Fatty acid biosynthesis and transcriptional regulation of Stearoyl-CoA Desaturase 1 (SCD1) in Buffalo milk

Zhipeng Li

Guangxi university

Suyu Lu

Guangxi university

Kuiqing Cui

Guangxi university

Laiba Shafique

Guangxi University

Saif ur Rehman

Guangxi University

Chan Luo

Guangxi University

Zhiqiang Wang

Guangxi University

Qingyou Liu (✉ 408659358@qq.com)

Guangxi University <https://orcid.org/0000-0003-3265-540X>

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Abstract

Background: Nutritionally, buffalo milk is of immense importance due to high contents of fatty acid (FA), highly energetic and nutritive value. The composition of FA has been regarded as functional food component, which facilitate to manufacture a variety of processed milk products. Moreover, Buffalo milk contains more unsaturated FAs (UFA) such as oleic and linolenic acids, which have been shown to exert a number of physiological effects. However, inadequate information reported about the chemical composition and mechanism of FA synthesis in buffalo milk. Our conducted research hypothesize that, the expression of SCD1 gene could alter the biosynthesis of fatty acid (FA) in epithelial cells of mammary gland and subsequently affect the FA contents in buffalo milk. Present study investigated transcriptional and biological role of Stearoyl-CoA Desaturase 1 (SCD1) in the buffalo mammary epithelial cells (BMECs) during FA and triacylglycerol (TAG) synthesis.

Results: Results showed that unsaturated fatty acid contents are much higher in concentration in buffalo milk as compared to Holstein cow. Significantly, higher expression level of FAS, ACACA, SREBP1, PPARG, GPAT and AGPAT genes were also been observed with the expression of the SCD1 in buffalo milk. Moreover, studies showed that, SCD1 gene alteration in BMECs also causes the change in the expression of FA synthesis related genes mentioned above and subsequently affect the FA composition in the cells. Overexpression of SCD1 significantly increased the expression of genes associated with FA and TAG synthesis and also improved the FA and unsaturated FA contents in BMECs, while down-regulation of SCD1 appear opposite consequences.

Conclusion: Thus, present study clearly demonstrated that, alteration in the expression of SCD1 plays an important role in FA and TAG synthesis and can directly or indirectly affect biosynthesis and metabolic pathways in BMECs and may be used as a symbol for important mechanism that contributes the characterization of buffalo milk.

Background

Buffalo is not considered as dairy animal in many regions but buffalo milk is placed at second most produced milk in the world, especially in Italy, India, Pakistan, Egypt and Nepal [1, 2]. Buffalo milk is rich source of fatty acid (FA), highly energetic and nutritive value. The composition of FA has been regarded as functional food component, which facilitate to manufacture a variety of processed milk products. Moreover, Buffalo milk contains more unsaturated FAs (UFA) such as oleic and linolenic acids, which have been shown to exert a number of physiological effects [3, 4]. However, inadequate information reported about the chemical composition and mechanism of FA synthesis in buffalo milk.

Milk FA is either de novo synthesized in the mammary gland or imported from the plasma. Though the signal mechanisms of the lipogenic process in rodent tissues have been described [5], but only a few studies have been reported in buffalo yet. However, it was suggested that fat synthesis in the mammary gland may share a similar mechanism [6, 7]. It was also observed that the main lipid metabolic pathways

were controlled by governing the DNA-binding activity and nuclear abundance of selected transcription factors regulating the expression of key genes [5, 8]. The major transcriptional factors involved in FA biosynthesis are sterol regulatory binding protein-1 (SREBP-1) and peroxisome proliferator-activated receptors (PPARs). FA or cholesterol can induce changes in the nuclear abundance of SREBP and bind to the nuclear receptors PPAR. SREBP can bind on sterol binding elements or on palindromic sequences with the promoter regions of its target genes such as *ACACA*, *FAS*, and *SCD* [8]. An implication of PPAR γ in the nutritional or insulin activation of lipogenesis has been observed by up regulation of *PPARG*, *FAS* and *ACACA* gene expression in bovine tissues [9]. The acetyl-CoA carboxylase (ACC) and FA synthase (FAS) enzymes (encoded by the *ACACA* and *FAS* genes, respectively) are involved in the metabolic pathway for *de novo* FA synthesis [8]. The ACCA protein provides cytoplasmic malonyl-CoA for FA synthesis, and the FAS protein is mainly responsible for the short- and medium-chain FA (C4-C16) synthesis [10].

In mammary gland, SCD is responsible for the synthesis of the major part of cis-9, trans-11- [11, 12] and of trans-7, cis-9- CLA isomers [13]. The FA can be desaturated by SCD and results in the synthesis of cis-9 monounsaturated FA (MUFA), and then esterified to glycerol sequentially by glycerol-3 phosphate acyl transferase (GPAT), acyl glycerol phosphate acyl transferase (AGPAT), and diacyl glycerol acyl transferase (DGAT) [8]. The promoter region of bovine SCD, especially the region containing the stearoyl-CoA desaturase transcriptional enhancer element, play a key role in the inhibitory effect on *SCD* gene transcription of trans-10, cis-12 CLA. It has been characterized as a core gene involved in the FA and triacylglycerol (TAG) synthesis [14] and also acts as study tool in biological system at transcriptional level and would be able to generate fundamental information for manipulating the quality of milk.

Several studies reported on FA compositions and its physico-chemical characteristics in buffalo and cow milk, while few studies have been focused on the molecular mechanisms. Thus, the experiments reported here were designed to test the hypothesis that the transcriptional and biological role of SCD1 during FA and TAG synthesis affect the FA constitutes in Buffalo milk. As an initial step, we characterized the FA composition of Buffalo and Holstein and analyzed the expression pattern of the genes related to FA synthesis. The relationship between SCD1 and selected genes were further studied by RNA interference (RNAi) and overexpression of SCD1 in Buffalo mammary epithelial cells (BMECs), and the effect on the FA compositions were also analyzed. The alteration of SCD1 may be used as a symbol for important mechanism that contributed in the characteristics of Buffalo milk.

Results

Routine Analysis of Milk Composition

Holstein cows and Buffalo milk composition were evaluated including, butter fat content, protein content, lactose content, and total solid and non-fat solid content (Table 1). Results showed that Holstein cows and Buffalo milk lactose contents was comparable, while the contents of FA, protein, total solids and non-

fat solids in Buffalo milk were significantly higher than Holstein milk. Among them, the butterfat content in buffalo milk showed 1.86 times higher than Holstein (7.88 ± 0.91 VS 4.24 ± 0.80).

FA Composition Analysis of Milk

Gas chromatograph was being employed to access the FA composition for two types of milk samples (Table 2) and the evaluated data showed that the FA composition of both milk samples were similar, except the CLA and EPA that beneficial to human health which are only found in Buffalo milk. The highest content of saturated FA (SFA) was C16:0, and the unsaturated FA (UFA) was C18:1. Both the FA and total FA in Buffalo milk are significantly higher than that of Holstein cows in terms of the FA content per 100 g of milk ($p < 0.05$).

Expression of Genes Related to FA Synthesis in Milk

In order to analyze the reasons of high milk fat ratio in Buffalo milk, the expression level of key genes related to milk fat synthesis for both milk categories were detected by QRT-PCR. Results illustrated that radically higher expression level of the fat synthesis related genes in Buffalo milk, including FA *de novo* synthesis related genes *FAS* and *ACACA*, Glyceride synthesis related genes *GPAT* and *Agpat6*, and transcriptional regulator related genes *SREBP1* and *PPARG* were examined than Holstein (Fig.1). In addition, the expression of *SCD1* in Buffalo milk was about 123 times higher than that of Holstein, which suggested that it may play a key role in the FA synthesis.

Effect of SCD1 Interference on FA Synthesis Related Genes Expression and FA Composition in BMECs

The interference efficiency of the siRNA was detected first to confirm the feasibility of the siRNA. Results confirmed that the transfection of siRNA1 and siRNA2 significantly decreased the expression of *SCD1* in comparison with the control group, while the siRNA3 and NC-siRNA have no significant affect on the expression level (Fig.2). The siRNA1 could interfere the expression of *SCD* gene to the greatest extent and so, was selected for the following study. The siRNA1 and NC-siRNA were transfected into BMECs respectively and QRT-PCR was used to spot out the expression of FA synthesis related genes (Fig.3). Results demonstrate that the expression of *SCD1* was significantly decreased one fifth compared to the blank control, indicating the siRNA transfection successfully interfere the *SCD1* gene expression. Subsequent to the interference, the expression of *FAS* and *PPARG* was considerably increased ($P < 0.05$), whereas *AGPAT6*, *ACACA* and *GPAT* gene was decreased ($P < 0.05$). The *SCD1* interfering did not significantly affect the expression of *SREBP1* ($P > 0.05$).

The FA contents extracted from cells were further analyzed by gas chromatography (Table 3). Results confirmed that, after the siRNA interference, SFA content including C16:0 and C18:0 were significantly increased ($P < 0.05$), while UFA contents including C16:1 and C18:1 decreased ($P < 0.05$). Moreover, the total SFA contents were also significantly increased ($P < 0.05$) and that of total UFA was markedly decreased ($P < 0.05$). It is interesting that no significant change ($P > 0.05$) was observed in total FA contents of the BMECs.

Effect of SCD1 over expression on FA Synthesis Related Gene Expression and FA Composition in BMECs

After SCD1 overexpression, the expression of SCD1 increased up to 8 times more as compared to the blank control. The expression level of both FAS and ACACA was significantly down regulated, whereas the expression of GPAT, SREBP1, PPARG and AGPAT was significantly enhanced (Fig.4). The composition of FA that extracted from BMECs was also analyzed by applying Gas chromatography (Table 4). With the overexpression of SCD1 in BMECs, the SFA contents including C16:0 and C18:0 were significantly decreased ($P < 0.05$), while that of UFA contents including C16:1, C18:1 and C18:2 were increased ($P < 0.05$). Meanwhile, the total FAs and UFA contents in the cells were increased significantly ($P < 0.05$), while the total SFA contents were decreased ($P < 0.05$).

Summary of Networks among Genes Involved in Milk Fat Synthesis

The networks between SCD1 and genes related to FA synthesis in BMECs were analyzed and summarized in Fig.5. Comparing with the control group, knockdown of SCD1 can significantly decrease the expression of TAG biosynthesis related genes (GPAT and Agpat6), while increase the expression of FA synthesis gene FAS. The expression of these genes altered to the opposed when SCD1 was overexpression, which was in accordance with the alteration of SCD1. While the expression of another FA synthesis gene ACACA was decreased with either SCD1 overexpression or siRNA treatment, though the change induced by siRNA was without significantly difference. The expression of the transcription factor PPARG were significantly up-regulated either the SCD1 was overexpression or knockdown. However, the other transcription factor SREBP was significantly up-regulated when SCD1 was overexpression, but not markedly affected by the reduction of SCD1.

Discussion

Milk is an essential nutritive component of human diet that provides nourishment to young ones and also the raw materials for various kinds of food. In our study, the protein and FA, especially the UFA, in buffalo milk are significantly higher than that of Holstein cow. Similar results have been reported in the previous literature [1, 2]. But our finding varies with previous studies, in terms of the lactose contents that are significantly higher in buffalo milk compared to the cow [2]. It is speculated that different environmental conditions for nourishment and feeding which play vital role of lactose content in milk, may lead to the inconsistency. However, this does not affect the buffalo milk quality and the low lactose content may even beneficent for the people having lactose digestion problems which referred to as lactose intolerance or malabsorption [15].

Milk fat is an important constituent of nutritional quality for dairy products and the UFA, such as oleic and linolenic acids, have been shown to exert a number of physiological effects like regulating blood fat, eliminating thrombus and regulating immunity [3, 4]. Additionally, fat contents and its composition have much more importance for technological and sensorial qualities of the dairy products. There are two sources of FA in milk, either come from de novo synthesis or uptake from blood [21] and the constituents of milk and their concentrations are linked to the genetic factors and nutritional factors. Thus, the

mechanism for the modification of milk fat content and FA composition should be investigated with particular focus on the effects of functional genomics. In the present study, we found that the expression level of genes related to FA *de novo* synthesis (*FAS* and *ACACA*), TAG synthesis (*GPAT* and *AGPAT*) and transcription factors (*SREBP1* and *PPARG*) in buffalo were significantly higher than that of Holstein cow. Thus, we can hypothesize that the high FA content in buffalo milk is related to the high expression level of these genes, which is consistent with the studies in goat and bovine [16–18].

Progress in lactation biology of bovine mammary gland has been extensively studied in the past century [19] and the major influential transcriptional factors involved are *SREBP1* and *PPARs*. *SREBPs* are the transcriptional factors that regulate lipid homeostasis through controlling the expression of enzymes required for FA *de novo* synthesis. The mature *SREBP* is translocated to the nucleus, where it bound with its target genes, including *ACACA*, *FAS*, *SCD1* and so on [20]. *SREBP1* is a central element in overall regulation of genes involved in milk fat synthesis [16, 21], and *SCD1* is involved in the *SREBP*-1-regulated pathway during milk fat synthesis [22, 23]. In our study, the effects of *SCD1* on *SREBP1* mRNA levels as a result of overexpressing or *SCD1* RNAi demonstrated that *SREBP1* could be regulated transcriptionally by *SCD1* in BMECs, which is consistent with the previous findings as mentioned above. *PPARs* belong to a superfamily of hormone receptors and regulate the transcription of genes that are involved in different metabolic pathways of lipids, including the transport of plasma triglycerides, the cellular uptake of FA, and the peroxisomal and mitochondrial β -oxidation [24].

It has also been reported that activation of *PPARG* can regulate lipogenesis and the expression of *FAS* and *ACACA* [9]. Although the activation of *PPARG* did not affect the expression of *SCD1* in rodents [25, 26], positive effect in ruminants has been stated earlier [18, 23, 27]. Our findings also confirmed that, there is a direct relationship between *SCD1* and *PPARG* in FA synthesis process that concurrence with the studies mentioned above. Further studies has reported that the expression of *SCD1* can be suppressed by the natural ligands of *PPARG*, palmitoleic acid and oleic acid, through a polyunsaturated FA response region (PUFA-RR) [28, 29]. As a result, an appropriate ratio of intracellular oleic to stearic acid should be maintained for proper regulation of *SCD1*, *SREBP1* and *PPARG*.

Several reports have found that *SCD1* polymorphism is associated with milk FA composition [30–32], that helps us to suppose that *SCD1* may play a crucial role in mammary FA and TAG synthesis. Although *SCD1* is mainly involved in the synthesis of MUFA and it may also affect the synthesis of TAG. The MUFA catalyzed by *SCD1* may even serve as the substrates for TAG synthesis in ruminant mammary gland [33]. In the present study, the alteration of the *SCD1* expression do affect the expression of genes related to FA and TAG synthesis and also their composition in BMECs, that agree with the previous findings mentioned above. In addition, the expression of enzymes involved in the TAG synthesis including *GPAT* and *AGPAT* were also affected due to the altered expression of *SCD1*. The increase of UFA, C16:1, C18:1 and C18:2 in the BMECs with the overexpression of *SCD1* may also be the combine action of *ACACA* and *FAS* down-regulation together with the up-regulation of *GPAT* and *AGPAT*, which is supported by the evidence that medium and long chain FA are potent inhibitors of *ACACA* in the mammary gland [18, 34]. It can be said that, *SCD1* may cooperate with proteins that may inhibit the FA synthesis to maintain an appropriate FA

synthesis and oxidation balance by altering its products. Evidence has been found that *SCD1* overexpression in CHO cells can led to significant increase of TAG synthesis, while decrease of FA oxidation [35, 36]. Moreover, down-regulation of *SCD1* can led to decrease in expression of genes related to TAG synthesis and results in lower cellular TAG content in 3T3-L1 preadipocytes [33], which are similar to our findings. And a general down-regulation of the gene expression in the mammary gland has been reported to contribute to the decrease in milk fat production [37].

Conclusion

In conclusion, our study indicated that *SCD1* plays an important role in FA and TAG synthesis and can directly or indirectly affect biosynthesis and metabolic pathways in BMECs. The overexpression of *SCD1* is favorable for TAG synthesis and increasing the TAG products that may suppress the *de novo* synthesis of FA by down-regulated expression of *ACACA* and *FAS*. The alteration of *SCD1* may be used as a symbol for important mechanism that contributes in the characterization of buffalo milk. Many studies have been done to explain the mechanism of FA synthesis in milk, but the molecular mechanisms are still not fully explored and need further studies.

Methods

Animals and Sampling

Holstein cows are indigenous and Italian Mediterranean Buffalo (*Bubalus bubalis*) are pure bred high-yielding dairy Buffaloes imported from Italy in 2015 by the farm (Guangxi HuaXu Buffalo Biotechnology co. LTD). All of them were kept under the uniform feeding and management conditions. Milk samples were collected from 30 Buffalos or Holstein cows in their 1st or 2nd parity and 100~150 days in lactation. Milk samples were collected in triplicate and kept on ice until the composition analysis including lactose content, fat percentage, protein percentage, total solids and non-dairy solids was performed by a milk composition analyzer (Milkscan FT120, Denmark) immediately after collection. All of the collected animal samples in this study have been permitted by the farm owner.

Milk Fat Extraction and Gas Chromatography Analysis

Milk fat was extracted following the method reported by Mele [38]. In brief, two grams of milk sample were mixed with 0.4 mL of ammonia 25%, 1 mL of ethyl alcohol 95%, and 5 mL of hexane, vortexed and centrifuged at $1600 \times g$ at 4°C. The upper layer was collected, and a second extraction with 1 mL of ethyl alcohol 95% and 5 mL of hexane was performed. A third extraction was made by using 5 mL of hexane. The extracted fat was dried, weighed, and finally dissolved in hexane. FA composition was determined by gas chromatography using a ShimadzuGC-2014C (Kyoto, Japan) gas-chromatograph equipped with an FID and a capillary column (Agilent DB23, California, USA; 30m $\times$ 0.32 mm i.d.; film thickness 0.25 μ m). Injection ported 230 °C and detector 280 °C. The column was kept at 180 °C for 5min and heated up to

230 °C at 3 °C min⁻¹. The carrier gas is kept in high-purity nitrogen and the injection volume is 1 µL. All milk FA compositions were expressed as g per 100 g of fat.

Total RNA Isolation

The total RNA in milk fat globules (MFG) was used for gene expression analysis based on the previous study [39]. Milk samples were centrifuged at 2000 × g for 10 min at 4°C to isolate milk fat. The supernatant fat layer was separated and 500 µL fat sample was taken with 1.5 mL of TRIzol LS solution (Invitrogen Life Technologies Inc., Carlsbad, CA). All the procedures were carried out at 4°C. RNA was dissolved in 30 µL of RNase-free water and stored at -80 °C.

Single-Strand cDNA Synthesis

RNA purity was evaluated by means of absorbance readings (ratio of A260/A230 and A260/A280) by using a NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Before First-strand cDNA synthesis contaminated genomic DNA was removed by DNase treatment. First-strand cDNA was synthesized using RevertAid First Strand cDNA Synthesis Kit (K1622, Thermo Fisher Scientific, Waltham, MA). The single-strand cDNA obtained was stored at -20°C.

Primer Design and QRT-PCR

Primers of the selected genes of interest were designed by using the oligo 7 software and only exon-exon junctions with great homology between sequences were chosen (Table 5). The expressions of selected genes were quantified by using SYBR Green supermix (4913914001, ROCHE) and fluorescence data were acquired using a fluorescence quantification PCR instrument (ABI 7500). A 20 µL mixture was performed in each run as follows: 1 µL cDNA, 8 µL H₂O, 10 µL Faststart Universal SYBR Green Master (ROX), and 0.5 µM aliquots of both forward and reverse primers. The thermal cycling profile started with a 3-min dwell temperature of 94°C, followed by 40 cycles of 30 sec at 94°C, 30 sec at the primer specific annealing temperature (60°C), 30 sec at 72°C, and a final step during which fluorescence was acquired. After 40 cycles, a melt curve was generated by temperature increments of 0.1°C starting from 60°C to 100°C, with fluorescence acquisition after each step. Each sample was repeated 3 times, and the relative gene expression was calculated using the 2^{-ΔΔCt} method with *GAPDH* as the reference gene. The selection of reference gene was based on a previous report [40].

Construction of Lentiviral Vector and Synthesis of siRNA

The SCD1 gene was cloned from Buffalo mammary epithelial cells according to the SCD1 sequence available in GenBank (No.AY241933) and inserted into the lentiviral vector. The siRNA targeting SCD1

gene were designed and synthesized by Gene Pharma (shanghai, China) with a control sequence (Table 6). Lentiviral vectors containing the SCD1 gene and the siRNA with a negative control (NC) were constructed by Gene Pharma (shanghai, China). The lentiviral vectors were packaged and propagated in 293T cell line with the packaging plasmid (Δ NRF) and the envelope plasmid encoding the vesicular stomatitis virus-G glycoprotein (VSVG).

Cell Culture

The BMECs were cultured and purified as devised previous method [41, 42]. Briefly, during lactation fresh tissue blocks from buffalo were obtained and washed 3 times and the acinus portion was extracted from the mammary gland tissue and transferred into high-resistance PBS (containing 400 IU mL⁻¹ of penicillin and 400 IU mL⁻¹ of streptomycin). Then were placed in culture dishes on a clean bench, cut into 1 to 2 mm pieces and tiled on the bottom of culture dish and cultured in incubator for 4 h. Then they were inverted and cultured in the upright position overnight. The epithelial cells started to grow after about 12 days and the epithelial cells were isolated by using trypsin digestion combined with a cell adherence speed method. The purification procedure was performed 3 times and the BMECs at generation 3 to 4 in the subculture were used for the following studies.

Infection and Transfection

Transfection of the lentiviral vectors were carried out by using the Lipofectamine™ 2000 (Invitrogen, USA). 100 μ M lentiviral vectors containing siRNA or SCD1 gene were used in each transfection and the transfected confluent cells were harvested for QRT-PCR analysis, 24h after transfection.

Statistical Analysis

All experiments were repeated three times. Results were expressed as mean $\pm$ standard error (SEM). Statistically significant differences were performed by using the SPSS 17.0 (IBM Corp., Armonk, NY). Differences of $P < 0.05$ were considered to be significant.

Abbreviations

ACACA: Acetyl-coenzyme A carboxylase alpha; AGPAT: Acyl glycerol phosphate acyl transferase; DGAT: Diacylglycerol acyltransferase; FAS: Fatty acid synthase; GPAT: Glycerol-3 phosphate acyl transferase; MUFA: Monounsaturated fatty acid; PPAR γ : Peroxisome proliferator activated receptor gamma; PUFA: Polyunsaturated fatty acid; SCD: Stearoyl-CoA desaturase; SCFA: Short chain fatty acid; SFA: Saturated fatty acid; UFA: Unsaturated fatty acid.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable

Availability of data and material

All data generated and analyzed during this study are included in this paper.

Competing interests

The authors declare no competing financial interest.

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Authors' contributions

LQY and CKQ designed the research studies; LZP and LSY performed the research, analyzed data and discussed the results and implications; LC and WZQ provided help in data analysis; LZP draft the paper, LS and SR provide help in language editing. All authors read and approved the final manuscript.

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Tables

Table 1. Routine composition of milk in different cows

Species	Fat %	Protein %	Lactose %	Total solids %	Non-dairy solids %
Holstein	4.24±0.80 ^a	3.39±0.55 ^a	4.92±0.32	13.40±1.36 ^a	9.17±0.58 ^a
Buffalo	7.88±0.91 ^b	5.37±0.32 ^b	4.99±0.41	18.16±1.08 ^b	10.23±0.47 ^b

Note: Different superscript letters means significantly different (P<0.05).

Table 2. FA composition of Holstein and buffalo milk

Fat acid		Holstein	Buffalo
Saturated	C4:0	36.96±10.92 ^a	94.49±13.39 ^b
Fat acid	C6:0	20.09±3.75 ^a	116.2±9.98 ^b
	C8:0	13.59±3 ^a	73±6.41 ^b
	C10:0	24.72±4.41 ^a	151.27±11.23 ^b
	C12:0	33.82±6.2 ^a	183.52±16.03 ^b
	C14:0	105.2±19.7 ^a	854.22±58.83 ^b
	C15:0	0.94±0.17 ^a	10±1.27 ^b
	C16:0	213.36±36.49^a	2986.31±178.62^b
	C17:0	7.37±1.74 ^a	81.52±8.62 ^b
	C18:0	51.77±8.47 ^a	957.3±114.08 ^b
	Total	507.82±94.85 ^a	5507.83±418.46 ^b
Unsaturated	C14:1	11.55±1.76 ^a	92.23±10.58 ^b
Fat acid	C16:1	22.06±4.7 ^a	247.68±33.77 ^b
	C17:1	2.89±0.53 ^a	29.58±3.2 ^b
	C18:1	343.33±62.99^a	2419.31±257.48^b
	C18:2	28.97±5.88 ^a	136.34±15.24 ^b
	C18:3	5.76±1.49 ^a	40.14±4.89 ^b
	C20:1	1.89±0.53 ^a	23.25±3.64 ^b
	C20:2	1.2±0.17 ^a	3.62±0.45 ^b
	C20:3	3.15±0.59 ^a	14.21±1.39 ^b
	CLA	—	19.4±1.68^a
	EPA	—	14.6±0.2^a
	Total	449.45±78.62 ^a	2959.94±327.72 ^a

Total	925.27±173.47 ^a	8467.77±746.18 ^b
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Note: “—” indicate undetectable; Different superscript letters means significantly different (P<0.05). All milk FA compositions were expressed as g per 100 g of fat.

Table 3. Effect of SCD1 interference on FA composition in BMECs

Fat Acid	Blank control (mg)	Negative control (mg)	RNA interfered (mg)
C16:0	0.614±0.11 ^a	0.591±0.11 ^a	0.759±0.18 ^{b↑}
C16:1	0.279±0.06 ^a	0.226±0.03 ^a	0.124±0.03 ^{b↓}
C18:0	0.657±0.16 ^a	0.547±0.07 ^a	0.875±0.17 ^{b↑}
C18:1	0.873±0.10 ^a	0.886±0.18 ^a	0.613±0.15 ^{b↓}
C18:2	0.212±0.06	0.203±0.02	0.216±0.02
Total SFA	1.271±0.27 ^a	1.138±0.17 ^a	1.634±0.36 ^{b↑}
Total UFA	1.364±0.22 ^a	1.315±0.24 ^a	0.953±0.20 ^{b↓}
Total	2.535±0.49	2.453±0.41	2.587±0.56

Note: Different superscript letters means significantly different (P<0.05). All milk FA compositions were expressed as g/100 g of fat. SFA: Saturated Fat acid; UFA: Unsaturated Fat acid.

Table 4. Effect of SCD1 overexpression on FA composition in BMECs

Fat Acid	Empty control	Negative control	Overexpress
	(mg)	(mg)	(mg)
C16:0	0.614±0.11 ^a	0.602±0.09 ^a	0.403±0.10 ^{b↓}
C16:1	0.279±0.05 ^a	0.256±0.06 ^a	0.486±0.09 ^{b↑}
C18:0	0.657±0.16 ^a	0.642±0.12 ^a	0.319±0.05 ^{b↓}
C18:1	0.873±0.11 ^a	0.821±0.15 ^a	1.276±0.17 ^{b↑}
C18:2	0.212±0.06 ^a	0.197±0.06 ^a	0.354±0.03 ^{b↑}
Total SFA	1.271±0.27 ^a	1.244±0.22 ^a	0.722±0.16 ^{b↓}
Total UFA	1.364±0.22 ^a	1.274±0.27 ^a	2.116±0.29 ^{b↑}
Total	2.535±0.50 ^a	2.518±0.48 ^a	2.838±0.45 ^{b↑}

Note: Different superscript letters means significantly different (P<0.05). SFA: Saturated Fat acid; UFA: Unsaturated Fat acid.

Table 5. Primers used for real-time quantitative PCR

Target genes	Primer Sequences(5'~3')	Tm (°C)	GenBank Accession No.
SCD1	F: TCCTGTTGTTGTGCTTCATCC R: GGCATAACGGAATAAGGTGGC	59	AY241933
FAS	F: GCAAAGTGGTCATTCAGGTACG R: CCCAGTGATGATGTAGCTCTTG	60	KM980092.1
PPARG	F: CAGTGTCTGCAAGGACCTCA R: GTAAAAGGCATGGGAGTGAT	59	HG270143.1
SREBP1	F: CTGACGACCGTGAAAACAGA R: AGACGGCAGATTTATTCAACTT	58	KU517672.1
GPAT	F: GCAGGTTTATCCAGTATGGCATT R: GGACTGATATCTTCCTGATCATCTTG	59	AY515690.1
AGPAT6	F: AAGCAAGTTGCCCATCCTCA R: AAAGTGTGGCTCCAATTTCTGA	59	DY208485
ACACA	F: TCCTGCTGCTATCGCTACTCCA R: CGCACTCACATAACCAACCAT	61	DQ773054.1
GAPDH	F: TGGAAAGGCCATCACCATCT R: CCCACTTGATGTTGGCAG	60	NM001034034.1

Table 6 siRNA sequence target SCD1

siRNA target SCD1	Sequence (5' to 3')
siRNA-1	GCCCAAGCUUGAGUAUGUUTT AACAUACUCAAGCUUGGGCTT
siRNA-2	GCCCUAUAUGGGAUCACAUTT AUGUGAUCCCAUAUAGGGCTT
siRNA-3	GGAGUCACCGAACCUACAATT UUGUAGGUUCGGUGACUCCTT
siRNA-NC	UUCUCCGAACGUGUCACGUTT ACGUGACACGUUCGGAGAATT

Figures

Figure 1

Expression of genes related to FA synthesis in milk by QRT-PCR analysis. Note: Different superscript letters means significantly different ($P<0.05$).

Figure 2

The relative expression of SCD1 after siRNA interference. Note: Different superscript letters means significantly different ($P<0.05$).

Figure 3

Expression of FA synthesis related genes after SCD1 interference. Note: Different superscript letters means significantly different ($P < 0.05$).

Figure 4

Expression of FA synthesis related genes after SCD1 overexpression. Note: Different superscript letters means significantly different ($P < 0.05$).

Figure 5

Summary of SCD1 associated FA synthesis and metabolism signal pathways in BMECs.