Stearoyl-CoA desaturase 1 deficiency increases insulin signaling and glycogen accumulation in brown adipose tissue

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Rahman, Shaikh Mizanoo, Agnieszka Dobrzyn, Seong-Ho Lee, Pawel Dobrzyn, Makoto Miyazaki, and James M. Ntambi. Stearoyl-CoA desaturase 1 deficiency increases insulin signaling and glycogen accumulation in brown adipose tissue. Am J Physiol Endocrinol Metab 288: E381–E387, 2005. First published October 19, 2004; doi:10.1152/ajpendo.00314.2004.—Stearoyl-CoA desaturase (SCD) catalyzes the synthesis of oleate (C18:1) and palmitoleate (C16:1), which are the main monounsaturated fatty acids of membrane phospholipids, triglycerides, wax esters, and cholesterol esters. Previously, we showed that SCD1 deficiency elevates insulin-signaling components and downregulates protein-tyrosine phosphatase-1B (PTP-1B) in muscle, a major insulin-sensitive tissue. Here we found that, in brown adipose tissue (BAT), another insulin-sensitive tissue, the basal tyrosine phosphorylations of insulin receptor (IR) and IR substrates (IRS-1 and IRS-2) were upregulated in SCD1−/− mice compared with wild-type mice. The association of IRS-1 and IRS-2 with the α-PS5 subunit of phosphatidylinositol 3-kinase as well as Akt-Ser73 and Akt-Thr308 phosphorylation is also elevated in the SCD1−/− mice. The mRNA expression, protein levels, and activity of PTP-1B implicated in the attenuation of the insulin signal are reduced in the SCD1−/− mice. The content of GLUT4 in the plasma membrane increased 2.5-fold, and this was accompanied by a 6-fold increase in glucose uptake in BAT of SCD1−/− mice. The increased glucose uptake was associated with higher glycogen synthase activity and glycogen accumulation. In the presence of insulin, [U-14C]glucose incorporation into glycogen was increased in BAT of SCD1−/− mice. Taken together, these studies illustrate increased insulin signaling and increased glycogen metabolism in BAT of SCD1−/− mice.

Stearoyl-CoA desaturase; brown adipose tissue; insulin signaling

STEAROYL-COA DESATURASE (SCD) is a microsomal enzyme that catalyzes the synthesis of monounsaturated fatty acids from saturated fatty acyl-CoA. The preferred substrates for SCD are palmitoyl- and stearoyl-CoA, which are converted to palmitoleoyl- and oleoyl-CoA, respectively (9). These monounsaturated fatty acids are components of membrane phospholipids, triglycerides, wax esters, and cholesterol esters (24–26). The saturated-to-monounsaturated fatty acid ratio affects membrane phospholipid composition, and alteration in this ratio has been implicated in a variety of disease states including cardiovascular disease, obesity, diabetes, neurological disease, skin disorders, and cancer (1, 14, 30, 31, 36, 37). For this reason, the expression of SCD is of physiological importance in normal and disease states. Two human and four mouse SCD isoforms (SCD1, SCD2, SCD3, and SCD4) have been characterized (3, 15, 23, 27, 28, 44). 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. However, new insights into the physiological role of the SCD1 gene and its endogenous products have come from recent studies of mouse models that have a disruption in the SCD1 gene (26, 44–46). Using these mouse models, we have shown that SCD1 expression is required in the synthesis of tissue triglycerides, cholesterol esters, wax esters, and 1-alkyl-2,3-diacylglycerol (24–26). The role of SCD1 expression in VLDL synthesis and secretion was also demonstrated (24).

A reduction in triglyceride synthesis and levels would lead to a decrease in generalized steatosis and improved glucose transport in insulin-sensitive tissues such as muscle and heart (39). Impairment of the glucose transport in the insulin-sensitive tissues contributes to the pathogenesis of type 2 (non-insulin-dependent) diabetes mellitus (4, 11). Brown adipose tissue (BAT) is an insulin-sensitive tissue in rodents and contains the main elements of the insulin signaling system (32, 40, 41). Thus, in these cells, the binding of insulin to its receptor leads to activation of the receptor kinase and tyrosine phosphorylation of several insulin receptor (IR) substrates (IRS), including IRS-1 and IRS-2 (42, 19). These, in turn, interact with and activate phosphatidylinositol 3-kinase (PI 3-kinase) (2), leading to activation of the main downstream effector Akt and stimulation of glucose uptake, glycogen synthesis, and protein synthesis (18, 38, 10). Previously, we (29) showed that mice with a targeted disruption in SCD1 gene are resistant to diet-induced weight gain and have increased insulin sensitivity compared with wild-type controls (29). The SCD1−/− mice also have increased energy expenditure (29) and basal thermogenesis (20).

In the current study, we have investigated the link between the loss of SCD1 function and insulin signaling in BAT. We provide evidence for a significant increase in insulin-signaling components in BAT of SCD1−/− mice, as demonstrated by an increase in basal tyrosine phosphorylation of the IR, IRS-1, and IRS-2; increased association of IRS-1 and IRS-2 with PI 3-kinase; increased phosphorylation of Akt/PKB; and reduction in expression and activity of protein-tyrosine phosphatase-1B (PTP-1B). The dramatic increase in glucose uptake leads to increased glycogen accumulation in BAT of the SCD1−/− mice.

MATERIALS AND METHODS

Animal experiments. The generation of targeted SCD1−/− mice has been previously described (26). Purebred homozygous (SCD1−/−) and wild-type (SCD1+/+) male mice on an SV129 background were

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used. Mice were maintained on a 12:12-h dark-light cycle and were fed a normal nonpurified diet (5008 test diet; PMI Nutrition International, Richmond, IN). Mice were housed and bred in a pathogen-free facility of the Department of Biochemistry (Univ. of Wisconsin, Madison, WI). The breeding of these animals was in accordance with the protocols approved by the Animal Care Research Committee of the University of Wisconsin-Madison. Male SCD1+/− and SCD1+/+ mice were killed at 12 wk of age without fasting. BAT was isolated and used throughout the study.

Evaluation of phosphorylation status of insulin-signaling proteins. The phosphorylation assays were carried out as described (17). BAT were homogenized and centrifuged at 12,000 g for 30 min in ice-cold 50 mM HEPES buffer (pH 7.4) containing 137 mM NaCl, 1 mM MgCl2, 1 mM CaCl2, 10 mM Na3PO4, 10 mM NaF, 2 mM EDTA, 2 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml aprotinin, and 10% glycerol. Supernatants were collected, and protein concentration was measured with Bradford protein assay reagent (Bio-Rad), using BSA as standard. Tissue homogenates (500 μg protein) were then immunoprecipitated with 4 μg of anti-IR, -IGF-1 receptor, -IRS-1, or -IRS-2 antibodies for 18 h. Immunoprecipitates were washed three times by brief centrifugation and gentle suspension in ice-cold homogenization buffer plus 0.1% SDS and then subjected to SDS-PAGE on 10% gradient gel. Proteins were transferred and immobilized on Immobilon-P transfer membrane. The membranes were immunoblotted with anti-phosphotyrosine antibodies, and bands were visualized using enhanced chemiluminescence and quantified by densitometry. To measure the IRS-1 or IRS-2-associated α-p85 subunit of PI 3-kinase, equal amounts of protein (500 μg) were immunoprecipitated with either IRS-1 or IRS-2 and then immunoblotted with an antibody specific to the α-p85 subunit of PI 3-kinase. Akt/PKB Ser and Thr phosphorylation was measured using phosphoSer473 and -Thr308 antibodies (Cell Signaling Technology, Beverly, MA). Immunoprecipitation and Western blotting procedures are the same as those described for IR, IRS-1, and IRS-2 tyrosine phosphorylations.

PTP-1B expression. The PTP-1B protein levels were assessed by immunoblotting, using polyclonal antibodies. PTP-1B mRNA levels were measured by RT-PCR, as described (33), and the activity of PTP-1B was measured by 10.220.33.6 on April 7, 2017 http://ajpendo.physiology.org/ Downloaded from

RESULTS

Increased basal tyrosine phosphorylation of IR and IRSs in BAT of SCD1+/− mice. To assess the phosphorylation status of the IR, immunoprecipitated IR were subjected to Western blotting with anti-phosphotyrosine antibodies (Fig. 1A). Densitometric analysis revealed that the basal IR tyrosine phosphorylation was 1.7-fold higher in BAT of SCD1+/− mice (n = 4 mice/group, P < 0.05) than in wild-type mice. To determine whether phosphorylation of the proximal elements of the insulin-signaling cascade was also increased in the basal state, we assessed the degree of IRS-1 and IRS-2 tyrosine phosphorylation as well as protein levels. IRS-1 tyrosine phosphorylation was 1.6-fold higher in BAT of SCD1+/− mice (n = 4 mice/group, P < 0.05) compared with wild-type mice (Fig. 1B). IRS-2 tyrosine phosphorylation was about threefold higher in the SCD1+/− mice (n = 4 mice/group, P < 0.001) than in controls (Fig. 1C). There was no significant difference in the IR, IRS-1, and IRS-2 protein levels between the two groups of mice. We also examined tyrosine phosphorylation of IGF-I receptor but did not find any changes between the two groups of mice (Fig. 1D).

Increased α-p85 association with the IRS in BAT of SCD1+/− mice. When tyrosine residues of IRS are phosphorylated, they associate with the α-p85 subunit of PI 3-kinase, resulting in its activation (43). The association of the α-p85 subunit of PI 3-kinase with IRS-1 and IRS-2 (Fig. 1E) was 1.3-fold (n = 4 mice/group, P < 0.05) and 1.6-fold higher (n = 4 mice/group, P < 0.01), respectively, in SCD1+/− mice than in SCD1+/+ mice. There was no change in the levels of α-p85 protein (Fig. 1D).

Reduced PTP-1B in BAT of SCD1+/− mice. Protein-tyrosine phosphatases, particularly PTP-1B, attenuate insulin signaling by dephosphorylating the IR and the IRSs. In muscle, PTP-1B protein levels and activity are reduced in SCD1+/− mice (33). To investigate the possible role of PTP-1B in the insulin signal transduction in BAT, we measured PTP-1B mRNA levels, protein content, and activity in BAT of SCD1+/− and SCD1+/+
mice. Figure 2A shows that the PTP-1B mRNA levels were decreased by 55%. Consistent with a reduction in PTP-1B mRNA levels, the PTP-1B protein levels were 75% lower (n = 4 mice/group; Fig. 2B), and the PTP-1B activity in BAT of SC1D1−/− mice was 51% lower (n = 4 mice/group; P < 0.001) compared with control mice (Fig. 2C). The protein levels of leukocyte common antigen-related protein (LAR) were similar in SC1D1+/+ and SC1D1−/− mice (Fig. 2B).

Increased phosphorylation of Akt/PKB in the SC1D1−/− mice. To investigate insulin-signaling status downstream of PI 3-kinase, we examined the phosphorylation of Ser473 and Thr308 of Akt/PKB, a key Ser/Thr kinase that mediates many metabolic effects of insulin, including activation of GLUT4 translocation to the plasma membrane (38). The immunoblot analysis and the densitometric analysis show that Ser473 and Thr308 (Fig. 3) phosphorylations were seven- (n = 4 mice/group; P < 0.001; Fig. 3A) and fivefold higher (n = 4 mice/group; P < 0.001), respectively, in SC1D1−/− mice compared with SC1D1+/+ mice. The GAPDH antibody was used as a control for loading, and, as shown, the GAPDH levels were not altered in the plasma membranes of the SC1D1−/− and SC1D1+/+ mice. We then measured in vivo DG uptake in BAT to determine whether the increase in GLUT4 levels in the plasma membrane of the SC1D1−/− mice results in increased glucose uptake. Radioactive DG was injected intravenously, and its distribution in BAT of SC1D1−/− and SC1D1+/+ mice was determined. Radioactive mannitol was used as an internal control. There was a sixfold increase in 2-DG content (n = 4

SC1D1+/+ mice (Fig. 4A). Densitometric analysis shows that the GLUT4 levels in the plasma membrane of SC1D1−/− mice are ~2.5-fold higher (n = 4 mice/group; P < 0.001) compared with SC1D1+/+ mice. The GAPDH antibody was used as a control for loading, and, as shown, the GAPDH levels were not altered in the plasma membranes of the SC1D1−/− and SC1D1+/+ mice. We then measured in vivo DG uptake in BAT to determine whether the increase in GLUT4 levels in the plasma membrane of the SC1D1−/− mice results in increased glucose uptake. Radioactive DG was injected intravenously, and its distribution in BAT of SC1D1−/− and SC1D1+/+ mice was determined. Radioactive mannitol was used as an internal control. There was a sixfold increase in 2-DG content (n = 4
mice/group, \( P < 0.001 \) in BAT of \( SCD1^{--} \) mice compared with \( SCD1^{++} \) mice (Fig. 4B).

**Increased glycogen synthesis in BAT of \( SCD1^{--} \) mice.** To determine whether increased glucose uptake leads to increased glycogen synthesis, we measured the activities of two key enzymes in glycogen metabolism: glycogen synthase and glycogen phosphorylase. Both the total and active forms of glycogen synthase were 1.8- (\( n = 4 \) mice/group, \( P < 0.05 \)) and 2.3-fold higher (\( n = 4 \) mice/group, \( P < 0.01 \)), respectively, in the BAT of \( SCD1^{--} \) mice (Fig. 5A). Total glycogen phosphorylase and the active form of glycogen phosphorylase activities in BAT were similar between the \( SCD1^{--} \) mice and wild-type mice (Fig. 5B). To determine whether increased glycogen synthesis resulted in net glycogen accumulation, we measured glycogen content in the BAT of \( SCD1^{--} \) and \( SCD1^{++} \) mice. Chemical determination of glycogen showed a

Fig. 5. Activity of glycogen synthase (A) and glycogen phosphorylase (B) and glycogen content (C) in BAT of \( SCD1^{++} \) and \( SCD1^{--} \) mice. Enzyme activities were assayed in homogenates as described in MATERIALS AND METHODS. G6P, glucose 6-phosphate. Data are means \( \pm SD; n = 4 \) mice/group. *\( P < 0.05 \), **\( P < 0.01 \), and ***\( P < 0.001 \) vs. \( SCD1^{++} \).
SCD deficiency elevates insulin-signaling components and downregulates PTP-1B in muscle (33). The present study investigated whether loss of the SCD1 gene has any impact on insulin-signaling components in BAT. We showed in this study that loss of SCD1 function in mice leads to increased basal state tyrosine phosphorylation of IR, IRS-1, and IRS-2 in BAT. The SCD1 deficiency does not influence IGF-I receptor phosphorylation and is therefore specific to the insulin-signaling pathway in the BAT of SCD1−/− mice.

There are several mechanisms by which SCD1 deficiency could lead to increased basal tyrosine autophosphorylation of the IR, despite lower levels of plasma insulin in the SCD1−/− mice. The mechanism that is consistent with our results is that loss of SCD1 function results in the downregulation of the expression of the protein-tyrosine phosphatase, an enzyme that would catalyze the rapid dephosphorylation of the IR and IRS-1, attenuating the insulin response. Indeed, we found reduced expression of PTP-1B mRNA, protein levels, and enzyme activity in the BAT of SCD1−/− mice. Consistent with our observations, PTP-1B knockout mice exhibit increased basal tyrosine phosphorylation of the IR and IRS-1 in muscle and have increased insulin sensitivity (8). Increased IR tyrosine phosphorylation and basal glucose uptake have been observed in the heart and white adipose tissue, which are insulin-sensitive tissues (Rahman SM and Ntambi JM, unpublished data). The results presented here and the recent results shown in muscle (33) show that the insulin-signaling pathway involving the downregulation of PTP-1B activity is partly responsible for the sustained IR autophosphorylation in insulin-sensitive tissues. PTP-1B−/− mice also show increased insulin sensitivity and, like the SCD1−/− mice, are resistant to diet-induced obesity (8). Thus the phenotypes exhibited by PTP-1B−/− mice in many ways are similar to those of the SCD1−/− mice (8, 29).

Further experiments will be required to determine how SCD1 deficiency leads to downregulation of PTP-1B expression in insulin-sensitive tissues.

The series of protein phosphorylations on the signaling molecules downstream of the IR culminates in uptake of glucose into cells by the glucose transporter GLUT4. The mechanism by which GLUT4-containing vesicles become activated and dock at the plasma membrane seems to involve Akt activation by PI 3-kinase phosphorylation (18). Akt also appears to participate in the insulin-signaling pathway by phosphorylating glycogen synthase kinase-3, which in turn activates glycogen synthase to promote glycogen synthesis (5, 22). We found increased association of PI 3-kinase with IRS-1 and IRS-2 and increased Ser and Thr phosphorylation of total Akt in the BAT of SCD1−/− mice. These results indicate that increased insulin signaling, followed by increased glucose transport and increased glycogen synthesis, ultimately leads to increased glycogen accumulation in the BAT of SCD1−/− mice. Unlike in muscle tissue, we found that BAT glycogen phosphorylase activity was similar between the SCD1−/− and wild-type mice. The physiological relevance for increased accumulation of glycogen in the SCD1−/− mice is not clear, but it may be that the presence of adequate stored carbohydrate is required to sustain the increased fatty acid oxidation observed in these mice (29). This hypothesis would be consistent with the concept that lipid metabolism depends on carbohydrate metabolism.

It has been reported that leanness is accompanied by increased energy expenditure and insulin sensitivity in mice (8). Therefore, it is also possible that the increased insulin sensitivity in the SCD1−/− mice may be due to the fact that they are leaner, with less body fat, than the wild-type control animals (24, 29). SCD1−/− mice do not gain weight even after being fed very high lipogenic (high fat and high carbohydrate) diets (29, 28). On the other hand, the experiments leading to weight loss in wild-type animals, to match them with SCD1−/− mice (e.g., exercise, caloric restriction), automatically led to increased insulin sensitivity and, more importantly, downregulated SCD (7, 28). These experiments could not provide a definitive answer regarding the primary cause of increased insulin sensitivity in the SCD1−/− mice. The only experimental model that could be used to demonstrate that SCD deficiency itself is enough to improve insulin sensitivity is the generation of tissue-specific SCD1 knockout mice. The hypothesis that SCD activity is coupled to the insulin-signaling pathway is supported by our study (16) showing that thiazolidinediones, antidiabetic compounds that are peroxisome proliferator-activated receptor-γ agonists and increase insulin sensitivity in mice, also repress SCD1 gene expression (16).

In summary, the present work demonstrates that SCD1 deficiency leads to increased insulin signaling and glycogen metabolism in BAT. Leanness that arises from SCD1 deficiency, as reported in our previous studies, might in part be responsible for increased insulin signaling. The increased tyrosine phosphorylation of IR and IRS-1 and activation of Akt/PKB are known events that lead to increased glucose uptake and glycogen accumulation in muscle and other insulin-sensitive tissues. The mechanism of sustained autophosphorylation of the IR in the BAT of SCD1−/− mice is similar to that in muscle and seems to be due to the downregulation of PTP-1B activity. Reduction in PTP-1B activity has been associated with increased insulin signaling and reduction in insulin resistance in mice. The results provide further evidence that the SCD1 gene may serve as a potential target in the treatment of insulin resistance and diabetes.

GRANTS

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