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ORIGINAL ARTICLE

High-level exogenous *trans*10, *cis*12 conjugated linoleic acid plays an anti-lipogenesis role in bovine mammary epithelial cells

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ABSTRACT

Primary bovine mammary epithelial cells (BMECs) were treated by 0, 37.5, 75, 112.5, 150 μmol/L *trans*10, *cis*12 conjugated linoleic acid (CLA) to evaluate the effects of different level *trans*10, *cis*12 CLA on lipogenesis in BMEC. Addition of 75–150 μmol/L *trans*10, *cis*12 CLA reduced significantly the triacylglycerol (TAG) content ($P < 0.05$), but did not have inhibiting action on cell proliferation ($P > 0.05$). Treatment with 150 μmol/L *trans*10, *cis*12 CLA for 48 h resulted in a 17.1% reduction ($P < 0.0001$) of medium chain fatty acids (MCFA, C14 < C < C16), a 26.5% reduction ($P < 0.0001$) of unsaturated fatty acids (UFA) and a corresponding reduction of the mRNA abundance of acetyl coenzyme A (acetylCoA) carboxylase (*ACC*) ($P = 0.046$), fatty acid synthase (*FAS*) ($P = 0.017$) and stearoylCoA desaturase1 (*SCD1*) ($P = 0.002$). Another finding was that *trans*10, *cis*12 CLA elevated expression of diacylglycerol acyltransferase2 (*DGAT2*) ($P = 0.020$) and long chain acylCoA synthetases (*ACSL*) ($P = 0.032$). In conclusion, higher *trans*10, *cis*12 CLA, not low *trans*10, *cis*12 CLA, inhibited milk fat synthesis and changed fatty acid composition by regulating the expression of *FAS*, *ACC*, *SCD*1, *DGAT2* and *ACSL*.

Key words: *bovine, fatty acid composition, mammary epithelial cells,* trans*10,* cis*12* CLA, *triacylglycerol.*

INTRODUCTION

Conjugated linoleic acids (CLAs) are a group of positional and geometric isomers of the conjugated dienoic derivatives of linoleic acid. In recent years, CLAs have received more and more attention because of their numerous potential human health-related effects, including effects on obesity (Bhattacharya *et al*. 2006), body composition, insulin sensitivity (Park *et al*. 1999), anticancer (Kelley *et al*. 2007), antiatherosclerosis (Lee *et al*. 1994) and immune modulation (O'Shea *et al*. 2004).

Although *trans*10, *cis*12 CLA is of benefit to human health, it has been deemed as one of these bioactive fatty acids (FAs) which can result in milk fat depression (MFD) (Piperova *et al*. 2000). MFD naturally occurs in dairy cows when milk fat synthesis is inhibited by intermediates of ruminal bio-hydrogenation. Some researchers have shown that *trans*10, *cis*12 CLA caused transcriptional downregulation of acetyl coenzyme A carboxylase (*ACC*) and fatty acid synthase (*FAS*) (Kadegowda *et al*. 2009). *ACC* and *FAS* are the key enzymes in the process of FA *de novo* synthesis. *ACC* is responsible for catalyzing acetylCoA carboxylation into malonylCoA that is a key mesostate in FA *de novo* synthesis. The *FAS*, which is a compound enzyme, is in charge of serial condensation reaction, decarboxylation, dehydration and reduction reaction. Some studies showed that downregulation of *ACC* and *FAS* induced by *trans*10, *cis*12 CLA was special in mammary tissue, but expressed upregulation in adipose tissue (Harvatine *et al*. 2009).

Triacylglycerol (TAG), the main component of milk fat, is synthesized from various FAs and α-phosphate glycerol. Therefore, the TAG content is not only related to FA synthesis, but also to the combination of FA and α-phosphate glycerol. Given the association between *trans*10, *cis*12 CLA and MFD, it was

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hypothesized that *trans*10, *cis*12 CLA might affect the esterification of FAs and α-phosphate glycerol. 1-acylglycerol-3-phosphate O-acyltransferases (*AGPAT*) and diacylglycerol acyltransferase (*DGAT*) are considered to be the key regulatory enzymes in TAG synthesis. *AGPAT* catalyzes the transfer of an acyl from acyl-CoA to 1-acylglycerol-3-phosphate to form *sn*-1, 2 diacylglycerol, which is then used as the substrate of *DGAT* to form TAG.

In the present work, primary bovine mammary epithelial cells (BMECs) were used to evaluate the effect of different levels of *trans*10, *cis*12 CLA on TAG synthesis at the cellular level and the highest level of *trans*10, *cis*12 CLA was chosen for studying its role in correlative messenger RNA (mRNA) transcriptional levels and FA composition. The results were hoped to be contributory to understanding of MFD molecular mechanism.

MATERIALS AND METHODS

Cell culture and treatments

The detailed sampling and cell culture procedures were as described in our previous studies (Zhao *et al*. 2010, 2012). Simply, mammary tissues from healthy mid-lactation Holstein dairy cows were cut into pieces (about 1 mm^3) and incubated in Dulbecco's modified Eagle's medium (DMEM)/F12 medium (Gibco, New York, NY, USA) containing 5 mg/mL transferrin, 5 mg/mL insulin, 5 mg/mL prolactin, 1 mg/mL hydrocortisone (Sigma, San Fransisco, CA, USA), 10 ng/mL epithelial growth factor (EGF), 1% glutamine, 1% penicillin, 1% streptomycin and 10% fetal bovine serum (FBS) (Sangon Biotech Shanghai Co., Ltd, Shanghai, China) at 37°C in a humidified atmosphere of 5% CO_2. After cells migrating from tissues covered 80% of the bottom, the tissues were removed. Pure primary BMECs were achieved by isolating and purifying the impure cells (a mixed growth of mammary gland epithelial cells and fibroblasts) using trypsin and ethylenediaminetetraacetic acid (EDTA) (Sangon Biotech Shanghai Co., Ltd, Shanghai, China). When the bottoms of the flasks were completely covered with pure BMEC, the BMECs were treated with trypsin-EDTA, harvested and transferred into six-well plastic cell culture plates. The cells were cultured in fat-free and hormone-free DMEM/F-12 media that was supplemented with bovine serum albumin (BSA), antibiotics, acetate and ascorbic acid (Hangzhou Amprotein Bioengineering Co., Ltd, Hangzhou, China) for 24 h before treatment with or without *trans*10, *cis*12 CLA (Sigma Agent in China, Hangzhou, China) for 48 h. The CLA used to treat the BMEC was complexed to BSA (CLA-BSA) as described by Ip *et al*. (1999). Briefly, BSA was used as a carrier for CLA in the whole experiment. BSA was mixed with CLA in ethanol to keep the molar ratio of CLA to BSA at 4/1. The CLA-BSA solution was added to the fat-free and hormone-free media to attain the required concentration.

Five treatments (0, 37.5, 75, 112.5, 150 μmol/L *trans*10, *cis*12 CLA) were designed to evaluate the effects of different levels of *trans*10, *cis*12 CLA on cells proliferation ($n = 6$) and TAG content ($n = 3$) and to choose an optimum CLA concentration used for studying its effect on relative gene mRNA abundance ($n = 4$) and FA composition ($n = 6$). Every test was performed at least three times. Within the five treatments tested, higher *trans*10, *cis*12 CLA (75–150 μmol/L) showed the obvious inhibiting effect on TAG synthesis. Thus, the highest dose (150 μmol/L) was chosen for studying the effect of high-dose *trans*10, *cis*12 CLA on expression patterns of genes involving lipogenesis and FA composition.

RNA extraction, reverse transcription and RT-PCR

The total RNA of the BMEC was extracted using Trizol reagent (Invitrogen Chinese Inc., Shanghai, China). The RNA extraction was immediately followed by reverse transcription to complementary DNA (cDNA) using a reverse transcription kit (Takara Biotechnology Dalian Co., Ltd, Dalian, China) in accordance with the manufacturer's protocol. The expression levels of the mRNA were detected by real-time quantitative PCR (ABI 7500; Applied Biosystems Chinese Inc., Shanghai, China) in a reaction volume of 20 μL using the SYBR Premix Ex Taq™ RT-PCR Kit (Takara Biotechnology Dalian Co., Ltd, Dalian, China). The following cycling conditions were used for all amplifications: 10 s at 94°C (enzyme activation), 5 s at 95°C (denaturation) and 34 s at 60°C (annealing/extension). A dissociation stage was added to the PCR procedure to ensure the specific amplification of each primer pair. In addition, a standard curve was generated from a dilution series of cDNA samples to detect the efficiency of amplification step for each primer pair. Takara Biotechnology Dalian Co., Ltd. was entrusted to synthesize the primers for the objective genes and reference gene (β-actin) according to the sequences of the primers, as shown in Table 1.

BMEC growth assay using methylthiotetrazole (MTT)

An MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, Sigma) assay was performed to evaluate the effects of *trans*10, *cis*12 CLA on BMEC proliferation. In 96-well plates, 10^4 cells were seeded per well and treated with *trans*10, *cis*12 CLA at concentrations of 0 μmol/L, 37.5 μmol/L, 75 μmol/L, 112.5 μmol/L and 150 μmol/L (six duplicates per concentrations, and the assay was repeated at least three times). After a 48 h incubation

Table 1 Primer sequences for RT-RCR

Target gene	Sequence
ACC	F: CATCTTGTCCGAAACGTCGAT
	R: CCCTTCGAACATAC*ACC*TCCA
FAS	F: *ACC*TCGTGAAGGCTGTGACTCA
	R: TGAGTCGAGGCCAAGGTCTGAA
SCD1	F: ACAATTCCCGACGTGGCTT
	R: GGCATAACGGAATAAGGTGGC
DGAT1	F: GAACTCCGAGTCCATC*ACC*TACTT
	R: TCTGATGC*ACC*ACTTGTGAACA
AGPAT	F: GGATCCGAGTGGAGGTACGA
	R: CATCATCCCAAGCAGGTCG
ACSL	F: GTGGGCTCCTTTGAAGAACTGT
	R: ATAGATGCCTTTG*ACC*TGTTCAAAT
DGAT2	F: GCCCTGCGCCATGGA
	R: TAC*ACC*TCATTCTCCCCAAAGG
β-actin	F: TAGACTTCGAGCAGGAGATG
	R: CC*ACC*AGACAGCACTGTGTT

with CLA media, 20 μL 0.5 mg/mL MTT, which can interact with living cells to form purple crystals, was added into each well for 4 h at 37°C. The media was removed from the wells, and then 150 μL dimethyl sulfoxide (Sangon Biotech Shanghai Co., Ltd, Shanghai, China) was added to dissolve the crystals. The absorbance of the solubilized crystals was detected using a microplate reader (Tecan sunrise, Tecan, Morrisville, NC, USA) at 570 nm. The absorbance correlates linearly with the number of viable cells.

Cytosolic TAG measurements

After stimulation by *trans*10, *cis*12 CLA, the cells were rinsed with ice-cold phosphate-buffered saline (pH = 7.4) and were scraped off into the cell lysis buffer. The partial cell lysates were assayed for their protein concentration using the Bradford protein content assay kit (KeyGen Biotech Inc., Nanjing, China). The TAG was extracted from the partial cell lysates using the same volume of chloroform/methanol (2:1 by volume) and was quantified using the TAG content assay kit (KeyGen Biotech Inc., Nanjing, China). The TAG content of each well was calibrated using the protein content measurement from the same well. All assays were performed at least three times in triplicate.

Lipid extraction and pre-treatment for gas chromatographic analysis

The FAs were analyzed using the protocol integrating methods of Tsiplakou *et al*. (2006) and Sørensen *et al*. (2008) The following reagents (Sangon Biotech Shanghai Co., Ltd, Shanghai, China) were added to 0.5 mL of cell lysis solution in sequence, followed by vortexing for 30 s after each addition: 1 mL 0.1 mol/L KCl, 0.8 mL methanol, 2 mL chloroform/methanol (1:1 by volume), 2.7 mL chloroform and 2.5 mL chloroform/methanol (2:1 by volume). The mixtures were placed overnight at 4°C. The phase position was separated by centrifugation at 2250 × *g* for 10 min. The lower liquid phase (the chloroform layer) was transferred into a clean glass vial and then dried under N_2. The methylation of the FAs was carried out according to the procedure of Tsiplakou *et al*. (2006). To the dried lipids, 1.7 mL hexane was added followed by 40 μL methyl acetate, vortexing for 30 s. Then, 100 μL $NaOCH_3$ (0.5 mol/L NaOH in methanol) was added into the mixture. The liquid mixture was vortexed for 2 min and incubated for 20 min at room temperature, after which 60 μL oxalic acid solutions (30 μL/mL oxalic acid in diethyl ether) was added to terminate the reaction. After vortexing, the sample was centrifuged at 2400 × *g* for 5 min at 5°C. The supernatant (containing the FAs) was transferred into Aglie vials for gas chromatographic analysis (Agilent 6890; Palo Alto, CA, USA).

The analysis of the FA composition of BMEC using gas chromatography

One microliter aliquots of the hexane phase were injected in split-mode (100:1) onto a fused 5% phenyl methyl polysiloxane column (DB-23; 30 m × 0.32 mm internal diameter and a film diameter of 0.25 μm; Aligent, Santa Clara, CA, USA). The injector temperature was set at 250°C, the detector at 260°C, and the oven was initially set at 100°C for 5 min followed by an increase from 100 to 130°C at the rate of 8°C per min, then changed to 130°C for 5 min followed by an increase from 130 to 170°C at the rate of 4°C per min, after that 170°C for 30 min followed by an increase from 170 to 215°C at a rate of 5°C per min, and ending with a setting of 215°C for 10 min. The carrier gas was nitrogen and the constant flow rate was approximately 20 cm/s. The fatty acid methyl esters (FAMEs) were identified by comparing the retention times of the peaks generated by the samples with those of the peaks obtained by injecting a standard mixture of 37 methyl esters (C4-C22) (Supelco, Philadelphia, PA, IDA: 47 885 U).

Statistical analysis

Statistical analysis was carried out using analysis of variance (ANOVA) function of SAS software (9.1: SAS Institute Inc., Cary, NC, USA). Student's *t*-test was used for pairwise comparison and Tukey's test was used for multiple comparison. The effects of *trans*10, *cis*12 CLA treatments were compared to the control treatment for all experiments. Data (mean ± standard error) are representative results derived from a minimum of three independent experiments. Values for $P < 0.05$ were considered statistically significant. The model included the main effects of treatments and replicates.

RESULTS

BMEC proliferation profile in response to *trans*10, *cis*12 CLA

The results of different levels of *trans*10, *cis*12 CLA on the growth of BMEC is presented in Table 2. Before the MTT assay, cells were exposed to *trans*10, *cis*12 CLA for 48 h. Compared to the cells exposed to media without *trans*10, *cis*12 CLA, the cell proliferation profile did not change ($P = 0.061$) with the increasing concentration from 37.5 to 150 μmol/L.

The accumulation of cytosolic TAG

The concentration of cytosolic TAG decreased with the increased *trans*10, *cis*12 CLA concentration (Table 2).

Table 2 The effects of different levels of *trans*10, *cis*12 CLA on cell proliferation and cytoplasmic TAG accumulation in BMEC

Index	Treatment (μmol/L)					SEM	*P*-value
	0	37.5	75	112.5	150		
Viable cells percentage (% control)	100.00	102.41	99.91	99.14	97.8	0.003	0.061
TAG concentration (mg/mg protein)	0.038	0.024	0.016*	0.013*	0.005*	0.001	0.014

*$P < 0.05$ represent significant differences between the treatment and the control. CLA, conjugated linoleic acid; BMECs, bovine mammary epithelial cells; TAG, triacylglycerol.

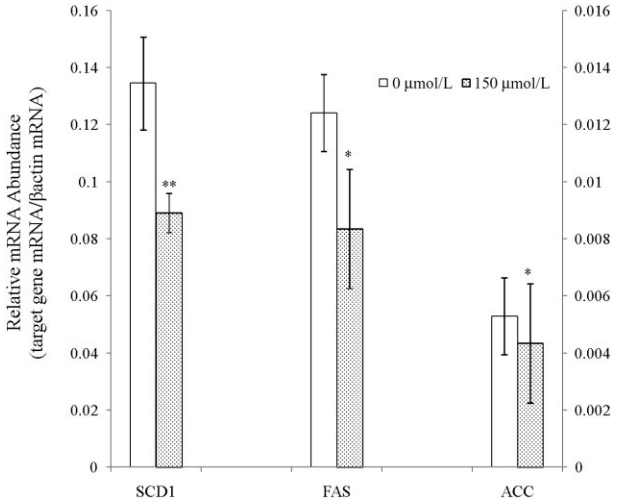

Figure 1 The effects of *trans*10, *cis*12 conjugated linoleic acid (CLA) on the messenger RNA (mRNA) expression of fatty acid synthase (*FAS*), acetyl coenzyme A carboxylase (*ACC*) and stearoylCoA desaturase1 (*SCD1*). The relative expression levels of the target genes were measured by RT-PCR, calculated using the 2–△△Ct method and expressed as target gene mRNA/β-actin mRNA. β-actin was used as an endogenous control gene to normalize the data. $*P < 0.05$, $**P < 0.01$ represent significant differences between the treatment and the control. All of the data are expressed as the means ± SE ($n = 4$). Note: the left axis refers to the *SCD1* mRNA level and the right axis refers to the *FAS* and *ACC* mRNA levels.

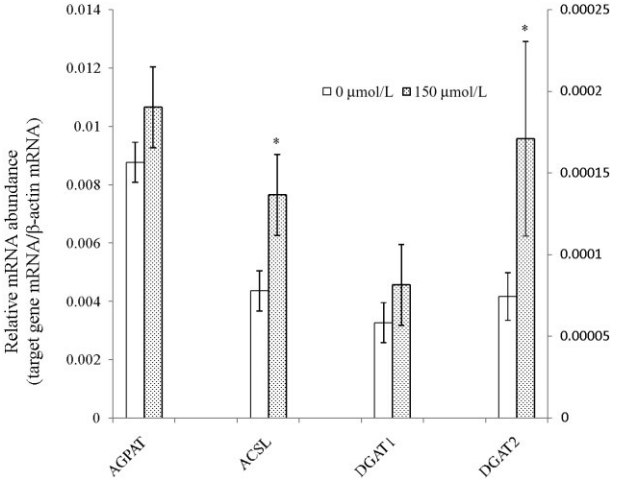

Figure 2 The effects of *trans*10, *cis*12 conjugated linoleic acid (CLA) on the messenger RNA (mRNA) expression of 1-acylglycerol-3-phosphate O-acyltransferases (*AGPAT*), diacylglycerol acyltransferase2 (*DGAT2*), *DGAT-2*, and acetyl coenzyme A synthetase(*ACSL*). The relative expression levels of the target genes were measured by RT-PCR, calculated using the 2-△△Ct method and expressed as target gene mRNA/β-actin mRNA. β-actin was used as an endogenous control gene to normalize the data. $*P < 0.05$, $**P < 0.01$ represent significant differences between the treatment and the control. All of the data are expressed as the means ± SE ($n = 4$).

Compared with the control, lipogenesis was significantly ($P = 0.014$) inhibited by 58.6%, 65.8% and 87.8% by *trans*10, *cis*12 CLA (75–150 μmol/L). The highest dose (150 μmol/L) was chosen for studying the effects of high *trans*10, *cis*12 CLA on lipogenesis.

The mRNA expression of the enzymes implicated in FA synthesis and TAG synthesis

The results of the mRNA abundance of *FAS*, *ACC* and stearoylCoA desaturase1 (*SCD1*) (genes involved in FA synthesis) are shown in Figure 1. Adding 150 μmol/L *trans*10, *cis*12 CLA decreased the mRNA expression of *FAS* ($P = 0.017$), *ACC* ($P = 0.046$) and *SCD1* ($P = 0.002$) by 32%, 10% and 34%, respectively. The mRNA expression of *AGPAT, DGAT1*, *DGAT2*, and long chain acylCoA synthetase (*ACSL*) (genes involved in TAG synthesis) is shown in Figure 2. The mRNA expressions of *DGAT2* ($P = 0.020$) and *ACSL* ($P = 0.032$) in BMECs were elevated 2.4-fold and 1.3-fold respectively by 150 μmol/L *trans*10, *cis*12 CLA. However, *AGPAT* ($P = 0.172$) and *DGAT1* ($P = 0.145$) had no obvious response to exogenous *trans*10, *cis*12 CLA.

The FA composition of the lipids extracted from the BMEC

The detectable FAs in BMEC included C14:0, C15:0, C15:1, C16:0, C16:1, C17:0, C17:1, C18:0, *trans*-C18:1, *cis*-C18:1, *cis*9, *trans*11 CLA, *trans*10, *cis*12 CLA, C18:2, C20:0, C20:1, C20:3n3 and C20:4n6. The effects of exogenous *trans*10, *cis*12 CLA on the FA profile of the lipids in BMEC was analyzed from two points: individual FAs and categorical FAs. For individual FAs (Table 3), the addition of 150 μmol/L *trans*10, *cis*12 CLA significantly increased the proportion of C15:0 ($P < 0.0001$), C17:0 ($P < 0.0001$), C18:0 ($P = 0.0003$), *trans*10, *cis*12 CLA ($P < 0.0001$) and C20:0 ($P < 0.0001$) and significantly decreased the proportion of C15:1 ($P < 0.0001$), C16:0 ($P = 0.003$), C16:1($P = 0.0006$), C17:1 ($P = 0.0300$), *trans*-C18:1 ($P < 0.0001$), *cis*-C18:1 ($P < 0.0001$) and C20:1 ($P = 0.015$). For categorical FAs (Table 3), MCFA (C14 < C < C16) ($P < 0.0001$), UFA ($P < 0.0001$) and monounsaturated fatty acid (MUFA) ($P < 0.0001$) were obviously decreased by 150 μmol/L *trans*10, *cis*12 CLA. Accordingly, the SFA proportion increased distinctly ($P < 0.0001$), but the polyunsaturated fatty acid (PUFA) ($P = 0.3471$) proportion did not change significantly after treating BMEC with *trans*10, *cis*12 CLA.

DISCUSSION

It was reported that 35–150 μmol/L *trans*10, *cis*12 CLA decreased MAC-T cell numbers in a dose-dependent way (Keating *et al*. 2008). According to this, *trans*10, *cis*12 CLA was speculated to be poisonous to cells. So the possible poisonousness of *trans*10, *cis*12 CLA to

Table 3 The fatty acid composition (mole %) of lipids extracted from BMEC that were treated with exogenous *trans*10, *cis*12 CLA

Index	Treatment		SEM	*P* value
	Control	*trans10, cis12* CLA		
C14:0	1.62	1.53	0.042	0.163
C15:0	0.22	1.52**	0.112	< 0.0001
C15:1	1.87	0.29**	0.150	< 0.0001
C16:0	19.37	17.43	0.341	0.003
C16:1	5.2	3.80**	0.202	0.0006
C17:0	0.19	1.44**	0.03	< 0.0001
C17:1	0.52	0.46*	0.015	0.030
C18:0	13.5	16.15**	0.347	0.0003
trans-C18:1	38.64	27.12**	0.413	< 0.0001
cis-C18:1	8.35	6.48**	0.217	< 0.0001
Cis9, *trans*11 CLA	0.16	0.15	0.027	0.476
*Trans*10, *cis*12 CLA	0.10	0.98**	0.036	< 0.0001
C18:2	1.29	1.27	0.04	0.659
C20:0	0.82	16.79**	0.395	< 0.0001
C20:1	3.46	2.32*	0.275	0.015
C20:3n3	2.35	2.35	0.068	0.958
C20:4n6	1.14	1.05	0.068	0.342
MCFA	29.69	24.61**	0.447	< 0.0001
UFA	63.45	46.61**	0.524	< 0.0001
SFA	36.44	53.35**	1.179	< 0.0001
PUFA	4.84	4.66	0.142	0.347
MUFA	58.62	41.95**	0.442	< 0.0001

*$P < 0.05$, **$P < 0.01$ represent significant differences between the treatment and the control. MCFA, medium chain fatty acids = C14:0 + C15:0 + C15:1 + C16:0 + C16:1; MUFA, monounsaturated fatty acids = C15:1 + C16:1 + C17:1 + C18:1 + C; PUFA, polyunsaturated fatty acids = C18:2 + C20:3n3+C20:4n6; SFA, saturated fatty acids = C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0; UFA, unsaturated fatty acids = PUFA+MUFA.

BMEC was evaluated by MTT method. The result showed that *trans*10, *cis*12 CLA (37.5–150 μmol/L) did not significantly inhibit primary BMEC proliferation. So the sequence results would not be influenced by the number of BMECs. The contradiction between the present study and previous studies may be relative to the different cell types (MAC-T cell line and primary BMEC).

In vivo, *trans*10, *cis*12 CLA has often been related to MFD in dairy cows (Loor & Herbein 2003; Peterson *et al*. 2003). The same conclusion was drawn from our present study *in vitro*. However, our present results were inconsistent with the results of Sørensen *et al*. (2008), who reported that 20 μmol/L *trans*10, *cis*12 CLA had no effect on the lipid accumulation of cultured MAC-T cells. The difference was mainly relative to the different CLA addition. In the present study, 37.5 μmol/L *trans*10, *cis*12 CLA also had no effect on lipogenesis in primary BMEC. These previous and present results indicated that only high levels of *trans*10, *cis*12 CLA can depress lipogenesis in BMEC.

The elevated expression of *DGAT2* and *ACLS* looked like a contradiction with the depressed TAG content caused by *trans*10, *cis*12 CLA. Yonezawa *et al*.'s (2004) results proclaimed that both saturated (palmitic and stearic) and unsaturated (oleic and linoleic) FAs could stimulate the accumulation of TAG in primary cultured BMEC. Based on this result, it was conjectured that all kinds of long chain FA (LCFA), including *trans*10, *cis*12 CLA, had potential to stimulate TAG accumulation in BMEC. The only difference is that some special LCFAs, for example *trans*10, *cis*12 CLA, not only exert a stimulative effect on TAG synthesis like other LCFA but also exert an inhibitory effect on the *de novo* synthesis of FA, which are the raw material for TAG synthesis, by down-regulating *FAS* and *ACC*. Meanwhile, *trans*10, *cis*12 CLA directly promoted TAG synthesis by up-regulating *ACSL* and *DGAT2*. The ultimate phenotype was a decreased TAG accumulation by *trans*10, *cis*12 CLA, which demonstrated that the down-regulating effect of *trans*10, *cis*12 CLA on the mRNA abundance of *FAS*, *ACC* and *SCD1* is stronger than the up-regulating effect on gene expressions of *ACSL* and *DGAT2*.

DGAT joins in the final step of triacylglycerol biosynthesis. The two known *DGATs*, *DGAT1* and *DGAT2*, share little if any, similar sequences despite their similar enzymatic properties in *in vitro* assays (Cases *et al*. 2001). Yen *et al*. (2005) reported that *DGAT2* was a more effective enzyme than *DGAT1*. Coincidently, in the present experiment, treatment with *trans*10, *cis*12 CLA elevated the expression of *DGAT2* but not *DGAT1*, which was in agreement with Sørensen *et al*. (2008). Data from Kadegowda *et al*. (2009) demonstrated that C18:0 induced increased mRNA of *AGPAT6* and *DGAT1* compared to the control

group in the MAC-T cell line. Our present study showed that the mRNA abundance of *AGPAT* and *DGAT1* also had a tendency to elevate in response to *trans*10, *cis*12 CLA, but this was not statistically significant. It was inferred that all kinds of C18 FA had potentiality to induce increased mRNA of *AGPAT* and *DGAT1* in BMEC; the increased degree of mRNA abundance would vary dependent on the saturation degree of fatty acids.

Cattle treated with abomasal infusion of *trans*10, *cis*12 CLA have shown a decrease in the expression of *SCD1* (Baumgard *et al*. 2002). Some studies *in vitro* also showed a down-regulation of *SCD1* mRNA expression in response to *trans*10, *cis*12 CLA (Peterson *et al*. 2004; Kadegowda *et al*. 2009). In the present study, *trans*10, *cis*12 CLA also decreased the expression of *SCD1* in BMEC, which was the main reason for decreased UFA and increased SFA caused by *trans*10, *cis*12 CLA in BMEC. SFA was reported to have an inhibitory effect on acetyl-CoA carboxylase (Ntambi *et al*. 2002) and suppressed carbon chain extending. Based on Ntambi *et al*.'s conclusion, the lessened MCFA proportion in the present study, which was consistent with the study *in vivo* (Maxin *et al*. 2010; Medeiros *et al*. 2010), may be induced by increased SFA. Just as we predicted, *trans*10, *cis*12 CLA proportion increased because of the supplement of exogenous *trans*10, *cis*12 CLA. But the increased *trans*10, *cis*12 CLA did not result in increased C18:2. The main reason may be related to the decreased *SCD1* expression which limited the desaturated process of C18:0. The increased C18:0 proportion response to the exogenous *trans*10, *cis*12 CLA just confirmed the speculation.

In summary, only high-dose *trans*10, *cis*12 CLA, but not low levels, can decrease TAG synthesis of BMEC through inhibiting the *de novo* fatty acid synthesis of BMEC by lowering the expression level of *FAS* and *ACC*, and changed the FA composition of milk fat through reducing MUFA proportion by suppressing the expression of *SCD1*. Further studies are required to evaluate protein expression of enzymes and modulatory factors which participate in lipid metabolism.

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