Lack of stearoyl-CoA desaturase-1 function induces a palmitoyl-CoA Δ6 desaturase and represses the stearoyl-CoA desaturase-3 gene in the preputial glands of the mouse

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Abstract **The mouse preputial gland (PG), a specialized sebaceous structure, is rich in wax esters, triglycerides, and alkyl-2,3-diacylglycerol. We have found that the mouse PG expresses the three gene isoforms (SCD1, SCD2, and SCD3) of the Δ9 stearoyl-CoA desaturase enzyme that catalyzes the biosynthesis of monounsaturated fatty acids mainly, C16:1n-7 and C18:1n-9. However, mice with a targeted disruption in the SCD1 isoform (SCD1$^{-/-}$) have undetectable SCD3 mRNA expression in the PG while the expression of SCD2 isoform was not altered. The levels of C16:1n-7 were reduced by greater than 70% while that of C18:1n-9 were reduced by 28%. The content of the C16:1n-10 (Δ6 hexadecenoic acid) isomer and a major fatty acid of the PG was increased by greater than 2-fold, mainly in the wax ester fraction of the SCD1$^{-/-}$ mouse. We demonstrate that the increase in C16:1n-10 is due to induction of a specific palmitoyl-CoA Δ6 desaturase activity. Testosterone administration to the SCD1$^{-/-}$ mouse induced SCD3 mRNA expression and resulted in an increase in the Δ9 desaturation of 16:0-CoA, but not of 18:0-CoA. These observations demonstrate that loss of SCD1 function alters the expression of SCD3 and reveal for the first time the presence and regulation of a palmitoyl-CoA Δ6 desaturase enzyme in mammals.**—Miyazaki, M., F. E. Gomez, and J. M. Ntambi. **Lack of stearoyl-CoA desaturase-1 function induces a palmitoyl-CoA Δ6 desaturase and represses the stearoyl-CoA desaturase-3 gene in the preputial glands of the mouse. *J. Lipid Res.* 2002.** 43: **2146–2154.**

Supplementary key words Δ6 hexadecenoic acid • wax esters • triglycerides • gene isoforms

Stearoyl-CoA desaturase (SCD) is a rate-limiting enzyme in the biosynthesis of monounsaturated fatty acids. It catalyzes the Δ9-*cis* desaturation of acyl-CoA substrates, the preferred substrates being palmitoyl-CoA and stearoyl-CoA, which are converted to palmitoleoyl-CoA and oleoyl-CoA, respectively (1). Palmitoleoyl-CoA and oleoyl-CoA, are substrates for incorporation into membrane phospholipids, triglycerides, cholesterol esters, and wax esters (2, 3). Several isoforms of SCD exist in the mouse genome. SCD1, SCD2, and SCD3, which are products of different genes, are the best characterized (4–6). Most organs of different mouse strains express SCD1 and SCD2 with the exception of liver (3) and skin, which express mainly the SCD1 and SCD3 isoforms, respectively (6). SCD2 is constitutively expressed in the brain (4, 6, 7) and like SCD1, is expressed at high levels in livers of mice that overexpress the truncated nuclear form of sterol regulatory element binding protein (SREBP)-1a (8). Despite the fact that the mouse SCD1, SCD2, and SCD3 genes are structurally similar, sharing ~87% nucleotide sequence identity in the coding regions, their 5′ flanking regions differ somewhat, resulting in divergent tissue-specific gene expression (4–6). However, in some tissues, such as the adipose and eyelid, both SCD1 and SCD2 isoforms are expressed, whereas in the skin and Harderian gland, all three gene isoforms are expressed (3, 7).

The preputial glands (PG) are large sebaceous glands, rich in wax esters, triglycerides, and alkyl-2,3-diacylglycerols and are believed to play a role in behavioral interactions through the release of pheromones (9). In humans, the PG is located in the corona glandis and prepuce and secretes smegma preputii. Many hormones, including sex steroids and pituitary and androgenic hormones, control the holocrine product of the gland (10–12). We have found that the PG of the mouse expresses the three SCD gene isoforms (SCD1, SCD2, and SCD3), but mice with a targeted disruption in the SCD1 isoform (SCD1$^{-/-}$) are deficient in the expression of SCD3 isoform as well, while

Manuscript received 15 July 2002.

Published, JLR Papers in Press, September 16, 2002.
DOI 10.1194/jlr.M200271-JLR200

Abbreviations: FAS, fatty acid synthase; PG, preputial gland; SCD, stearoyl-CoA desaturase; SREBP, sterol regulatory element binding protein.

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the expression of SCD2 isoform was not altered. The levels of 16:1n-7 were reduced by greater than 70%, while the C16:1n-10 isomer was increased by greater than 2-fold and incorporated mainly in the wax ester fraction of the PG of the SCD1$^{-/-}$ mouse. The increase in 16:1n-10 is due to induction of a specific palmitoyl-CoA Δ6 desaturase activity in the PG of the SCD1$^{-/-}$ mouse. When testosterone was administered to the SCD1$^{-/-}$ mouse, the SCD3 mRNA expression was derepressed, resulting in an increase of the Δ9 desaturation of 16:0-CoA, but not of 18:0-CoA. Our studies demonstrate that loss of SCD1 function represses the expression of SCD3 and induces a palmitoyl-CoA Δ6 desaturase enzyme reported here for the first time to be present in mammals.

MATERIALS AND METHODS

Animals and diets

The generation of targeted SCD1$^{-/-}$ mice has been previously described (3). Pre-bred homozygous (SCD1$^{-/-}$) and wild-type (SCD1$^{+/+}$) mice on an SV129 background were used. Mice were maintained on a 12 h dark/light cycle and were fed a normal chow diet (5008 test diet; PMI Nutrition International Inc., Richmond, IN) or a high-carbohydrate diet (2). The SCD1$^{-/-}$ and SCD1$^{+/+}$ mice are housed and bred in a pathogen-free barrier facility of the Department of Biochemistry. The breeding of these animals is in accordance with the protocols approved by the animal care research committee (ACRC) of the University of Wisconsin-Madison.

Materials

All radioactive chemicals were obtained from American Radiolabeled Chemicals, Inc. (St. Louis, MO). Thin layer chromatography plates (TLC Silica Gel G60) were from Whatman (Darmstadt, Germany). The cDNA probes for FAS was obtained from Dr. H. Sul, University of California, Berkeley. The cDNA probes for SREBP-1 and SREBP-2 were provided by Dr. Ezaki O, National Institute of Health and Nutrition, Japan. The probe for PPARγ2 was from Dr. Bruce Spiegelman, Harvard University and that of C/EBP-α was from Dr. Daniel Lane, Johns Hopkins University. All other chemicals were purchased from Sigma (St Louis, MO).

Lipid analysis

Total lipids were extracted from the PG according to the method of Bligh and Dyer (13), and were separated by silca gel TLC. The plate was developed until 10 cm, in petroleum ether-diethyl ether-acetic acid (80:30:1, v/v/v) as solvent. After air drying, the plates were further developed until 18 cm, in benzene-hexane (65:35, v/v) as solvent. The lipids were visualized by cupric sulfate in 8% phosphoric acid. The lipids were scraped, methylated, and analyzed by gas-liquid chromatography as previously described (7). The 16:1n-10 fatty acid was purified from the seed of *Thunbergia alata* (Burpees), which contains more than 80% of 16:1n-10 and was used as standard (14). To confirm the identity of 16:1n-10 in PG, the fatty acid methyl esters of PG were separated by 10% $AgNO_3$ TLC in hexane-diethyl ether (9:1 v/v) and converted to pyrrolidide derivatives with 10% acetic acid in pyrrolidide (15). The pyrrolidide derivatives of fatty acid were extracted and analyzed by tandem GC-MS (15). The wax ester, triglycerides, alkyl-2,3-diacylglycerol, and phospholipids were determined by gas-liquid chromatography using heptadecanoic acid and a 1,2-dihepatadecanoyl-L-α-phosphatidylcholine as internal standards.

Isolation and analysis of RNA

Total RNA was isolated from PG using Trizol reagent. Ten micrograms of total RNA were separated by 1.0% agarose-2.2 M formaldehyde gel electrophoresis and transferred to nylon membranes. The membranes were hybridized with ^{32}P-labeled FAS, GPAT, SREBP-1, SREBP-2, SCD1, SCD2, SCD3, Δ6-desaturase (Δ6D), PPAR-γ2, and C/EBP-α cDNA probes (7, 16, 17). Reverse transcriptase PCR for SCD3 was performed using a Promega Kit as the manufacturer recommended. The SCD3 and GAPDH primers were previously described (6, 18). Real time quantitative PCR assays were performed with a Cephied Smart Cycler. Briefly, the first strand cDNA was synthesized from 2 μg of DNase-treated total RNA with oligo-dT primer using the Omniscript^{TM} reverse transcriptase (Qiagen, Valencia, CA). Each amplification mixture (20 μl) contained 50 ng cDNA, 250 nM forward and reverse primers, and 2 μl of 10× Light Cycler-DNA Master SYBR Green PCR (Roche Molecular Biochemicals). GAPDH mRNA was used as the invariant control for all studies. SCD3 forward: 5′CTTGGATAACCACCCTGGGTG3′, reverse: 5′CATGCTGGTTCTTGGAGGC3′; Δ6D forward: GGAC ATAAAGAGCCTGCATG3′, reverse: 5′ACTGGAAGTACATAGG GATG3′; GAPDH forward: 5′TCCCGTTGATGACAAGCTTC3′, reverse: 5′ATGGTGAAGGTCGGTGTGAA3′.

Acyl-CoA desaturase assays

PG microsomes were isolated from wild-type and SCD1$^{-/-}$ mice by differential centrifugation. The microsome fractions were resuspened in a 0.1 M potassium phosphate buffer (pH 7.2). Specific Δ9-desaturase activity was determined from the production ^3H_2O using 30 μM [9, 10-^{3}H]stearoyl-CoA or [9,10-^{3}H] palmitoyl-CoA and 2 mM NADH as described (19). Acyl-CoA Δ6 desaturase was assayed at 23°C with 30 μM [1-^{14}C] fatty acid substrates and cofactors including 2 mM NADH, 42 mM NaF, 0.33 mM niacinamide, 1.57 mM ATP, and 0.09 mM CoA (20). The assay was stopped with the addition of 2.5 M KOH in ethanol and then heated at 85°C for 1 h. Following acidification with formic acid, the resulting fatty acids were recovered by extractions with hexane. Fatty acids were converted to methyl ester derivatives with 5% HCl-methanol. Reaction products were then analyzed on 15% $AgNO_3$ TLC plates in toluene at −20°C (14). Radioactivity was detected by autoradiography using Packard Instant Imager.

RESULTS

Figure 1 shows quantitation of the lipids extracted from the PG of SCD1$^{+/+}$ and SCD1$^{-/-}$ mice by gas liquid chromatography (GLC). As reported previously the main neutral lipids of the mouse PG consist of wax esters, triglycerides, and alkyl-2,3-diacylglcerol (11). The triglycerides were reduced in PG of the SCD1$^{-/-}$ mice by 36% compared with the wild-type control mice. The wax esters were reduced by 17% while the levels of alkyl-2,3-diacylglycerol and phospholipids were similar between SCD1$^{+/+}$ and SCD1$^{-/-}$ mice.

Figure 2A shows the GLC analysis of the fatty acid composition of total lipid extracted from the PG of the SCD1$^{+/+}$ and SCD1$^{-/-}$ mice. This profile shows a 73% decrease in C16:1n-7 and a 28% decrease in C18:1n-9 fatty acids in the total lipid fraction of SCD1$^{-/-}$ mice. In the wax ester fraction, 16:1n-7 was reduced by 73%, while 18:1n-9 was reduced by 39% (Fig. 2B). The content of 16:1n-7 was reduced by greater than 65% in the triglyceride fraction, while the con-

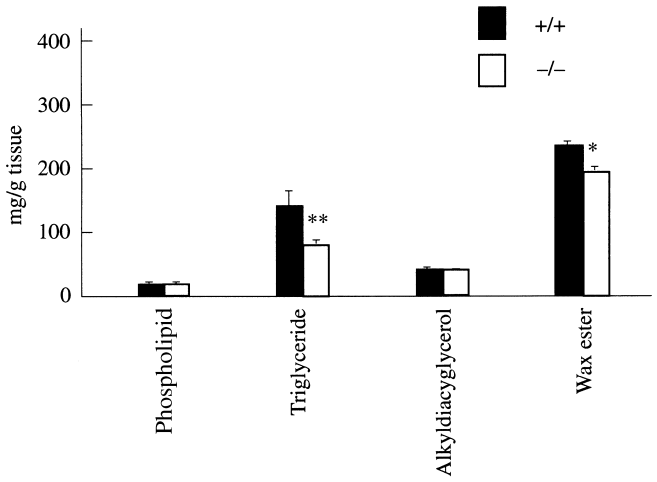

Fig. 1. Lipid levels in preputial gland (PG). Lipid was extracted from the PG of SCD1$^{+/+}$ and SCD1$^{-/-}$ mice and was further separated by thin layer chromatography and quantitated by GLC. Each value denotes the mean ± SD (n = 6). All mice were 20 weeks old. *$P <$ 0.05 and **$P <$ 0.01 between SCD1$^{+/+}$ and SCD1$^{-/-}$ mice.

tent of 18:1n-9 was reduced by 47% (Fig. 2C). There were corresponding increases in the saturated fatty acids in the total lipid as well as the triglyceride fractions, but small changes in the levels of the saturated fatty acids in the wax ester fraction were observed.

When conducting the GC analysis to obtain the fatty acid composition reported in Fig. 2, we noticed that the chromatogram had a 2-fold increase in a peak with retention time very close to that of C16:1n-7 (**Fig. 3A**). The methyl esters of 16:1n-7 and the unknown fatty acid also had same mobility on argentation TLC plates. To determine the structure of the unknown fatty acid in PG, the fatty acid methyl esters were converted to pyrrolidide derivatives and analyzed by tandem GC-MS. The molecular weights of these pyrrolidide derivatives were the same at 307. However, fragmentation patterns were different: C16:1n-7 pyrrolidine derivative has a series of ions at *m/e* 292, 278, 264, 250, 236, 222, 208, 196, 182, 168, 154, 140, 126, and 113, while the fragmentation pattern of unknown fatty acid was at 292, 278, 264, 250, 236, 222, 208, 194, 180, 166, 154, 140, 126, and 113 (Fig. 3B). The 12 atomic mass unit derived from the double bond in C16:1n-7 was observed between C8 and C9 fragments (unpublished observations), whereas that of an unknown fatty acid was observed between C5 and C6 fragments (Fig. 3B). We confirmed that the unknown fatty acid was C16:1n-10 because the double bond occurred between carbon n and n+1. In addition, the fragmentation pattern of the unknown fatty acid was consistent with that of C16:1n-10 from the seed oil of *Thunbergia alata*, which is composed of nearly 80 weight % of 16:1n-10 fatty acid (14). The PG contained several unknown fatty acids, but the levels of these fatty acids were not different between SCD1$^{+/+}$ and SCD1$^{-/-}$ mice. Figure 3C shows the contents of the 16:1n-10 fatty acid measured in the total lipid, triglyceride, wax ester, phospholipid, and 1-alkyl-2,3-diacylglycerol fractions of the PG of SCD1$^{+/+}$ and SCD1$^{-/-}$ mice. In the total lipid as well as the wax ester fraction the 16:1n-10 fatty acid was increased by greater than 2-fold in the SCD1$^{-/-}$ mouse compared with the wild-type mouse. In the alkyl-2,3-diacylglycerol and phospholipid fractions, C16:1n-10 content was increased by less than 1.5-fold. The low levels of C16:1n-10 observed in the triglyceride fraction were similar between the wild-type and SCD1$^{-/-}$ mice.

To determine the metabolic origin of C16:1n-10 fatty acid, the microsomes from the SCD1$^{+/+}$ and SCD1$^{-/-}$ PG were incubated with [1-^{14}C]16:0-CoA and Δ6 and Δ9 desaturase cofactors. The products of the reactions were separated using a TLC system at −20°C. This TLC system has been used for the separation of 16:1n-7 from 16:1n-10 (14). **Figure 4A** shows that there was no production of C16:1n-7 by the PG (lanes 3 and 5) and liver (lane 7) of SCD1$^{-/-}$ mice. C16:1n-10 was produced in PG of both the SCD1$^{-/-}$ and SCD1$^{+/+}$ mice (lanes 2 and 4) but with increased levels in the SCD1$^{-/-}$ mice (lanes 3 and 5). The greater than 50% increase in the content of 16:1n-10 is consistent with an increase in the activity of the palmitoyl-CoA Δ6 desaturase activity. Figure 4B shows that the rate of conversion of [^{14}C]16:0-CoA to [^{14}C]16:1n-10 was 2.6-fold higher in the microsome from PG of SCD1$^{-/-}$ mice than SCD1$^{+/+}$ mice. Palmitoyl-CoA Δ9 desaturase or SCD activity was high in both the liver and PG of the SCD1$^{+/+}$

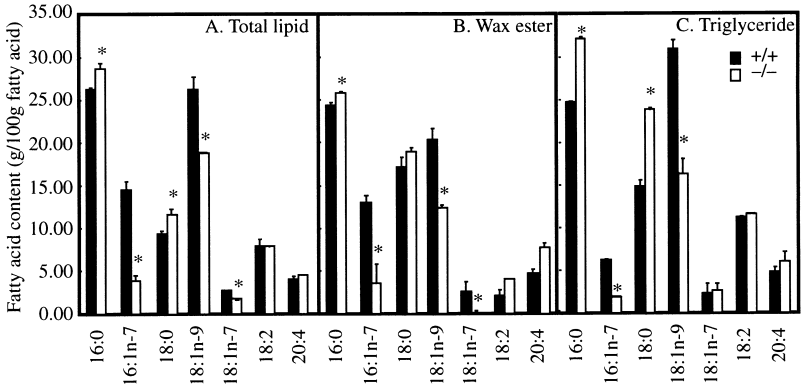

Fig. 2. Fatty acid composition of the lipid fractions of the PG. Lipid was extracted from the PG of SCD1$^{+/+}$ and SCD1$^{-/-}$ mice and was methyl-esterified, and quantitated by GLC. Data represent mean ± SD (n = 6).

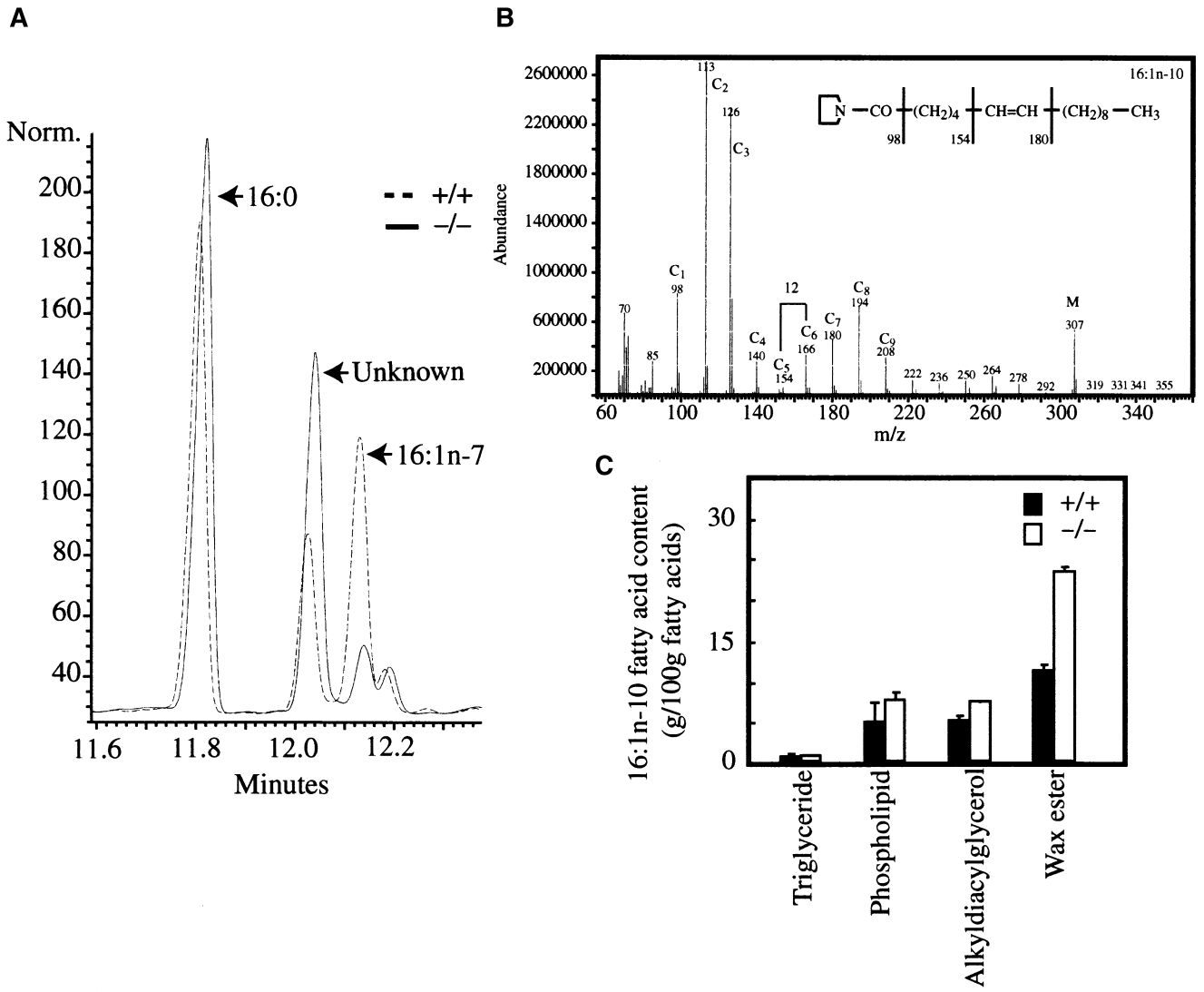

Fig. 3. A: Chromatogram of C16 fatty acids from SCD1$^{+/+}$ and SCD1$^{-/-}$ mice. Lipid was extracted from the PG of SCD1$^{+/+}$ and SCD1$^{-/-}$ mice, and the C16 fatty acids were further separated by argentation. TLC were separated by 15% $AgNO_3$-TLC in toluene at −20°C, methyl esterified, and analyzed by GLC. B: Mass spectrum of pyrrolidide derivative of 16:1n-10. The 16:1n-10 methyl esters were converted to pyrrolidide derivative prior to mass spectral analysis as described under Materials and Methods. C: The content of 16:1n-10 in the PG of SCD1$^{+/+}$ and SCD1$^{-/-}$ mice. Lipid was extracted from the PG of SCD1$^{+/+}$ and SCD1$^{-/-}$ mice and was further separated by TLC, methyl esterified, and quantitated by GLC. Data represent mean ± SD (n = 6).

mice but was not detected in the PG of the SCD1$^{-/-}$ mice. The microsomes from liver of both SCD1$^{+/+}$ and SCD1$^{-/-}$ had very low 16:0-CoA Δ6 desaturase activity.

To confirm that the increase in C16:1n-10 in the PG was due to an increase in a specific palmitoyl-CoA Δ6 desaturase activity, assays were conducted using 18:0-CoA and 18:2-CoA as substrates of Δ9 desaturase and Δ6D or fatty acid desaturase 2 (FADS2) (16), respectively. **Figure 5A** shows that the palmitoyl-CoA Δ6 activity was higher using 16:0-CoA as a substrate than with C18:0-CoA. The FADS2 activity as measured by the rate of conversion of [1-^{14}C]linoleic acid to labeled C18:3n-6 (GLA) was almost equal in the PG microsomes of both SCD1$^{+/+}$ and SCD1$^{-/-}$ mice. Further, the mRNA level of the FADS2 was not altered between SCD1$^{+/+}$ and SCD1$^{-/-}$ mice as shown by Northern blot analysis (Fig. 5B) and real-time PCR (Fig. 5C). Assays were also conducted in the liver microsomes of mice that had been fed with a high-carbohydrate diet known to induce Δ9 desaturase (2) and FADS2 (16). Figure 5D shows that the FADS2 activity was induced by high-carbohydrate feeding in the liver of both SCD1$^{+/+}$ and SCD1$^{-/-}$ mice, but no induction was observed when palmitoyl-CoA was used as substrate. These results indicate that the PG has a specific palmitoyl-CoA Δ6 desaturase activity that converts C16:0 to 16:1n-10 and is distinct from Δ6D or FADS2.

Figure 6A shows a Northern blot of total RNA isolated from the PG of wild-type and SCD1$^{-/-}$ mice and analyzed for the expression of SCD isoforms (SCD1, SCD2, and SCD3), FAS, SREBP-1, SREBP2, PPAR-γ, and C/EBP-α mRNAs. We found that the PG of the wild-type mice expressed all of the three SCD isoform mRNAs. As expected, the SCD1 was not expressed in the SCD1$^{-/-}$ mice. However, while the SCD2 isoform was expressed in the SCD1$^{-/-}$ mice,

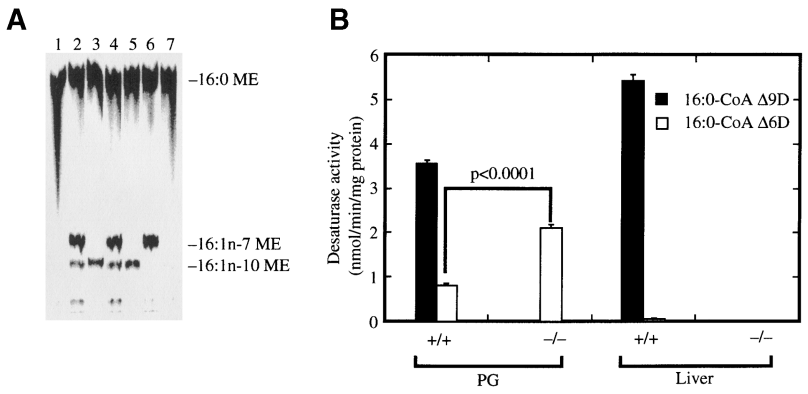

Fig. 4. 16:0-CoA Δ6 and Δ9 desaturase activities in PG. A: Auto radiograms of products of [1-^{14}C]16:0-CoA desaturase assays. Assays were conducted with microsomes (100 μg) from PG and liver. The products were saponified and methyl-esterified, and the fatty acid methyl esters were separated by 15% $AgNO_3$ TLC. Lane 1, no microsome; lanes 2 and 4, PG microsome from SCD1$^{+/+}$ mice; lanes 3 and 5, PG microsome from SCD1$^{-/-}$ mice, lane 6, liver microsome from SCD1$^{+/+}$ mice; lane 7, liver microsome from SCD1$^{-/-}$ mice. B: Δ6 and Δ9 desaturase enzyme activities. Data represent mean ± SD (n = 3).

the expression of SCD3 isoform mRNA was undetectable in the SCD1$^{-/-}$ mice (Fig. 6B) even when the more sensitive and quantitative RT-PCR at 40 cycles was used for detection (Fig. 6C). The mRNA levels of FAS, SREBP-1, SREBP-2, PPARγ2, and C/EBPα were similar between SCD1$^{+/+}$ and SCD1$^{-/-}$ mice. The 18S and 28S mRNAs used as a loading control were also similar between SCD1$^{+/+}$ and SCD1$^{-/-}$ mice. These results suggest that deletion of SCD1 gene results in a specific loss of SCD3 expression in the PG of the SCD1$^{-/-}$ mice.

Because androgens such as testosterone have been known to induce sebocyte differentiation and lipid synthesis in PG (11, 21), we examined whether testosterone affects the expression of SCD isoforms in PG. **Figure 7A** shows a Northern blot of total RNA from PG of wild-type and SCD1$^{-/-}$ mice with or without testosterone administration. Testosterone administration to the wild-type mice increased SCD1 and SCD3 mRNAs. SCD2 was not increased. In the SCD1$^{-/-}$ mice, testosterone induced SCD3 mRNA, suggesting that although normal SCD3 expression is undetectable in the PG of the SCD1$^{-/-}$ mice, the SCD3 gene is still functional, and its expression can be induced with the appropriate stimulus. The SCD2 was only slightly increased in the PG of the SCD1$^{-/-}$ mice. Figure 7B shows

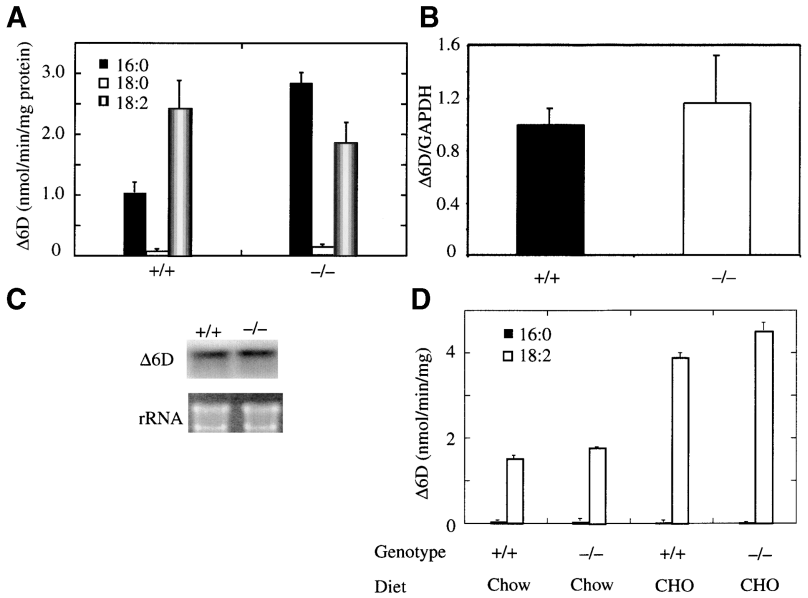

Fig. 5. A: The substrate specificity for Δ6D. Microsomal protein (100 μg) was incubated with a reaction mixture containing [^{14}C]fatty acid and essential cofactors as described under Materials and Methods. Data represent mean ± SD (n = 3). B: Real time PCR analysis for Δ6D. Total RNA from PG of each mouse was subjected to real time PCR using Δ6D-specific primers and the mRNA levels quantified as described under Materials and Methods. Data represent mean ± SD (n = 4). C: Northern blot and real time PCR analysis for the Δ6D. Total RNA (10 μg) pooled from the PG of four mice of each group was subjected to Northern blot analysis using a specific cDNA probe for the Δ6D. D: Palmitoyl-CoA Δ6 desaturase and FADS2 (Δ6D) in microsomes of livers of mice fed a high-carbohydrate diet (CHO); data represent mean ± SD (n = 4).

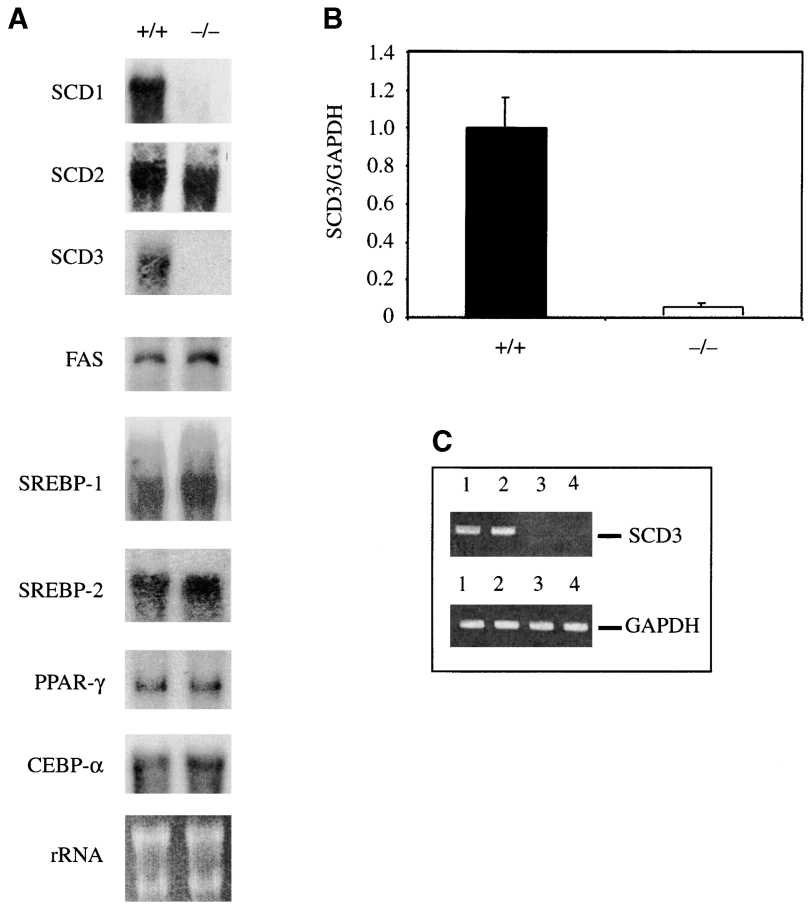

Fig. 6. A: Northern blot analysis for the expression of SCD mRNA isoforms in PG of SCD1$^{+/+}$ and SCD1$^{-/-}$ mice. Total RNA (10 μg) pooled from six mice of each group was subjected to Northern blot analysis followed by hybridization with labeled probes specific for SCD1, SCD2, SCD3, fatty acid synthase (FAS), sterol regulatory element binding protein-1 (SREBP-1), SREBP-2, PPAR-γ, and CEBP-α. B: Real time PCR for SCD3 expression. Total RNA from PG of each mouse was subjected to real time PCR, and the quantification of the mRNA levels was as described under Materials and Methods. Data represent mean ± SD (n = 4, $P < 0.0001$). C: RT-PCR for SCD3 expression at 40 cycles. Lanes 1 and 2, SCD1$^{+/+}$ PG; lanes 3 and 4, SCD1$^{-/-}$ PG.

the specific Δ9-desaturase activity in the PG as measured by the rate of production of $^3H_20$ from [9,10-^{3}H]palmitoyl-CoA and [9,10-^{3}H]stearoyl-CoA. The SCD activity on [9,10-^{3}H]stearoyl-CoA was higher in the wild-type mice and was reduced by 90% in the SCD1$^{-/-}$ mice. The SCD activity on [9,10-^{3}H]palmitoyl-CoA was very high in the SCD1$^{+/+}$ microsome but was almost undetectable in the SCD1$^{-/-}$ microsomes, consistent with the results shown in Fig. 3. Testosterone administration increased Δ9-desaturase activities with both palmitoyl-CoA and stearoyl-CoA substrates in the SCD1$^{+/+}$ mice, while in the microsomes of the SCD1$^{-/-}$ mice, the Δ9-desaturase activity was increased only with palmitoyl-CoA as a substrate. These results suggest that SCD3 prefers C16:0-CoA as a substrate of Δ9 desaturation over 18:0-CoA.

DISCUSSION

We describe for the first time the presence of a palmitoyl-CoA Δ6 desaturase activity in mammals that is involved in the desaturation of 16:0 into 16:1n-10. The results presented here also demonstrate that the palmitoyl-CoA Δ6 desaturase is induced in the PG of mice lacking a functional SCD1 isoform. The main neutral lipids of the PG are wax esters, triglycerides, and alkyl-2,3-diacylglycerol (11). In contrast to the Harderian glands and skin (2, 3, 7), the wax ester levels of the PG of the SCD1$^{-/-}$ mice were not dramatically decreased despite a greater than 70% and 25% decrease in the levels of 16:1n-7 and 18:1n-9, respectively. However, as shown in Fig. 3, the SCD1$^{-/-}$ mice increased 16:1n-10 by greater than 2-fold in the wax ester fraction. Because C16:1n-7 and C16:1n-10 are isomers with an alteration in the positioning of the double bond, it is possible that the increase in C16:1n-10 in the SCD1$^{-/-}$ mice is to compensate for C16:1n-7. This would allow the PG to maintain the physical properties of the wax esters and other fluids for proper secretion out of the glands.

The mouse genome contains three well-characterized structural genes (SCD1, SCD2, and SCD3) that are highly homologous at the nucleotide and amino acid level and encode the same functional protein (4–6). We found that

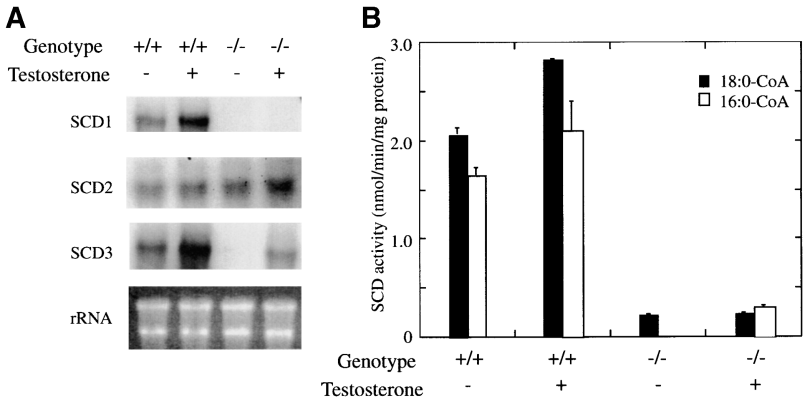

Fig. 7. Effect of testosterone on the expression and activities of SCD isoforms. A: Northern blot analysis for the expression of SCD isoforms. Testosterone propionate (10 μg/g mouse) was injected subcutaneously for 2 weeks. Total RNA (10 μg) pooled from three mice of each group was subjected to Northern analysis by hybridization with labeled probes for SCD1, SCD2, and SCD 3. B: Effect of testosterone on the SCD-specific activity. Microsomes (100 μg protein) from PG of each group were incubated in a reaction mixture containing either [^{3}H]stearoyl-CoA or [^{3}H]palmitoyl-CoA as substrates. Data represent mean ± SD (n = 3).

the mouse PG, like the mouse Harderian gland and skin (7), expresses three SCD isoforms. In addition we noted that there were differences in the levels of their expression between the SCD1$^{+/+}$ and SCD1$^{-/-}$ mice (Figs. 6 and 7). While the expression of SCD2 and that of several other lipogenic genes was similar in the SCD1$^{+/+}$ and SCD1$^{-/-}$ mice, the expression of SCD3 was eliminated in the SCD1$^{-/-}$ mice (Fig. 6). However, the induction of SCD3 gene expression by testosterone (Fig. 6) indicated that the SCD3 gene in the SCD1$^{-/-}$ mice is still functional and can be derepressed. In the skin of asebia mouse lacking SCD1, SCD3 expression was decreased, while the expression of the SCD2 was not altered (6). SCD1 is located in presebocytes of the sebaceous gland of the skin whereas the mature sebocytes express SCD3. On the other hand, SCD2 is expressed in hair follicles (6, 22). The expression pattern of the SCD1 and SCD3 in skin seems to be a reflection of sebocytes in different stages of differentiation. Because the skin and PG have a similar structure and both contain sebocytes, it is possible that the pattern of expression of the SCD1 and SCD3 isoforms in the PG is similar to that in the skin. In addition, the loss of expression of SCD3 in the SCD3 mouse suggests that the normal expression of SCD3 is dependent on SCD1 expression. However, the mechanism of how SCD1 deletion leads to repression of SCD3 gene is presently unknown.

We previously suggested that the SCD isoforms exhibit different substrate specificities in addition to exhibiting tissue-specific expression (6, 7, 23). The microsomes isolated from PG of SCD1$^{-/-}$ mice had very low desaturase activity toward

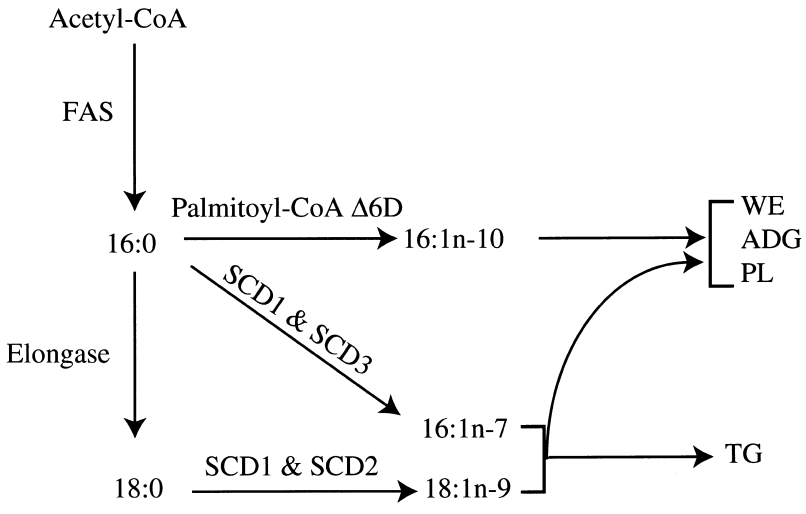

Fig. 8. Proposed scheme for the synthesis of monounsaturated fatty acid in the PG. SCD1 synthesizes 16:1n-7 and 18:1n-9, while SCD2 synthesizes mainly 18:1n-9. SCD3 synthesizes mainly 16:1n-7. C16:1n-7 and C18:1n-9 are then used in the synthesis of wax esters (WE), triglycerides (TG), alkyl-2,3-diacylglycerol (ADG), and phospholipids (PL). The PG is proposed to have a palmitoyl-CoA Δ6-desaturase that catalyzes the synthesis of 16:1n-10. C16:1n-10 is incorporated mainly in WE, ADG, and PL.

C16:0-CoA compared with C18:0-CoA (Fig. 5). The levels of C16:1n-7 derived from desaturation of C16:0-CoA were decreased by 70% in the SCD1$^{-/-}$ mouse compared with a decrease of 28% in the levels of C18:1n-9 that would be derived from the desaturation of C18:0-CoA. We found that younger SCD1$^{-/-}$ mice express SCD3 (unpublished observations), and it is possible that the 30% 16:1n-7 we detect in the PG is due to the developmental expression of the SCD3 in the SCD1$^{-/-}$ mice. Treatment of the mice with testosterone induced SCD3 gene expression (Fig. 7A) in the PG and led to an increase in the desaturation of 16:0-CoA (Fig. 7B). These studies along with a recent report of a palmitoyl-CoA-specific Δ9 desaturase from *Caenorhabditis elegans* (24) strongly suggest that C16:0-CoA is the main substrate of SCD3 isoform. However, confirmation of the substrate specificities will require the assays of recombinant desaturase enzymes in the presence of specific acyl-CoA substrates.

In plants, a specific 16:0-acyl carrier protein (ACP) Δ6 desaturase was identified and characterized (14) and was found to have extensive homology with the 18:0-ACP Δ9 and 16:0-ACP Δ9 desaturases (14). Our results now indicate that mammals also have a palmitoyl-CoA Δ6 desaturase that inserts a double bond between positions 5 and 6 in C16:0 to synthesize C16:1n-10. The palmitoyl-CoA Δ6 desaturase may be specific to the PG because we could not detect C16:1n-10 in any other tissue so far examined. In addition, the palmitoyl-CoA Δ-6 desaturase in the PG exhibited higher substrate specificity for C16:0-CoA, whereas the FADS2 could not utilize C16:0-CoA either in the PG (Fig. 4B) or liver (Fig. 5D). It is not presently known whether this palmitoyl-CoA Δ6 desaturase we have described is an isoform of FADS cluster consisting of FADS1, FADS2, and FADS3 (16) or whether it is related to any of the Δ9 SCD isoforms. The differences in the catalytic selectivity of the Δ9 SCD isoforms and the FADS family, in addition to the existence of structurally related acyl-CoA desaturases with different substrate recognition and double bond-positioning properties, would be to further refine changes in the fatty acid composition of various lipids.

We propose as depicted in **Fig. 8** that in the PG, palmitate is synthesized de novo by the FAS complex from acetyl-CoA. Palmitate then serves as a substrate for the microsomal malonyl-CoA-dependent elongase to produce stearate, which then serves as the main substrate of SCD1 and SCD2 to produce C18:1n-9. The C16:1n-7 would then be synthesized mainly by the SCD3 isoform and further incorporated into lipid fractions. An active palmitoyl-CoA Δ6 desaturase is already present in the PG and is responsible for the synthesis of the normal levels of C16:1n-10 from palmitate. Because SCD3 expression is repressed in the SCD1$^{-/-}$ mice, the levels of C16:1n-7 are reduced and C16:0 builds up. The PG cells then respond by inducing or increasing the activity of the palmitoyl-CoA Δ6 desaturase to convert more of the accumulating C16:0 into C16:1n-10. The mechanism of induction of the palmitoyl-CoA Δ6 desaturase activity is currently unknown. The C16:1n-10 isomer then compensates for the decreased levels of 16:1n-7 for esterification to fatty alcohols and the 2,3 positions of glycerol by enzymes of synthesis of wax esters, ADG, and phospholipids.

In conclusion, these studies have revealed that SCD1 gene expression is required for the synthesis of triglycerides in the PG. The loss of SCD1 function results in the loss of expression of SCD3 isoform and an induction of a palmitoyl-CoA Δ6 desaturase. The SCD1 knockout mouse may be a useful model to study the role of the various desaturases in lipid metabolism.

The authors thank Yeonhwa Park for useful discussions. This work was supported in part from a grant from the American Heart Association and in part from Xenon Genetics, Inc. (Vancouver, Canada) to J.M.N. F.E.G. received partial support from the National Council of Science and Technology (CONACYT-Mexico).

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