Extraction and Quantitative Analysis of Stearoyl-Coenzyme A Desaturase mRNA from Dairy Cow Milk Somatic Cells

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ABSTRACT

Study of stearoyl-coenzyme A desaturase (SCD) gene expression in the bovine mammary gland is limited by restricted availability of mammary tissue samples from biopsy or postmortem sampling of cows during temporal experiments. A technique was developed to isolate total RNA from somatic cells in bovine milk and to analyze SCD mRNA expression by quantitative reverse-transcription PCR. Total RNA yield was lower than in a previous goat study and was related to numbers of viable somatic cells. To obtain sufficient total RNA, 1-L milk samples were taken and stored for up to 24 h at 4°C. Complementary DNA prepared from somatic cells showed a 99% match with the published sequence for SCD mRNA in bovine adipose tissue. Stearoyl-CoA desaturase mRNA abundance relative to β -actin mRNA for 12 cows sampled across 4 time points varied (mean $\pm$ SE) from 0.88 ± 0.17 to 4.40 ± 0.50 . Fifty-five percent of variation was due to individual cows and 42% was due to daily variation within cows. Relative abundance of SCD mRNA was not related to the number of viable somatic cells or total RNA extracted from samples, but it was related to mammary desaturase activity, as indicated by changes in milk C₁₄ fatty acid concentrations. We concluded that somatic cells provide a noninvasive and repeatable alternative to mammary tissue samples obtained by biopsy or postmortem.

Key words: stearoyl-coenzyme A desaturase mRNA, milk somatic cell, reverse-transcription polymerase chain reaction, dairy cow

INTRODUCTION

Stearoyl-coenzyme A desaturase (SCD or Δ^9 -desaturase; EC 1.14.19.1) is the enzyme responsible for Δ^9 -desaturation of fatty acids in the bovine mammary gland and other tissues. Stearoyl-coenzyme A desaturase introduces a *cis*-double bond between carbons 9 and

10 of fatty acids, thereby converting saturated fatty acids into monounsaturated fatty acids. The main biological function of SCD in the mammary gland is to maintain fluidity of milk by converting stearic acid to oleic acid and, to a lesser extent, palmitic acid to palmitoleic acid. Another consequence of the action of SCD is to produce *cis*-9, *trans*-11 conjugated linoleic acid from vaccenic acid. Conjugated linoleic acid has several human health benefits, including actions against cancer, coronary heart disease, and diabetes (Parodi, 2004).

Selection of breeding animals with high SCD activity may lead to long-term improvements in milk fatty acid profiles by reducing saturated fatty acids and increasing oleic acid and conjugated linoleic acid concentrations. Studies of product:precursor relationships in milk fatty acids have shown that SCD activity shows considerable variation among individual cows (Lock and Garnsworthy, 2003) and has an estimated heritability of 30% (Royal and Garnsworthy, 2005). To identify superior cows, it would be desirable to measure expression of the gene regulating SCD in the mammary gland and to examine its relationship with SCD activity. Nutritional studies of milk fatty acid composition can also benefit from assessment of how nutrient supply influences SCD gene expression (e.g., Bernard et al., 2005, in goats; Baumgard et al., 2002, in dairy cows).

Bovine mammary SCD gene expression has been assessed previously by examining mRNA abundance in samples of mammary tissue taken postmortem (e.g., Beswick and Kennelly, 2000), although, obviously, this procedure is not appropriate for use in selection programs or temporal experiments. Stearoyl-coenzyme A desaturase mRNA has also been examined in tissue samples taken by mammary biopsy (e.g., Baumgard et al., 2002; Peterson et al., 2003). Even though this procedure is nondestructive, it is invasive and presents a risk of infection or udder damage.

A noninvasive alternative to biopsy is analysis of milk somatic cells, which have been used in a few studies of dynamic changes in mammary mRNA. Milk protein mRNA, for example, has been extracted from epithelial cells in caprine milk to study CN gene variants (Boutinaud et al., 2002). In a recent study, Murrieta et al.

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(2006) measured mRNA for SCD and other enzymes involved in lipogenesis by using milk somatic cells obtained postmortem from lactating beef cows; they found high correlations between mammary tissue and milk somatic cell data. However, we are not aware of any report of SCD mRNA expression in milk somatic cells from dairy cows. The objective of the study reported here was to develop a method for extraction and quantitative analysis of SCD mRNA from milk of high-yielding dairy cows, which could be used in future studies of SCD expression.

Particular areas for investigation were milk sample volume and sample storage conditions. Milk sample volume needs to be sufficient to provide adequate quantities of total RNA. Previous studies have reported using sample volumes of 200 mL in beef cows (Murrieta et al., 2006) and 300 mL in goats (Boutinaud et al., 2002). Because dairy cows yield up to 20 times as much milk as beef cattle and goats, however, the concentration of somatic cells may be expected to be diluted. Species differences in milk secretion also give rise to differences in somatic cell numbers and types, which could affect their mRNA content (Boutinaud and Jammes, 2002). Ribonucleic acid is sensitive to storage conditions, and freezing and thawing of milk have previously been shown to degrade mRNA (Boutinaud and Jammes, 2002). However, in large-scale studies it may be impractical to process milk samples immediately after collection.

In the present study, somatic cell numbers and viability were determined under different storage conditions. Total RNA yield from somatic cells was then determined to calculate the optimum sample volume. Stearoyl-coenzyme A desaturase cDNA was sequenced and SCD mRNA abundance was estimated by using the quantitative Taqman (Applied Biosystems, Foster City, CA) real-time reverse transcription PCR (RT-PCR) method. Stearoyl-coenzyme A desaturase mRNA abundance was then compared with the product:precursor relationship for C₁₄ fatty acids in milk as an indicator of mammary desaturase activity.

MATERIALS AND METHODS

Cows and Milk Samples

Batches of Holstein cows were selected from the University of Nottingham Dairy Center for this sequence of tests. The number of cows selected varied among tests, as indicated in later sections. Selection criteria were 1) parity 2 to 4; 2) DIM 40 to 80 d; 3) milk yield 30 to 50 L/d; and 4) free from mastitis. Milk samples (300 mL at first, and later increased to 1 L) were collected at the morning milking from each cow and trans-

ferred to the laboratory in autoclaved glass bottles (1 L) in a cooler (approximately 4°C) within 10 min.

Cows were fed a TMR that contained (percentage of total DM) 42% grass silage, 12% corn silage, 20% wheat, 11% sugar beet pulp, 7% soybean meal, 6% rapeseed meal, 1% Megalac (Volac International, Royston, UK), and 1% mineral-vitamin supplement.

Somatic Cell Isolation

Milk somatic cells were extracted by the method of Boutinaud et al. (2002), with modifications. Each milk sample was decanted into sterile 50-mL conical tubes (6 or 20 tubes per sample) and 50 µL of 0.5 mM EDTA in PBS was added to each tube to limit formation of CN micelles. Somatic cells were pelleted by centrifugation at 2,700 × *g* for 10 min at 4°C. Cream and skim-milk layers were removed, and cell pellets were washed twice in 5 mL of ice-cold PBS (pH = 7.2) with 0.5 mM EDTA, and centrifuged at 2,700 × *g* at 4°C for 15 min. The 6- or 20-cell pellets from each sample were combined in 4 mL of PBS and centrifuged at 2,700 × *g* at 4°C for 15 min. All the supernatant was discarded, apart from 200 µL, which was used to resuspend the pellet. This was transferred to a 1.5-mL tube and centrifuged at 2,700 × *g* at 4°C for 15 min. The upper phase (≈100 µL) was discarded and the cell pellet was resuspended in 1 mL of PBS. A 40-µL aliquot of this cell suspension was removed into a fresh 1-mL tube for determination of cell counts and viability by light microscopy. The remaining cell suspension was treated with 1.2 mL of Trizol reagent (Invitrogen, Carlsbad, CA) and stored at -80°C until used for total RNA extraction.

Cell Viability and Storage Temperature

To determine cell viability, somatic cells were stained with 0.4% trypan blue solution and counted in a hemocytometer. Nonviable cells were stained blue. All counts were performed in duplicate.

To investigate effects of storage temperature on cell viability, 4 samples of milk (300 mL) were collected from each of 4 cows at the morning milking and stored for 24 h at 0°C (ice), 4°C (refrigerator), -20°C (freezer), and -80°C (freezer) before testing cell viability.

To investigate the effects of storage time on cell viability, 5 samples of milk (300 mL) were collected from each of 4 cows at the morning milking and stored at 4°C for 24, 30, 48, and 60 h before testing cell viability.

Total RNA Preparation and cDNA Synthesis

Total RNA was extracted from isolated somatic cells using TRIzol reagent according to the manufacturer's

Table 1. Primers and probes for basic PCR and quantitative PCR of stearoyl-CoA desaturase (SCD) and β -actin transcripts

Enzyme target	Primer	Sequence (5'-3')	Amplicon length, bp	Accession number
SCD (basic PCR)	F	CGACGTGGCTTTTCTTCTC	158	AF188710
	R	CACAACAACAGGACACCAGG		
SCD (TaqMan)	F	CTGCGGGTCTTCCTGATCA	62	AF188710
	R	GACCATTCAAAAACGTCATT		
	Probe	TGGCAACACCATGGCGTTCCA		
β -Actin (TaqMan)	F	CGCCATGGATGATGATATTGC	66	AY141970
	R	AAGCCGGCCTTGACACAT		
	Probe	CGCTCGTGGTCGACAACGGC		

instructions. To remove genomic DNA contamination, samples were treated with DNase (M610A, Promega, Southampton, UK) according to the manufacturer's instructions. The concentration of isolated nucleic acid was determined by GeneQuant Pro RNA/DNA Calculator (Amersham Pharmacia Biotech, Little Chalfont, UK); A_{260}/A_{280} was >1.85 in all samples. Quality of total RNA was estimated by nondenaturing gel electrophoresis (5 μ g of total RNA separated on 1% wt/vol agarose gel stained with ethidium bromide).

Total RNA (0.5 μ g) was used as a template for single-stranded cDNA synthesis (Parr et al., 2004), with random hexamers (0.5 μ g; C1181, Promega) and Moloney Murine Leukemia Virus reverse transcriptase enzyme (200 units; M1701, Promega). The single-stranded cDNA was diluted to 100 μ L of final volume for subsequent PCR-based analysis. For comparison, single-stranded cDNA was also prepared with total RNA (0.5 μ g) extracted from bovine mammary and liver tissue samples obtained from 6 cows immediately postmortem, frozen in liquid nitrogen, and stored at -70°C .

Basic RT-PCR

Primers used for RT-PCR were designed with Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) with the cow adipose tissue SCD mRNA sequence (AF188710; Chung et al., 2000) and are listed in Table 1.

The PCR reaction, containing 10 μ L of single-stranded cDNA template, 5 μ L of $10\times$ PCR buffer, 5 μ L of dNTPs (10 mM), 1.25 μ L of forward primer and reverse primer (10 pmol/ μ L; Table 1), 2.5 U of *Taq* DNA polymerase (Promega), and 3.0 μ L of MgCl_2 (25 mM), was carried out in a final volume of 50 μ L on a thermal cycler (GeneAmp PCR system 2400, PerkinElmer, Beaconsfield, UK). Polymerase chain reaction conditions were 94°C for 3 min, 40 cycles at 94°C for 30 s, 56°C for 30 s, and 72°C for 1.5 min, followed by a 7-min incubation at 72°C . Amplification products were fractionated using nondenaturing electrophoresis (1% wt/

vol agarose gels), and selected DNA bands were extracted and purified using Sigma GenElute PCR clean-up kits (Sigma, Gillingham, Dorset, UK).

cDNA Nucleotide Sequence Analysis

Concentration of cDNA was determined with a NanoDrop ND-1000 fluorospectrometer (NanoDrop Technologies, Wilmington, DE). The nucleotide sequence of cDNA was determined with a CEQ 8000 genetic analysis system (Beckman Coulter, High Wycombe, UK) in both directions by using the basic PCR primers listed in Table 1 (10 pmol/ μ L).

The nucleotide sequence of cDNA was compared with sequences in the public database NCBI-BLAST (<http://www.ncbi.nlm.nih.gov/blast>). The BLAST sequence analysis program was used for initial sequence comparison with accession numbers NM173959.3, BC112700, AF188710, AY241933.1, and AB075020.1.

Quantitative RT-PCR

Primers and probes for SCD and β -actin for quantitative PCR were designed with Primer Express software (Version 1.5, ABI Applied Biosystems). Reference sequences were SCD mRNA in cow adipose tissue (AF188710; Chung et al., 2000) and cow mammary gland β -actin mRNA (AY141970; Suchyta et al., 2003). For the SCD amplicon, to ensure discrimination between cDNA and genomic DNA PCR amplification, the forward primer was in exon 3, whereas the reverse was in exon 4 of the SCD genomic sequence (AY241932). Because the gene structure of the cow β -actin was not available, a similar approach could not be carried out for this amplicon. Each probe (Sigma-Genosys Ltd., Haverhill, UK) was labeled at the 5'-end with reporter dye FAM (6-carboxyfluorescein) and at the 3'-end with quencher dye TAMRA (6-carboxytetramethyl-rhodamine). Sequences of primers and probes are listed in Table 1.

After total RNA was extracted and single-stranded cDNA was synthesized by reverse transcription using random hexamers, as described above, single-stranded cDNA was used as the template for quantitative PCR to evaluate relative expression levels for SCD and β -actin. Stearoyl-coenzyme A desaturase and β -actin were run in separate wells for each sample. Each PCR reaction was performed in triplicate with a final volume of 25 μ L containing 12.5 μ L of PCR Mastermix (2 $\times$, Taqman Universal PCR Mastermix, Applied Biosystems), 0.75 μ L of each primer (10 pmol/ μ L), 0.5 μ L of probe (10 pmol/ μ L), 2 μ L of single-stranded cDNA (generated from the equivalent of 10 ng of total RNA), and 8.5 μ L of RNase-free water. Samples were placed in 96-well plates and amplified in an automated sequence detector (ABI Prism 7700 Sequence Detection System, Applied Biosystems) with the default Taqman real-time PCR program of 2 min at 50°C and 10 min at 95°C; each cycle included denaturing at 95°C for 15 s and annealing at 60°C for 1 min.

Relative quantities of SCD and β -actin mRNA were determined by the relative standard curve method (a 5-point standard curve) as described by Parr et al. (2004) from which the Ct (threshold cycle) value of a particular variant could be converted to nanograms of total RNA equivalent used for first-strand synthesis. Calibration curves for SCD and β -actin were generated by using single-stranded cDNA prepared from the total RNA of milk somatic cells from 8 cows mixed together and diluted over a range of 10:1 to 10:5. Samples without cDNA were used for negative control. Calibration curves were generated by plotting the Ct value against nanograms of total RNA equivalent used to synthesize single-stranded cDNA (nanograms). The amplification efficiencies of the SCD and β -actin amplicons were 88 and 93%, respectively. Relative abundance of SCD mRNA was determined in 4 samples from each of 12 cows, which were collected at intervals of 9 ± 0.6 d.

Desaturase Activity

Milk 14:0 and 14:1 concentrations (as a percentage of total fatty acids) were determined by the rapid extraction method of Feng et al. (2004). A milk fatty acid index of desaturase product:precursor relationships was calculated as 14:1/(14:0 + 14:1). This index was used as a proxy for desaturase activity to compare with SCD mRNA abundance.

Statistical Analysis

Statistical analysis was performed by using Genstat 8 (Lawes Agricultural Trust, Harpenden, UK). Effects of storage temperature and time on cell viability were

tested by using logistic regression with a binomial distribution and logit link function. Variance components for relative abundance of SCD mRNA were examined using a general linear model (GLM) with a Poisson distribution and log link function. Variance was partitioned into effects attributable to cow, sample replicate within cow, and residual. The model used to describe the data was therefore

$$\text{SCD}_{ijk} = m + C_i + S_{ij} + \text{residual},$$

where m is the overall constant, C_i is the effect of cow i ($i = 1 \dots 12$), and S_{ij} is the effect of sample number j ($j = 1 \dots 4$). Relative contributions of these factors were assessed by cumulative analysis of deviance. Viable cell count and RNA yield were added to the model individually to examine their relationships with SCD mRNA. The relationship between SCD mRNA and the C_{14} desaturase index was examined by GLM after taking the natural logarithm of SCD mRNA.

RESULTS

Somatic Cell Numbers and Viability

The average number of somatic cells in fresh milk samples from 8 cows was $90 \pm 7.4 \times 10^3/\text{mL}$. Somatic cell counts determined by microscopy showed good agreement with values determined by midinfrared analysis of samples at the National Milk Records Laboratory, (Harrogate, Yorkshire, UK; $r = 0.74$; $P < 0.05$). Somatic cell viability averaged $67 \pm 4.3\%$ in fresh milk samples.

Compared with fresh samples, cell viability decreased by 2% (NS) when milk samples were stored at 4°C for 24 h, decreased by 12% ($P < 0.05$) when stored on ice, and decreased by up to 100% ($P < 0.001$) at lower temperatures (Figure 1A). Only a few viable cells were observed after storage at -20°C , and no viable cells were observed after storage at -80°C . This suggests that the best temperature tested in this experiment at which to store milk samples was 4°C.

Compared with fresh samples, cell viability in milk samples stored at 4°C decreased by 2% (NS) after 24 h, 10% (NS) after 30 h, 17% ($P < 0.05$) after 48 h, and 45% ($P < 0.001$) after 60 h (Figure 1B). This suggests that samples can be stored for 24 to 30 h without significant loss of cell viability.

Total RNA Yield from Milk Somatic Cells

When total RNA was extracted from 300-mL fresh milk samples from 8 cows, RNA yield was 1.2 to 13.5 μg per sample. A yield of 1.2 μg of total RNA was not considered sufficient, so total RNA was extracted from

Figure 1. Effect of storage temperature and time on viability of somatic cells extracted from milk samples. A) samples stored for 24 h at different temperatures (4 cows per treatment); B) samples stored at 4°C for different time periods (4 cows per treatment). Viability was reduced by storage at ≤0°C and for ≥48 h (* $P < 0.05$; *** $P < 0.001$). Effects were tested using logistic regression with a binomial distribution and logit link function. Bars and error bars indicate back-transformed means ± SE.

a further 1 L of fresh milk from the same 8 cows. Total RNA yield varied from 3.3 to 26.9 $\mu\text{g/L}$ (Table 2) and was related to the number of viable somatic cells (per liter) by the equation

$$\log(\text{RNA}) = 0.27 \times \log(\text{viable cells}) - 2.85 \quad (R^2 = 0.64; P = 0.017).$$

Nondenaturing gel electrophoresis was performed on total RNA samples from 6 cows with total RNA yields above 5 μg to examine the quality of the RNA. The 18S and 28S rRNA bands (the most abundant RNA species)

could be seen clearly and a significant quantity of high molecular weight genomic DNA was not present in the samples (Figure 2). There was some variability in the intensity of staining between lanes; this was probably caused by the quantity to be loaded per lane being derived from an estimation of nucleic acid concentration, rather than a specific estimate of total RNA concentration (see the materials and methods section).

Basic RT-PCR

Polymerase chain reaction, with SCD-specific primers, was performed on cDNA prepared from total RNA extracted from milk somatic cells, mammary tissue, and liver tissue (Figure 3). As expected, an amplicon of 158 bp was present in the positive control samples (mammary tissue and liver tissue) and milk somatic cells. To confirm the amplicon generated from milk somatic cells encoded SCD, the 158-bp band was gel purified and subjected to nucleotide sequence analysis. The nucleotide sequence obtained with the forward primer showed a 99% match with the published sequence in the NCBI-BLAST gene bank, and the nucleotide sequence obtained by using the reverse primer showed a 98% match to the same database sequences. This indicated that SCD was expressed in the isolated milk somatic cells.

Expression Analysis with Quantitative PCR

Polymerase chain reaction products amplified with quantitative primers and probes, from single-stranded cDNA synthesized from total RNA extracted from milk somatic cells, gave a single 62-bp band on gels, indicating there was no contamination from genomic DNA. The abundance of SCD mRNA relative to β -actin mRNA for 12 individual cows (Figure 4) varied (mean ± SE) from 0.88 (±0.17) to 4.40 (±0.50). Analysis of deviance in the GLM analysis indicated that 55% of total deviance in relative abundance of SCD mRNA was due to variation among cows and 42% was due to variation among samples within cows. Relative abundance of SCD mRNA was not related to the number of viable somatic cells or total RNA extracted from samples.

Comparison with Desaturase Activity

A significant relationship ($P < 0.05$) was observed between relative abundance of SCD mRNA and the C_{14} index of desaturase activity (Figure 5).

DISCUSSION

The objective of this study was to develop a method for extraction and quantitative analysis of SCD mRNA

Table 2. Cell count, viability, and RNA yield from 1-L fresh bovine milk samples

Item	Cow							
	1	2	3	4	5	6	7	8
Total cells ($\times 1,000$)	101	127	108	756	21	334	208	36
Viability (%)	89	66	72	93	61	87	81	56
RNA yield (μg)	12.6	10.0	13.5	26.9	3.3	14.1	16.6	4.2
A ₂₆₀ /A ₂₈₀	2.10	1.96	1.90	2.20	1.89	1.87	1.93	1.91

from milk of high-yielding dairy cows, which could be used in future studies of SCD gene expression. To achieve this objective, total RNA yield and quality, and effects of storage conditions on cell viability were determined. The results were compared with the proportions of 14:1 and 14:0 in milk, which are commonly used as an index of mammary desaturase activity.

Somatic cell number and type varies among species because of differences in the physiology of milk secretion and immune defense mechanisms (Boutinaud and Jammes, 2002). Milk from goats has a somatic cell count 15 times greater than milk from cows. Apocrine secretion of milk in goats results in a much greater proportion of exfoliated epithelial cells and cytoplasmic particles than milk from cows, which secrete a greater proportion of leukocytes (Boutinaud and Jammes, 2002).

Because these results indicated that RNA yield correlated directly with viability of cells recovered, it was necessary to maximize the survival rate of somatic cells. Boutinaud et al. (2002) found a significant correlation between the number of viable cells and epithelial cell count, and also a significant correlation between epithelial cell count and total RNA yield from somatic cells in goat milk. This emphasizes the importance of maximizing retention of cell viability until RNA can be extracted. Maximizing retention of cell viability is crucial

also because milk has been shown to contain ribonuclease, with bovine milk having greater activity than caprine milk (Liu and Williams, 1982).

It has been suggested that the viability of somatic cells is sensitive to milk storage temperature, because ice crystals can damage cells during freezing and thawing (Barkema et al., 1997). The findings of the current study support this notion, because viability was severely impaired when samples were frozen. Under ideal circumstances, total RNA would be extracted from somatic cells in milk samples immediately after collection. This is not always practical in large studies, but it appears that adequate viability can be maintained for 24 h by storing samples at 4°C. A similar conclusion was drawn for milk epithelial cells by Buehring (1990), who found that viability, as indicated by the ability of epithelial cells to proliferate in culture, was reduced by 23% after 24 h of storage at 4°C, but was reduced by 92% after 48 h. Following total RNA extraction, samples can be frozen to await subsequent analysis.

Total RNA yield in this study varied from 3.3 to 26.9 $\mu\text{g/L}$ of milk in individual samples. This is considerably lower than total RNA yield (1.2 mg/L) from caprine milk (Boutinaud et al. 2002). Yield of total RNA averaged 0.20 $\mu\text{g}/1,000$ viable somatic cells in this study, compared with 0.35 μg in the study of Boutinaud et al. (2002). Most of the difference in total RNA yield can be explained by differences in the numbers of total and viable cells, although differences in cell type and extraction methods might also have contributed. To obtain sufficient total RNA for further processing, under the conditions of this study a sample size of 1 L was needed. Murrieta et al. (2006) used approximately 200 mL of milk as a source of somatic cells from beef cows. In their study, milk was collected from the excised mammary gland immediately postmortem. These authors did not report cell counts or total RNA yield, but it is likely that both would have been higher than from milk sampled during milking. Boutinaud et al. (2002) found that, in goats, total and epithelial cell counts in postmilking samples were more than twice as high as in samples obtained during milking.

Cells rich in cytoplasmic organelles and larger than 10 μm were considered as epithelial cells by Boutinaud

Figure 2. Nondenaturing gel electrophoresis (1%) of total RNA (5 μg) from milk somatic cells of 6 cows stained with ethidium bromide. The positions of 28S and 18S ribosomal RNA are indicated.

Figure 3. Nondenaturing gel electrophoresis (1.5%) of PCR products amplified using single-stranded cDNA synthesized with total RNA extracted from milk somatic cells, mammary tissue, and liver tissue. Position of the 158-bp amplicon encoding stearoyl-CoA desaturase is indicated. Lane 3: positive control, cDNA prepared from bovine mammary gland RNA; lane 10: positive control, cDNA prepared from bovine liver tissue; lane 6: negative control, no cDNA template in PCR reaction; lane 7: 100-bp DNA ladder; other lanes: cDNA prepared from milk somatic cells of 6 cows.

et al. (2002), who found that epithelial cells constituted approximately 27% of somatic cells in caprine milk. In the current study, approximately 2% of cells fit this description, but they could not be counted accurately because of clumping and cell debris, as observed by Lee et al. (1980) and Buehring (1990). When culturing somatic cells, Buehring (1990) identified a second type of epithelial cell that was smaller. Unlike large epithelial cells, small cells underwent cell division for up to 2 mo in culture. However, small epithelial cells could not be counted accurately in milk because they could not be adequately distinguished from lymphocytes.

Figure 4. Abundance of stearoyl-CoA desaturase (SCD) mRNA relative to β -actin mRNA in milk somatic cells from 12 cows. Bars and error bars show means $\pm$ SE of 4 samples per cow (assayed in triplicate). Effect of cow ($P < 0.001$) was tested using a generalized linear model with Poisson distribution and log link function.

Gel electrophoresis confirmed that PCR products amplified by using SCD-specific primers were similar for RNA isolated from milk somatic cells and for RNA prepared from bovine mammary gland and liver tissue. Sequence analysis showed a 99% match with the SCD sequence in bovine adipose tissue (Chung et al., 2000). These findings indicate that milk somatic cells are a reliable source of mRNA for examining SCD gene expression. Similar conclusions have been reached when comparing somatic cells and tissue samples for milk

Figure 5. Relationship between relative abundance of stearoyl-CoA desaturase mRNA (log SCD mRNA: β -actin mRNA) in milk somatic cells and C_{14} desaturase index [milk *cis*-9 14:1/(14:0 + *cis*-9 14:1)] from 12 cows. Line shows relationship ($y = 0.01x - 0.09$; SE 0.002; $P < 0.001$) after adjusting for individual cow effects ($P < 0.001$) with a generalized linear model. Data points are means of triplicate assays for each of 4 samples per cow.

protein genes in goats (Boutinaud et al. 2002) and for lipogenic enzymes in beef cattle (Murrieta et al. 2006).

The quantitative RT-PCR results show that relative abundance of SCD mRNA can be measured precisely with somatic cells. The results also indicate that SCD expression is variable among individual cows and among samples from the same cow. Using ratios of milk fatty acids that are precursors and products of SCD activity, Lock and Garnsworthy (2002) found that SCD activity varied among cows independently of diet, suggesting a strong genetic influence. This was confirmed by Royal and Garnsworthy (2005), who estimated the heritability of SCD activity to be 0.30. Furthermore, Lock et al. (2005) reported that SCD activity assessed by fatty acid ratios was not related to milk yield, milk fat content, or DIM.

Variation in relative abundance of SCD mRNA among samples taken from individual cows on different days cannot be explained with the data available from this study. Variation was random and was not related to milk yield or DIM. All cows consumed the same diet throughout the sampling period. Although somatic cell count is known to vary on a daily basis (Harman, 1994; Boutinaud et al., 2002), relative abundance of SCD mRNA was not related to the number of viable somatic cells or total RNA extracted from samples in the current study. One possible influence is variation among samples in the proportion of epithelial cells. To correct for total RNA loading, β -actin was used as a standard housekeeping gene. β -Actin would be expressed in leukocytes as well as epithelial cells. One cannot assume that SCD is not expressed in leukocytes, but the level of expression is likely to be lower than in epithelial cells. Therefore, it is possible that relative abundance of SCD might vary with the epithelial cell content of milk samples. In future studies, this could be tested by examining expression of additional genes expressed only in epithelial cells or by cytochemical determination of epithelial cell content.

Ideally, SCD expression measured in somatic cells would be compared with SCD expression measured in tissue samples obtained by biopsy, but biopsy samples could not be taken in the current study. As an alternative, product:precursor relationships for milk C_{14} fatty acids are commonly used as a proxy for desaturase activity. The C_{14} desaturase index is considered to be a better indicator of desaturase activity than indices produced from other fatty acids because virtually all 14:0 and *cis*-9 14:1 are synthesized in the mammary gland. The significant relationship between the C_{14} desaturase index and relative abundance of SCD mRNA in milk somatic cells indicates that both produce similar estimates of relative SCD activity in the mammary gland.

CONCLUSIONS

This study showed that milk somatic cells can be used as a source of mRNA to study SCD expression in high-yielding dairy cows. Somatic cells provide a noninvasive and repeatable alternative to mammary tissue samples obtained by biopsy or postmortem. Compared with studies of somatic cells from goats and beef cattle, total RNA yield was lower in dairy cows, so a relatively large quantity (1 L) of milk was required. Because total RNA yield is related directly to cell viability, samples have to be processed as soon as possible and not frozen before total RNA extraction. Variation in SCD expression is due to individual cow variation and daily variation within cows. Initial results showed that SCD expression assessed by mRNA extracted from somatic cells was reflected in greater apparent SCD activity in the mammary gland, as indicated by changes in milk fatty acids.

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