte G and Valacchi G** (2010) Cigarette smoke affects ABCAl expression *Via* Liver X receptor nuclear translocation in human keratinocytes. *International Journal of Molecular Sciences* **11**, 3375–3386.
**Talukdar S and Hillgartner FB** (2006) The mechanism mediating the activation of acetyl-coenzyme A carboxylase-alpha gene transcription by the liver X receptor agonist T0-901317. *Journal of Lipid Research* **47**, 2451–2461.
**Taylor S, Wakem M, Dijkman G, Alsarraj M and Nguyen M** (2010) A practical approach to RT-qPCR-Publishing data that conform to the MIQE guidelines. *Methods (San Diego, Calif.)* **50**, S1–S5.
**Ventto L, Leskinen H, Kairenius P, Stefański T, Bayat AR, Vilkki J and Shingfield KJ** (2017) Diet-induced milk fat depression is associated with alterations in ruminal biohydrogenation pathways and formation of novel fatty acid intermediates in lactating cows. *British Journal of Nutrition* **117**, 364–376.
**Wang Y, Zhang X, Wei Z, Wang J, Zhang Y, Shi M, Yang Z and Fu Y** (2017) Platycodin D suppressed LPS-induced inflammatory response by activating LXRα in LPS-stimulated primary bovine mammary epithelial cells. *European Journal of Pharmacology* **814**, 138–143.
**Wang JF, Zhang X, He XJ, Yang B, Wang H, Shan XF, Li CQ, Sun DB and Wu R** (2018) LPS-induced reduction of triglyceride synthesis and secretion in dairy cow mammary epithelial cells *via* decreased SREBP1 expression and activity. *Journal of Dairy Research* **85**, 439–444.
**Xu DD, Wang G, He XJ, Wang JF, Yang B, Sun ZP, Sun DB, He QY, Zhang X and Wu R** (2017) 17β-Estradiol and progesterone decrease MDP induced NOD2 expression in bovine mammary epithelial cells. *Veterinary Immunology and Immunopathology* **188**, 59–64.
**Yao DW, Luo J, He QY, Xu HF, Li J, Shi HB, Wang H, Chen Z and Loor JJ** (2016) Liver X receptor α promotes the synthesis of monounsaturated fatty acids in goat mammary epithelial cells *via* the control of stearoyl-coenzyme A desaturase 1 in an SREBP-1-dependent manner. *Journal of Dairy Science* **99**, 6391–6402.
**Zebeli Q and Ametaj BN** (2009) Relationships between rumen lipopolysaccharide and mediators of inflammatory response with milk fat production and efficiency in dairy cows. *Journal of Dairy Science* **92**, 3800–3809.
**Zhao XJ, Li ZP, Wang JH, Xing XM, Wang ZY, Wang L and Wang ZH** (2015) Effects of chelated Zn/Cu/Mn on redox status, immune responses and hoof health in lactating Holstein cows. *Journal of Veterinary Science* **16**, 439–446.
**Zhou J, Febbraio M, Wada T, Zhai Y, Kuruba R, He J, Lee JH, Khadem S, Ren S, Li S, Silverstein RL and Xie W** (2008) Hepatic fatty acid transporter CD36 is a common target of LXR, PXR, and PPARgamma in promoting steatosis. *Gastroenterology* **134**, 556–567.
**Zhou J, Dong G, Ao C, Zhang S, Qiu M, Wang X, Wu Y, Erdene K, Jin L, Lei C and Zhang Z** (2014) Feeding a high-concentrate corn straw diet increased the release of endotoxin in the rumen and pro-inflammatory cytokines in the mammary gland of dairy cows. *BMC Veterinary Research* **10**, 172.

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