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Short Communication: Effect of Stearoyl-Coenzyme A Desaturase Polymorphism on Fatty Acid Composition of Milk

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ABSTRACT

The effect of the stearoyl-CoA desaturase (SCD) gene on milk fatty acid composition was tested. Cows of 3 breeds of northern Italy, Piedmontese, Valdostana, and Jersey, were genotyped at exon 5 of the SCD gene. This has been suggested as a primary candidate gene to change the proportion of saturated vs. unsaturated fatty acids in milk, wherein a single nucleotide polymorphism (C/T) gives rise to a different AA codon. It was possible to ascribe a reduced desaturase activity to the T allele only in the case of caproleic and myristoleic fatty acids. In contrast with the findings of SCD effects on carcass fat, it was not possible to confirm the higher desaturation activity of this single nucleotide polymorphism on long-chain fatty acids, due to the different pathways that originate milk fatty acids of different carbon length; long-chain fatty acids are highly influenced by the complex metabolic events that affect the ingested nutrients during their transfer to milk fat.
Key words: fatty acids, breed, stearoyl-CoA desaturase

The fatty acid (**FA**) composition of milk fat is an important variable that affects both the processing characteristics and the nutritional value of dairy products (Jensen et al., 1990). Modifying milk composition could increase the efficiency of manufacturing dairy products, either by leading to the production of dairy products that conform more closely to current dietary recommendations, or to the production of more tasteful cheeses. Collins et al. (2003) concluded that flavors of ripened cheese are directly dependent on the proportion of FFA, mainly the short-chain FA. Those short-chain FA were highly correlated (r = 0.70 to 0.99) with corresponding FA in the milk of origin (Lucas et al., 2006).

Factors such as genetic effect and stage of lactation have been considered of minor importance in influencing milk FA composition (Palmquist et al., 1993), but significant heritability and repeatability estimates for milk FA composition (Karijord et al., 1982; Bobe et al., 1999, 2003) suggest that sufficient variation exists among cows fed the same diet to produce a more healthful FA composition. Karijord et al. (1982) found that heritabilities of each single FA are low and conclude that, although genetic variation exists within breed with respect to fatty acid composition of milk fat, composition might be changed by selection, provided that it is considered important enough to receive major emphasis. Until now, genetic selection has not been used on FA, because of the complexity of FA analysis through gas chromatography. Recently, Soyeurt et al. (2006) showed that the prediction of FA concentrations in cow's milk was possible by mid-infrared spectrometry, a tool used during the routine milk recording to predict different components of milk. It is likely, therefore, that the FA composition of milk will be taken into account in selection schemes in the future. However, the identification of genetic markers or gene polymorphisms affecting milk fat composition would be a faster method for the selection of dairy animals for specific dairy products.

Stearoyl-CoA desaturase (**SCD**) is a key enzyme in the cellular biosynthesis of monounsaturated fatty acids (**MUFA**). It is located in the endoplasmic reticulum and catalyzes the insertion of a double bond between carbon atoms 9 and 10 in a spectrum of saturated fatty acids (Enoch et al., 1976; Palmquist et al., 1993). Mammary activity of SCD is noted to be lower in the Jersey cow compared with Holstein (Beaulieu and Palmquist, 1995; Drackley et al., 2001). The SCD gene is a key component in the leptin-signaling pathway, and it is the most important leptin-regulated gene (Cohen et al., 2002). The SCD gene was also indicated as a primary candidate gene to change the proportion of saturated vs. unsaturated fatty FA in milk, also increasing the conjugated linoleic FA content, a FA purported to possess anticarcinogenic properties (Islas-Trejo et al., 2002). The complete bovine SCD mRNA, which spans 5.1 kb and codes for a protein of 355 AA, has been cloned and sequenced (GenBank accession

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no. AY241932). Eight single nucleotide polymorphisms (**SNP**) have been identified in Holstein, Jersey, and Brown Swiss cattle, forming 2 distinct haplotypes referred to as A and B (Medrano et al., 1999). Three SNP were found in exon 5, and 5 were found in the 3′ untranslated region of the gene. The 3 SNP of exon 5 are in linkage disequilibrium (Taniguchi et al., 2004); only one of them (position 10329) codes for a different AA codon: alanine (allele C) or valine (allele T). The Val variant was identified as the ancestral allele by Taniguchi et al. (2004), who found that frequency was 0.4 in Japanese Black cattle. Moioli et al. (2005) calculated the allele frequency of the Val/Ala variant in 4 Italian breeds and found that frequency of the Val allele was maximal in Simmental (47.62%) and minimal in Chianina (22.73%).

Taniguchi et al. (2004) found that the Val residue may change the enzyme catalytic activity compared with Ala. They showed that, in Japanese Black cattle, allele C was more frequently associated with a higher content of MUFA in carcasses, and suggested that genotyping for this region would be a useful tool for selection of favorable beef carcasses. Zhang (2005) tested the effect of the Val/Ala variant on the individual fatty acid composition of fat extracted from beef muscle (longissimus dorsi) of 172 bulls. They found that the Val allele was more frequent (0.83) in the analyzed cattle, but agreed with Taniguchi et al. (2004) in that heterozygous Val/Ala (C/T) animals had higher C16:1/C16:0 in intramuscular fat than did homozygous Val (TT) animals.

The purpose of the present research was to test SCD gene effects on milk FA composition in 3 dairy breeds, Jersey, Piedmontese, and Valdostana, reared on 3 farms in northern Italy. The study was conducted on 79 lactating cows (25 Jersey, 27 Piedmontese, and 27 Valdostana), by collecting milk samples at 3 milk records of the same lactation, so that the first record was at d 60 from calving; the second record between the d 60 and 140; and the third record around d 210. The cows were reared on 3 nearby farms in the province of Turin (northwest Italy). Animals that calved between April and May 2005 were chosen; they were of either second or third calving to avoid season and age effect on the parameters to be estimated. The 3 farms were registered in the National Herdbook of each breed and performed the official milk recording: one monthly record along the whole lactation with collection of milk sample for analysis of milk quality, following the regulation of the International Committee for Animal Recording (ICAR). Individual results of the milk recording (milk yield, fat and protein content) at the test day, where the milk sample for FA analysis was collected, were used in this analysis. The Piedmontese and Valdostana are indigenous breeds of northwest Italy, where they are still the most popular cattle; their milk is mainly processed into cheese and they are preferred over Holsteins for the higher content of milk total solids. The Jersey breed was introduced to Italy only few decades ago. This study was not conducted in an experimental station but on commercial dairy farms, so that each farm used its own feeding system, which consisted of corn silage, hay, and concentrates in different proportions, depending on the herd; the main features are summarized in Table 1. Pasture was not used in any of the herds.

Table 1. Feeding parameters in each herd/breed

Herd/breed	DMI/BW (%)	CP (g/kg of DM)	NE_L (MFU/head per d)[1]
Jersey	3.2	183	16.6
Piedmontese	2.2	131	10.2
Valdostana	3.0	109	12.2

[1]MFU = milk forage unit; INRA, 1978.

Milk samples (50 mL) were digested with 10 mL of NH_3 (25% vol/vol) and mixed with 40 mL of ethanol (96% vol/vol). The extraction was performed with 100 mL of a mixture of diethyl ether:pentane (1:1 vol/vol). The solvent phase was filtered through 25 g of anhydrous Na_2SO_4 and evaporated under vacuum. This procedure was based on an ISO standard method (ISO, 2001). Milk fat (100 mg) was diluted in 5 mL of hexane and derivatized as methyl esters by addition of 0.25 mL of 2 *N* KOH in methanol (ISO, 2002). One milliliter of the upper phase containing fatty acid methyl esters was diluted with 7 mL of diethyl ether and 2 mL of hexane for the on-column injection. Gas chromatography analysis of fatty acid methyl esters was performed by a HP6890 (Agilent Technologies, Palo Alto, CA) and a DB23 low-bleed (Agilent Technologies) capillary column (30 m length, 0.32 mm i.d., 0.25 μm film thickness). On-column injection was adopted, and hydrogen (1 mL/min) was used as the carrier gas. The temperature program was as follows: 40°C for 3 min, 25°C/min to 120°C for 1 min, 4°C/min to 162°C for 2 min, 8°C/min to 220°C for 3 min; a flame-ionization detector was used, held at 250°C. The characteristics of the capillary column did not allow *trans* FA to be separated. Consequently, the peak indicated as C18:1 includes *trans*-11, together with *cis*-9 and *cis*-7 isomers.

DNA of all animals was amplified with primers designed on the cattle sequence (GenBank accession no. AY241932) to produce an amplicon of 212 bp, spanning from 10,232 to 10,443 bp, encoding the portion of exon 5 containing the targeted C/T SNP, as well as part of the 3′ region. The primers used were ACCTGGCTGGT-GAATAGTGC (forward) and TGACATATGGAGAGGG-GTCA (reverse). The PCR amplification was performed

in a final volume of 50 μL, containing 250 ng of genomic DNA, 0.2 m*M* of each dNTP, 40 pmol of each primer, 2 m*M* $MgCl_2$, and 2.5 U of AmpliTaq Gold (Applied Biosystems, Foster City, CA). Thirty-five PCR cycles at and annealing temperature of 58°C were performed. The detection of sequence variation was performed on the denaturing HPLC (**DHPLC**) Transgenomic WAVE system (Transgenomic, Omaha, NE), which allows the detection of heterozygous samples through the resolution of DNA fragments based on differential retention of double-stranded vs. single-stranded DNA (Hecker, 2002). At a given temperature, the difference in the melting between homo- and heteroduplexes is revealed by differences in retention times. The DNA profiles of all animals were compared at the partial denaturation temperature of 60.6°C. Direct sequencing was performed on 15 samples of the 3 putative genotypes detected on the DHPLC system to confirm the detection results, by a Perkin Elmer ABI Prism 310 DNA sequencer. The PCR for sequencing was obtained by using ABI Prism BigDye Terminator Cycle Sequencing, Ready Reaction kits (version 1.1, Applied Biosystems). The protocol for single- and double-stranded DNA was optimized in 20 μL of final volume containing 4 μL of Terminator Ready Reaction mix, 10 to 15 ng of PCR product, and 5 pmol of single primer. The product of the sequencing reaction was purified with Nucleoseq kit (M-Medical, Milan, Italy).

Mean values at the 3 milk records for each cow of the following variables (Y), Y1 = C4:0; Y2 = C6:0; Y3 = C8:0; Y4 = C10:0; Y5 = C10:1; Y6 = C12:0; Y7 = C14:0; Y8 = C14:1; Y9 = C16:0; Y10 = C16:1; Y11 = C18:0; Y12 = C18:1; Y13 = conjugated linoleic acid (CLA); Y14 = C10:1/C10:0; Y15 = C14:1/C14:0; Y16 = C16:1/C16:0; Y17 = C18:1/C18:0; Y18 = short-chain FA; Y19 = saturated FA; Y20 = polyunsaturated FA; Y21 = MUFA; Y22 = daily milk yield (kg); Y23 = fat percentage; and Y24 = protein percentage were analyzed according to the GLM procedure (SAS Institute, 1985) with the following univariate model:

$$Y1_{ij} \text{ to } Y21_{ij} = \mu + b1\, x_{ij} + b2\, z_{ij} + b3\, a_{ij}\, (H_i) + H_i + e_{ij}$$

where μ is the overall mean; *b1* is the covariate of milk yield (x) on the variable; *b2* is the covariate of the fat content (z) on the variable; *b3* is the covariate of the SCD allele (a) on the variable, within herd/breed; H is the herd/breed effect; and *e* is the residual effect.

The SCD allele effect was estimated as covariate by assuming the effect of allele C = 1 and allele T = 0. Using the same model and procedure, milk yield at the test day (Y22), not including covariates *b1* and *b2*, and fat (Y23) and protein (Y24) content at the test day, not including covariate *b2*, were analyzed. Because of the low genotype frequency in the Jersey breed, this breed was excluded from the analysis.

In Table 2, mean values for each herd and each analyzed variable are reported. Data of milk composition and yield are consistent with the results for Jersey, Piedmontese, and Valdostana breeds, as reported in the Official Statistics of the milk-recorded cows, produced every year by the Italian Breeders Association (AIA, 2005). The FA composition of the milk of the Valdostana breed is similar to that reported by Battaglini et al. (2004); the FA composition of the Jersey milk reflects the standard composition of FA in milk fat of this breed, as reported in North American studies (DePeters et al., 1995; White et al., 2000); however, no reference in the literature was found on the FA composition of milk of the Piedmontese.

Frequencies of SCD allele in each herd and breed are reported in Table 3. Allele T, which is reported as having a negative effect on the desaturase activity of the enzyme (Taniguchi et al., 2004), had a very low frequency (0.06) in the herd of Jersey cows; therefore, the SCD allelic effect was analyzed only in the Piedmontese and Valdostana herds. To avoid confounding breed or herd effect with SCD allelic effect, the herd was included in the statistical model as a fixed factor and the allelic effect was nested within the herd or breed factor. The variables that were significantly affected by SCD allele C are reported in Table 4. A positive effect of allele C was found on caproleic (+0.03; +0.033) and myristoleic (+0.149; +0.181) acids, respectively, in the Piedmontese and Valdostana cows. Also, the ratios of caproleic and myristoleic acids on the corresponding saturated FA were positively affected by allele C: C10:1/C10:0 was +0.012 and +0.014; C14:1/C14:0 was +0.014 and +0.015, respectively, in Piedmontese and Valdostana cows.

An unfavorable effect of allele C was found on C16:1 in both breeds; moreover, in the Valdostana cows, an unfavorable effect of this allele was found on C18:1 as well as on the ratio C16:1/C16:0. No effect was evident on CLA, polyunsaturated FA, and MUFA in either breed.

These results, in contrast with those obtained on carcass FA by Taniguchi et al. (2004), who associated the effect of C allele to a higher content of all MUFA, might be explained by the origin of milk FA. Evidence suggests that almost all C4:0 to C14:0 fatty acids and approximately one-half of C16:0 in milk are derived from de novo FA synthesis (Palmquist et al., 1969). The remaining C16 and almost all of the longer chain fatty acids are thought to be derived from circulating blood lipids (Bauman and Davis, 1977). Circulating blood lipids may be derived from digestion and absorption of dietary fatty acids or from mobilization of fatty acids

Table 2. Mean values and standard deviations for each analyzed variable

	Herd 1/Jersey (n = 75)		Herd 2/Piedmontese (n = 81)		Herd 3/Valdostana (n = 730)		Overall (n = 229)	
Variable	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Milk yield (kg)	23.895	5.560	10.863	3.347	12.038	4.994	15.547	7.540
Fat (%)	4.379	1.017	3.830	0.968	3.699	0.635	3.968	0.937
Protein (%)	3.750	0.454	3.668	0.435	3.389	0.432	3.606	0.464
C4:0	4.030	0.855	3.997	0.637	3.704	0.773	3.914	0.768
C6:0	2.657	0.313	2.409	0.484	2.053	0.326	2.377	0.455
C8:0	1.628	0.146	1.403	0.273	1.189	0.167	1.408	0.271
C10:0	3.715	0.467	3.080	0.664	2.471	0.440	3.094	0.733
C10:1	0.296	0.051	0.272	0.065	0.253	0.052	0.274	0.059
C12:0	4.466	0.783	3.822	0.903	3.065	0.569	3.792	0.952
C13:0	0.141	0.070	0.158	0.046	0.122	0.054	0.141	0.059
C14:0	11.889	1.072	11.955	1.665	11.264	1.168	11.713	1.368
C14:1	0.971	0.198	1.024	0.292	1.076	0.262	1.023	0.257
C15 iso	0.257	0.047	0.397	0.079	0.398	0.104	0.351	0.103
C15 anteiso	0.419	0.051	0.601	0.082	0.610	0.107	0.544	0.120
C15:0	1.068	0.283	1.275	0.131	1.284	0.152	1.210	0.222
C16:0	32.374	4.091	27.587	2.540	27.419	3.269	29.101	4.039
C16 iso	0.256	0.047	0.446	0.083	0.251	0.046	0.322	0.111
C16:1	1.288	0.310	1.425	0.313	1.751	0.347	1.484	0.374
C17 iso	0.513	0.057	0.577	0.081	0.673	0.101	0.586	0.103
C17 anteiso	0.431	0.066	0.578	0.110	0.544	0.093	0.519	0.111
C17:0	0.531	0.093	0.687	0.138	0.773	0.097	0.663	0.149
C17:1	0.201	0.055	0.321	0.093	0.432	0.128	0.317	0.134
C18:0	11.004	1.899	9.875	1.758	10.333	1.414	10.391	1.763
C18:1	18.314	3.037	24.718	3.639	25.960	3.520	23.016	4.757
C18:2	2.778	0.707	2.190	0.487	1.896	0.478	2.289	0.670
C18:3	0.317	0.053	0.370	0.100	0.809	0.137	0.493	0.241
Conjugated linoleic acid	0.451	0.087	0.831	0.207	1.668	0.695	0.973	0.645

from adipose tissue (Grummer, 1991). Results from the current study showed a positive effect of SCD allele on C14:1 and C10:1 FA, the only FA that are fully derived from de novo synthesis.

In agreement with the findings on FA composition of carcass fat, the C allele positively influenced the desaturation of C14:0 into C14:1, as well as of C10:0 into C10:1; however, we cannot confirm the higher desaturation activity of this allele on all MUFA, as proposed by Medrano et al. (1999).

The SCD C allele had no positive effect on the desaturation of C16:0 and C18:0; as regards C18:1/C18:0, results from this study should be taken as preliminary, because we did not separate any of the isomers of C18:1, which also includes vaccenic acid.

Wahle et al. (2004) noted that the main form of CLA can be produced by SCD desaturation of vaccenic acid (*trans*-11 C18:1) in most mammalian tissues, and Lock et al. (2004) noted that vaccenic acid is converted to *cis*-9, *trans*-11 CLA via the SCD enzyme. On the contrary, our results show no positive effect of SCD allele C on CLA content.

Long-chain FA originate mainly from the diet, and their characteristics are altered by biohydrogenation of unsaturated FA by ruminal microorganisms and digestion in the intestinal tract (Palmquist and Jenkins, 1980). The efficiency of this transfer from the diet to the endoplasmic reticulum of mammary secretory cells is difficult to estimate (Grummer, 1991).

Our results confirm the complex interaction between dietary lipids and mammary SCD activity, as suggested by Bernard et al. (2005), who showed that lipid supple-

Table 3. Stearoyl-CoA desaturase allele frequency in each herd/breed

	Allele	
Herd/breed	C	T
Jersey	0.94	0.06
Piedmontese	0.42	0.58
Valdostana	0.65	0.35

Table 4. Variation of milk yield, fat and protein content, and fatty acid percentage due to stearoyl-CoA desaturase allele C (b_{SCD})

	$b_{SCD\ Piedmontese\ herd}$		$b_{SCD\ Valdostana\ herd}$	
Variable	Estimate	$P <$	Estimate	$P <$
C10:1	0.030	0.007	0.033	0.01
C14:1	0.149	0.01	0.181	0.009
C16:1	–0.239	0.05	–0.182	0.02
C18:1	0.557	NS	–1.455	0.05
C10:1/C10:0	0.012	0.0008	0.014	0.0006
C14:1/C14:0	0.014	0.006	0.015	0.01
C16:1/C16:0	–0.003	NS	–0.009	0.001

mentation led to a decrease in both mammary SCD mRNA level and enzyme activity. On the contrary, Delbecchi et al. (2001) hypothesized that mammary SCD gene expression may be regulated by the different *cis*-9 C18:1/C18:0 ratios brought to the mammary gland by the diet.

We conclude therefore that the higher desaturase activity of SCD allele C (which, in carcass FA, was made evident on all the MUFA) in the composition of milk fat can only be appreciated on myristic and caproleic FA because of the different pathways from which milk FA derive.

The very low frequency of the T allele in the Jersey breed, which is characterized by a lower mammary activity of SCD compared with Holstein (Beaulieu and Palmquist, 1995; Drackley et al., 2001), might suggest that the activity of this enzyme is not merely affected by the AA sequence, but by more complex regulation factors or interaction with other genes (Cohen et al., 2002). Recently, particular attention has been focused on the genes involved in the regulation and activation of SCD, such as sterol regulatory element binding protein and its cleavage activation protein (Medrano, 2005).

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