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Isolation and characterisation of the bovine *Stearoyl-CoA desaturase* promoter and analysis of polymorphisms in the promoter region in dairy cows.

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The nucleotide sequence data reported in this paper have been submitted to GenBank and has been assigned the accession number AJ555480.

ABSTRACT

Conjugated linoleic acid (CLA) in milk arises through microbial biohydrogenation of dietary polyunsaturated fatty acids (PUFA) in the rumen, and by the action of mammary Stearoyl-CoA desaturase (Scd). A large variation (up to ten-fold) in the concentration of this fatty acid in milk have been observed, even in cows receiving the same diet. The reasons for this variation are not well understood. In this study, the bovine core promoter region was isolated by a genome walking strategy from genomic DNA GenomeWalker libraries and then cloned and characterised. This core promoter sequence extended approximately 600bp upstream of the translation start site. The presence of putative transcription factor binding sites conserved in bovine, human, and mouse promoters were observed. Evidence that this promoter fragment was functional *in vivo* was obtained from expression studies in a mammary cell line. The promoter sequence of the *scd* gene was compared between cows selected for the ability to produce high fatty acid methyl esters (FAME) (2.22-2.72) in their milk, with the same promoter region of low FAME producing cows (0.81-1.12). However, such comparisons of the sequences of the *scd* promoter region of cows producing high milk CLA compared with low CLA revealed no polymorphisms in this promoter segment. Furthermore, no sequence polymorphisms were observed between the *scd* promoter region of Holstein Friesian, Montbeliarde, Normande, Norwegian Red, Charlois, Limousin and Kerry breeds.

(**Key words**: Conjugated Linoleic acid, Stearoyl-CoA desaturase, Promoter, Polymorphisms).

Abbreviation key: CLA = Conjugated Linoleic Acid, **scd** = Stearoyl-CoA desaturase, **PCR** = polymerase chain reaction, **SNPs** = Single Nucleotide Polymorphisms, **PUFA** = Polyunsaturated Fatty Acids, **FAME** = fatty acid methyl esters.

INTRODUCTION

Conjugated Linoleic acid (CLA) is a collective term to describe one or more positional and geometric isomers of linoleic acid (*cis*-9, *cis*-12-$C_{18:2}$). The *cis*-9, *trans*-11-$C_{18:2}$ isomer is the predominant one in the human diet as a result of microbial biohydrogenation in the rumen. This isomer is produced in ruminants directly as an intermediate during the microbial biohydrogenation of dietary linoleic acid, and endogeneously from *trans*-vaccenic (*trans*-11-$C_{18:1}$) acid in mammary tissue by the action of Stearoyl-CoA desaturase (Scd) (Griinari *et al.,* 2000). Animal-fat containing foods including dairy products, beef and lamb are rich sources of CLA (Chin *et al.,* 1992; Fritsche and Steinhart, 1998; O'Shea *et al.,* 2000).

CLA has attracted much attention in recent years, due to its many potential health benefits. Studies have shown that CLA exhibits anti-carcinogenic activity in animal models (Belury *et al.,* 1995; Ha *et al.,* 1990; Ip *et al.,* 1996; Liew *et al.,* 1995) and in *in vitro* studies using a range of human cancer cell lines including mammary (Miller *et al.,* 2001; Park *et al.,* 2000; Visonneau *et al.,* 1997), prostate (Cesano *et al.,* 1998; Palombo *et al.,* 2002) and colon, (Miller *et al.,* 2001; Palombo *et al.,* 2002). Other properties of CLA include anti-atherogenic activity (Lee *et al.,* 1994; Nicolosi *et al.,* 1993), the ability to reduce the catabolic effects of immune stimulation (Cook *et al.,* 1993; Millar *et al.,* 1994), the ability to enhance growth promotion (Chin *et al.,* 1994) and the ability to reduce body fat (Pariza *et al.,* 1996).

During biohydrogenation of linoleic acid in the rumen, CLA is produced. This CLA is largely a transient intermediate and is involved as an intermediate in the biohydrogenation pathway to produce stearic acid. A build up of *trans*-vaccenic acid

occurs (Harfoot and Hazelwood, 1988). *Trans*-vaccenic acid is also an intermediate in the biohydrogenation of other PUFA (Griinari and Bauman, 1999). Several studies have shown that substantial amounts of *trans*-vaccenic acid (60-300g/day) reach the duodenum of lactating cows (Kalscheur *et al.*, 1997a, 1997b; Wonsil *et al.*, 1994). Infusion studies using *trans*-vaccenic acid post-ruminally resulted in elevated *cis*-9, *trans*-11 CLA in milk-fat, indicating conversion in the mammary gland (Griinari and Bauman, 1999). Several studies have found substantial Scd activity in both mammary and adipose tissue of ruminant animals (Kinsella *et al.*, 1972; Martin *et al.*, 1999; St John *et al.*, 1991, Ward *et al.*, 1998). Mammary Scd enzyme, which converts *trans*-vaccenic acid to *cis*-9, *trans*-11 CLA is believed to be responsible for the formation of the majority of CLA in milk (Griinari and Bauman, 1999).

Animal diet is a major factor affecting the milk-fat content of CLA, with dietary supplements containing oils rich in PUFA, such as linoleic and linolenic acids, being the most effective for CLA enrichment of milk. However, substantial variations in the CLA content of milk-fat (ranging from 3 to 10-fold) of cows on the same dietary treatment have been observed in a number of studies (Jiang *et al.*, 1996; Stanton *et al.*, 1997; Kelly *et al.*, 1998a; 1998b; Lawless *et al.*, 1998; 1999; Peterson *et al.*, 2002; Solomon *et al.*, 2000; White *et al.*, 2001). The reasons for this variation are not well understood. Perhaps ruminally derived CLA is a more important contributor to milk CLA in grass-fed cows. The variation in the CLA content of milk-fat may also be due to a variation in rumen conditions leading to differences in the availability of CLA (or CLA precursors) that escape from the rumen. It might also be caused by differences in mammary Scd activity associated with either regulation of *scd* gene expression, differences in structure of the enzyme due to gene polymorphisms, or differences in

89 downstream factors that would affect interaction between enzyme and substrate (e.g.
90 phosphorylation) (Peterson *et al.,* 2002).
91
92 The promoter region of the human (Bene *et al.,* 2001; Zhang *et al.,* 2001), chicken
93 (Lefevre *et al.,* 2001), and mouse (Ntambi *et al.,* 1988; Kaestner *et al.,* 1989; Mihara,
94 1990) *scd* genes have been isolated, cloned and characterized. It has been shown in
95 these studies that there is a conserved PUFA response region in all three, and that this
96 includes critical binding sites for Sterol Response Element Binding Protein (SREBP)
97 and Nuclear Factor-Y (NF-Y) transcription factors. Sequence comparison of the
98 human and mouse promoters indicated a second region of high homology including
99 the 5'UTR and basal/proximal promoter. It has been suggested that there are two
100 different transcription start sites in the human promoter, and that these may be
101 dependent on tissue-specific factors (Zhang *et al.,* 2001).
102
103 In this study, the role that polymorphisms in the core promoter region of the bovine
104 *scd* gene might play in influencing regulation of the *scd* gene was investigated. The
105 bovine core promoter was therefore isolated from genomic DNA by a genome
106 walking approach using primers designed to a genomic database sequence for the
107 bovine *Stearoyl-CoA desaturase* gene (AF481915) that contained 162 bases of
108 5'UTR. Analysis of this promoter sequence identified a number of conserved
109 potential transcription factor binding sites based on comparison with the human and
110 mouse *scd* promoter regions and interrogation of the TRANSFAC 4.0 database of
111 transcription factor sequences (Wingender *et al.,* 2000**)** with the MatInspector V2.2
112 program (Quandt *et al.,* 1995). Importantly, and for the first time for bovine *scd*, this
113 core promoter was shown to drive transcription of the reporter gene luciferase in an *in*

vitro mammalian culture system, confirming its presumed function. A number of animals were screened for possible polymorphisms in this promoter region. These included cows producing high milk-fat CLA as a % of fatty acid methyl ester (% FAME) content (2.22-2.72), compared with cows yielding a low milk-fat CLA as a % of FAME content (0.81-1.12). Additionally, sequence comparisons of the *scd* promoter region from a number of different breeds were made.

A longer bovine scd promoter sequence (Acc No. AY241932) is now included in the Genbank database but this was not available at the time of this study: the two promoter sequences were submitted within a very short time of each other.

MATERIALS AND METHODS

Construction of GenomeWalker (GW) libraries

To obtain the 5' flanking sequence of the *scd* gene, GenomeWalker (GW) libraries were constructed from bovine total genomic DNA using a Universal GenomeWalker kit (Clontech, UK) according to the manufacturer's instructions. High quality genomic DNA was first extracted from cultured lymphoblast cells from a Holstein-Friesian animal. Briefly, a cell suspension (15 ml) was centrifuged at 1200 g for 5min and the pellets mixed and incubated overnight at 37^{o}C in 900 µl 0.2M EDTA 0.5% sodium-*n*-lauroyl sarcosine and 25µl of proteinase K (20mg/ml). 10µl of RNase (2mg/ml) was added to each tube and incubated at 37^{o}C for 1.5 hr. The mix was split in two, 200 µl phenol added, mixed and incubated for 30 min at 37^{o}C in a rotary mixer. Chloroform (200 µl) was added, tubes were shaken vigorously and incubated at 37^{o}C for 1 hr in a rotary mixer. Tubes were centrifuged at 16000 g for 15 min to form two layers. The upper layer was transferred to a fresh tube and two volumes of ice-cold 100% ethanol added. Tubes were inverted abruptly four times, and at this point a DNA precipitate was obtained. The supernatant was decanted, 100 µl 70% ethanol added and incubated overnight at 21^{o}C in a rotary mixer. Ethanol was decanted off and the pellet was allowed to air-dry. The DNA pellets were resuspended in 50 µl 10mM Tris-HCl, pH8.0 containing 1mM EDTA (TE) buffer and aliquots were checked for integrity on a 0.7% w/v agarose gel containing ethidium bromide (EtBr) (2µg/ml) gel.

The genomic DNA was digested with four restriction enzymes, *Dra*I, *Stu*I, *Eco*RV, and *Pvu*II, to create four pools of DNA fragments. Adaptors were ligated onto both

ends of these fragments to create four GW libraries and these libraries were designated DL1 (*Dra*I), DL2 (*Stu*I), DL3 (*Eco*RV), and DL4 (*Pvu*II).

Isolation and characterisation of the *scd* promoter from GW libraries

To specifically isolate the *scd* promoter, genome walking primers pAKGW1 (5'-GCTCTCAGACACTGGGATCACTTTCTCGGG-3') and pAKGW2 (5'-AACTGAGTGTAGACTAGTTCCTGAGCCTGC-3') were designed using Vector NTI software (InforMax, Inc.) to a genomic database sequence for the bovine *scd* gene (AF481915) and synthesized by MWG Biotech (Ger). The primary PCR was carried out using 1μl of the GenomeWalker libraries with the gene-specific primer, pAKGW1, and adaptor primer, AP1 (from kit). The primary PCR products were diluted 1:50 and used as template for nested PCR with a second gene-specific primer, pAKGW2, and a second adaptor primer AP2 (again from kit). The reaction final volume was 50 μl containing *Taq* DNA polymerase buffer (Invitrogen), 1.5mM $MgCl_2$, 200μM dNTPs (Promega), 0.3μM each primer, and 1U *Taq* polymerase (Invitrogen). Immediately prior to cycling 2.5 μl of DMSO was added to the mix. The reaction was amplified for 35 cycles for both the first and second PCR. Cycling was performed in a DNA Engine thermal cycler (MJ Research) and conditions were 95^{o}C for 2min, followed by 35 cycles of 95^{o}C for 30s, 60^{o}C for 30s and 72^{o}C for 3 min. This was followed by a 72^{o}C final extension step for 7 min.

PCR products were directly cloned into the pCR2.1 vector (Invitrogen) and transformed using One Shot Top-10 chemically competent *E. coli* cells according to the manufacturer's instructions (Invitrogen). Colony PCR was performed to identify clones that potentially carried the *scd* promoter. Small-scale preparations of plasmid

DNA were made from these colonies using the procedure outlined by the manufacturer (Sigma). Sequencing of three plasmids (DL1.2, DL1.4 and DL1.8) was performed by MWG Biotech (Ger). The resulting sequences were analysed using Vector NTI software (InforMax, Inc). Potential transcription factor binding sites were identified using the MatInspector V2.2 program (Quandt *et al.,* 1995) by interrogation of the TRANSFAC 4.0 database of transcription factor sequences (Wingender *et al.,* 2000).

Luciferase reporter vector construction

pCR2.1-based plasmids containing the putative *scd* promoter were restriction digested at 37^{o}C to release the fragment and create compatible ends for sub-cloning into the pGL3-Basic (Promega) promoter-less expression vector. The pGL3-Basic vector was digested in the same manner. Ligation reactions were transformed into TAM Ultracomp chemically competent *E. coli* cells (Active Motif Europe), using instructions recommended by the manufacturer.

Cell culture

Chinese Hamster Ovary K1 (CHO-K1) cells obtained from ATCC (CCL-61) were cultured in Dulbecco's Modified Eagles Medium F12 (Biowhittaker, UK) containing 10% (v/v) foetal bovine serum (Invitrogen). Human mammary MCF-7 cells (ATCC – HTB22) were cultured in Eagles Minimum Essential Media (Invitrogen) containing 10% (v/v) foetal bovine serum (Invitrogen) and 1% (v/v) non-essential amino acids (Invitrogen). Cells were routinely passaged every 3-4 days by washing with phosphate buffered saline (PBS) and treating with 3ml EDTA-trypsin (Sigma) to remove adherent cells. Cells were maintained in a humidified incubator at 37^{o}C and

198 5% CO_2. Cells were grown to 80% confluency and both CHO-K1 and MCF-7 cells
199 were transiently co-transfected with luciferase reporter vectors using Fugene 6
200 transfection reagent (Roche Diagnostics). Six-well transfection plates were seeded at
201 a concentration of 3 x 10^5 cells per well and incubated overnight at 37^{o}C and 5% CO_2.
202 pGL3-Scd experimental constructs (1μg) were co-transfected with 25ng of pRL-SV40
203 plasmid (Promega) to control for transfection efficiency. Forty-eight hours after
204 transfection, media was removed from the wells, 300μl Passive Lysis Buffer
205 (Promega) was added followed by incubation at room temperature for 10min.
206
207 **Luciferase assay**
208 The Dual Luciferase Assay Kit (Promega) was used to measure both Renilla and
209 Firefly luciferase expression by the reporter vectors on a Tecan Spectrafluor Plus
210 luminometer using the Magellan software (Tecan). Mean Firefly luciferase activity
211 values were corrected for variations in transfection efficiency using the corresponding
212 mean Renilla luciferase figures. The corrected values were expressed as a percentage
213 of the positive control value (pGL3-Control). Transfection values were a result of
214 three independent transfections, with n=6, for both cell types.
215
216 **Analysis of *scd* promoter polymorphism incidence**
217 A dairy herd (n=75) had been on a ryegrass diet for a period of six months and their
218 milk was analysed for fatty acid methyl esters content (% FAME) using the gas liquid
219 chromatography (GLC) method described previously (Stanton *et al.,* 1997). FAME
220 content was measured in milk samples from 59 animals taken at the evening milking
221 on two occasions during the grazing season, in July and September. Nine of these 75
222 cows with consistently low and high milk CLA as a % of FAME values over both

sampling times were selected for this study. The CLA isomer measured was the *cis*-9, *trans*-11 CLA isomer. High CLA animals produced greater than 2.0% FAME (2.22-2.72) in their milk, compared with low milk CLA producers of ~ 1.0% FAME (0.81-1.12). The CLA as a % of FAME ranged from 0.81 to 2.72 as quoted in Table I and are the mean values of both sampling times.

Genomic DNA, for PCR amplification of the *scd* promoter fragment, was extracted from whole blood (200µl) collected in heparinised tubes from the coccygeal vein from these nine Holstein Friesian cows and in addition ten cows of different bovine breeds using a Gentra capture column™ (Gentra). Amplification of the bovine promoter was performed using primers D9Dfor (5'-TGATGGGGTAGTGAGGAGC-3') and D9Drev (5'-GTTCCTGAGCCTGCTTTTGC-3') with 1µl of genomic DNA as template (~200ng), in a final volume of 50µl, containing *Taq* DNA polymerase buffer (Invitrogen), 1.5mM $MgCl_2$, 200µM dNTPs (Promega), 0.3µM each primer, and 1U *Taq* polymerase (Invitrogen). Cycling was performed in a DNA Engine thermal cycler (MJ Research) and conditions were 95^{o}C for 1min, followed by 35 cycles of 93^{o}C for 1min, 58.5^{o}C for 1min, 72^{o}C for 2min and a final extension step of 72^{o}C for 10min. PCR products were purified using a PCR purification kit (Qiagen). DNA was eluted in 30µl of PCR grade water (Sigma).

RESULTS and DISCUSSION

Isolation of the bovine *scd* promoter

Isolation of the bovine *scd* promoter was achieved using a genome walking strategy, which involved digestion of bovine genomic DNA with four restriction enzymes, *Dra*I, *Stu*I, *Eco*RV, and *Pvu*II, to create pools of short DNA fragments of varying lengths. Adaptors were ligated onto the ends of these pools of DNA and PCR employed to "walk" along the genome. Primers for PCR were designed to a bovine genomic sequence for the *scd* gene (AF481915) that extends approximately 160bp upstream of the translation initiation codon. PCR products were obtained for libraries DL1 (*Dra*I), DL2 (*Stu*I) and DL3 (*Eco*RV), but not for the DL4 (*Pvu*II) library. Library DL1 yielded a mixture of fragments but as these were also the longest, these fragments were purified as a mixed pool and cloned into the pCR2.1 vector. Colony PCR was performed and three plasmids (DL1.2, DL1.4 and DL1.8) were sequenced. BlastN analysis of the three sequences indicated that they were all fragments of the bovine *scd* gene and the DL1.4 sequence extended upstream of the bovine genomic database sequence, AF481915.

Comparison of bovine, human and mouse *scd* promoters

An alignment of the DL1.4 sequence was performed with a human promoter sequence (AF320307) (Zhang *et al.,* 2001) and a mouse promoter sequence (M21280) (Figure I). The bovine sequence displayed 67% identity with the human *scd* sequence and 59% homology with the murine promoter sequence *scd*1. The bovine sequence has been submitted to the Genbank database (Acc. No. AJ555480). Transcription start sites have been identified in both the mouse (Ntambi *et al*., 1988) and human (Zhang

et al., 2001) genes at 28bp and 37bp respectively from the proximal TATA box in the bovine sequence. The bovine promoter sequence presented in Figure I is numbered relative to the human transcription start site, and extends to -407.

Potential transcription factor binding sites were compared with those predicted for the human promoter (Zhang *et al.,* 2001). Putative transcription-factor binding sites identified using the MatInspector program (Quandt *et al.,* 1995) included Nuclear factor Y, Octamer binding factor 1, and muscle-specific Mt-binding sites. Comparison of the mouse, human and bovine sequences identified a number of conserved sequences in two particular regions of the promoters. The first area of conservation occurs between -37 to –119, where there are two conserved TATA sequences (5'-TTTAAAT-3' and 5'-TAAAA-3'), a fat-specific element (FSE) (5'-CTGAGGAAA-3') (-77 to –86), and binding sites for the transcription factors AP-1 (-64 to – 68), NF-1 (-102 to –109) and HNF4 (-116 to –119). Analysis of the human *SCD* promoter has shown that for liver and hair follicles, there was a major transcription initiation site 35 nucleotides downstream from the proximal TATA box (Zhang *et al*., 2001). It was also shown that another transcription initiation site was present 37 nucleotides downstream from the distal TATA box (Bene *et al*., 2001), and it was suggested that the human *SCD* gene has different start sites that depend on different tissue-specific factors (Zhang *et al*., 2001). The TATA sequence, TTTAAAT, is somewhat unusual: this sequence, where the A in the second position is replaced by a C, T, or G, has been shown to reduce the efficiency of a promoter in *in vitro* studies (Conchino *et al*., 1983).

The second conserved region occurs at –313 to –390 and has been designated the PUFA response region. Putative binding sites for transcription factors NF-Y (–313 to –317), NF-1 (-333 to –336), NF-Y/NF-1 (-351 to –361), SREBP (-366 to –376), and SP-1 (-386 to –390) are conserved between the three promoter sequences. The PUFA response region present in the bovine sequence is an element previously shown to mediate the down-regulation of mouse *scd* expression in response to PUFA (Waters *et al.,* 1997). The Sterol Response Element binding protein (SREBP) has been shown to activate *scd* genes in the mouse (Tabor *et al.,* 1999; Shimomura *et al.,* 1998), and it has also been shown that PUFA negatively regulate SREBP mRNA and protein activation (Xu *et al.,* 1999; Yahagi *et al.,* 1999). This bovine promoter fragment also contains the CCAAT box at -356 to –360 previously shown to be critical for transcriptional activation in the human promoter (Zhang *et al.,* 2001). This CCAAT element is also required for full activation of mouse *scd*1 and *scd*2 promoters and binds the NF-Y transcription factor (Tabor *et al.,* 1999).

Transcription studies in CHO-K1 and MCF-7 cells

To determine whether this 407bp putative promoter sequence was sufficient to direct transcription, luciferase reporter gene constructs containing the putative promoter cloned into the promoter site of the promoter-less expression vector pGL3-Basic were constructed. A plasmid (pRL-SV40) providing constitutive expression of Renilla luciferase was co-transfected to serve as transfection efficiency control. Two cell types were transfected, a human mammary cell line, MCF-7, and a Chinese hamster ovary cell line, CHO-K1. The CHO-K1 cell line is most commonly used to express mammalian genes *in vitro* and should contain the general transcription factors necessary for activating transcription of most genes. The human mammary cell line

contains more tissue-specific transcription factors, and is a more suitable system for studying regulation of a gene expressed in the mammary gland.

The putative promoter showed promoter activity in both CHO-K1 and MCF-7 cell lines (Figure II). Promoter activity was greatest in the mammary MCF-7 cell line being 6-fold higher in MCF-7 cells than in CHO-K1 cells. These results indicate that the 407bp region upstream of the proposed transcription start site is sufficient to direct transcription.

Promoter polymorphism screen

Animals receiving an identical dietary treatment of ryegrass for a 6-month period were seen to have milk CLA as a % of FAME varying from 0.81 to 2.72. The sample size selected was initially 59 Holstein Friesian cows, from which milk was analysed for CLA content and subsequently 9 cows were selected and divided into two groups of high and low CLA producers. There were no differences in the milk yields, feed intake and milk fat content of the high v's low CLA animals. However, we did observe that blood glucose and non-essential fatty acids (NEFA) differed significantly between high and low CLA cows, with glucose being higher in the blood of the low CLA group whereas NEFA was lower in the low CLA group compared to the high CLA group. To investigate whether polymorphisms in the bovine *scd* promoter could explain the considerably large inter-cow variation in milk fat CLA content, the nucleotide sequences of the promoter region of nine cows (Table I) were analysed to identify sequence polymorphisms which may be related to elevation of milk fat CLA.

In an attempt to also include an increased genetically diverse element, the promoter region of the *scd* gene was amplified and sequenced from ten animals of different breeds (high genetic merit Holstein Friesian, low genetic merit Holstein Friesian, Montbeliarde, Normande, Norwegian Red, Irish Friesian, Dutch Freisian, Charlois, Limousin, Kerry). No information regarding the milk CLA status of these animals was available.

The *scd* promoter region sequences were aligned for these nineteen animals (nine animals showing high and low milk CLA as a % of FAME and ten animals of varying bovine breeds) and compared to search for any polymorphisms in the area (Data not shown). This alignment showed the total absence of any polymorphic sites between the bovine *scd* promoters of 19 (nine high and low milk CLA and ten animals of different breeds). This high conservation may be significant in indicating that the regulation of this gene is under extremely rigid control by transcription factors, and a high conservation is necessary for full activation.

CONCLUSION

In conclusion we have isolated and partially characterised the bovine *scd* promoter. This promoter fragment showed 59% and 67% similarity to both the mouse and human sequences, respectively. Differences in the CLA content of milk from these animals were not a consequence of polymorphisms within the core promoter of this gene. The *scd* promoter sequence exhibits remarkable sequence conservation not just across high and low CLA yielding Holstein Friesians but also across ten different breeds. In contrast considerable differences were seen between human and mouse sequences which included variation in non-binding regions and binding regions for

AP-1, SP-1, and NF-KB transcription factors. Consequently the observed variation in the levels of milk CLA produced in the Holstein Friesian animals may be explained by differences in ruminant synthesis of CLA or CLA precursors, polymorphisms in the coding sequences of the bovine *scd* gene, or differences in the regulatory proteins themselves, an area that requires further investigation.

ACKNOWLEDGEMENTS:

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TABLES and FIGURES

Table I: High and low CLA animals.

Animal	Breed	% FAME
330	Holstein Friesian	0.81 +/- 0.49
385	Holstein Friesian	0.88 +/- 0.49
387	Holstein Friesian	1.04 +/- 0.49
44	Holstein Friesian	1.12 +/- 0.49
728	Holstein Friesian	2.22 +/- 0.49
67	Holstein Friesian	2.27 +/- 0.49
249	Holstein Friesian	2.32 +/- 0.49
376	Holstein Friesian	2.34 +/- 0.49
225	Holstein Friesian	2.72 +/- 0.49

Figure I: Alignment of Human and Mouse Stearoyl-CoA desaturase promoters with DL1.4 (Bovine) sequence.

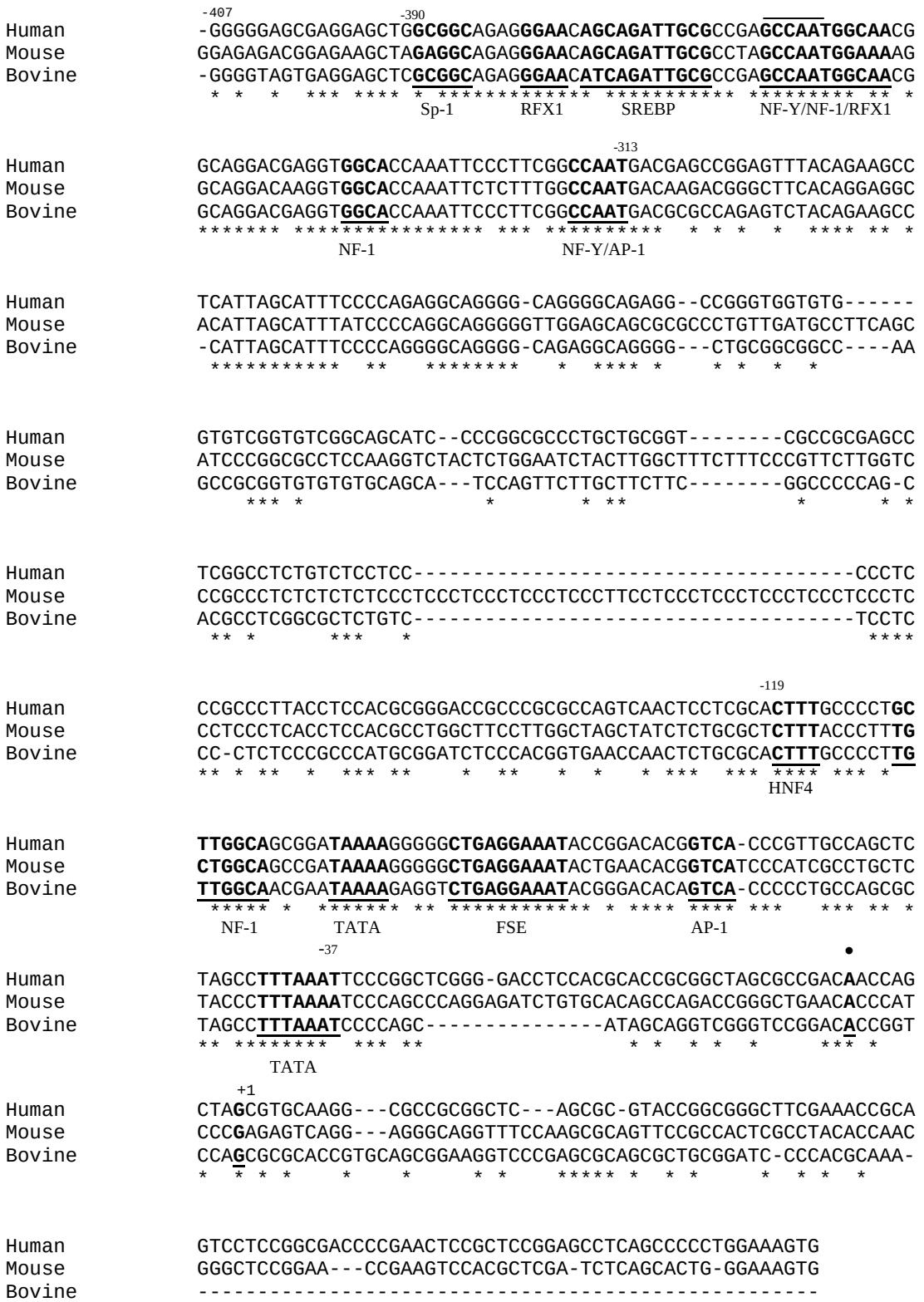
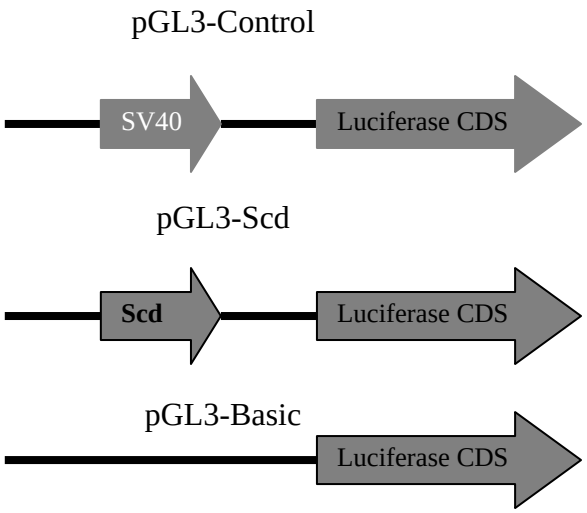
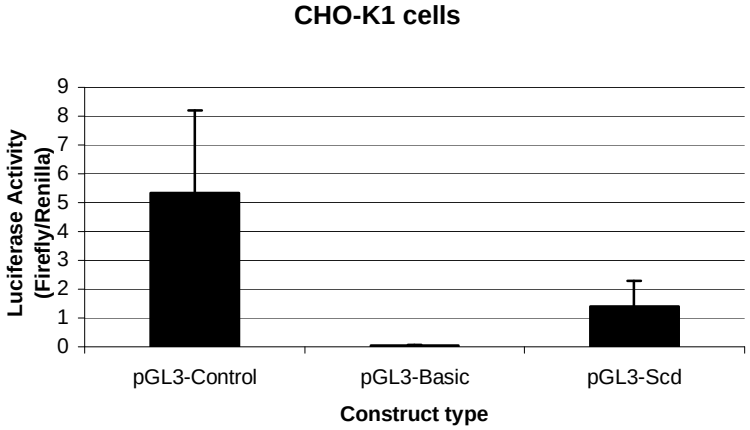


Figure II: Transient transfection of mammalian cell lines with promoter constructs.



(a)



(b)

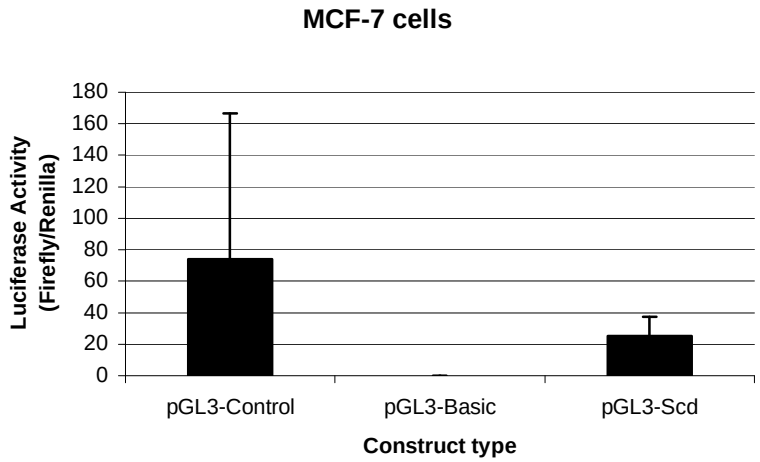


FIGURE LEGENDS

Figure I: Sequence alignment of the DL1.4 sequence with database entries for the human (AF320307) and murine (M21280), *scd*1 promoters. Numbering is indicated above sequence. Transcription factor binding sites are underlined and bold and these sites are based on TRANSFAC 4.0 database search results and comparison of human and mouse *scd* gene promoters. The critical CCAAT box from human studies is overlined. Numbering is relative to the human transcription start site and is indicated by +1. The transcription start site in the mouse promoter is indicated by the symbol •. Abbreviations: RFX-1=X-box-binding regulatory factor 1, SREBP=Sterol response element binding protein, NF-Nuclear factor.

Figure II: Transient transfection of mammalian cells with the putative bovine *scd* promoter sequence. (a) Luciferase assay following transient co-transfection of CHO-K1 cells with promoter construct. (b) Luciferase assay following transient co-transfection of MCF-7 cells with promoter construct. Luciferase activities are given as a ratio of Firefly to Renilla (pRL-SV40) values

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