Stearoyl-CoA desaturase (SCD) is a rate-limiting enzyme catalyzing the synthesis of monounsaturated fatty acids, mainly oleate (18:1) and palmitoleate (16:1), which are a major component of tissue lipids. SCD1 deficient mice reveal increased energy expenditure and decreased body adiposity due to the upregulation of genes of fatty acid oxidation and the downregulation of genes of lipid synthesis in liver. In this review, we examine data showing that SCD is an important component in the regulation of skeletal muscle metabolism, which affects insulin sensitivity, mitochondrial fatty acid oxidation and ceramide de novo synthesis in oxidative myofibers. The lack of SCD1 gene increases the rate of fatty acid β-oxidation through activation of the AMP-activated protein kinase (AMPK) pathway and by upregulating genes of fatty acid oxidation in soleus and red gastrocnemius muscles. Consistent with increased β-oxidation, the contents of free fatty acids and long-chain acyl-CoAs are significantly decreased, which together with reduced mRNA level and activity of serine palmitoyltransferase led to reduced ceramide synthesis in oxidative muscles of SCD1-/- mice. Thus, reduced contents of free fatty acids, acyl-CoAs and ceramides as well as increased AMPK phosphorylation, might contribute to increased insulin sensitivity observed in muscle of SCD1-/- mice. SCD1 deficiency also results in downregulation of the expression of the protein-tyrosine phosphatase 1B, which is responsible for the sustained insulin receptor autophosphorylation despite reduced levels of plasma insulin in the SCD1-/- mice. SCD1 deficiency reduced ceramide synthesis, increased AMPK phosphorylation and carnitine palmitoyltransferase 1 activity also in soleus and red gastrocnemius muscles of leptin deficient ob/ob mice. These findings raise the possibility that SCD1 may be a downstream component of the leptin signaling pathway in skeletal muscle.

Key words: stearoyl-CoA desaturase, insulin, ceramide, oxidative muscles, lipid metabolism
INTRODUCTION

Mammalian stearoyl-CoA desaturase (SCD) belongs to a family of desaturases that has been highly conserved throughout evolution in animals, plants, and yeast (1). SCD is a microsomal enzyme that in conjunction with nicotinamide adenine dinucleotide, the flavoprotein cytochrome b
2\textsubscript{5} reductase, and the electron acceptor cytochrome b
2\textsubscript{5} and, in the presence of molecular oxygen, introduces a single double bond in a spectrum of methylene-interrupted fatty acyl-CoA substrates. The preferred substrates for SCD are palmitoyl- and stearoyl-CoA, which are converted to palmitoleoyl- and oleoyl-CoA, respectively (1). These products are the most abundant monounsaturated fatty acids in the various kinds of lipids, including phospholipids, triglycerides, cholesteryl esters, wax esters, and alkyl-diacylglycerols (2, 3). Apart from being components of lipids, monounsaturated fatty acids also serve as mediators of signal transduction and cellular differentiation, including neuronal differentiation (4). Monounsaturated fatty acids influence apoptosis (5, 6) and may play a significant role in mutagenesis of some tumors (7). Oleate has also been shown to regulate food intake (8).

Four mouse SCD isoforms (SCD1, SCD2, SCD3, and SCD4) have been characterized (9 - 12). The physiological role of each SCD isoform and the reason for having four or more SCD gene isoforms in the rodent genome are currently unknown. Under normal dietary conditions, SCD1 mRNA is highly expressed in white adipose tissue, brown adipose tissue, meibomian gland, Harderian and preputial glands (13) and is dramatically induced in liver in response to high carbohydrate diet (12). SCD2 is predominantly expressed in the brain (9). Similar to SCD1, SCD2 mRNA is expressed to a lesser extent in kidney, spleen, skeletal muscles, heart and lung where it is induced in response to a high carbohydrate diet (9, 14). In some tissues, such as the adipose and eyelid, both SCD1 and SCD2 genes are expressed whereas in the skin, Harderian and preputial glands a three gene isoforms (SCD1, SCD2, SCD3) are expressed (2, 13, 14). In skin, SCD1 expression is restricted to the undifferentiated sebocytes, while SCD3 is expressed mainly in the differentiated sebocytes (14). SCD4, which is expressed exclusively in the heart, demonstrates tissue-specific regulation by leptin (11). Humans have two characterized SCD genes, which show 85% homology to the murine SCD1 (10, 15).

Recent studies of asebia (ab\textsuperscript{1} and ab\textsuperscript{2}) mouse strains that have a natural mutation in the SCD1 gene (10) and a mouse model with a targeted disruption of the SCD1 gene (SCD1\textsuperscript{-/-}) (13) have provided evidence that SCD is a critical control point in lipid partitioning and body weight regulation. SCD1 deficiency results in increased energy expenditure (16) and decreased body adiposity due to the upregulation of genes of fatty acid oxidation (16, 17) and the downregulation of genes of lipid synthesis in liver (reviewed in (1)). SCD1 knockout mice are considerably leaner than their wild-type littermates and have decreased hepatic triglyceride synthesis and increased rate of β-oxidation in liver (17), brown adipose tissue (18) and skeletal muscles (19). SCD1 deficient animals have also higher basal thermogenesis
and are resistant to diet-induced weight gain and fat accumulation, despite increased food intake (16). Recent studies show that SCD1 is required for the fully developed obese phenotype of leptin deficient ob/ob mice (20) and suggest that a significant proportion of leptin's metabolic effect results from inhibition of this enzyme (20). SCD1 deficiency also attenuates overaccumulation of lipids in liver in peroxisome proliferator-activated receptor-α (PPARα)-deficient mice (21).

The molecular mechanism underlying the antisteatotic action of SCD1 deficiency is not completely understood. However, our study established that one mechanism is via increased activation of AMP-activated protein kinase (AMPK) (17, 19), that leads to phosphorylation and inactivation of acetyl-CoA carboxylase resulting in decreased malonyl-CoA content (22). Malonyl-CoA is both an intermediate in de novo synthesis of fatty acids and an allosteric inhibitor of carnitine palmitoyltransferase 1 (CPT1), the enzyme that transfers long-chain acyl-CoA molecules from the cytosol to the mitochondria where they are oxidized (23). A decrease in the cellular levels of malonyl-CoA in the liver and skeletal muscles of SCD1-/- mice would thus derepress CPT1, resulting in increased fatty acid oxidation and downregulation of fatty acid synthesis (23). The findings in the SCD1-/- mice are therefore similar to those observed in mice lacking acetyl-CoA carboxylase 2, which also have increased fatty acid oxidation in skeletal muscle and exhibit a lean phenotype (24).

The antisteatotic action of SCD1 deficiency involves also transcriptional effects. Loss of SCD1 function downregulates sterol regulatory binding protein 1c, a lipogenic transcription factor (25), thereby reducing the expression of lipogenic enzymes like fatty acid synthase, acetyl-CoA carboxylase and glycerol-3-phosphate acyltransferase in liver. SCD1 deficiency also upregulates the expression of hepatic genes involved in fatty acid β-oxidation (e.g. CPT 1, acyl-CoA oxidase, very long-chain acyl-CoA dehydrogenase) (16, 17).

In this review, we examine recent data showing that SCD1 is an important component in the regulation of skeletal muscle metabolism, which deficiency/downregulation increases insulin sensitivity and mitochondrial fatty acid oxidation but decreases ceramide de novo synthesis in oxidative muscles.

**REGULATION OF SCD1 EXPRESSION/ACTIVITY IN SKELETAL MUSCLES**

SCD expression is modulated by dietary factors (e.g. PUFA, cholesterol, vitamin A), hormonal signals (e.g. insulin, glucagon), environmental factors (temperature changes, metals, alcohol, thiazolidinediones), peroxisomal proliferators, and developmental processes (reviewed in (12)). Most of these factors have been shown to regulate SCD expression/activity in liver and adipose tissue, however some of them control also muscle SCD.

Among the dietary factors glucose seems to be the most important in regulation of SCD expression in skeletal muscle. As established by Houdali and co-workers (26) long-term glucose oversupply induces a rapid increase in SCD expression and
enzyme activity which leads to fast and specific changes in fatty acid metabolism. Treatment of muscle cells with high glucose (20 mmol/l) even mimicked the phenotype of SCD1 overexpression (27). Similar effect appeared when myotobules were incubated with high palmitate (200 mmol/l) (27). The other dietary factor affecting SCD1 expression is sucrose, which induces a time-dependent typical noninsulin-dependent diabetes syndrome characterized by insulin resistance in rats. Short time (3 weeks) of sucrose feeding decreased SCD1 mRNA and activity, whereas long term (6 months) of sucrose feeding increased the desaturase activity and mRNA level. This increase was consistent with an increase in oleic acid, the 20:4/18:2 ratio, and 22:4n-6 and 22:5n-6 acids in liver and muscle lipids (28). Neither blood insulin levels nor insulin resistance was a factor affecting SCD1 changes in mRNA and activity found with the sucrose-rich diet (28). On the other hand, Yechoor et al. (29) established that pure deficiency of insulin action present in muscle of insulin receptor knockout mice results in downregulation of SCD1.

In pig muscles a reduced protein diet is known to increase the level of intramuscular lipid. The reduced protein diet significantly increased SCD protein expression and activity as well as the level of monounsaturated and total fatty acids in muscle (30). Surprisingly, conjugated linoleic acid that was shown to decrease SCD1 gene expression in 3T3-L1 adipocytes (31) and in liver (32) has no significant effect on SCD activity in pig muscles (33, 34).

Two classes of nuclear receptor ligands, thiazolidinediones (TZDs) and rexinoids, have been shown to lower hyperglycemia and hyperinsulinemia in diabetic rodents (35). TZDs are ligands for PPAR-γ, a nuclear hormone receptor that has been demonstrated to be critically involved in regulating both the differentiation and metabolism of adipocytes (36). In skeletal muscle both rosiglitazone and roxinoid (LG100268) increased SCD1 mRNA level affecting insulin sensitivity in muscle of Zucker Diabetic Fatty (ZDF) rats (37). In cultured human muscle cells also agonist of liver X receptor - T0901317 strongly increased expression of genes encoding lipogenic enzymes, including SCD1, fatty acid synthase and sterol regulatory binding protein 1c, and promoted triglyceride accumulation in the presence of a high glucose concentration as well (38).

Furthermore, Ikeda and co-workers (39) established that training induces SCD1 mRNA level in muscles. Mice exercised by 2-week swimming had increased sterol regulatory element-binding protein 1 mRNA level in skeletal muscles (gastrocnemius, quadriceps), which led to increase in expression of lipogenic genes, such as SCD1, acetyl-CoA carboxylase 1 and diacylglycerol acyltransferase 1.

SCD1 DEFICIENCY INCREASES FATTY ACID OXIDATION IN MUSCLES

The studies showing that SCD1 regulates the rate of β-oxidation in the liver (17) together with findings that SCD1 expression in skeletal muscle is most
abundant in the muscles with high oxidative capacity (A. Dobrzyń, J.M. Ntambi, data not published), suggested that SCD could also be involved in regulation of muscle fatty acid oxidation. Indeed, our recent study has shown that SCD1 deficiency increases the rate of β-oxidation in soleus and red gastrocnemius muscles by activating of AMPK pathway (19). Consistent with increased β-oxidation, the contents of free fatty acids and long-chain acyl-CoAs were significantly lower in soleus and red gastrocnemius muscles of SCD1-/- mice. Interestingly, in white gastrocnemius of SCD1-/-, which is a glycolytic muscle, the rate of fatty acid oxidation was similar to that in wild type mice (19). The effect of SCD1 deficiency on β-oxidation and the AMPK pathway is thus similar to that observed after leptin treatment, because leptin was shown to activate AMPK in oxidative muscles (soleus and red gastrocnemius) but not in glycolytic muscles (white gastrocnemius or extensor digitorum longus) (40). Further, SCD1 deficiency increases AMPK phosphorylation and CPT1 activity also in the oxidative muscles of ob/ob mice. These findings raise the possibility that SCD1 may be a downstream component of the leptin signaling pathway in muscle as it has been previously shown in the liver (20). Further studies are underway to determine the role of SCD1 in leptin signaling in skeletal muscle.

The relationship between SCD1 expression and rate of β-oxidation was confirmed by using of other physiological models. In muscle of obese humans, high expression of SCD1 corresponded with low rates of fatty acid oxidation (decreased AMPK activity), increased triglyceride synthesis and increased monounsaturation of muscle lipids. Contrary, overexpression of human SCD1 in myotubes from lean subjects was sufficient to mimic the obese phenotype (41). Furthermore, in mice with inactivation of the gene perilipin, an adipocyte lipid droplet surface protein, SCD1 gene is significantly downregulated (42). It produced lean and obesity resistant phenotype by a coordinated upregulation of genes involved in β-oxidation, the Krebs cycle, and the electron transport chain concomitant with a downregulation of genes involved in lipid biosynthesis. The parallel observation between SCD1-/- and plin-/- mice confirmed that SCD1 expression directly or indirectly e.g. by increase of the relative or absolute amount of saturated fatty acids, concomitant with a decrease of unsaturated fatty acids in the tissue, contribute to enhanced fatty acid oxidation in skeletal muscles.

SCD1 AND MUSCLE CERAMIDE METABOLISM

It is now appreciated that ceramide is a regulated bioactive lipid whose metabolism and actions are closely related to lipoapoptosis and insulin resistance in muscle cells (43, 44). The signaling pool of ceramide is generated by sphingomyelin hydrolysis and/or by de novo synthesis. The first committed step in de novo ceramide synthesis, the esterification of palmitoyl-CoA and serine, is catalyzed by serine palmitoyl transferase (SPT). Transcriptional regulation of
SPT has been observed in response to several types of inflammatory and stress stimuli (45), leptin-receptor mutation (43) and activation of AMPK (44), and has been suggested to be in response to changes in intracellular fatty acid content. A study on CHO cells has shown that oleic acid, the major product of SCD, may play an important role in regulation of intracellular ceramide synthesis (6).

Recently we have shown that loss of SCD1 function decreases de novo ceramide synthesis by downregulating SPT activity and the expression of both SPT subunits (LCB1 and LCB2) in the oxidative skeletal muscles. It caused the reduction in the total content of ceramide in the soleus and red gastrocnemius by 42 and 48%, respectively, in SCD1-/- mice compared with wild-type controls. The contents of fatty acyl-CoAs were also reduced (19). Other pathway of ceramide formation is through sphingomyelin hydrolysis by neutral and/or acid sphingomyelinases. SCD1 deficiency decreased the sphingomyelin content in soleus and red gastrocnemius muscles of SCD1-/- mice by 24 and 29%, respectively, compared with wild-type mice, but did not affect gene expression nor activities of sphingomyelinases in the three muscle types. It suggests that rate of ceramide formation by sphingomyelin hydrolysis is not regulated by SCD (19).

As mentioned above, the rate of fatty acid β-oxidation was increased in oxidative muscles of SCD1-/- mice. Because the effect of SCD1 deficiency on ceramide content and the rate of β-oxidation was less pronounced in white gastrocnemius, reduction of ceramide synthesis in oxidative muscles of SCD1-/- mice appears to be largely the result of increased rates of β-oxidation. AMPK has been shown to downregulate various ATP consuming anabolic pathways, including fatty acid and cholesterol synthesis (22), and might also participate in the regulation of ceramide synthesis. Indeed, 5-aminoimidazole- 4-carboxamide (AICA) riboside, which enters cells and is converted to AICA ribotide, an ATP analog, has been shown to inhibit palmitate-induced SPT activity, LCB2 gene expression, and de novo ceramide synthesis in rat astrocytes (44) and bovine retinal pericytes (46). Consequently, in both oxidative muscles of SCD1-/- mice, changes in AMPK phosphorylation parallel the downregulation of SPT and decrease in the total ceramide content. SCD1 deficiency also decreased the activity of SPT and the total content of ceramide in oxidative muscles, but not in the white gastrocnemius of double-mutant ab+/ab+/ob/ob mice relative to ab+/ab+;ob/ob mice (19). Taken together, these data indicate that activation of AMPK and an increase in β-oxidation, via decreasing intramuscular palmitoyl-CoA content, are the main factors causing downregulation of SPT activity and reduction in ceramide synthesis in skeletal muscle of SCD1-deficient mice.

SCD1 AND INSULIN SIGNALING IN SKELETAL MUSCLES

As mentioned above, long-term glucose oversupply induces SCD expression and enzyme activity in skeletal muscle which leads to fast and specific changes in fatty
acid metabolism possibly contributing to the insulin resistance (26). In humans, an abundance of the SCD1 product palmitoleic acid has been described in insulin resistance and obesity (47). Reduced adipose tissue mass could either elicit insulin resistance or insulin sensitivity as demonstrated in several animal models (48).

The whole body glucose tolerance is much greater in the SCD1-/- mice than in control animals. Fasting insulin levels are lower in the male SCD1-/- on chow diet compared with the wild type mice (16). On a high-fat diet, insulin levels are similar in the two groups. However, after a 30-min glucose load both male and female SCD1-/- mice tend to have lower fasting plasma glucose levels and show improved glucose tolerance compared with the wild type mice (16). In addition, the glucose-lowering effect of insulin as demonstrated by the insulin tolerance test is greater in the SCD1-/- mice than wild type mice. However, when the SCD1 mutation was introduced in mice with lipodystrophy, the mutant mice had reduced insulin levels coupled with increased glucose levels (49) suggesting that there might be a β-cell failure. The mechanism of reduced insulin levels and a possible β-cell failure associated with SCD1 deficiency are still being investigated.

Increased fatty acid oxidation leads to decrease in free fatty acids, fatty acyl-CoAs and ceramide levels in the skeletal muscles of SCD1-/- mice (19). Accumulation of free fatty acids, fatty acyl-CoAs, ceramides and 1,2 diacylglycerols, lead to insulin resistance in skeletal muscle (50). By activating protein kinase C, the lipid molecules seem to reduce insulin receptor substrate 1 (IRS1) phosphorylation and the activity of Akt, leading to impaired GLUT4 translocation to the plasma membrane (50). Recent studies have shown that AMPK activation ameliorates insulin resistance induced by high-fat diet in muscle (51). In fat-fed rats a single injection of 5-amino-4-imidazolecarboxamide riboside (AICAR) (51) or exercise (52), both of which increase AMPK activity, cause increase in insulin stimulated glucose uptake in muscle 24 h later. AICAR has also been shown to increase insulin-stimulated glucose uptake by the muscle of control rats (51). Thus, reduced contents of free fatty acids, long-chain acyl-CoAs and ceramides as well as increased AMPK phosphorylation (19), might contribute to increased insulin sensitivity in muscle of SCD1-/- mice.

SCD1 deficiency also results in the downregulation of the expression of the protein-tyrosine phosphatase 1B (PTP-1B), an enzyme that catalyzes the rapid dephosphorylation of the insulin receptor (IR) and IRS1 and IRS2 (53, 54). The downregulation of the PTP-1B expression and activity is responsible for the sustained insulin receptor autophosphorylation despite reduced levels of plasma insulin in the SCD1-/- mice. Insulin mediated glucose uptake was also higher in the soleus muscle from SCD1-/- suggesting that the IR is more responsive to insulin in the SCD1-/- than in SCD1+/+ mice. Consistent with these observations, PTP-1B knockout mice exhibit increased tyrosine phosphorylation of the IR and IRS-1 in muscle (55). The PTP-1B-/- mice also show increased insulin sensitivity and are also resistant to diet induced obesity. Thus, the phenotypes exhibited by the PTP-1B-/- mice in many ways are similar to those
of the SCD1-/- mice. It is not known at present whether PTP-1B is a downstream target of SCD1 expression or whether the decrease observed in expression is a secondary consequence of altered lipid homeostasis, owing to changes in intracellular lipid levels, as a result of SCD1 deficiency.

The other possible mechanism that could lead to increased insulin signaling is an alteration in the properties of the cell membrane, which is composed largely of lipid and activates the IR. Oleate is the major monounsaturated fatty acid found in membrane phospholipids and the ratio of saturated to monounsaturated fatty acids has been implicated in alteration of membrane fluidity (12). It is proposed that the decrease in the monounsaturated fatty acid content of the membrane phospholipids in the SCD1-/- mice is compensated by polyunsaturated fatty acids causing a greater increase in membrane fluidity owing to the presence of more double bonds in the fatty acyl chain. Recent data show that the degree of insulin resistance in rodents and humans is inversely correlated to the amount of polyunsaturated fatty acids within skeletal muscle phospholipid (56). The increased membrane fluidity would enhance IR aggregation thus increasing its phosphorylation upon insulin binding. More studies will, however, be required to demonstrate a direct correlation between insulin sensitivity and membrane fluidity.

Connection between SCD1 and insulin signaling pathway was also observed in muscle insulin receptor knockout mice, where pure deficiency of insulin action present in these animals results in downregulation of SCD1, as well as upregulation of some signaling related genes, such as Akt2, and the fatty acid transporter CD36 (29). Reverse, Voss et al. (27) showed that stable overexpression of SCD1 in muscle cells decreased tyrosine-phosphorylation of IRS1 and serine473 phosphorylation of Akt1/protein kinase B and is sufficient to impair glucose uptake and insulin signaling. Moreover, insulin-resistant skeletal muscle of ZDF rats is characterized by a specific gene expression profile with increased levels of SCD1 (27). This supports the hypothesis that elevated SCD1 expression is a possible cause of insulin resistance and type 2 diabetes.

CONCLUSION

As described in the present paper, SCD appears to be an important component in the regulation of skeletal muscle metabolism. The lack of SCD1 gene increases the rate of fatty acid β-oxidation through activation of the AMPK pathway and by upregulating genes of fatty acid oxidation in oxidative muscles (Fig. 1). As a result of increased β-oxidation, the contents of free fatty acids and long-chain fatty acyl-CoAs are significantly decreased, which together with reduced mRNA level and activity of serine palmitoyltransferase led to reduced de novo ceramide synthesis in soleus and red gastrocnemius of SCD1-/- mice. Thus, decreased contents of free fatty acids, acyl-CoAs and ceramides as well as increased AMPK phosphorylation, might contribute to increased insulin sensitivity observed in
muscle of SCD1-/- mice. SCD1 deficiency also results in the downregulation of the expression of PTP-1B, which is responsible for the sustained insulin receptor autophosphorylation. We propose that SCD might be a potential therapeutic target in the treatment of the insulin resistance, especially that SCD1 expression in skeletal muscle is regulated by factors that also affect insulin signaling pathway.

Acknowledgement: This paper has been supported by Medical University of Białystok grant: 3-18623.

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Received: October 13, 2006
Accepted: November 20, 2006

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