Effects of sex and age on the expression of stearoyl-CoA desaturase in bovine muscle and adipose tissue

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Abstract— **The objective of the present study was to measure the mRNA expression of stearoyl-CoA desaturase (SCD1) in muscle, internal and subcutaneous adipose tissue in crossbred bulls and heifers raised under identical production conditions and slaughtered at 14 or 18 months of age. In addition, we investigated the relationship between SCD1 expression levels and phenotypic fatty acid (FA) data. The relative expression of SCD1 was determined by real-time quantitative PCR. The SCD1 mRNA levels in muscle were similar between the sexes (P>0.05), whereas they were 4.1-fold and 2.5-fold (P<0.05) higher in heifers when measured in internal and subcutaneous adipose tissue, respectively. The animals slaughtered at 18 months had lower SCD1 mRNA levels in MLL (P=0.048), while no differences were found in internal and subcutaneous adipose tissue. The accumulation of SCD1 transcripts was highest in subcutaneous adipose tissue and lowest in muscle regardless of sex and slaughter age. Desaturation indices for muscle FA were positively and significantly correlated with SCD1 mRNA levels in internal and subcutaneous adipose tissue, but not in muscle. It was concluded that SCD1 mRNA was more abundant in the internal and subcutaneous adipose tissue of heifers compared to bulls and that its variation partly contributed to sex- and age-differences in the FA composition of bovine fat.**

Keywords— **Beef, fatty acid, SCD1 mRNA.**

I. INTRODUCTION

Stearoyl-CoA desaturase (SCD) is the enzyme responsible for the conversion of SFA into MUFA and *trans*-vaccenic acid into its corresponding conjugated linoleic acid isomer (CLA *c9*, *t11*) [1]. Two different SCD gene isoforms have been characterised in cattle – SCD1, mainly expressed in adipose tissue, and SCD5, mainly expressed in the brain [2]. The expression of SCD1 in bovine adipose tissue is regulated by a number of factors, e.g. diet [3], breed [4] or age [5].

The objective of this study was to measure the expression of SCD1 in muscle, internal and subcutaneous adipose tissue and to investigate its relationship with phenotypic fatty acid (FA) composition of adipose tissue from bulls and heifers slaughtered at 14 or 18 months of age.

II. MATERIAL AND METHODS

A total of 24 Charolais × Simmental crossbred animals were used (12 bulls and 12 heifers). They were reared and finished under identical housing and feeding conditions. Both bulls and heifers were assigned according to their initial live weight and age to one of the two groups and slaughtered at approximately 14 or 18 months of age.

Twenty-four h *post mortem*, muscle and subcutaneous adipose tissue samples were collected from the *musculus longissimus lumborum* (MLL), adjacent to the quartering site between the 8th and 9th ribs. The FA composition was determined after the extraction of total lipids in accordance with [6]. Alkaline trans-methylation of FA was performed in accordance with [7]. Gas chromatography of FA methyl esters was performed using the HP 6890 gas chromatograph (Agilent Technologies, Inc., Santa Clara, CA, USA) with a programmed 60 m DB-23 capillary column (150 to 230°C). FA were identified on the basis of retention times corresponding to standards (PUFA 1, PUFA 2, PUFA 3, 37 Component FAME Mix; Supelco, Bellefonte, PA, USA).

The muscle (MLL), internal (cod or udder adipose tissue) and subcutaneous adipose tissue samples (2 – 4 mg) were collected immediately after slaughter and submerged in 200 μl of 2 × Nucleic Acid Purification Lysis Solution (Applied Biosystems, Foster City, CA, USA), homogenized using a rotor-stator homogenizer and stored at -20 °C until analysed. After defrosting, 200 μl of PBS and 5 μl of proteinase K were added to

the samples and left at room temperature for 1 h. RNA was isolated using 6100 Nucleic Acid PrepStation (Applied Biosystems, Foster City, CA, USA) following the manufacturer's protocol. In addition, a step with Absolute RNA Wash Solution was included. All RNA samples were reverse transcribed to cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) following the manufacturer's protocol. Real-time PCR was performed with the 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Gene expression levels were detected for SCD1, while β-actin was used as an internal reference gene. Primer and probe sequences for SCD1 and β-actin were designed using Primer Express 3.0 software (Applied Biosystems, Foster City, CA, USA). SCD1 sequences (AY241933) were as follows: forward, 5'-CCGACGTGGCTTTTTCTTCT-3'; reverse, 5'-TGGGTGTTTGCGCACAAG-3'; TaqMan MGB, FAM 5'-TCACGTGGGTTGGCT-3'. β-actin sequences (AY141970) were as follows: forward, 5'-TCACGGAGCGTGGCTACAG-3'; reverse, 5'-TTGATGTCACGGACGATTTCC-3'; TaqMan MGB, VIC 5'-TTCACCACCACGGGC-3'. Each PCR reaction was performed in triplicate in a total volume of 10 µl with gene specific primers concentration of 500 nM and TaqMan MGB probes concentration of 200 nM, 5 µl Fast TaqMan Universal Master Mix (2×) (Applied Biosystems, Foster City, CA, USA), 1 µl cDNA, and nuclease-free water up to volume. The PCR programme (Fast protocol) was as follows: 95 °C for 20 s followed by 40 cycles of 95 °C for 2 s and 60 °C for 20 s. Relative transcript levels of the target gene (SCD1) were calculated for each sample as $2^{-\Delta Ct}$, where ΔCt (Ct – threshold cycle) was calculated as $Ct_{(SCD1)} - Ct_{(\beta\text{-actin})}$.

FA data and SCD1 transcript levels were analysed using the GLM procedure of SAS (SAS Institute Inc., 2006), with the model including sex, age at slaughter, and their interaction as the fixed effects. Pearson correlation coefficients were calculated to evaluate the association between SCD1 transcript levels and desaturation indices in MLL and subcutaneous adipose tissue using the CORR procedure of SAS [8]. Desaturation indices used as a proxy for the SCD enzyme activity were calculated according to [9]:

C14 index = C14:1/(C14:0+C14:1) . 100
C16 index = C16:1/(C16:0+C16:1) . 100
C18 index = C18:1/(C18:0+C18:1) . 100

III. RESULTS AND DISCUSSION

Table 1 shows the relative levels of SCD1 mRNA measured in MLL, internal and subcutaneous adipose tissue of bulls and heifers slaughtered at 14 or 18 months. No significant sex × age interaction was present for SCD1 mRNA levels in any of the tissues examined and, therefore, the main effect results are presented. Heifers exhibited higher SCD1 mRNA levels than bulls in internal and subcutaneous adipose tissue ($P<0.05$), but not in muscle. The animals slaughtered at 18 months had lower SCD1 mRNA levels in MLL ($P=0.048$), while no differences were found in internal and subcutaneous adipose tissue. Although not statistically tested, the accumulation of SCD1 transcripts was numerically highest in subcutaneous adipose tissue and lowest in MLL regardless of sex and slaughter age.

Table 1 Relative levels of SCD1 mRNA measured in muscle, internal and subcutaneous adipose tissue (AT) (LSM ± *SE*)

Tissue	Sex		Age at slaughter		Signif.[a]
	Bulls	Heifers	14 m	18 m	
Muscle	13.3	10.1	14.7	8.8	A
	±1.9	*±2.0*	*±2.0*	*±1.9*	
Internal AT	35.5	142.3	100.7	76.1	S
	±22.4	*±23.5*	*±23.4*	*±22.4*	
Subcutaneous AT	123.4	306.5	201.7	228.2	S
	±56.4	*±56.4*	*±56.4*	*±56.4*	

[a] A – significant effect of age at slaughter; S - significant effect of sex

Similarly to the present study, threefold higher SCD1 mRNA levels were previously obtained in the subcutaneous adipose tissue of 48-month-old cows compared to 24-month-old bulls [10]. It has also been previously reported that SCD1 mRNA reached a peak at 12 months of age in the subcutaneous adipose tissue of Angus steers [11] and in the MLL of Hanwoo steers [12], and then maintained its level or somewhat declined.

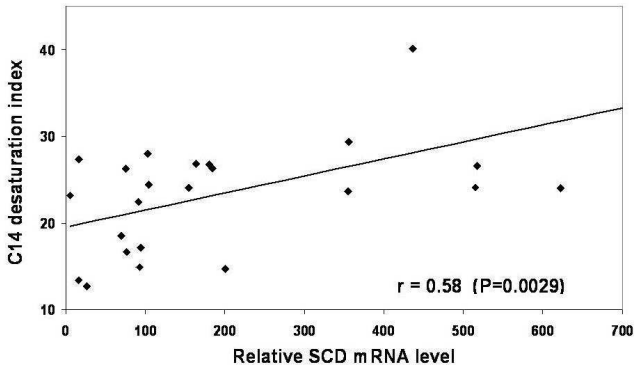

Fig. 1: Relationship between SCD1 mRNA expression measured in subcutaneous AT and C 14 desaturation index calculated for FA in subcutaneous AT

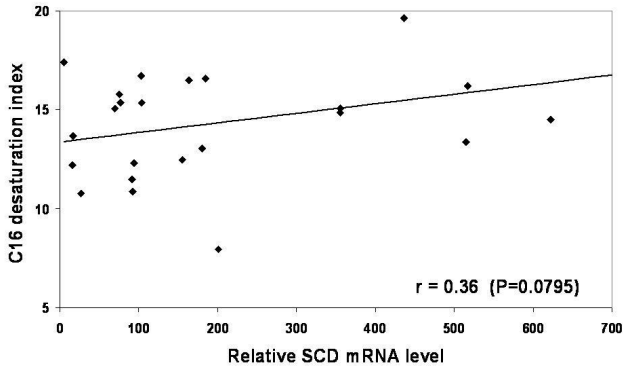

Fig. 2: Relationship between SCD1 mRNA expression measured in subcutaneous AT and C 16 desaturation index calculated for FA in subcutaneous AT

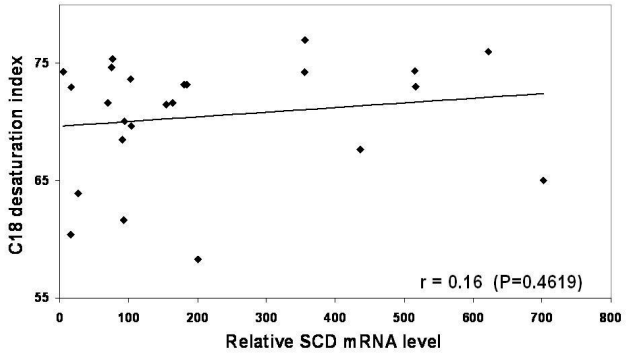

Fig. 3: Relationship between SCD1 mRNA expression measured in subcutaneous AT and C 18 desaturation index calculated for FA in subcutaneous AT

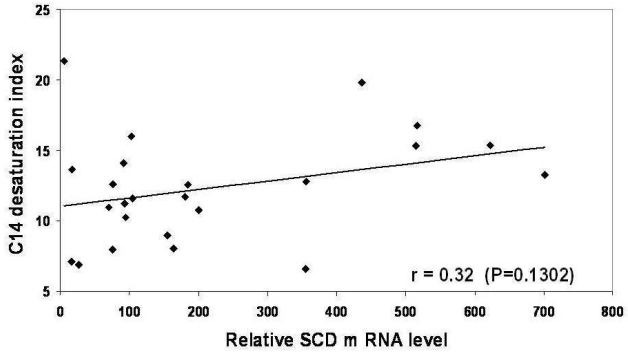

Fig. 4: Relationship between SCD1 mRNA expression measured in subcutaneous AT and C 14 desaturation index calculated for FA in muscle

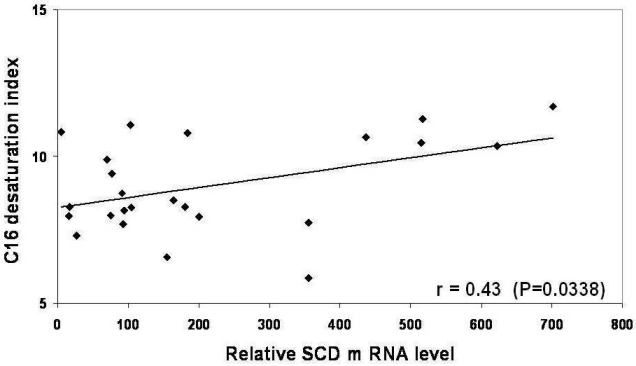

Fig. 5: Relationship between SCD1 mRNA expression measured in subcutaneous AT and C 16 desaturation index calculated for FA in muscle

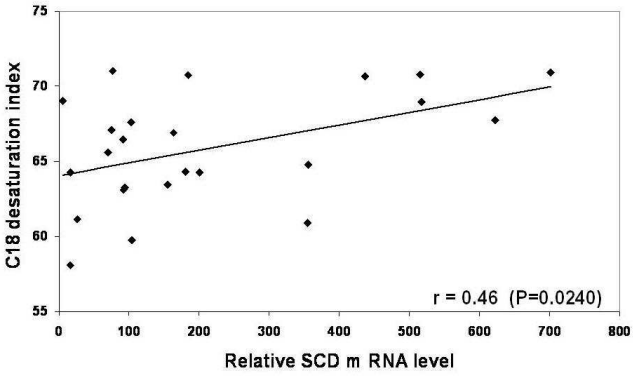

Fig. 6: Relationship between SCD1 mRNA expression measured in subcutaneous AT and C 18 desaturation index calculated for FA in muscle

The relationships between relative SCD1 mRNA levels measured in subcutaneous fat and C14, C16 and C18 desaturation indices [9] calculated for MLL and subcutaneous adipose tissue are demonstrated in Fig. 1, 2, 3, 4, 5 and 6. The indices were positively correlated with SCD1 mRNA levels determined in subcutaneous (but also internal) adipose tissue. No such relationship was, however, observed for the SCD1 expression in muscle (data not shown). In agreement with our study, positive correlation was established between SCD1 gene expression and the C16:1/C18:0 ratio in the subcutaneous adipose tissue of corn-fed Angus and Wagyu steers [13].

IV. CONCLUSIONS

Relative SCD1 mRNA levels were higher in the internal and subcutaneous adipose tissues of heifers compared to bulls. The transcript levels measured in subcutaneous adipose tissue may have been partly related to the desaturation indices calculated for both MLL and subcutaneous adipose tissue.

ACKNOWLEDGMENT

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