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Effects of lipopolysaccharide on the stearoyl-coenzyme A desaturase mRNA level in bovine primary hepatic cells

Q.D. Jiang[1], H.P. Li[2], F.J. Liu[2], X.J. Wang[2], Y.J. Guo[2], L.F. Wang[2], W.F. Lu[2], H.J. Li[2], X.P. Li[1] and Y.Y. Wang[2]

[1]College of Veterinary Medicine, Northwest A & F University, Yangling, Shaanxi, China
[2]Key Laboratory of Animal Biochemistry and Nutrition, Ministry of Agriculture, Henan Agricultural University, Zhengzhou, Henan, China

Corresponding authors: X.P. Li / Y.Y. Wang
E-mail: jiangqd2000@126.com / wangyueying1@126.com

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ABSTRACT. This study aimed to compare the effects of lipopolysaccharide (LPS) on stearoyl-coenzyme A desaturase (*SCD*) gene expression in mouse primary hepatic cells. To obtain sufficient total RNA, primary hepatic cells were plated on 6-cm diameter-type collagen 1-coated dishes (1 x 10^6 cells per dish). The test was divided into 6 groups with 6 replications per group. The 6 groups were treated with the following volumes of LPS (0.1 mg/mL): 0, 1, 1.5, 2, 4, and 8 µL. The cells were cultured for 24 h, and the total RNA was extracted from samples. Reverse transcription polymerase chain reaction was used to analyze *SCD* mRNA levels. With increasing LPS amounts, the *SCD* mRNA expression first decreased and then increased slightly; the expression was the lowest in the 2-µL LPS condition. The *SCD* mRNA levels from the 4- and 8-µL LPS conditions were slightly higher than that from the 2-µL LPS condition, but the difference was not significant ($P > 0.05$). The *SCD* mRNA level from the 2-µL LPS condition was obviously

lower than that from the 0-, 1-, and 1.5-µL LPS condition, and the differences were significant ($P < 0.05$), and the *SCD* mRNA levels from the 0-, 1-, and 1.5-µL LPS conditions were not significantly different ($P > 0.05$). The *SCD* mRNA levels from the 4- and 8-µL LPS conditions were obviously lower than those from the 0- and 1-µL LPS conditions, and the differences were significant ($P < 0.05$).

Key words: Reverse transcription polymerase chain reaction; Bovine; Stearoyl-coenzyme A desaturase mRNA; Primary hepatic cells

INTRODUCTION

Stearoyl-coenzyme A desaturase (SCD) is the enzyme responsible for the conversion of saturated fatty acids into monounsaturated fatty acids (MUFA) in mammalian tissues. The composition of fatty acids that are stored in the fat depots reflects the previous action of SCD on substrates such as stearic acid and palmitic acid (Kim and Ntambi, 1999). MUFAs have been implicated as mediators in signal transduction and cellular differentiation, including neuronal differentiation (Bradley et al., 2008; Yonezawa et al., 2008). Recently, oleate has been shown to regulate food intake in the brain (Obici et al., 2002), and MUFAs may also influence apoptosis and mutagenesis in some tumors (Hardy et al., 2000). Thus, variation in SCD activity in mammals would be expected to affect a variety of key physiological variables, including cellular differentiation, metabolic rate, adiposity, atherosclerosis, and obesity.

The host responds to infection with multiple changes in intermediary metabolism (Beisel, 1975; Goldstein and Elwyn, 1989). The most prominent feature of this pathological state can be attributed to a reduction in lipoprotein lipase (LPL) activity (Kaufmann et al., 1976), an increase in *de novo* synthesis of fatty acids (Guckian, 1973), and an increase in the hepatic re-esterification of fatty acids derived from peripheral tissues (Wolfe et al., 1985). Most of these changes can also be reproduced by administering the purified endotoxin lipopolysaccharide (LPS), a complex glycolipid component of the outer capsule of gram-negative bacteria.

Lactation is a physiological situation that is characterized by profound changes in lipid metabolism in mammary glands and adipose tissue. During lactation, lipogenesis (Agius et al., 1979) and dietary lipid uptake (Oller do Nascimento and Williamson, 1986) are decreased in adipose tissue, and these metabolic activities are increased in the mammary gland (Williamson, 1986). The mammary gland of fed lactating rats has a high rate of lipogenesis (5-fold higher on a per gram basis than that in the liver) that is very sensitive to food intake (Robinson et al., 1978). Infection in women and cattle can have an adverse effect on lactation. The initial aim of this work was to examine whether acute administration of LPS stimulated lipogenesis in the lactating mammary gland as it does in livers of non-lactating rats and mice (Feingold et al., 1992; Adi et al., 1992). Contrary to expectations, LPS inhibited lipogenesis in the mammary gland, and this led us to examine its effects on other aspects of lipid metabolism in this tissue.

MATERIAL AND METHODS

Hepatocyte isolation

Hepatocytes were isolated between 8:00 and l0:00 a.m. according to the following proce-

dure: scrub bovine abdomen with 75% alcohol, open abdomen, expose liver portal vein, intubate and ligate, and perfuse the inferior vena cava with 37°C D-Hank's liquid at a flow rate of l0-20 mL/min until the liver surface redness disappears. Then, quickly cut through the inferior vena cava to allow fluid outflow until the liver surface appears soiled yellow. Clamp the inferior vena cava and perfuse the liver bulge with 0.05% collagenase at 1-2 mL/min. Later, perfuse for 4 min with 4°C phosphate-buffered saline (PBS). Finally, split the liver film gently with a glass needle to disperse the cells. Collect the cell suspension in 400-mesh nylon web by filtering. Wash hepatocytes 3 times with 4°C PBS, and separate cells with 60% Percoll. First, centrifuge cells at 4°C and 200 rpm for 15 min to collect the precipitate, and then centrifuge the precipitate at 4°C and 800 rpm for 4 min. Finally, examine a hepatocyte by trypan blue staining, microscopic examination of morphology, and activity. All procedures were approved by the Animal Ethics Committee.

Primary hepatic cell culture

Primary hepatic cells were suspended in Dulbecco's modified Eagle's medium/F12 (Sigma-Aldrich, St. Louis, MO, USA) with B27 (Gibco, Grand Island, NY, USA), insulin-transferrin-selenium-ethanolamine (ITS-X, Gibco), 10 mM 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (Nacalai Tesque Inc., Kyoto, Japan), antibiotics, 20 ng/mL epidermal growth factor (Sigma-Aldrich), 20 ng/mL basic fibroblast growth factor bFGF (R&D Systems Inc., Minneapolis, MN, USA), and 10 ng/mL hepatocyte growth factor (Peprotech, Rocky Hill, NJ, USA). Cells were plated onto 6-cm-diameter type collagen 1-coated dishes (1 x 10^6 cells per dish). A total of 6 groups of primary hepatic cells were selected for this study, and each group included six 6-cm-diameter type collagen-coated dishes. The groups received the following volumes of LPS (0.1 mg/mL, Sigma): 0, 1, 1.5, 2, 4, and 8 µL. Cells were cultured for 24 h and harvested to extract total RNA.

RNA extraction and reverse transcription

Total RNA was extracted from primary hepatic cells with the TRIzol reagent kit (Invitrogen Inc., Minneapolis, MN, USA) and used to determine the *SCD* mRNA levels by reverse transcription polymerase chain reaction (RT-PCR). All extracted RNA samples were finally dissolved in 20 µL RNase-free water. The purity of the dissolved RNA was assessed by the A260 nm/A280 nm ratio that was measured using an ultraviolet/visible spectrophotometer (NanoDrop 2000/2000C, Rocky Hill, USA). The integrity of the RNA was determined by denaturing agarose gel electrophoresis.

RNA was reverse transcribed with a reaction mix containing 6.5 µL diethylpyrocarbonate-H_2O, 4 µL 5X buffer, 4 µL 2.5 mM dNTP mix, 1 µL 50 pM Oligo(dT)18, 2 µL 5 U/µL AMV, 0.5 µL 40 U/µL RNase inhibitor, and 2 µL RNA. The total reaction volume was 20 µL. After gently mixing, the solution was incubated for 60 min at 42°C, 15 min at 72°C, and 2 min in an ice bath. Two controls were performed: 1 control was prepared using all reagents except the RNA sample, for which an equivalent volume of water was substituted, and the other control was prepared using all reagents except reverse transcriptase. The controls underwent identical PCR procedures as experimental samples. The cDNA product was stored at -20°C.

RT-PCR

The primers used for amplification of *SCD* mRNA were designed using the Primer Pre-

mier TM Version 5.0 software (PREMIER Biosoft International, Victoria, Canada) and checked by basic local alignment search tool searches. All the primers, including those for the β-actin gene that was used as an internal reference, were synthesized by Shanghai Sango Biological Engineering Technology & Services Co. Ltd. (China). Electrophoresis on 1.2% (w/v) agarose gels was conducted to determine the quality and integrity of the primers. The sequence of primers and product size were as follows: SCD (NM_009127) sense 5'-tcttgtccctatagcccaatccag-3' and antisense 5'-agctcagagcgcgtgttcaa-3', product 130 bp; β-actin sense 5'-ctccctatggctccttcatgc-3' and antisense 5'-cccttctggtgctgctgctt-3', product 520 bp.

The cDNAs were further amplified by PCR in a 25-μL mix consisting of 1 μL RT reaction solution, 12.5 μL 2X Master mix, 1 μL 20 pM forward primer, 1 μL 20 pM reverse primer, and 9.5 μL sterilized H_2O. The reaction substrates were mixed by gently flicking the bottom of each tube. PCR amplification was carried out for 35 cycles (95°C, 10 s; 53°C, 20 s; and 72°C, 30 s) for β-actin and 35 cycles (95°C, 10 s; 52°C, 20 s; and 72°C, 30 s) for *SCD*. Reactions were completed with a final extension at 72°C for 10 min. For each 5 μL sample, the PCR amplification products were mixed gently with 3 μL fluorochrome before protection from light for 10 min, and then they were visualized on a 1.2% agarose gel with the MultiImage Light system.

Statistical analysis

All results are reported as means ± standard deviation and were analyzed using the Statistical Package for the Social Sciences (SPSS) statistical software (version 13.0). Differences between group data were analyzed using the Student-Newman-Keuls post hoc test of one-way analysis of variance (ANOVA), and differences between groups were evaluated using the paired-samples <0.01 in 2-tailed tests.

RESULTS

The integrity of the total RNA that was extracted from primary hepatic cells and assessed by gel electrophoresis is shown in Figure 1. The relative expression level of *SCD* mRNA in primary hepatic cells from the 6 different groups is shown in Table 1 and Figure 2.

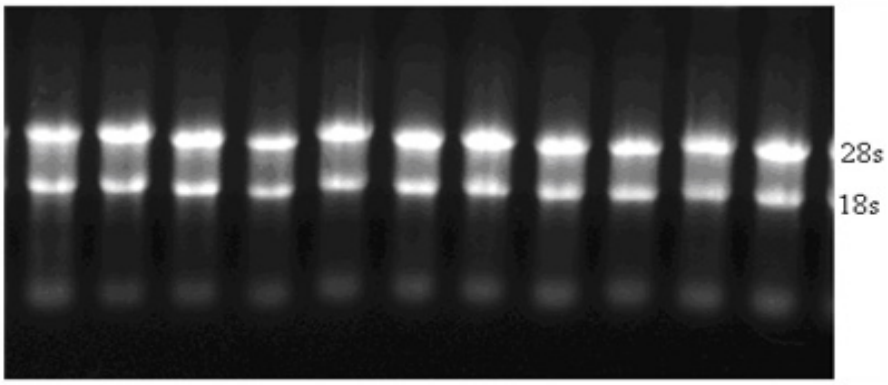

Figure1. Electrophoresis of RNA formaldehyde denaturing gel.

Table 1. Dose effect of lipopolysaccharide on the transcription level of SCDmRNA level in bovine primary hepatic cell (N = 6).

Group	D0	D1	D2	D3	D4	D5
LPS (μL)	0	1	1.5	2	4	8
SCD mRNA	0.67 ± 0.14[a]	0.63 ± 0.09[a]	0.53 ± 0.09[ac]	0.40 ± 0.05[b]	0.45 ± 0.17[c]	0.49 ± 0.15[c]

Data in the same column with different letter mean significant differences, $P < 0.05$.

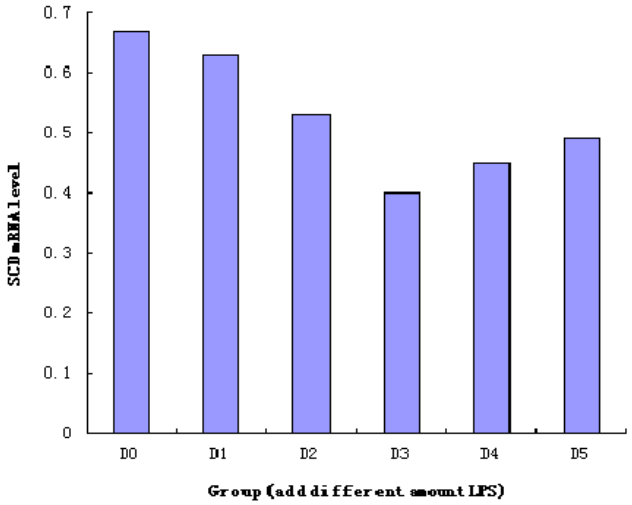

Figure 2. Dose effect of lipopolysaccharide on the transcription level of SCDmRNA level in bovine primary hepatic cell.

With increasing LPS amounts, the mRNA level first decreased and then increased slightly, and it was the lowest with 2 μL LPS. The *SCD* mRNA levels were slightly higher in the 4 and 8 μL LPS conditions than in the 2 μL LPS condition, but the differences were not significant ($P > 0.05$). The *SCD* mRNA level was obviously lower in the 2 μL LPS condition than in the 0, 1, and 1.5 μL LPS conditions, and the differences were significant ($P < 0.05$). At the same time, the *SCD* mRNA levels in the 0, 1, and 1.5 μL LPS conditions were not significant different ($P > 0.05$). The *SCD* mRNA levels in the 4 and 8 μL LPS conditions were obviously lower than that in the 0 and 1 μL LPS conditions, and the differences were significant ($P < 0.05$).

DISCUSSION

SCD is the enzyme responsible for the desaturation of fatty acids in the bovine mammary gland and other tissues. The main biological function of SCD in the mammary gland is to maintain the fluidity of milk by converting stearic acid to oleic acid and, to a lesser extent, palmitic acid to palmitoleic acid. Conjugated linoleic acid has several human health benefits, including actions against cancer, coronary heart disease, and diabetes (Parodi, 2004).

Studies of product-precursor relationships in milk fatty acids have demonstrated that SCD activity shows considerable variation among individual cows (Lock and Garnsworthy, 2003) and has an estimated heritability of 30% (Royal and Garnsworthy, 2005). Nutritional

studies of milk fatty acid composition can also benefit from an assessment of how nutrient supply influences *SCD* gene expression (Baumgard et al., 2002, in dairy cows).

Bovine mammary gene expression has been previously assessed by examining its mRNA abundance in samples of mammary tissue that were taken postmortem (e.g., Beswick and Kennelly, 2000). *SCD* mRNA has also been examined in tissue samples that were taken by mammary biopsy (e.g., Baumgard et al., 2002; Peterson et al., 2003). A noninvasive alternative to biopsy is analysis of milk somatic cells, which have been used in a few studies of dynamic changes in mammary mRNA. In a recent study, Murrieta et al. (2006) measured the mRNA levels of *SCD* and other enzymes involved in lipogenesis using milk somatic cells that were obtained postmortem from lactating beef cows. They found high correlations between mammary tissue and milk somatic cell data.

However, we are not aware of any report about the effect of LPS on *SCD* mRNA expression in primary hepatic cells. In this study, different amounts of LPS were added to primary hepatic cells, and *SCD* mRNA expression was analyzed by RT-PCR. The results of this study could be used in future studies about the relationship between LPS and fatty acids in milk as an indicator of mammary desaturase activity.

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REFERENCES

Adi S, Pollock AS, Shigenaga JK, Moser AH, et al. (1992). Role for monokines in the metabolic effects of endotoxin. Interferon-gamma restores responsiveness of C3H/HeJ mice *in vivo*. *J. Clin. Invest* 89: 1603-1609.

Agius L, Robinson AM, Girard JR and Williamson DH (1979). Alterations in the rate of lipogenesis *in vivo* in maternal liver and adipose tissue on premature weaning of lactating rats: a possible regulatory role of prolactin. *Biochem. J.* 180: 689-692.

Baumgard LH, Matitashvili E, Corl BA, Dwyer DA, et al. (2002). Trans-10, cis-12 conjugated linoleic acid decreases lipogenic rates and expression of genes involved in milk lipid synthesis in dairy cows. *J. Dairy Sci.* 85: 2155-2163.

Beisel WR (1975). Metabolic response to infection. *Annu. Rev. Med.* 26: 9-20.

Beswick NS and Kennelly JJ (2000). Influence of bovine growth hormone and growth hormone-releasing factor on messenger RNA abundance of lipoprotein lipase and stearoyl-CoA desaturase in the bovine mammary gland and adipose tissue. *J. Anim. Sci.* 78: 412-419.

Bradley RL, Fisher FF and Maratos-Flier E (2008). Dietary fatty acids differentially regulate production of TNF-α and IL-10 by murine 3T3-L1 adipocytes. *Obesity* (*Silver. Spring*) 16: 938-944.

Feingold KR, Staprans I, Memon RA, Moser AH, et al. (1992). Endotoxin rapidly induces changes in lipid metabolism that produce hypertriglyceridemia: low doses stimulate hepatic triglyceride production while high doses inhibit clearance. *J. Lipid. Res.* 33: 1765-1776.

Goldstein SA and Elwyn DH (1989). The effects of injury and sepsis on fuel utilization. *Annu. Rev. Nutr.* 9: 445-473.

Guckian JC (1973). Role of metabolism in pathogenesis of bacteremia due to Diplococcus pneumoniae in rabbits. *J. Infect. Dis.* 127: 1-8.

Hardy S, Langelier Y and Prentki M (2000). Oleate activates phosphatidylinositol 3-kinase and promotes proliferation and reduces apoptosis of MDA-MB-231 breast cancer cells, whereas palmitate has opposite effects. *Cancer Res.* 60: 6353-6358.

Kaufmann RL, Matson CF and Beisel WR (1976). Hypertriglyceridemia produced by endotoxin: role of impaired triglyceride disposal mechanisms. *J. Infect. Dis.* 133: 548-555.

Kim YC and Ntambi JM (1999). Regulation of stearoyl-CoA desaturase genes: role in cellular metabolism and preadipocyte differentiation. *Biochem. Biophys. Res. Commun.* 266: 1-4.

Lock AL and Garnsworthy PC (2003). Seasonal variation in milk conjugated linoleic acid and **Δ**9-desaturase activity in dairy cows. *Prod. Sci.* 79: 47-59.

Murrieta CM, Hess BW, Scholljegerdes EJ, Engle TE, et al. (2006). Evaluation of milk somatic cells as a source of mRNA for study of lipogenesis in the mammary gland of lactating beef cows supplemented with dietary high-linoleate safflower seeds. *J. Anim. Sci.* 84: 2399-2405.

Obici S, Feng Z, Morgan K, Stein D, et al. (2002). Central administration of oleic acid inhibits glucose production and food intake. *Diabetes* 51: 271-275.

Oller do Nascimento CM and Williamson DH (1986). Evidence for conservation of dietary lipid in the rat during lactation and the immediate period after removal of the litter. Decreased oxidation of oral [1-14C]triolein. *Biochem. J.* 239: 233-236.

Parodi PW (2004). Milk fat in human nutrition. *Aust. J. Dairy Technol.* 59: 3-59.

Peterson DG, Matitashvili EA and Bauman DE (2003). Diet-induced milk fat depression in dairy cows results in increased trans-10, cis-12 CLA in milk fat and coordinate suppression of mRNA abundance for mammary enzymes involved in milk fat synthesis. *J Nutr* 133: 3098-3102.

Robinson AM, Girard JR and Williamson DH (1978). Evidence for a role of insulin in the regulation of lipogenesis in lactating rat mammary gland. Measurements of lipogenesis *in vivo* and plasma hormone concentrations in response to starvation and refeeding. *Biochem. J.* 176: 343-346.

Royal MD and Garnsworthy PC (2005). Estimation of genetic variation in **Δ**9-desaturase enzyme activity in dairy cows. *Proc. Br. Soc. Anim. Sci.*52.

Williamson DH (1986). Regulation of metabolism during lactation in the rat. *Reprod. Nutr. Dev.* 26: 597-603.

Wolfe RR, Shaw JH and Durkot MJ (1985). Effect of sepsis on VLDL kinetics: responses in basal state and during glucose infusion. *Am. J. Physiol.* 248: E732-E740.

Yonezawa T, Haga S, Kobayashi Y, Katoh K, et al. (2008). Unsaturated fatty acids promote proliferation via ERK1/2 and Akt pathway in bovine mammary epithelial cells. *Biochem. Biophys. Res. Commun.* 367: 729-735.