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Regulation of stearoyl-CoA desaturases and role in metabolism

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Abstract

Stearoyl-CoA desaturase (SCD) is the rate-limiting enzyme catalyzing the synthesis of monounsaturated fatty acids, mainly oleate (18:1) and palmitoleate (16:1). These represent the major monounsaturated fatty acids of membrane phospholipids, triglycerides, wax esters and cholesterol esters. The ratio of saturated to monounsaturated fatty acids affects phospholipid composition and alteration in this ratio has been implicated in a variety of disease states including cardiovascular disease, obesity, diabetes, neurological disease, and cancer. For this reason, the expression of SCD is of physiological significance in both normal and disease states. Several SCD gene isoforms (SCD1, SCD2, SCD3) exist in the mouse and one SCD isoform that is highly homologous to the mouse SCD1 is well characterized in human. The physiological role of each SCD isoform and the reason for having three or more SCD gene isoforms in the rodent genome are currently unknown but could be related the substrate specificities of the isomers and their regulation through tissue-specific expression. The recent studies of asebia mouse strains that have a natural mutation in the SCD1 gene and a mouse model with a targeted disruption of the SCD1 gene have provided clues concerning the role that SCD1 and its endogenous products play in the regulation of metabolism.

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1. Introduction

Stearoyl-CoA desaturase (SCD) is the rate-limiting enzyme in the biosynthesis of monounsaturated fatty acids (MUFAs). SCD in conjuction with NADPH, cytochrome b_5 reductase, and cytochrome b_5 and in the presence of molecular oxygen introduces a single double bond (between carbons 9 and 10) into saturated fatty acyl-CoAs [1–4]. Based on recent kinetic isotope data of the plant desaturase, the current hypothesis for the desaturation reaction is that the enzyme removes hydrogen atoms starting with the one at the C-9 position, followed by the removal of the second hydrogen atom from the C-10 position. This stepwise mechanism is highly specific in the position at which the double bond is introduced and implies that the C-9 and C-10 bond is accurately positioned with respect to the diiron center [5–7]. The desaturation of a fatty acid is an oxidation reaction and requires molecular oxygen and two electrons. However, oxygen itself is not incorporated into the fatty acid chain but is released in the form of water [2]. Although the insertion of a double bond occurs in a spectrum of methylene-interrupted fatty acyl-CoA substrates including *trans*-11 octadecenoic acid [8], the preferred substrates are palmitoyl- and stearoyl-CoA, which get converted into palmitoleoyl- and oleoyl-CoA, respectively [3,9,10]. The MUFAs synthesized by SCD are then used as major substrates for the synthesis of various kinds of lipids including phospholipids, triglycerides and cholesteryl esters (Fig. 1). Oleic acid is the preferred substrate for acyl-CoA:cholesterol acyltransferase (ACAT) and diacylglycerol acyltransferase (DGAT), the enzymes responsible for cholesteryl ester and triglyceride synthesis, respectively. In addition, oleate is the major monounsaturated fatty acid in human adipose tissue and in the membrane phospholipid of the redblood cell. The ratio of stearic acid to oleic acid has been implicated in the regulation of cell growth and differentiation through effects on membrane fluidity [11–14] and signal transduction [15]. Monounsaturated fatty acids also influence apoptosis and may have some role in mutagenesis of some tumors [15–17]. Overall, SCD expression affects the fatty acid composition of membrane phospholipids, triglycerides and cholesterol ester, resulting in changes in membrane fluidity, lipid metabolism and obesity. In this review we focus on our current understanding of the physiological role of the SCD1 isoform in lipid metabolism. These studies have advanced due to the availability of the mouse model with a natural mutation in the SCD1 gene [18–22] and the mouse with a targeted disruption in the SCD1 gene that was recently generated [18,25–28].

2. Expression and regulation of SCD genes

The genes for SCD have been cloned from different species including yeast [29], *C. elegans* [30], sheep [31], hamster [32], rat [33], mice [22,34,35] and human [36,37]. In the mouse, three isoforms

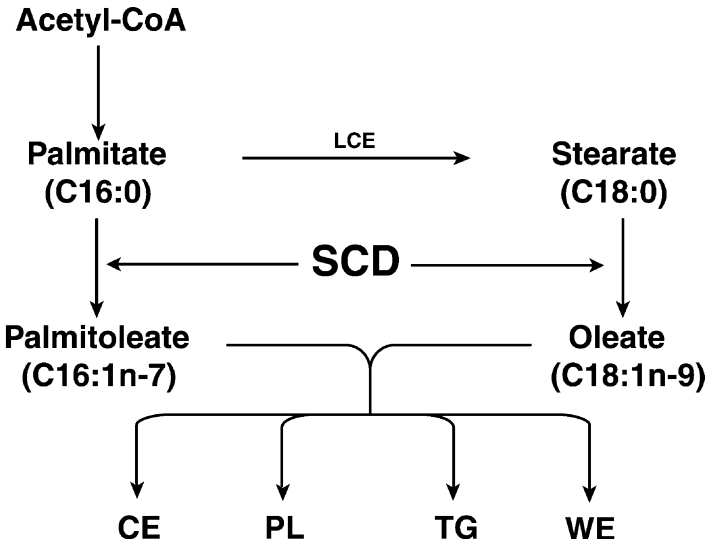

Fig. 1. Role of SCD1 in lipid synthesis. CE, cholesterol ester; TG, triglyceride; WE, wax esters; PL, phospholipids; SCD, stearoyl-CoA desaturase; LCE, long chain elongase.

(SCD1, SCD2 and SCD3) have been identified [22,34,35] whereas in the rat, two isoforms have been characterized [33,38]. In many different mouse strains, all of the SCD genes are localized in close proximity on chromosome 19 [22,39] and code for a transcript of about 4.9 kb. In humans, only a single functional SCD gene on chromosome 10 has so far been characterized. The second SCD locus on chromosome 17 is a fully processed pseudogene [36]. Like human, ovine [31], sheep and cows [40] so far have only one SCD gene isofom identified and characterized. Although the mouse isoforms share 85–88% identity at their amino acid sequence [22,34,35], their 5′-flanking regions differ somewhat [22,41] resulting in divergent tissue-specific gene expression. Under normal dietary conditions, SCD1 mRNA is highly expressed in white adipose tissue (WAT) brown adipose tissue (BAT), meibomian gland, Harderian and preputial glands [27] and is dramatically induced in liver in response to high carbohydrate diet [42]. SCD2 is predominantly expressed in the brain [34] and is developmentally induced during the neonatal myelinating period [43–45]. Similar to SCD1, SCD2 mRNA is expressed to a lesser extent in kidney, spleen, heart and lung where it is induced in response to a high carbohydrate diet [22,34,46]. In addition, SCD2 mRNA is expressed in B-lymphocytrs and is down regulated during T-lymphocyte development [47–49]. In some tissues, such as the adipose and eyelid, both SCD1 and SCD2 genes are expressed whereas in the skin, Harderian and preputial glands all the three gene isoforms are expressed [22,25,27]. In skin, SCD1 expression is restricted to the undifferentiated sebocytes, while SCD3 is expressed mainly in the differentiated sebocytes [22]. SCD2 is also expressed in hair follicles [21]. Expression of the human SCD gene gives rise to two mRNA transcripts of 3.9 and 5.2 kb which arise as a consequence of two polyadenlyation signals, indicating that the two differently expressed transcripts encode the same SCD polypeptide [36]. The function of the polyadenylation is not known but could be, in addition to the transcriptional control, a means by which the two transcripts differ in stability or translatability thus allowing for rapid and efficient changes in cellular environment [50].

Many developmental, dietary, hormonal and environmental factors regulate SCD1 expression (Table 1). High-carbohydrate diets [35,42,50], insulin [51], glucose and fructose [51,52], cholesterol [53–55], cold temperatures [56,57], light [58], some drugs (fibrates, peroxisome proliferators and LXRα agonists) [14,55,59,60], retinoic acid [55,61,62] induce hepatic SCD1 gene expression. Polyunsaturated fatty acids (PUFA) especially of the n-6 and n-3 families [42,63], conjugated linoleic acid (CLA) [64,65], cAMP (or drugs that increase its intracellular levels) [35,66], tumor necrosis factor-α [67] and thyroid hormone [68], however, inhibit SCD1 mRNA transcription in the liver. Previous studies in 3T3-L1 adipocytes have shown that thiazolidinediones [69] and some steroid hormones [70] decrease SCD1 mRNA levels together with reduced SCD specific activity. Sulfur-substituted (thia) fatty acids [71] inhibit SCD either at the transcription level or at the SCD enzyme activity, while cyclopropene fatty acids (sterculic acid) directly inhibit SCD activity in vivo and in vitro [72–76]. The SCD protein undergoes rapid degradation producing transient elevations of SCD enzyme activity in response to physiological demands [118]. The regulation of SCD is therefore very diverse and its expression could affect a variety of key physiological variables, including insulin sensitivity, metabolic rate, adiposity, atherosclerosis and cancer.

2.1. SCD expression and lipid synthesis

The clue as to the physiological role of the SCD1 gene and its endogenous products has come from recent studies of the asebia mouse strains (ab^{j} and ab^{2j}) that have a naturally-occurring mutation in SCD1 [21] as well as a laboratory mouse model with a targeted disruption ($SCD1^{-/-}$) [26]. These mouse models have revealed that SCD1 gene expression is required in the de novo synthesis of a number of lipids including triglycerides, cholesterol esters, wax esters and alkyl diacylglycerol (Fig. 1). $SCD1^{-/-}$ mice are deficient in hepatic triglycerides and cholesterol esters [18], despite the presence of normal activities of ACAT and DGAT, the enzymes responsible for cholesterol ester and triglyceride synthesis, respectively. The levels of 16:1 and 18:1 are reduced in the tissue lipid fractions of $SCD1^{-/-}$ mice while 16:0 and 18:0 are increased [18]. Normally, a high carbohydrate diet fed to mice or rats induces the hepatic SCD1 gene and other lipogenic genes through the sterol regulatory element binding protein (SREBP-1) dependent mechanism, resulting in an increase in monounsaturated fatty acids (MUFAs) and hepatic triglycerides. One of our

Table 1
Reglation of stearoyl-CoA desasturase

Dietary factors	Hormones	Other
▲ Glucose [51,52]	▲ Insulin [51]	▲ Peroxisome [60]
▲ Fructose A [52]	▲ Growth hormone [103,104]	▲ Temperature [56,57]
▲ Vitamin A [55,61,62]	▲ Estrogen [105]	▲ Iron [109]
▲ Cholesterol [53–55]	▲ Androgen [25,106]	▲ Liver-X-receptor agonist [55,59,60]
▲ Vitamin D[100]	▼ Leptin [24]	▲ TGF-β [110]
▼ Polyunsaturated fatty acids [42,63]	▼ Glugacon [107]	▲ Light [58]
▲▼ Alcohol [101,102]	▼ Thyroid hormone [68]	▲ β-Amyloid [111]
▲▼ Conjugated linoleic acid [64,65]	▲▼ Dehydroepiandrosterone [70,108]	▼ Thiazolidinediones [69,112]
		▼ Cadmium [113]
		▼ TNF-α [114]

recent observations [23] is that SCD1$^{-/-}$ mice on a high carbohydrate diet fail to accumulate hepatic triglycerides. Supplementation of the high carbohydrate diet with high levels of triolein or tripalmitolein can normalize cholesterol ester levels, however, the triglyceride levels are not returned to the levels found in the wildtype mouse [23]. The SCD1$^{-/-}$ mice have very low levels of triglycerides in the very low density lipoprotein (VLDL) and low density lipoprotein (LDL) fractions compared to the wildtype counterparts [18,77]. Furthermore, the rate of VLDL-triglyceride secretion, as measured by inhibition of VLDL clearance using Triton WR1339, was dramatically reduced in the SCD1$^{-/-}$ mice [24]. An experiment using [^{3}H]-glycerol to determine lipid synthesis levels showed a very low rate of triglyceride synthesis in liver of SCD1$^{-/-}$ mice [23]. Transient transfections of an SCD1 expression vector into Chinese hamster ovary (CHO) cells resulted in increased SCD1 activity and esterification of cholesterol to cholesterol esters [18]. These observations reveal that endogenously synthesized MUFAs by SCD most likely serve as the main substrates for the synthesis of hepatic triglycerides and cholesterol esters [18,23]. The enzymes involved in the de novo synthesis of triglycerides and cholesterol ester including SCD, ACAT, DGAT and microsomal glycerol phosphate acyl transferase (GPAT), are located in the endoplasmic reticulum membrane. A possible physiological explanation for the requirement of SCD expression is for the production of more easily accessible MUFAs within the vicinity of ACAT, DGAT and microsomal GPAT for the synthesis of the triglycerides and cholesterol esters.

Hypertriglyceridemia (HTG) syndromes are among the most common lipid disorders in humans. Although there is strong evidence that many of these syndromes are heritable [77], the genetics of these disorders is not well understood. It is most likely that HTG is a complex trait; i.e. multiple genes influence the expression of the phenotype. In addition, the onset of HTG is affected by diet, insulin sensitivity, and obesity [78–80]. Given the strong correlation between the in vivo triglyceride and cholesterol ester synthesis and the activity of SCD, Attie et al. [77] validated and applied a simple plasma marker of SCD activity, the ratio of plasma oleate to stearate (18;1/18:0 ratio, the ''desaturation index''), to test the hypothesis that in vivo SCD activity accounts for a large fraction of the variation in human plasma triglycerides. In addition, the desaturation index in human subjects exposed to a regimen known to raise serum triglyceride levels, i.e. high-carbohydrate diets was studied. The results supported an important role of SCD in human serum triglyceride levels. Thus, the human SCD gene may be another checkpoint in the process of cholesterol, triglyceride and lipoprotein metabolism in humans and has broad implication for the potential use as a target in the treatment of human HTG.

The SCD1$^{-/-}$ mice show cutaneous abnormalities with atrophic sebaceous glands and narrow eye fissure with atrophic meibomian glands, suggesting an important role for monounsaturated fatty acids in skin homeostasis. It is known that the major function of sebaceous gland and meibomian gland is to secrete lipid complex-lubricants, termed sebum and mebum. These contain respectively, wax ester, triglyceride, and cholesterol ester. These fluids prevent the evaporation of moisture from skin and the eyeball. The skin and eyelid of SCD1$^{-/-}$ mice are deficient in triglyceride, cholesterol ester, and wax esters [19–21, 26]. The levels of free cholesterol, however, are increased [26]. Thus under conditions of high cellular cholesterol, SCD1 gene expression would indirectly protect the cell from the harmful effects of free cholesterol by converting it to cholesterol ester by ACAT for storage. The benefit of expressing SCD1 would be to provide oleoyl-CoA, the preferred substrate for ACAT-mediated cholesterol esterification [81], DGAT for triglyceride

synthesis [82,83] and wax synthase for wax ester synthesis. In addition, the presence of normal levels of MUFAs would maintain a more appropriate ratio of cholesterol to other lipids and maintain cell membrane integrity. Since excess free cholesterol has been known to lead to cell death [84–88], it is tempting to speculate that atrophy of the sebaceous and meibomian glands observed in the SCD1$^{-/-}$ mouse may be due to an increase in the amount of cellular free cholesterol in these glands rather than the reduced levels of sebum and meibum.

The studies of SCD gene expression in the mouse Harderian gland revealed the role of SCD1 in the biosynthesis of another class of lipids: the alky-2,3-diacylglycerol. The Harderian gland that was first described by Johann J Harder towards the end of 17th century is located in the orbit of the eye and, in most species, is the largest tissue. The major products from the gland vary between the different species of mammals. In rodents, the gland synthesizes lipids, indoles, and porphyrins, which are secreted by an exocytotic mechanism. The major lipid synthesized by the mouse Harderian gland is 1-alkyl-2,3-diacylglycerol (ADG) [89,90]. ADG is a lubricant of the eyeball and is crucial in assisting the movement of the eyelid, along with mebum from the meibomian gland. In our recent study [27], SCD1$^{-/-}$ mice exhibited a deficiency in ADG and n-9 eicosenoate (20:1n-9), which is the main MUFA of ADG. We found that 20:1n-9 is an elongation product of 18:1n-9. Feeding diets of high levels of oleate or eicosenoate did not result in an increase of 20:1n-9 and failed to restore the deficiency in ADG. Therefore, endogenously synthesized oleate by SCD1, as was demonstrated in liver, is essential for the biosynthesis of ADG and eicosenoate in the mouse Harderian gland. The scheme depicted in Fig. 2 is used as an example to show how SCD may be involved in the biosynthesis of ADG in the mouse Harderian gland. It is proposed that 16:0 in the Harderian gland is synthesized de novo by the fatty acid synthase complex (FAS) from acetyl-CoA producing 16:0 as the major end product. The 16:0 then serves as a substrate for the microsomal malonyl-CoA dependent elongase to produce 18:0, which then serves as the main substrate of either SCD1 or SCD2. The 16:1n-7 synthesized by either SCD2 or SCD3 is first converted to 18:1n-7 by a general elongase, and then both 18:1n-9 and 18:1n-7 are converted to 20:1n-9 and 20:1n-7, respectively, by the Harderian gland elongase. Desaturation of 20:0 to 20:1 is unlikely since 20:1n-11 was not detected. This elongase may be specific to the Harderian gland because we cannot detect 20:1n-9 or 20:1n-7 fatty acids in other tissues. In addition, the elongase in Harderian gland exhibited high substrate specificity for 18:1n-9 and 16:1n-7-CoA, whereas in other tissues such as liver and adipose tissues high substrate specificity for 16:0-CoA was found. The 20:1n-9 and 20:1n-7 then become the preferred substrates for esterification to the 2,3 positions of alkylglycerol by the 1-alkylglycerol: acyltransferase for the synthesis of ADG. The reasons for incorporating specific very long chain MUFAs in the 1-alkyl-2,3-diacyglycerol of the mouse Harderian gland are not clear but it may be that they are required to maintain the correct physical properties of the fluids for normal eye function. We found very low levels of n-3 and n-6 polyunsaturated fatty acids (PUFAs) in the Harderian gland indicating that the lipids of this gland are mainly composed of saturated fatty acids and MUFAs of the n-9 series. 18:1 is a major component of the Harderian gland membranes and decrease in levels would reduce membrane fluidity of the Harderian gland. Consistent with this observation we noted that the Harderian gland isolated from the SCD1$^{-/-}$ mice was very rigid possibly due to decreased membrane fluidity. The Harderian gland could therefore be a useful model in studying the metabolism MUFAs of the n-9 series and the roles they play in physiological processes. Humans do not have the Harderian gland, but because this structure is part of the retinal axis, it is likely that this tissue

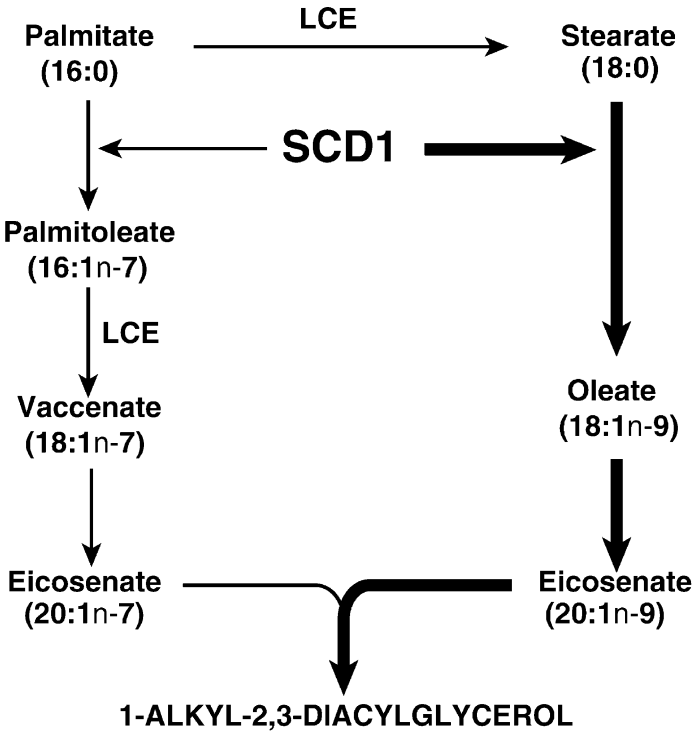

Fig. 2. Proposed scheme showing the possible involvement of SCD1 in the biosynthesis of the 1-alkyl 2,3-diacylglycerol of the HG. The HG is proposed to have an elongase, which catalyzes the elongation of C18:1n-7 and C18:1n-9 the products of SCD to 20:1n-7 and 20:1n-9 respectively [27].

has evolved into the retina in humans. Consistent with this stipulation, high SCD expression has been reported in human retinal pigment epithelical cells and its expression may play an important role in the pathophysiology of these cells [62].

2.2. *Effect of SCD1 expression on substrate specificities and lipid composition*

The use of the mouse models has led to the recent finding that the three SCD isoforms exhibit different specificity for substrates. Harderian gland microsomes of SCD1$^{-/-}$ mice still show high desaturase activity toward 16:0-CoA whereas the SCD activity toward 18:0-CoA was reduced by more than 90%. These studies suggested that 18:0-CoA is the main substrate of the SCD1 isoform. Consistent with this notion, the level of n-7 MUFAs (18:1n-7 and 20:1n-7) derived from elongation and desaturation of 16:0-CoA were decreased by only 30% in the SCD1$^{-/-}$ mice compared to a decrease of greater than 90% in the levels of n-9 MUFA (18:1n-9 and 20:1n-9) derived from elongation and desaturation of 18:0-CoA. The preputial gland (PG) of the mouse, similar to the skin and Harderian gland, also expresses the three SCD isoforms [25]. The main neutral lipids of the PG are wax esters, triglycerides and alkyl-2,3-diacylglycerol [91,92]. In contrast to the Harderian glands and skin [20,26,27], 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, the SCD1$^{-/-}$ mice increased 16:1n-10 by greater than

2-fold in the wax ester fraction. Since 16:1n-7 and 16:1n-10 are positional isomers, it is possible that the increase in 16:1n-10 in the SCD1$^{-/-}$ mice is to compensate for 16:1n-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. Interestingly, PG of SCD1$^{-/-}$ mice lacked SCD3 expression while the expression of the SCD2 was not altered. When SCD activity was measured in the PG, the desaturase activity towards 16:0-CoA was extremely low compared to 18:0-CoA. However, treatment of the SCD1$^{-/-}$ mice with testosterone induced SCD3 expression and led to an increase in desaturase activity toward 16:0-CoA. Consistent with these notions, the levels of 16:1n-7 derived from desaturation of 16:0-CoA were decreased by 70% in the SCD1$^{-/-}$ mice compared to a decrease of 30% in the levels of 18:1n-9 that are derived from the desaturation of C18:0. Therefore, these studies strongly suggested that 16:0-CoA is the main substrate for the SCD3 isoform. The SCD1 and SCD2 preferentially utilized 18:0-CoA as the substrate for desaturation. The different substrate specificity may explain why there are several SCD isoforms in the mouse genome. The differences in the catalytic selectivity of the SCD isoforms may be to 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 proposed [25] that in the preputial glands, palmitate is synthesized de novo by the FAS from acetyl-CoA, which then serves as a substrate for the microsomal malonyl-CoA dependent elongase to produce stearate, which ultimately then serves as the main substrate of SCD1 and SCD2 to produce 18:1n-9. The 16:1n-7 would then be synthesized mainly by the SCD3 isoform and further incorporated into lipid fractions. Because SCD3 expression is lost in the SCD1$^{-/-}$ mice the levels of 16:1n-7 are reduced and those of 16:0 increase. The preputial gland cells then respond by inducing or increasing the activity, due to substrate flux of the palmitoyl-CoA $\Delta 6$ desaturase to convert more of the accumulating 16:0 into 16:1n-10. The mechanism of induction of this activity as a result of SCD1 deficiency is currently unknown. The 16:1n-10 then substitutes for the decreased levels of 16:1n-7 for esterification to fatty alcohols and the 2,3 positions of glycerol by enzymes synthesizing wax esters, ADG, and phospholipids.

2.3. Role of SCD1 in regulation of lipogenesis and fatty acid oxidation

In a recent study Ntambi et al. [28] employed DNA microarrays to identify genes whose expression was altered in the liver of SCD1$^{-/-}$ mice. Two hundred mRNAs that were significantly different between the livers of SCD1$^{-/-}$ and SCD1$^{+/+}$ mice were identified. The most striking pattern was genes involved in lipogenesis and fatty acid β-oxidation. Lipid oxidation genes and targets of peroxisome proliferator activated receptor-alpha (PPARα) such as acyl-CoA oxidase (ACO), very long chain acyl-CoA dehydrogenase (VLCAD), carnitine palmitoyltransferase-1 (CPT-1), and fasting-induced adipocyte factor (FIAF) were up regulated while lipid synthesis genes such as SREBP-1, FAS, and mitochondrial GPAT were down regulated in the SCD1$^{-/-}$ mice. SREBP-1c is the main SREBP-1 isoform expressed in liver and regulates the expression of lipogenic genes [28]. Insulin levels, dietary carbohydrate, fatty acids, and cholesterol regulate the SREBP-1 gene expression and protein maturation. Thus, the down regulation of SREBP-1 gene expression in the SCD1$^{-/-}$ could have numerous effects on various metabolic pathways regulated by SREBP-1. For instance, the induction of SREBP-1 by insulin and cholesterol

greatly enhances the synthesis and secretion of triglycerides by the liver [55,93,94]. However, in the SCD1$^{-/-}$ mice, carbohydrate feeding failed to induce triglyceride synthesis and secretion by the liver [23]. In addition the finding of reduced adiposity and increased energy expenditure [28] in the setting of increased food intake is suggestive of increased fatty acid oxidation. CPT, ACO, VLCAD and FIAF are known targets of PPAR-α and contain PPARα response regions in their promoters [95,96]. Since PPARα mRNA is unchanged (unpublished), the up regulation of enzymes of fatty acid β-oxidation in the SCD1$^{-/-}$ mice must be downstream of PPARα transcription. Thus, the characteristics exhibited by the SCD1$^{-/-}$ mice are consistent with presence of a PPARα activator with reduced activity in wild type mice. Decreased SCD activity could also alter the levels of ligands for other nuclear hormone receptors important in lipid homeostasis. The SCD1$^{-/-}$ mice exhibit increase in the contents of saturated fatty acids (16:0 and 18:0) while the contents of the PUFAs of the n-6 and n-3 are not changed [18,26]. One possible mechanism is that the saturated fatty acids induce the signal in the SCD1$^{-/-}$ mice that activates the PPARα but this has yet to be determined. Alternatively, the increased levels of 18:0- or 16:0-CoAs as suggested by Cohen et al. [24] could inhibit acetyl-CoA carboxylase (ACC) through a well known feed back mechanism of lipogenesis, and the reduction in the levels of malonyl-CoA can lead to activation of CPT1, resulting in increased transport of fatty acids into the mitochondria for oxidation. In this mechanism, it is hypothesized that in the absence of SCD1 a reduced rate of triglyceride and VLDL synthesis increases the intracellular pool of saturated fatty acids leading to an increase in fatty acid oxidation. MUFAs are known to be necessary for normal rates of synthesis of triglycerides and cholesterol esters, which in turn are required for hepatic lipid storage and VLDL synthesis [18,23,24]. When lipid storage and VLDL production are reduced, the intracellular pool of saturated fatty acyl CoAs would be expected to increase. Saturated fatty acyl CoAs, but not monunsaturated fatty acyl CoAs, are known to allosterically inhibit acetyl CoA carboxylase-1(ACC), thus reducing cellular levels of malonyl CoA [97–99]. Malonyl CoA is required for fatty acid biosynthesis and also inhibits the mitochondrial CPT-1 shuttle system, the rate-limiting step in the import and oxidation of fatty acids in mitochondria [97–99]. Thus, reduced levels of SCD1 would lead to a decrease in the cellular levels of malonyl CoA and derepresses fatty acid oxidation. These findings are similar to those seen in mice lacking ACC-2, which also have increased fatty acid oxidation in skeletal muscle and have a lean phenotype [99]. Other mechanisms could account for the increased energy expenditure in the SCD1 deficient mice. For instance SCD1 deficiency could be associated with increased activity of AMPK, an enzyme that has been shown to stimulate fatty acid oxidation following leptin administration. Another possibility is that a deficiency in SCD1 would alter phospholipid composition thereby impacting on membrane properties or signal transduction. SCD1 deficiency could also be associated with direct or indirect effects on uncoupling proteins (UCPs) that are involved in thermogenesis. Finally, the hair and lipid abnormalities associated with defects in SCD1 deficient mice could be associated with increased heat dissipation. Experiments are under way to evaluate the various mechanistic possibilities.

2.4. Leptin regulation of SCD1

SCD activity has been shown to be elevated in the adipose tissue of various animal models of obesity [24]. A positive correlation between SCD activity in skeletal muscle and the percentage of

body weight has recently been reported in human subjects [115]. Leptin deficient mice (ob/ob) are characterized by a five-fold higher deposition of body fat than their lean counterparts. The consistent change in the fatty acid composition in these mice, is an increase in MUFAs as a result of increased SCD activity [24]. When leptin is injected into ob/ob mice the obese phenotype is normalized. Leptin also elicits a novel metabolic response in a number of tissues including liver. To elucidate the underlying mechanism, Cohen et al. [24] used oligonucleotide microarrays to identify genes specifically regulated by leptin in the livers of ob/ob mice. K-means clustering identified a large number of leptin-regulated genes, the most robust of which SCD1. SCD1 mRNA levels, enzymatic activity, and the levels of MUFAs were markedly increased in ob/ob mice and are all specifically reduced by leptin administration. The percentages of monounsaturated fats were also reduced in the leptin treated livers. These data suggested the possibility that leptin specific down regulation of SCD1 mRNA and activity might mediate some of its metabolic effects. To explore the effects of SCD1 deficiency on the ob/ob phenotype, heterozygous asebia ($ab^J/+$) mice were intercrossed to ob/+ mice. Double mutant ab^J/ab^J; ob/ob mice were born at the expected frequency and were noticeably thinner than ob/ob controls. The ab^J/ab^J; ob/ob mice showed a dramatic reduction in body weight at all ages compared to littermate ob/ob control. Fat mass in the 16 week old double mutant mice was decreased from 51.0 to 32.1% in females and from 49.9 to 28.1% in males compared to ob/ob controls. Mice of both sexes also showed a significant increase in percent lean mass relative to ob/ob littermates. Energy balance was analyzed by measuring food intake and energy expenditure in ob/ob and lean littermates with or without homozygous SCD1 mutations. As expected, ob/ob mice were hyperphagic compared to lean littermate controls. The ab^J/ab^J;ob/ob mice however, consumed more food than ob/ob littermates, suggesting that SCD1 deficiency may modulate CNS pathways that regulate food intake, perhaps secondary to the increased oxygen consumption. Ob/ob mice are known to be markedly hypometabolic, since in the absence of leptin, they exist in a state of perceived starvation and activate a metabolic program to conserve energy. We found that the ab^j/ab^j;ob/ob mice had a complete correction of their hypometabolic phenotype, with energy expenditure equivalent to, or even greater than, wild-type littermates.

Ob/ob mice have massively enlarged livers that are engorged with lipid (fatty liver). Gross inspection revealed that both the hepatomegaly and steatosis of ob/ob mice were normalized in ab^J/ab^J; ob/ob mice. Histological sections of ob/ob liver showed characteristic, large lipid-filled vacuoles, while those of double mutant mice showed little or no vacuolation with a histological appearance that is indistinguishable from wild-type mice. Consistent with this histologic appearance, the levels of liver triglyceride were substantially increased in ob/ob mice whereas triglycerides in ab^J/ab^J; ob/ob mice were reduced to levels comparable to lean controls. Triglyceride levels in lean ab^J/ab^J mice are reduced even further below that of lean controls. These data show that SCD1 is required for the development of the obese phenotype of ob/ob mice and further suggest that a significant proportion of leptin's metabolic effects may result from inhibition of this enzyme. The metabolic effects of leptin on SCD1 in liver, however, are likely to be the result of central action, as mice lacking the leptin receptor in the brain display enlarged, fatty livers, while livers from mice with a liver-specific knockout of the leptin receptor appear normal. Leptin also reduces hepatic SCD1 activity when administered intracerebroventricularly. However, the nature of the CNS signals that modulate liver metabolism in response to leptin is unknown.

3. Conclusion

The recent studies using the knockout mouse models have revealed the phenotypes generated as a result of SCD1 gene deficiency. We have learned that this enzyme is critical in the biosynthesis of neutral lipids; triglycerides, cholesterol esters, wax esters and 1-alkyl-2,3-diacylglycerol [18,23,25,27]. We have learn here and from other recent reviews on SCD [116-118] that the SCD isoforms exhibit tissue-specific expression, have different substrate specificity and the SCD1 in particular is regulated by numerous factors. We have also learned that SCD1 deficiency either directly or indirectly induces a signal that partitions fatty acids towards oxidation rather than synthesis. Furthermore SCD1 deficiency leads to leanness, increased insulin sensitivity and increased metabolic rate [28]. SCD1 is a target of leptin signaling [24]. Thus, in addition to playing a role in lipid metabolism, the SCD1 is a promising therapeutic target in the treatment of the metabolic syndrome.

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References

[1] Ntambi JM. Prog Lipid Res 1995;34:139–50.
[2] Ntambi JM. J Lipid Res 1999;40:1549–58.
[3] Enoch HG, Catala A, Strittmatter P. J Biol Chem 1976;251:5095–103.
[4] Enoch HG, Strittmatter P. Biochemistry 1978;17:4927–32.
[5] Broadwater JA, Haas JA, Fox BJ. The fundamental versatile role of diiron enzymes in lipid metabolism. Fett/Lipid 1994:100.
[6] Shanklin J, Whittle E, Fox BG. Biochemistry 1994;33:12787–94.
[7] Shanklin J, Achim C, Schmidt H, Fox BG, Munck E. Proc Natl Acad Sci USA 1997;94:2981–6.
[8] Mahfouz MM, Valicenti AJ, Holman RT. Biochim Biophys Acta 1980;618:1–12.
[9] Prasad MR, Joshi VC. J Biol Chem 1979;254:6362–8.
[10] Strittmatter P, Enoch HG. Methods Enzymol 1978;52:188–93.
[11] Ntambi JM. Prostaglins Leukot Essent Fatty Acids 1995;52:117–20.
[12] Casimir DA, Miller CW, Ntambi JM. Differentiation 1996;60:203–10.
[13] Ntambi JM, Takova T. Differentiation 1996;60:151–8.
[14] Sun Y, Hao M, Luo Y, Liang CP, Silver DL, Cheng C, et al. J Biol Chem 2003;278:5813–20.
[15] Kasai T, Ohguchi K, Nakashima S, Ito Y, Naganawa T, Kondo N, et al. J Immunol 1998;161:6469–74.
[16] Falvella FS, Pascale RM, Gariboldi M, Manenti G, De Miglio MR, Simile MM, et al. Carcinogenesis 2002;23:1933–6.
[17] Lu J, Pei H, Kaeck M, Thompson HJ. Mol Carcinog 1997;20:204–15.
[18] Miyazaki M, Kim YC, Gray-Keller MP, Attie AD, Ntambi JM. J Biol Chem 2000;275:30132–8.
[19] Parimoo S, Zheng Y, Eilertsen K, Ge L, Prouty S, Sundberg JP, et al. J Investig Dermatol Symp Proc 1999;4:320–2.

[20] Sundberg JP, Boggess D, Sundberg BA, Eilertsen K, Parimoo S, Filippi M, et al. Am J Pathol 2000;156:2067–75.
[21] Zheng Y, Eilertsen KJ, Ge L, Zhang L, Sundberg JP, Prouty SM, et al. Nat Genet 1999;23:268–70.
[22] Zheng Y, Prouty SM, Harmon A, Sundberg JP, Stenn KS, Parimoo S. Genomics 2001;71:182–91.
[23] Miyazaki M, Kim YC, Ntambi JM. J Lipid Res 2001;42:1018–24.
[24] Cohen P, Miyazaki M, Socci ND, Hagge-Greenberg A, Liedtke W, Soukas AA, Friedman JM, et al. Science 2002;297:240–3.
[25] Miyazaki M, Gomez FE, Ntambi JM. J Lipid Res 2002;43:2146–54.
[26] Miyazaki M, Man WC, Ntambi JM. J Nutr 2001;131:2260–8.
[27] Miyazaki M, Kim H-J, Man WC, Ntambi JM. J Biol Chem 2001;276:39455–61.
[28] Ntambi JM, Miyazaki M, Stoehr JP, Lan H, Kendziorski CM, Yell BS, et al. Proc Natl Acad Sci USA 2002; 99:11482–6.
[29] Stukey JE, McDonough VM, Martin CE. J Biol Chem 1990;265:20144–9.
[30] Watts JL, Browse J. Biochem Biophys Res Commun 2000;272:263–9.
[31] Ward RJ, Travers MT, Vernon RG, Salter AM, Buttery PJ, Barber MC. Biochem Soc Trans 1997;25:S673.
[32] Ideta R, Seki T, Adachi K, Nakayama Y. Dermatology 1998;196:47–50.
[33] Mihara K. J Biochem (Tokyo) 1990;108:1022–9.
[34] Kaestner KH, Ntambi JM, Kelly TJ, Lane MD. J Biol Chem 1989;264:14755–61.
[35] Ntambi JM, Buhrow SA, Kaestner KH, Christy RJ, Sibley E, Kelly TJ, et al. J Biol Chem 1988;263:17291–300.
[36] Zhang L, Ge L, Parimoo S, Stenn K, Prouty SM. Biochem J 1999;340:255–64.
[37] Zhang L, Ge L, Tran T, Stenn K, Prouty SM. Biochem J 2001;357:183–93.
[38] Thiede MA, Ozols J, Strittmatter P. J Biol Chem 1986;261:13230–5.
[39] Tabor DE, Xia YR, Mehrabian M, Edwards PA, Lusis AJ. Mamm Genome 1998;9:341–2.
[40] Chung M, Ha S, Jeong S, Bok J, Cho K, Baik M, et al. Biosci Biotechnol Biochem 2000;64:1526–30.
[41] Waters KM, Miller CW, Ntambi JM. Biochim Biophys Acta 1997;1349:33–42.
[42] Ntambi JM. J Biol Chem 1992;267:10925–30.
[43] DeWille JW, Farmer SJ. Neurosci Lett 1992;141:195–8.
[44] Garbay B, Boiron-Sargueil F, Shy M, Chbihi T, Jiang H, Kamholz J, et al. J Neurochem 1998;71:1719–26.
[45] DeWille JW, Farmer SJ. Dev Neurosci 1992;14:61–8.
[46] Kim YC, Ntambi JM. Biochem Biophys Res Commun 1999;266:1–4.
[47] Buttke TM, Van Cleave S, Steelman L, McCubrey JA. Proc Natl Acad Sci U S A 1989;86:6133–7.
[48] Tebbey PW, Buttke TM. Immunology 1990;70:379–86.
[49] Tebbey PW, Buttke TM. Biochem Biophys Res Commun 1992;186:531–6.
[50] Sessler AM, Kaur N, Palta JP, Ntambi JM. J Biol Chem 1996;271:29854–8.
[51] Waters KM, Ntambi JM. J Biol Chem 1994;269:27773–7.
[52] Jones BH, Stridge MK, Claycombe KJ, Smith PJ, Moustaid-Moussa N. Biochem J 1998;335:405–8.
[53] Kim HJ, Miyazaki M, Man WC, Ntambi JM. Ann N Y Acad Sci 2002;967:34–42.
[54] Kim HJ, Miyazaki M, Ntambi JM. J Lipid Res 2002;43:1750–7.
[55] Repa JJ, Liang G, Ou J, Bashmakov Y, Lobaccaro JM, Shimomura I, et al. Genes Dev 2000;14:2819–30.
[56] Goldman SS. J Neurochem 1978;30:397–400.
[57] Tiku PE, Gracey AY, Macartney AI, Beynon RJ, Cossins AR. Science 1996;271:815–8.
[58] Kis M, Zsiros O, Farkas T, Wada H, Nagy F, Gombos Z. Proc Natl Acad Sci U S A 1998;95:4209–14.
[59] Liang G, Yang J, Horton JD, Hammer RE, Goldstein JL, Brown MS. J Biol Chem 2002;277:9520–8.
[60] Miller CW, Ntambi JM. Proc Natl Acad Sci USA 1996;93:9443–8.
[61] Miller CW, Waters KM, Ntambi JM. Biochem Biophys Res Commun 1997;231:206–10.
[62] Samuel W, Kutty RK, Nagineni S, Gordon JS, Prouty SM, Chraratna RA, et al. J Biol Chem 2001;276:28744–50.
[63] Waters KM, Ntambi JM. Lipids 1996;31(Suppl):S33–0S36.
[64] Choi Y, Kim YC, Han YB, Park Y, Pariza MW, Ntambi JM. J Nutr 2000;130:1920–4.
[65] Lee KN, Pariza MW, Ntambi JM. Biochem Biophys Res Commun 1998;248:817–21.
[66] Storlazzi A, Maresca B, Gargano S. Mol Cell Biol Res Commun 1999;2:172–7.

[67] Weiner FR, Smith PJ, Wertheimer S, Rubin CS. J Biol Chem 1991;266:23525–8.
[68] Waters KM, Miller CW, Ntambi JM. Biochem Biophys Res Commun 1997;233:838–43.
[69] Kim YC, Gomez FE, Fox BG, Ntambi JM. J Lipid Res 2000;41:1310–6.
[70] Gomez FE, Miyazaki M, Kim YC, Marwah P, Lardy HA, Ntambi JM, et al. Biochemistry 2002;41:5473–82.
[71] Hovik KE, Spydevold OS, Bremer J. Biochim Biophys Acta 1997;1349:251–6.
[72] Gomez EF, Baum E, Ntambi JM, Fox BG. Biochem Biophys Res Commun 2003;300:316–26.
[73] Khoo DE, Fermor B, Miller J, Wood CB, Apostolov K, Barker W, et al. Br J Cancer 1991;63:97–101.
[74] P'Ullarkat RK, Maddow J, Reha H. Lipids 1976;11:802–7.
[75] Waltermann M, Steinbuchel A. FEMS Microbiol Lett 2000;190:45–50.
[76] Zoeller RA, Wood R. Biochim Biophys Acta 1985;845:380–8.
[77] Attie AD, Krauss RM, Gray-Keller MP, Brownlie A, Miyazaki M, Kastelein JJ, et al. J Lipid Res 2002; 43:1899–907.
[78] Goldstein JL, Schrott HG, Hazzard WR, Bierman EL, Motulsky AG. J Clin Invest 1973;52:1544–68.
[79] Pajukanta P, Nuotio I, Terwilliger JD, Porkka KV, Ylitalo K, Pihlajamaki J, et al. Nat Genet 1998;18:369–73.
[80] Pajukanta P, Terwilliger JD, Perola M, Hiekkalinna T, Nuotio I, Ellonen P, et al. Am J Hum Genet 1999; 64:1453–63.
[81] Cases S, Novak S, Zheng YW, Myers HM, Lear SR, Se E, et al. J Biol Chem 1998;273:26755–64.
[82] Cases S, Smith SJ, Zheng YW, Myers HM, Lear SR, Se E, et al. Proc Natl Acad Sci U S A 1998;95:13018–23.
[83] Cases S, Stone SJ, Zhou P, Yen E, Tow B, Lardizabal KD, et al. J Biol Chem 2001;276:38870–6.
[84] Zhang D, Tang W, Yao PM, Yang C, Xie B, Jackowski S, Tabas I. J Biol Chem 2000;275:35368–76.
[85] Tabas I. Biochim Biophys Acta 2000;1529:164–74.
[86] Yao PM, Tabas I. J Biol Chem 2000;275:23807–13.
[87] Yao PM, Tabas I. J Biol Chem 2001;276:42468–76.
[88] Feng B, Tabas I. J Biol Chem 2002;277:43271–80.
[89] Payne AP. J Anat 1994;185:1–49.
[90] Seyama Y, Kasama T, Yasugi E, Park S-H, Kano K. Lipids in harderian glands their significance. In: Webb SM, Hoffman RA, PuigDomingo ML, Reiter RJ, editors. Harderian glands. Porphyrin metabolism, behavioral endocrine effects. Berlin: Springer-Verlag; 1992.
[91] Sansone G, Hamilton JG. Lipids 1969;4:435–40.
[92] Thody AJ, Cooper MF, Bowden PE, Meddis D, Shuster S. J Endocrinol 1976;71:279–88.
[93] Shimomura I, Bashmakov Y, Ikemoto S, Horton JD, Brown MS, Goldstein JL. Proc Natl Acad Sci USA 1999; 96:13656–61.
[94] Shimomura I, Bashmakov Y, Horton JD. J Biol Chem 1999;274:30028–32.
[95] Kersten S, Seydoux J, Peters JM, Gonzalez FJ, Desvergne B, Wahli W. J Clin Invest 1999;103:1489–98.
[96] Kersten S, Mard S, Tan NS, Escher P, Metzger D, Chambon P, et al. J Biol Chem 2000;275:28488–93.
[97] McGarry JD, Mannaerts GP, Foster DW. J Clin Invest 1977;60:265–70.
[98] McGarry JD, Leatherman GF, Foster DW. J Biol Chem 1978;253:4128–36.
[99] Abu-Elheiga L, Matzuk MM, Abo-Hashema KA, Wakil SJ. Science 2001;291:2613–6.
[100] Katayama ML, Pasini FS, Folgueira MA, Snitcovsky IM, Brentani MM. J Steroid Biochem Mol Biol 2003; 84:57–69.
[101] Rao GA, Lew G, Larkin EC. Lipids 1984;19:151–3.
[102] Umeki S, Shiojiri H, Nozawa Y. FEBS Lett 1984;169:274–8.
[103] Beswick NS, Kennelly JJ. J Anim Sci 2000;78:412–9.
[104] Frick F, Linden D, Ameen C, Eden S, Mode A, Oscarsson J. Am J Physiol Endocrinol Metab 2002;283:E1023–0E1031.
[105] Hermier D, Catheline D, Legr P. Comp Biochem Physiol A Physiol 1996;115:259–64.
[106] Ideta R, Seki T, Adachi K, Nakayama Y. Dermatology 1998;196:47–50.
[107] Lefevre P, Diot C, Legr P, Douaire M. Arch Biochem Biophys 1999;368:329–37.
[108] Imai K, Kudo N, Koyam M, Shirahata A, Kawashim Y. Jpn J Pharmacol 2001;86:437–47.
[109] Pigeon C, Legr P, Leroyer P, Bouriel M, Turlin B, Brissot P, Loreal O. Biochim Biophys Acta 2001;1535:275–84.

[110] Samuel W, Nagineni CN, Kutty RK, Parks WT, Gordon JS, Prouty SM, et al. J Biol Chem 2002;277:59–66.
[111] Uryu S, Tokuhiro S, Oda T. Biochem Biophys Res Commun 2003;303:302–5.
[112] Kurebayashi S, Hirose T, Miyashita Y, Kasayama S, Kishimoto T. Diabetes 1997;46:2115–8.
[113] Kudo N, Nakagawa Y, Waku K, Kawashima Y, Kozuka H. Toxicology 1991;68:133–42.
[114] Weiner FR, Smith PJ, Wertheimer S, Rubin CS. J Biol Chem 1991;266:23425–8.
[115] Jones B, Maher MA, Banz WJ, Zemel MB, Whelan J, Smith P, et al. Am J Physiol 1996;34:E44–0E49.
[116] Miyazaki M, Ntambi JM. Prostaglins, leukotrienes, Essential Fatty Acids 2003;68:113–21.
[117] Ntambi JM, Makoto M. Curr Opin Lipidol 2003;14:255–61.
[118] Heinemann FS, Ozols J. Prostaglins. Leukotrienes Essential Fatty Acids 2003;68:123–33.