Oleoyl-CoA Is the Major de Novo Product of Stearyl-CoA Desaturase 1 Gene Isoform and Substrate for the Biosynthesis of the Harderian Gland 1-Alkyl-2,3-diacylglycerol*

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1-Alkyl-2,3-diacylglycerol (ADG) is a unique neutral lipid found in the eyelid-associated Harderian gland (HG) of the mouse and acts as a lubricant to facilitate eyelid movement. We found that the HG of the mice with a disruption in the gene for stearyl-CoA desaturase 1 (SCD1) (SCD1−/−) is deficient in ADG. The amount of C20:1n-9, which is a major fatty acid of ADG, was reduced by greater than 90% despite normal elongase enzyme activity proposed to elongate it from C18:1n-9. HG from SCD1−/− mice exhibited high desaturase activity toward C16:0-CoA as substrate but had very low desaturase activity toward C18:0-CoA. Feeding diets containing high levels of oleate to the SCD1−/− mice did not increase the levels of C18:1n-9 or C20:1n-9 in the HG and failed to restore the ADG to the levels found in the HG of the wild-type mouse. De novo ADG synthesis as measured by the incorporation of [3H]glycerol and [14C]glucose was high in the SCD1+/+ mouse but was reduced by greater than 90% in the HG of SCD1−/− mouse. The deficiencies in the levels of ADG and C20:1n-9 were not compensated for by the expression of SCD2 and SCD3 isoforms in the HG of the SCD1−/− mouse. These observations demonstrate that SCD1-synthesized oleoyl-CoA is a major substrate required for the biosynthesis of normal levels of ADG and that the SCD isoforms present in the HG have different substrate specificity.

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 stearyl-CoA, which are converted to palmitoleoyl-CoA and oleoyl-CoA, respectively (1). The resulting monounsaturated fatty acids are substrates for incorporation into membrane phospholipids, triglycerides, and cholesterol esters (2). Several isoforms of SCD exist in the mouse genome. SCD1, SCD2, and SCD3, which are products of different genes, are the most well characterized (3–5). Most organs of different mouse strains express both SCD1 and -2 with the exception of liver, which expresses mainly the SCD1 isoform (3). SCD2 is constitutively expressed in the brain (3) 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 (6). 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. However, in some tissues such as the adipose and eyelid both SCD1 and SCD2 genes are expressed whereas in the skin all the three SCD gene isoforms are expressed (5). The reason for having two or more SCD isoforms in the same tissue is not known but could be related to the substrate specificity of the isoforms and their regulation through tissue-specific expression.

The existence of multiple SCD genes in mice and rat tissues makes it difficult to determine the role of each gene in lipid metabolism. Most previous studies have assessed SCD gene function by measuring mRNA expression but have not differentiated which SCD isoform is responsible for the altered total SCD activity. The clue as to what the physiological role of the SCD1 gene and its endogenous products (the monounsaturated fatty acids) is has come from recent studies of the asebia mutant mouse strains (ab1 and ab2) that have a natural mutation in the SCD1 gene (5, 8–9). Most recently we have generated a mouse model with a targeted disruption of the SCD1 gene (7) and have shown that it has phenotypes similar but not identical to those present in the natural models. The 5′-boundary of the natural deletion in the SCD1 gene of the asebia mutant mouse has not been mapped within a 10-kilobase promoter region suggesting that this deletion could extend into other genes. Consequently, it has not yet been possible to generate appropriate DNA primers to distinguish the heterozygous asebia mice from the wild-type mice by genotyping. The basis for the difference in some phenotype between the laboratory and the natural mutant mouse strains could also be because of strain background modifying gene effects.

The Harderian gland (HG) that was first described by Johann J. Harder in 1694 (10) occurs in most terrestrial vertebrates and is located within the orbit of the eye where in most species it is the largest structure. The chief products of the gland vary between different groups of vertebrates. In rodents the gland synthesizes lipids, porphyrins, and indoles (11). The lipids are excreted by an exocytotic mechanism (12). The major secretory lipids of the HG of the mouse has been identified as 1-alkyl-2,3-diacylglycerol (ADG) whereas in the rat the chief products are wax esters (11). The ADG acts as a lubricant of the eyeball and is therefore important in facilitating the movement of the eyelid (13). In addition it has been proposed that the HG can act as a site of immune response, a source of pheromones, a source of thermoregulatory lipids, a photoprotective organ, a part of a retinal-pineal axis, a site of osmoregulation, and a
source of growth factors (11, 12). The HG is also a target of hormonal agents including gonadal, thyroid, and pituitary hormones, in addition to the many neuropeptides that have been shown to regulate the activity of this gland (12).

In this study, we found that the HG of the mouse with a targeted disruption of the SCD1 gene isoform has decreased levels of ADG, the major lipid of this gland. The amount of C20:1n-9, which is the major fatty acid in the ADG, was reduced by greater than 90% despite the presence in both SCD1/−/− and SCD1+/+ mice of normal elongase activity, the enzyme proposed to elongate C18:1n-9 to C20:1n-9. HG microsomes isolated from SCD1/−/− mice had very low desaturase activity toward C18:0-CoA as a substrate compared with C16:0-CoA. Feeding diets containing high levels of oleate to the SCD+/− mice did not increase the levels of C18:1 or C20:1n-9 in the HG and failed to restore the levels of ADG to the levels found in the HG of the wild-type mouse. De novo ADG synthesis as measured by the incorporation of [3H]glycerol and [14C]glucose was reduced by greater than 80% in the HG of SCD1/−/− mice. Taken together, the observations demonstrate that SCD1 isoform has a preference of C18:0-CoA as a substrate that SCD1-synthesized oleoyl-CoA is the major substrate required for the biosynthesis of very long chain monounsaturated fatty acids of HG 1-alkyl-2,3-diacylglycerol.

EXPERIMENTAL PROCEDURES

Animals and Diets—The generation of targeted SCD1/−/− mice has been described previously (7). Pre-bred homozygous (SCD1/−/−) and wild-type (SCD1+/+) male mice on an SV129 background were used. Mice were maintained on a 12-h dark/light cycle and were fed a normal nonpurified diet (5008 test diet; PMI Nutrition International, Richmond, IN) or a high carbohydrate diet (TD99252; Harlan Teklad, Madison, WI) supplemented with 5% soybean oil (control oil), 5% or 30% high oleate oil. In some experiments the high carbohydrate diet was supplemented with 5% C20:1n-9. The fatty acid composition of soybean oil is 11% palmitic acid (16:0), 5% stearic acid (18:0), 23% oleic acid (18:1n-9), 53% linoleic acid (18:2n-6), and 8% linoleic acid (18:3n-3). The fatty acid composition of high oleate diet is 2% 16:0, 12% 18:1n-9, 26% 18:2n-6, 6% 18:3n-3, and 50% eicosenoic acid (20:1n-9). SCD1/−/− and SCD1+/+ mice are housed and bred in a pathogen-free barrier facility of the Department of Biochemistry, University of Wisconsin, Madison. The breeding of these animals is in accordance with the protocols approved by the Animal Care Research Committee of the University of Wisconsin, Madison.

Materials—Radioactive [32P]dCTP (3000 Ci/mmol) was obtained from Dupont. Thin layer chromatography plates (TLC: Silica Gel G60) were from Merck. [1,2,3-3H]Glycerol and [14C]Glucose were obtained from American Radiolabeled Chemicals Inc. (St. Louis, MO). The cDNA probes for FAS were obtained from Dr. H. Sui, University of California, Berkeley. The cDNA probes for SREBP-1 and SREBP-2 were provided by Dr. O. Ezaki, National Institute of Health and Nutrition, Japan. Rabbit anti-rat SCD, which was raised against purified rat liver SCD, was a gift from Dr. Juris Ozols, University of Connecticut Health Center, Farmington, CT. All other chemicals were purchased from Sigma.

Lipid Analysis—Total lipids were extracted from HG according to the method of Bligh and Dyer (14) and separated by silica gel TLC using petroleum ether:dichloroethane:acetic acid (80:30:1) as the developing solvent. The lipids were visualized by cupric sulfate in 8% phosphoric acid. The lipids were scraped, methylated, and analyzed by gas-liquid chromatography on a capillary column coated with DB-225 (30-m length, 0.25 mm internal diameter, 0.25 μm; Agilent Technologies, Inc., Wilmington, DE). Column temperature was kept at 70 °C for 1 min, increased to 180 °C at a rate of 20 °C/min and then to 220 °C at a rate of 3 °C/min. The temperature was kept at 220 °C for 15 min. 20:1n-9 and 20:1n-7 fatty acids were identified by comparison of retention times with authentic standards (Sigma). A heptadecanoic acid and a 1,2-diheptadecanoyl-sn-glycerol standard were added as internal standards for the quantitation of 1-alkyl-2,3-diacylglycerol and phospholipids, respectively.

Isolation and Analysis of RNA—Total RNA was isolated from each tissue using the acid guanidine-phenol-chloroform extraction method (8). 20 μg of total RNA were separated by 1.0% agarose/2.2 M formaldehyde gel electrophoresis and transferred onto nylon membrane. The membrane was hybridized with 32P-labeled FAS (15), SREBP-1 (16), SREBP-2 (12), SCD2 (3), and SCD1 (4) cDNA probes. Northern blot analysis was performed by reverse transcription polymerase chain reaction using mouse skin RNA and specific primers (forward, 5′-CTTGAGATAACCCATCGGTG-3′; reverse, 5′-CATGCTGTCTTGAGGGC-3′) (5).

Enzyme Assays—Microsomes were isolated from HGs of SCD1+/+ and SCD1/−/− mice by differential centrifugation and suspended in a 0.1 M potassium phosphate buffer (pH 7.2). Seoaroyl-CoA desaturase was assayed at 23 °C with 30 μM [14C]oleoyl-CoA or [14C]palmitoyl-CoA and 2 mM NADH (7). Microsomal elongase activity was assayed at 37 °C with 1 mM dithiothreitol, 2 mM NADPH, 2 mM NADH, 30 μM acyl-CoA, and 100 μM [3-14C]malonyl-CoA (17).

Immunoblotting—Total protein was prepared from pooled HG from three mice of each group. The HG (100 μg) was rinsed with phosphate-buffered saline and homogenized in 2 ml of 50 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, 0.25% sodium deoxycholate, 0.1% SDS, 1 mM Na3VO4, 10 mM Na2MoO4, 40 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 2 μM aprotinin, and 1 μM leupeptin. The homogenate was centrifuged at 10,000 × g for 10 min at 4 °C. The same amount of protein (25 μg) from each fraction was subjected to 10% SDS-polyacrylamide gel electrophoresis and transferred to Immobilon-P transfer membranes at 4 °C. After blocking with 10% non-fat milk in Triss-buffered saline buffer (pH 8.0) plus Tween at 4 °C for 2 h, the membrane was washed and incubated with rabbit anti-rat SCD as primary antibody and goat anti-rabbit IgG-horseadish peroxidase conjugate as the secondary antibody. Visualization of the SCD protein was performed with an enhanced chemiluminescence Western blot detection kit (18).

In Vivo Assay for 1-Alkyl-2,3-diacylglycerol Synthesis—[3H]Glycerol or [14C]Glucose was dissolved in 0.9% NaCl at a concentration of 5 μCi/0.2 ml and injected into mice 1 h before being sacrificed (19–21). HG lipids were extracted as described by Bligh and Dyer (14) and separated by TLC using hexane:ether:acetic acid (90:30:1) as developing solvent. The 1-alkyl-2,3-diacylglycerol fraction was scraped off the plate, and the radioactivity was measured using a liquid scintillation counter.

RESULTS

Fig. 1 shows a Northern blot of total RNA isolated from various tissues of wild-type mice and analyzed for the expression of SCD1, SREBP-1, and FAS mRNAs. Compared with several mouse lipogenic tissues including liver and white adipose tissue, the HG expresses much higher mRNA levels of SCD1, SREBP-1, and FAS indicating that the HG is highly lipogenic. 28 S mRNA expression used as loading control was similar in the tissues tested.

Fig. 2 shows TLC analysis of lipids extracted from HG of...
Total lipids were extracted from livers of SCD1+/+ and SCD1−/− mice, respectively. Cholesteryl ester, and triglycerides, re-
novated as standards for ADG, palmitoylglycerol, cholesteryl oleate, and triolein were used as standards for ADG, cholesteryl ester, and triglycerides, respectively. Equivalent amounts of lipid extract (from 0.1 mg of tissue homogenate) were loaded in each lane. 1-Octadecyl-2,3-di-palmitoylglycerol, cholesteryl oleate, and triolein were used as standards for ADG, cholesteryl ester, and triglycerides, respectively. TLC. Equivalent amounts of lipid extract extracts were pooled and analyzed by TLC. Each value denotes the mean ± S.D. (n = 6). All mice were 8 weeks old. Bold values denote a statistical significance of p < 0.001 between wild-type and SCD1−/− mice. ADG, 1-alkyl-2,3-diacylglycerol. PL, phospholipid.

| Genotype         | +/+      | −/−      |
|------------------|----------|----------|
| Harderian gland  |          |          |
| Total fatty acids (mg/g tissue) | 423.7 ± 26.3 | 236.1 ± 11.6 |
| ADG (mg/g tissue) | 280.6 ± 27.57 | 132.7 ± 8.9  |
| PL (mg/g tissue)  | 109.7 ± 5.5 | 82.8 ± 9.3 |

SCD1+/+ and SCD1−/− mice. As reported previously (22, 23), the main lipid present in the HG of the mouse is ADG. The ADG was markedly reduced in HG of the SCD1−/− mice compared with the SCD1+/+ control mice. The free cholesterol levels were not changed. Triglyceride levels were very low, and cholesterol esters were undetectable. ADG and phospholipids were also measured quantitatively by GLC using heptadecanoic acid as an internal standard. Table I shows that the total ADG content in HG of SCD1−/− mice was decreased by 53% whereas the phospholipid content was decreased by 24%.

Fig. 3 shows the contents (mg/g) of the major fatty acids measured in the total lipid, 1-alkyl-2,3-diacylglycerol, and phospholipid fractions of the HG of SCD1+/+ and SCD1−/− mice. The most abundant monounsaturated fatty acid in the total lipid of the SCD1+/+ mice is eicosenate (C20:1n-9) (148 mg/g), which was decreased by greater than 90% in the SCD1−/− mice. The content of C20:1n-7 was decreased by 34% whereas that of C18:1n-9 was decreased by 60%. In the ADG fraction, C20:1n-9 and C20:1n-7 were decreased by 93 and 44%, respectively. The phospholipid fraction did not contain C20:1n-9 or C20:1n-7, but the content of C18:1n-9 was decreased by 57%. The HG lipids contained very low levels of w-6 and w-3 polyunsaturated fatty acids.

To determine whether dietary oleate could substitute for the endogenously synthesized oleate and restore the HG ADG levels of the SCD1−/− mice, we supplemented the semipurified diet with high levels of C18:1n-9 (75% of total fat) as high oleate oil and fed it to the SCD1−/− mice for 1 month, a long enough feeding regimen to ensure equilibration of lipid pools. The food intake was not very different between the SCD1+/+ and SCD1−/− mice controls and the SCD1−/− mice. Total HG extracts were prepared, the lipid fractions were analyzed by TLC, and the fatty acid composition was analyzed by GLC. Feeding diets supplemented with 5 or 30% oleate-rich diet to the SCD1−/− mice did not result in an increase in the levels of ADG (Fig. 4A). The content in (mg/g tissue) of ADG was not increased (Fig. 4B). GC analysis showed that content s of C18:1n-9 and 20:1n-9 were not increased in the ADG fraction of the SCD1−/− mouse (Fig. 4C). The content

### Table I

| Genotype         | +/+      | −/−      |
|------------------|----------|----------|
| Harderian gland  |          |          |
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**FIG. 2.** TLC of lipid extracts from HG of SCD1+/+ and SCD1−/− mice.

**FIG. 3.** The contents of fatty acids in the total lipid, ADG, and phospholipid (PL) fractions of HG of SCD1+/+ and SCD1−/− mice. Lipid extracts were pooled and separated by TLC, methyl-esterified, and quantitated by GLC as described under “Experimental Procedures.” Data represent mean ± S.D. (n = 6). *, p < 0.05 versus SCD1+/+ mice (Student’s t test).
of C20:1n-9 was not increased in the total lipid or ADG fraction when SCD1−/− mice were fed diets supplemented with high levels of C20:1n-9 as trieicosanoin (data not shown). These observations suggest strongly that the normal levels of C20:1n-9 in 1-alkyl-2,3-diacylglycerol are largely dependent on endogenously synthesized C18:1n-9.

The low levels of ADG observed in the SCD1−/− mice could have resulted from reduced levels of the elongase activity, the enzyme that would catalyze the elongation of C18:1n-9 to C20:1n-9. Fig. 5A shows that the elongase activity as measured by rate of conversion of [14C]malonyl-CoA to labeled C20:1n-9 was almost equal in HG microsomes of both SCD1+/+ and SCD1−/− mice. Compared with elongase activities in liver and white adipose tissue, the HG elongase activity was highest when either palmitoleoyl-CoA or oleoyl-CoA were used as substrates (Fig. 5B). These observations demonstrate the requirement of palmitoleoyl-CoA and oleoyl-CoA as substrates for elongation into very long monounsaturated fatty acids that subsequently become incorporated into ADG and the HG.

To establish that the low levels of ADG in the SCD1−/− mice is because of lower de novo synthesis rates, we used [3H]glycerol or [14C]glucose as precursors of lipid synthesis to measure directly newly synthesized ADG in the HG of SCD1+/+ and SCD1−/− mice. Fig. 6 shows that the ADG synthetic rate both from glucose and glycerol was decreased by greater than 80% in the HG of the SCD1−/− mice. These results indicate that the normal levels of ADG in the HG are dependent on SCD1 gene expression.
_activity was determined by liquid scintillation counting. *, extraction of the lipids, ADG was separated using TLC, and the radioactivity was determined by liquid scintillation counting. 

To determine whether the HG of the SC1D–/– mouse expresses other SCD isoforms in addition to SCD1, total RNA isolated from SC1D+/+ and SC1D–/– mice was analyzed by Northern blot using as specific DNA probes SCD2 and SCD3 isoforms. Fig. 7A shows that the HG of SC1D–/– mice as expected did not express SC1D but that SCD2 and SCD3 isoforms are expressed in both SC1D+/+ and SC1D–/– mice. Interestingly, SCD2 mRNA level in SC1D+/+ was lower than that in the SC1D–/– mouse whereas that of SC3 was higher in the SC1D+/+ than in the SC1D–/– mouse. FAS, SREBP-1, and SREBP-2 mRNA levels were similar between SC1D+/+ and SC1D–/– mice. The 28S mRNA expression used as a loading control was also similar in the SC1D+/+ and SC1D–/– mice. Consistent with the Northern blot results of Fig. 7A, Western blot analysis showed high immunoreactive SCD protein in HG from the SC1D+/+ with a decreased protein level in the SC1D–/– mice (Fig. 7B). To determine whether the SCD isoforms have different substrate specificity, we assayed microsomes from HG of SC1D+/+ and SC1D–/– mice for desaturase activity toward the main substrates of SCD, C16:0-CoA, and C18:0-CoA. Fig. 7C shows that SCD enzyme activity in the HG as measured by the rate of conversion of [1-14C]palmitoyl-CoA to [1-14C]palmitoleoyl-CoA was higher in the wild-type mice and was reduced by 35% in the SC1D–/– mouse. However, the SCD activity as measured by the rate of conversion of [1-14C]stearyloyl-CoA to [1-14C]oleoyl-CoA was lower in the wild-type mice and was reduced by 50% in the SC1D–/– mouse. These results indicate that SC1D can use C16:0-CoA (the 35%) as a substrate but uses C18:0-CoA (the 50%) as the preferred substrate for desaturation. On the other hand SCD2 or SCD3 isoform uses C16:0-CoA as a specific substrate of desaturation. These results suggest that SCD isoforms present in the HG have different substrate specificity.

**DISCUSSION**

We found that the eyeball-associated HG of the mouse has a higher expression of SC1D mRNA than in other tissues analyzed previously, including liver and white adipose tissue (Fig. 1). The expression of other lipogenic genes (FAS and SREBP-1) was also higher than the levels observed previously in liver and white adipose tissue. These observations indicated to us that the HG is a highly lipogenic gland. The major neutral lipid found in the HG of the mouse is ADG with lower levels of phospholipid and free cholesterol. As indicated in Fig. 2 and Table I, the SC1D–/– mice have a deficiency in ADG and C20:1n-9; the main monounsaturated fatty acid in ADG was reduced by greater than 90% (Fig. 3). The phospholipid levels were also decreased in the SC1D–/– mice. Feeding diets supplemented with high levels of oleate did not result in an increase in the levels of C18:1 and C20:1n-9 and could not repair the deficiency in 1-alkyl-2,3-diacylglycerol (Fig. 4). Feeding diets supplemented with high levels of C20:1n-9 itself to the SC1D–/– mice did not correct the deficiency in ADG either. Further, we demonstrated that the deficiency of ADG in the SC1D–/– mice was because of a decreased rate of de novo synthesis (Fig. 5). However, the ADG synthesis occurred at a much lower level (20%) whereas the level of ADG was reduced by 50% suggesting a possible defect in the secretion of ADG from the HG of the SC1D–/– mice. The deficiencies in the levels of ADG and C20:1n-9 were not compensated for by the expression of SCD2 and SCD3 isoforms in the HG of the SC1D–/– mouse. These observations suggest that endogenously synthesized C18:1 as a result of SCD1 gene expression is required for the biosynthesis of ADG in the HG. The endogenously synthesized oleate is also required for synthesis of normal levels of phospholipids.

The mouse genome contains three well characterized structural genes (SC1D, SCD2, and SCD3) that are highly homologous at the nucleotide and amino acid level and encode the same functional protein (5). Although the difference in physiological function between SC1D, -2, and -3 has not been well addressed, we suggested previously that the SC1D and SCD2 isoforms might exhibit different specificity for substrates (18), in addition to exhibiting tissue-specific expression (5, 7). We found that the mouse HG expresses three SCD isoforms (SC1D, SCD2, and SCD3) and, in addition, noted that there were differences in the levels of their expression between the SC1D+/+ and SC1D–/– mice (Fig. 7). Although SCD2 was expressed at lower levels in the SC1D+/+ mice its expression was elevated in the SC1D–/– mice. Expression of SCD3 was higher in the SC1D+/+ but decreased in the SC1D–/– mice. Similarly in the skin of asebia mouse lacking SC1D, SCD3 expression was decreased (6). In addition, the three SCD isoforms were expressed in different cell types in the skin (6). SC1D is located in pre-sebocytes of the sebaceous gland of the skin whereas the mature sebocytes expressed SCD3. On the other hand SCD2 is expressed in hair follicles. It is not known whether the HG SCD isoforms are expressed in different cell types, as well, but it is possible that their pattern of expression is a reflection of HG cell types in different stages of differentiation. Despite the expression of SCD2 and SCD3 in the HG of SC1D–/–, the deficiencies in the levels of ADG and C20:1n-9 were not compensated for suggesting a distinct role of each SCD isoform in the synthesis of monounsaturated fatty acids of the HG.

The observation that microsomes isolated from HG of SC1D–/– mice had very low desaturase activity toward C18:0-CoA compared with C16:0-CoA (Fig. 7) strongly suggests that C18:0-CoA is the main substrate of SCD1 isoform. The other isoforms (SCD2 or -3) preferentially utilize C16:0-CoA as the substrate of desaturation. Consistent with this notion the levels of C18:1n-7 and C20:1n-7 derived from elongation and desaturation of C16:0-CoA were decreased by 30% in the SC1D–/– mouse compared with a decrease of greater than 90% in the levels of C20:1n-9 that would be derived from the elongation of C18:1n-9. These studies, along with a recent report of a palmitoyl-CoA-specific Δ9 desaturase from Caenorhabditis elegans (24), strongly suggest that the SCD isoforms have different substrate specificity and may explain why there are several SCD isoforms in the mouse genome. The differences in the catalytic selectivity of the SCD isoforms may also contribute to the establishing of the lipid composition of the cell. A finer control can be provided by regulated expression of several isoforms with differing selectivity than by expression of either one or two with the same substrate selectivity.

We propose, as depicted in Fig. 8, that in the HG palmitate is synthesized de novo by FAS from acetyl-CoA producing palmi-
FIG. 7. A, Northern blot analysis for the expression of SCD mRNA isoforms in HG of SCD1+/+ and SCD1−/− mice. Total RNA (10 μg) pooled from three mice of each group was subjected to Northern analysis followed by hybridization with labeled probes specific for SCD1, SCD2, SCD3, SREBP-1, SREBP-2, and FAS. 28 S mRNA is used as a loading control. B, immunoblot analysis for the expression of SCD protein from HG of SCD1+/+ and SCD1−/− mice. Total protein (25 μg) of each group was subjected to 10% SDS-polyacrylamide gel electrophoresis followed by detection with SCD antibody. C, SCD activity in the HG of SCD1+/+ and SCD1−/− mice. Microsomal fractions (100 μg) from HG of each group were incubated with a reaction mixture containing [14C]stearoyl-CoA or [14C]palmitoyl-CoA for 10 min. The products were saponified, and the fatty acids were separated by AgNO3-impregnated TLC plates. Each value represents the mean ± S.D. (n = 6). *p < 0.001 versus SCD1+/+ mice (Student’s t test).

Fig. 8. Proposed scheme for the synthesis of monounsaturated fatty acids in mice that become incorporated into the 1-alkyl-2,3-diacylglycerol of the HG. The HG is proposed to have an elongase, which catalyzes the elongation of C18:1n-7 and C18:1n-9 to 20:1n-7 and 20:1n-9, respectively.

In conclusion, the studies have revealed that SCD1 gene expression is required for the synthesis of another class of neutral lipid, the ADG. The Harderian-specific elongase that synthesizes the very long chain monounsaturated fatty acids of ADG requires C18:1 and C16:1, the endogenous products of SCD isoforms, as substrates. The failure of dietary fatty acids to repair the deficiency in phospholipids and ADG levels strongly suggests the HG relies on de novo monounsaturated fatty acids for lipid synthesis. Presently, the SCD1 knockout mouse may be a useful model to study the role of endogenous oleoyl-CoA in lipid metabolism, and the studies described here may have broad implications for the potential use of SCD1 as a target in the treatment of some eye diseases.

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