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Characterization and regulation of the bovine *stearoyl-CoA* desaturase gene promoter $\stackrel{\stackrel{_{\scriptstyle \leftrightarrow}}{\scriptstyle \leftarrow}}{\sim}$

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Abstract

The bovine stearoyl-CoA desaturase (*Scd*) gene plays an important role in the bovine mammary gland where substrates such as stearic and vaccenic acids are converted to oleic acid and conjugated linoleic acid (CLA), respectively. Up to 90% of the CLA in bovine milk is formed due to the action of this enzyme in the mammary gland. The areas of the bovine promoter of importance in regulating this key enzyme were examined and an area of 36 bp in length was identified as having a critical role in transcriptional activation and is designated the *Scd* transcriptional enhancer element (STE). Electrophoretic mobility shift assay detected three binding complexes on this area in Mac-T cell nuclear extracts. Treatment of cells with CLA caused a significant reduction in transcriptional activity, with this effect being mediated through the STE region. The bovine *Scd* gene promoter was up-regulated by insulin and down-regulated by oleic acid. © 2006 Elsevier Inc. All rights reserved.

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Stearoyl-CoA desaturase (*Scd*) is the rate-limiting enzyme in the biosynthesis of monounsaturated fatty acids (MUFA). In rodents, the *Scd* enzyme is predominantly located in the liver [1]. In contrast, adipose tissue is the major source of *Scd* in growing ruminants, and mammary tissue is the major site in lactating ruminants [2–4]. The preferred substrates of the *Scd* enzyme are palmitoyl- and stearoyl-CoA, which are converted to palmitoleoyl- and oleoyl-CoA (palmitate and oleate), respectively [5,6]. The MUFAs synthesized by *Scd* are used as major substrates for the synthesis of phospholipids, triglycerides, and cholesterol esters. *Scd* expression has effects on the fatty acid composition of membrane phospholipids, triglycerides, and cholesterol esters, resulting in changes in membrane fluidity, lipid metabolism, and obesity [1].

Conjugated linoleic acid (CLA) is a term that refers to a collection of positional and geometrical isomers of octadecadienoic acid with conjugated double bonds. Although its existence has been known for decades, CLA became the object of intense research after the discovery that it has anti-mutagenic activity [7] and anti-carcinogenic activity [8–12]. Since this discovery, it has also been shown to have anti-atherogenic actions [13–17], to affect fat partitioning in the body [18,19], and to normalize impaired glucose tolerance and improve hyperinsulinemia in the pre-diabetic rat [20,21].

In ruminant animals, CLA is formed in the rumen as an intermediate product in the digestion of dietary fat [22]. The next step is the conversion of this transient CLA to vaccenic acid (*trans*-11 18:1). These initial steps occur rapidly. The conversion of *trans*-11 18:1 to 18:0 appears to involve a different group of organisms and occurs at a slower rate [23]. *Trans*-11 18:1 accumulates in the rumen and is transported to the mammary gland where the action

^{*} Abbreviations: CLA, conjugated linoleic acid; EMSA, electrophoretic mobility shift assay; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; Scd, stearoyl-CoA desaturase; SREBP, sterol response element binding protein; STE, *Scd* transcriptional enhancer element.

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of the *Scd* enzyme (also known as δ^9 desaturase) converts it to CLA. Endogenous synthesis in the mammary gland is estimated to account for up to 78% of CLA in milk from cows on total mixed ration (TMR) diets [24,25] and for up to 91% of milk CLA from pasture-fed cows [26].

The promoter regions of the human [27,28], chicken [29], mouse [30–32], and bovine [33] *Scd* genes have been isolated. It has been shown that there is a conserved PUFA response region in all, and that this includes critical binding sites for SREBP and NF-Y transcription factors. It has been suggested that PUFAs down-regulate activity of the *Scd* gene, by interfering with the SREBP function [34]. It has also been demonstrated that, in the human [28] and mouse [34] promoters, the CCAAT box at -501, which is also a possible NF-Y binding site, is required for transcriptional activation. Sequence comparison of the human and mouse and bovine promoters indicated that there are two regions of high homology: (1) the 5'-UTR and basal/proximal promoter (-37 to -134 in bovine), and (2) a region containing the PUFA-response element (-313 to -390 in bovine) [33] (Fig. 1A). The human promoter study also indicated that there are two different transcription start sites, and the transcription initiation at these sites may be dependent on tissue-specific factors [28].

In the present study, we characterized the bovine *Scd* gene promoter. The basal promoter required for transcriptional activation was investigated by creating truncated promoter reporter constructs and analysing their expression in a bovine mammary cell line, Mac-T. The effects of substrates for the *Scd* enzyme on transcriptional activity of the gene were investigated. The effects of hormones involved in lactation were also examined. The potential transcription factors binding to sites of interest were explored.



Fig. 1. Scd promoter reporter construction and basal transcriptional activity. (A) Different lengths of the Scd gene promoter fragments (F1–F6) were cloned into a luciferase reporter vector. The potential regions of importance for transcription are indicated, along with promoter fragment sizes. (B) Scd promoter constructs (F1–F6) were transiently transfected into Mac-T cells. The experimental Firefly luciferase activities (F) were normalized to the internal *Renilla* luciferase activity (R) to give the relative luciferase activities (R.L.U.). Different letters above histograms represent differences among treatments (P < 0.05).

Materials and methods

Promoter reporter construction. Genomic DNA was extracted from bovine lymphocytes using a genomic DNA extraction kit (Qiagen). Varying length fragments of the Scd gene promoter (GenBank Accession No. AY241932) were amplified by PCR using primers (Table 1) containing a recognition site for either restriction enzyme XhoI or KpnI at their 5' ends. PCR amplification was carried out from 200 ng of genomic DNA in a mix which contained final concentrations of the following constituents: 1× Taq DNA polymerase buffer, 1.5 mM MgCl₂, 200 µM dNTPs, 0.3 mM each primer, 10% DMSO, and 1 U Taq polymerase (Invitrogen) in a final volume of 50 µl. PCR conditions were 94 °C for 2 min, followed by 35 cycles of denaturing at 95 °C for 1 min, 58 °C for 20 s, and elongation at 72 °C for 1 min. Promoter fragments were cloned into a promoter-less luciferase reporter vector, pGL3-basic (Promega), by digesting both fragments and vector with KpnI and XhoI. Ligation reactions for cloning into the pGL3-Basic vector were set up at a ratio of 1:3 and 1:4 of vector to insert, using 50 ng of vector in either case. T4 DNA ligase and 1× ligase buffer (Invitrogen) were used according to manufacturer's instructions. Plasmids were sequenced to confirm promoter insertion and orientation.

Cell culture and transfection. Mac-T cells were obtained from Nexia Biotechnologies and cultured routinely in Dulbecco's modified Eagle's media (DMEM) (Invitrogen) containing 5% fetal calf serum (Invitrogen) and 5 mg/ml bovine insulin (Sigma) at 37 °C in a humidified incubator with 5% CO2. Twenty-four-well cell culture plates were seeded at a concentration of 2×10^4 cells per well and incubated overnight to 80% confluency. Cells were transiently transfected with luciferase reporter vectors by using Fugene 6[™] transfection reagent (Roche). On the day of transfection, 0.0125 µg of a pRL-TK Renilla (Promega) vector was mixed with 0.2 µg of the pGL3-experimental constructs balanced with pGL3 basic vector to correct for differences in length in DNA fragment. Fugene 6™ transfection reagent (0.6 µl) was added to 20 µl of serum-free media. The plasmid DNA mix was then added slowly to the media-Fugene 6[™] mix and incubated at room temperature for 20 min. This mix was then added to cells dropwise, swirled gently to disperse the mix, and incubated. Fatty acids (99% pure isomers) (Matreya) were conjugated to fatty acid-free BSA (Sigma) at 4:1 ratio using 1 mM BSA stocks (M. McIntosh, personal communication). Media were changed to the experimental treatment (30 µM fatty acid concentrations) 5 h after transfection and further incubated. In the case of hormonal treatments, media were changed to serum-free starvation media 5 h after transfection and incubated for 12 h. Hormonal treatment media were added at this point (10 mg/ml insulin, 5 µg/ml prolactin, 200 ng/ml dexamethasone or 10 ng/ml leptin) and cells re-incubated. After 72 h, media were removed from the wells and 100 µl of 1× passive lysis buffer (Promega) added. All transfection experiments were repeated three times independently and the data from one representative experiment are presented here.

Table 1

Oligonucleotide sequences used in PCR and EMSA

Luciferase assay. The Promega Dual Luciferase Assay Kit was used according to the manufacturer's instructions to measure both *Renilla* and firefly luciferase activities by the reporter vectors on a luminometer (Turner Biosystems, Promega). Firefly luciferase values were normalized to the *Renilla* luciferase activities.

Nuclear extract preparation and electrophoretic mobility shift assay (EMSA). The preparation of nuclear extracts from the Mac-T cells was carried out according to a standard method [35]. Protein concentrations of nuclear extracts were measured using the Bio-Rad Protein assay kit (Bio-Rad, Hercules, CA, USA). Single-stranded oligonucleotides (Table 1) were synthesized (Invitrogen). The sense and anti-sense oligonucleotides were annealed to form a double-stranded probe using 10× annealing buffer and incubating at 95 °C for 5 min and 65 °C for 15 min followed by slowly cool down to room temperature. The double-stranded probe was end-labeled with [³²P]ATP (Amersham). EMSA was carried out by incubating 5 µg nuclear protein with labeled probe with/without unlabeled competitor oligonucleotides in 10× gel shift buffer (Promega) for 30 min at room temperature. Protein-DNA complexes were resolved on a 4% polyacrylamide gel run at ~150 V for 3 h. Gels were dried and exposed to a phosphoimaging screen (Bio-Rad) overnight. Screens were developed using Molecular Imager FX and Quantity One software (Bio-Rad).

Statistical analysis. Statistical analysis was carried out using the oneway ANOVA function of Minitab software. Significance was declared at p < 0.05.

Results

Scd promoter reporter construction and basal promoter activity

The areas required for basal transcriptional activity of the *Scd* gene promoter were investigated by amplifying different lengths of the gene promoter (Fig. 1A) and cloning into a luciferase reporter system. These fragments extended from a common 3' start point (+14) in the promoter and extended 5' upstream of the transcriptional start site. These fragments were designed to include specific areas of interest in the promoter sequence. Fragment F1 (212 bp) contained the first area of conservation designated the fat specific element among the mouse, human, and bovine promoters. Fragment F2 (380 bp) extended to contain the second conserved area (PUFA response region) minus the CCAAT motif previously

Name	Sequence	Location in AY241932
	Primer	
pSCDR	CTCGGGACCTTCCGCTGCAC	1899–1880
pSCDF1	TCGGCGCTCTGTCTCCTCC	1687–1704
pSCDF2	AACGGCAGGACGAGGTGG	1519–1536
pSCDF3	GGCAGAGGGAACAGCAGATTG	1483–1503
pSCDF4	CAGAGGGAGGCGGGGC	1139–1154
PSCDF5	CCTACCTCACAGGGCCACAAG	635–655
PSCDF6	GCCTGCCTGGTTCCCAGC	376–393
	Oligonucleotide (EMSA)	
P1 for	GGCAGAGGGAACATCAGATTGCGCCGAGCCAATGGC	
P1 rev	GCCATTGGCTCGGCGCAATCTGATGTTCCCTCTGCC	
P2 for	GGCAGAGGGAACATCAGATTGCG	
P2 rev	CGCAATCTGATGTTCCCTCTGCC	
P3 for	TTGCGCCGAGCCAATGGC	
P3 rev	GCCATTGGCTCGGCGCAA	

shown to be important in the human promoter [28]. Fragment F3 (414 bp) further extended by 36 bp longer than F2 to contain this CCAAT motif and SREBP binding sites. Fragment F4 (760 bp), F5 (1264 bp), and F6 (1523 bp) extended further into the promoter to analyze the full-length promoter activity. These reporter constructs were transiently transfected into a bovine mammary epithelial cell line (Mac-T) to assess the areas of most importance in regulating transcription of the gene. The basal transcriptional activities of these constructs are shown in Fig. 1B. While both the short 212 bp F1 fragment and the 380 bp F2 fragment did show modest transcriptional activity, inclusion of the additional 36 bp in the F3 fragment had a significant effect on the basal transcriptional activity. There was an 8- to 10-fold increase in luciferase activity with the addition of this area. This indicates that this 36 bp fragment contains transcription activation element(s) of critical importance to the expression of the Scd gene and thus designated the transcription enhancer element (STE). In addition, a further twofold increase in promoter activity was seen by the extra 346 bp included in the 760 bp F4 fragment. Further extension (F5, F6) of the promoter fragments did not have any effect on the overall transcriptional activity.

Effect of CLA on Scd gene transcription

Previous studies have shown that supplemental feeding of CLA to dairy cows can have effects on the desaturase ratio. For this reason we were interested in the effect that CLA would have on activation of the bovine *Scd* gene promoter. Mac-T cells were transfected with a number of luciferase reporter vectors under the control of varying lengths of the *Scd* gene promoter, and cells treated with either the *cis*-9, *trans*-11 or *trans*-10, *cis*-12 CLA isomer. The CLA isomers had been fused to BSA to act as a carrier, thus BSA was used as a control for this experiment. In addition, the effect of CLA on the *Scd* gene promoter was compared to that of vaccenic acid.

In the promoter fragment that did not contain the STE (F2), CLA had the effect of increasing transcriptional activity of the promoter, with the *cis*-9, *trans*-11 isomer having the highest transcriptional activity (50% increase compared to no CLA control DMEM group), while the BSA and vaccenic acid had no effect (Fig. 2A). When the STE enhancer region was included, however, the effect of both CLA isomers was dramatic. The promoter activity was reduced more than 10-fold with CLA treatments, and there was also a difference between isomers, with the trans-10, cis-12 isomer having a greater effect on the promoter (Fig. 2B). The down-regulation effect of CLA on the Scd promoter begins with the inclusion of the 36 bp enhancer element and is maintained as promoter length increased (Fig. 3B). This indicates that it is this enhancer region that is critical for mediating the effect of CLA on the Scd gene promoter.



Fig. 2. Effect of CLA on *Scd* gene transcription. (A) Activities of the F2 (380 bp) *Scd* promoter fragment in Mac-T cells treated with 30 μ M of BSA, both CLA isomers, or vaccenic acid. Different letters above histograms represent differences among treatments (P < 0.05). (B) Activities of the F3 (416 bp), F4 (760 bp) and F6 (1523 bp) *Scd* promoter fragments in Mac-T cells treated with 30 μ M BSA, both CLA isomers, or vaccenic acid. * and ** above histograms represent differences among treatments (P < 0.05) in each promoter construct group.

Effect of fatty acids on Scd transcriptional activity

In Fig. 3, the 1523 bp F6 promoter fragment was employed to determine any effect of other *Scd* enzyme substrate fatty acids on the bovine *Scd* promoter. Thirty micromolar concentrations of different fatty acids were used to treat the cells in Figs. 3A and B. As seen in Fig. 3A, there was no effect of the polyunsaturated fatty acids (PUFA), linoleic and linolenic acids, on *Scd* transcription. Fig. 3B shows the effect of stearic, oleic, and vaccenic acids on the promoter activity. Both stearic and vaccenic acids did not substantially up-regulate the *Scd* transcription rate but oleic acid did significantly downregulate the transcription rate. In addition, increasing concentrations (10, 50, and 100 μ M) of vaccenic acid (*trans*-11 18:1) did not have any effect on promoter activity (Fig. 3C).

Effect of lactogenic hormones on Scd transcription

The effects of prolactin, dexamethasone (a synthetic glucocorticoid), insulin, and leptin, both alone and in combination on the *Scd* promoter activity were investigated in vitro using Mac-T cells (Fig. 4). Insulin alone showed a significant increase on activation of the *Scd* gene promoter, further evidence of its pro-lipogenic role. The other hormones alone



Fig. 3. Effect of linoleic acid, linolenic acid, vaccenic acid, stearic acid or oleic acid on *Scd* promoter activity. (A) Effect of PUFA on *Scd* promoter activity. Scd-F6 promoter construct was transfected into Mac-T cells treated with 30 μ M of control (BSA only), linoleic acid, or linolenic acid. (B) Effect of *Scd* substrates on Scd promoter activity. Scd-F6 promoter construct was transfected into Mac-T cells treated with 30 μ M of control (BSA only), vaccenic, stearic or oleic acids. (C) Effect of increasing concentrations of vaccenic (*trans*-11 18:1) on *Scd* promoter activity. Scd-F6 promoter construct was transfected into Mac-T cells treated with either control (BSA only) or 10, 50, and 100 μ M of vaccenic acid. Different letters above histograms represent differences among treatments (P < 0.05).

or in combination with insulin did not have any significant effect on transcriptional activity, however there was a consistent trend of reduction in transcriptional activity when all four hormones were supplied to the cells in combination, but this was not statistically significant.

Nuclear proteins binding to 36 bp STE region

To examine the potential binding of nuclear proteins in Mac-T cells to the 36 bp STE region, nuclear extracts



Fig. 4. Effect of lactogenic hormones on *Scd* promoter activity. Scd-F6 promoter construct was transfected into Mac-T cells and treated with lactogenic hormones, insulin (I) (10 mg/ml), prolactin (P) (5 μ g/ml), dexamethasone (D) (200 ng/ml), and leptin (L) (10 ng/ml), both alone and in combination. Different letters above histograms represent differences among treatments (P < 0.05).

were isolated from the Mac-T cells and used in EMSAs with ³²P-labeled STE oligonucleotide probe. The EMSA showed three binding complexes formed on the STE region (Fig. 5), indicating that potentially three nuclear proteins bind to this area. Interestingly when we used two separate fragments (P2 and P3) (Fig. 5A), which split the 36 bp STE region in half but covered the entire region, in EMSA, the binding pattern was completely changed, with the loss of the smaller binding complexes and presence of different binding doublets binding to both of the fragments.



Fig. 5. Binding of nuclear proteins from Mac-T cells to the 36 bp STE by EMSA. (A) Sequence and predicted transcription factor binding sites of the STE region. (B) The STE (lanes 1 and 2) and P1 (lane 3) and P2 (lane 4) oligonucleotides were ³²P-labelled, incubated with the nuclear extracts of Mac-T cells, and subjected to EMSA. In lane 1, 100-fold unlabeled STE oligonucleotide was added into the reaction.

Discussion

The Scd gene plays a critical role in lipid synthesis in the bovine mammary gland, however, regulation of its expression has up to this point been poorly understood. In a previous study, the bovine Scd gene promoter was isolated and analyzed by promoter analysis software [33]. In particular, two areas of conservation between the human, mouse and bovine gene promoters were identified and the potential transcription factors with binding sites in this area were identified by screening the TRANSFAC 4.0 database of transcription factor sequences [36] with the MatInspector V2.2 program [37]. The first conserved area was situated from -37 to -134 and contained the TATA box and binding sites for AP-1, SP-1, HNF4, and a fat-specific element (FSE). The second conserved region occurred at -313 to -390 and has been designated the PUFA response region. Putative binding sites in this region include the sites for transcription factors NF-Y, NF-1, NF-Y/NF-1, SREBP, and SP-1. In the current study, we characterized the bovine Scd gene promoter for its basal transcriptional activity through cloning different lengths of the Scd gene promoter into a luciferase reporter system and analyzing the basal activity of these promoter fragments in a bovine mammary epithelial cell line (Mac-T). Our data showed that the F1 (212 bp) fragment which contains the first conserved region and the F2 (380 bp) which contains the first conserved region plus the partial second conserved PUFA region have only a modest basal transcriptional activity, while inclusion of an additional 36 bp of the PUFA response region in the F3 fragment (416 bp) had a significant increase (8- to 10-fold) on the promoter activity. This indicates that this 36 bp fragment is of critical importance to the expression of the Scd gene and contains binding sites for the transcriptional activators of the Scd gene. We designate this critical 36 bp area as the Scd transcriptional enhancer element (STE). In addition, a further twofold increase in promoter activity was seen by the extra 346 bp included in the 760 bp F4 fragment, indicating presence of important transcriptional activators also in this region. Further extension (F5, F6) of the promoter fragments did not have any effect on the overall transcriptional activity. Thus, the 760 bp upstream sequence may represent the full promoter region required for the basal promoter activity.

Sequence analysis of the STE region reveals potential binding sites for SREBP (sterol response element binding protein), RFX1 (regulatory factor for X box), and NF-Y. The SREBP has been shown to activate *scd* genes in the mouse [34,38] and transcriptional activation of SREBP to the target genes requires an additional transcription factor(s), so far identified as the Sp1 and the CCAAT-binding factor/NF-Y [39–42]. The bovine STE also contains the CCAAT box, implying a potential interaction of SREBP with the CCAAT-binding factor in this region. The CCAAT-binding factor (or NF-Y) has previously been shown to be critical for transcriptional activation in the human promoter [28] and the CCAAT element is also required for full activation of mouse scd1 and scd2 promoters [34]. As a first step to explore the transcription factors which bind to the STE region and are important in activation of the Scd promoter, we performed EMSA using nuclear extracts from Mac-T cells and a ³²P-labeled STE oligonucleotide. Three binding complexes were detected. However, the binding patterns were completely changed when we used two fragments (P2 and P3), which split the full STE sequence, as probes (Fig. 4A). Division of the STE resulted in larger binding doublets and the loss of the smaller binding complexes. This indicates that the binding property of the entire STE sequences is different from the short sequences and the entire STE sequence may be required for the binding of critical transcriptional activators in this region. The nature of these binding complexes remains to be identified.

A number of studies have reported that de novo fatty acid synthesis is reduced with increased trans-10, cis-12 CLA treatment [43-45] and either the Scd mRNA is reduced or the desaturase activity indicators are altered with trans-10, cis-12 CLA supplementation [43,44]. These data suggest some effect of CLA on Scd gene expression. Our current study demonstrated this effect. In the promoter fragment that did not contain the 36 bp enhancer region, CLA had the effect of increasing transcriptional activity of the promoter, however, when the enhancer region was included, CLA isomers dramatically inhibited the promoter activity and there was also a difference between isomers, with the trans-10, cis-12 isomer having a greater effect on the promoter. The down-regulation effect of CLA on the Scd promoter is maintained as promoter length increased. This indicates that it is this STE enhancer region that is also critical for mediating the effect of CLA on the Scd gene promoter.

The identification of *Scd* promoter down-regulation by CLA may explain the previously observed reduction of Scd mRNA by supplemental feeding of dairy cows with the trans-10, cis-12 CLA isomer in particular being a result of direct transcriptional down-regulation. Since the STE region plays a critical role in the down-regulation, identification of transcription factors binding to this region will help us understand the mechanisms of this regulation. A previous study by Peterson et al. [46] in cultured Mac-T cells showed that the active form of the SREBP protein was not present when cells were incubated in the presence of trans-10, cis-12 CLA. This may explain the effect seen in our study, as the SREBP protein is predicted to bind in this region. It seems likely that the SREBP protein is not activated during CLA treatment, resulting in a loss of its binding to the STE region and inhibition of the Scd promoter.

The murine *Scd*-1 gene has been shown to be responsive to PUFA, which down-regulate the gene in both liver and primary hepatocytes by affecting their transcription rate. *Scd*-1 expression in mature adipocytes,

however, is down-regulated through effects on the mRNA half-life stability [47]. In the bovine promoter, we did not find any significant effect of addition of PUFA to the media on the Scd transcription rate in a mammary epithelial cell-line. This may reflect a tissue specific effect. In addition, the murine and bovine differ in the number of Scd genes present, thus, the effect may also be isoform specific.

Key substrates for the Scd enzyme in the lactating ruminant are stearic and vaccenic acids. Stearic and vaccenic acids are converted to oleic and conjugated linoleic acid (CLA), respectively. Oleic acid is the preferred substrate for the cholesterol ester and triglyceride synthesis enzymes acyl-CoA:cholesterol transferase (ACAT) and diacylglycerol transferase (DGAT). The ratio of stearic acid to oleic acid (also known as the desaturase index) has been implicated in the regulation of cell growth and differentiation due to its effect on membrane fluidity [1,48] and signal transduction [49]. The conversion of vaccenic acid to CLA by the action of the Scd enzyme accounts for the majority of CLA in bovine milk. In this study, we investigated the effects of 30 µM concentrations of these substrates on the action of the Scd promoter. Our results show that both stearic and vaccenic acids do not affect the Scd transcription rate but that oleic acid does downregulate the transcription rate, perhaps indicating that there is a negative feedback effect of oleic acid on Scd expression which regulates the desaturase index and maintains membrane fluidity, a key factor in maintaining normal cell function.

The mammary gland is the major site of Scd expression in lactating ruminants [2-4]. Scd has also been shown to have a role in energy expenditure through its co-operation with the hormone Leptin. Cohen et al. [50] identified a number of genes as being leptin-regulated in liver tissue in leptin-deficient mice, the most robust of these being Scd. Scd mRNA levels, enzyme activity, and MUFA levels were all markedly increased in leptin knockout mice and specifically reduced by leptin administration. To study the effect of the lactogenic hormones, insulin, glucocorticoid and prolactin, and leptin on Scd promoter activity, we examined the Scd promoter activity in Mac-T cells treated by individual hormones or their combinations. Insulin alone showed a significant stimulative effect, consistent with its pro-lipogenic role. The remaining hormones did not have any effect on transcriptional activity in Mac-T cells.

In conclusion, in this study, we characterized the bovine *Scd* gene promoter for its basal transcriptional activity and identified a region of critical importance, designated the stearoyl-CoA desaturase transcriptional enhancer element (STE). This element is 36 bp in length, located from -366 to -402 upstream of the putative transcription initiation site and is bound by nuclear proteins from bovine mammary cells to form three complexes. This STE region also plays a key role in the inhibitory effect of CLA on *Scd* transcription.

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