Biochemical and Biophysical Research Communications **266,** 1–4 (1999)
Article ID bbrc.1999.1704, available online at http://www.idealibrary.com on IDEAL®

BREAKTHROUGHS AND VIEWS

Regulation of Stearoyl-CoA Desaturase Genes: Role in Cellular Metabolism and Preadipocyte Differentiation

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Received October 6, 1999

The degree of fatty acid unsaturation in cell membrane lipids determines membrane fluidity, whose alteration has been implicated in a variety of disease states including diabetes, obesity, hypertension, cancer, and neurological and heart diseases. Stearoyl-CoA desaturase (SCD) is a key rate-limiting enzyme in the synthesis of unsaturated fatty acids by insertion of a *cis*-double bond in the Δ9 position of fatty acid substrates. Palmitate and stearate are the preferred substrates, which are converted to palmitoleate and oleate, respectively. These monounsaturated fatty acids are the major constituents of cellular membrane phospholipids and triacylglycerol stores found in adipose tissue. Two mouse and rat *SCD* genes (*SCD1* and *SCD2*) have been cloned and characterized. During the differentiation of 3T3-L1 preadipocytes into adipocytes, *SCD1* and *SCD2* mRNAs are induced concomitant with increased *de novo* synthesis of palmitoleate and oleate. The physiological significance of expressing the two isoforms in the adipocytes is currently unknown. In this review we discuss the role of the SCD isoforms in metabolism and the recent findings on the differential regulation of mouse *SCD* genes by the antidiabetic thiazolidinediones (TZDs), during preadipocyte differentiation. © 1999 Academic Press

The rate-limiting step in the biosynthesis of monounsaturated fatty acids is the insertion of a *cis*-double bond in the Δ9 position (between carbons 9 and 10 atoms) of methylene-interrupted fatty acyl-CoA substrates. This oxidative reaction is catalyzed by the stearoyl-CoA desaturase (SCD) and involves cytochrome b_5, NADP(H)-dependent cytochrome b_5 reductase and molecular oxygen (1–3). Although several substrates for the desaturase including vaccenic acid (4) are known, the preferred substrates are palmitoyl- and stearoyl-CoA which are converted to palmitoleoyl- and oleoyl-CoA, respectively (3). Palmitoleate (C16:1Δ9) and oleate (C18:Δ9) are the major (58%) constituents of membrane phospholipids and triacylglycerol stores found in differentiated 3T3-L1 adipocytes and in mouse adipose tissue *in vivo* (5). The balance and/or the ratio between saturated and monounsaturated fatty acids directly influence the membrane fluidity and its physical properties, and alterations in the ratio of these fatty acids have been implicated in a range of disease states including diabetes, obesity, hypertension, cancer, neurological, vascular and heart diseases (6). The regulation of the SCD is therefore of considerable physiological importance and its activity is sensitive to hormonal, developmental and environmental factors (7).

The mouse and rat genome contain two well characterized structural genes (*SCD1* and *SCD2*) that are highly homologous at the nucleotide and amino acid level and encode the same functional protein (8–11). Recently, a single human *SCD* gene was cloned and characterized (12). Other *SCD* cDNAs and genes have been isolated from different species including yeast (13), ovine (14), and hamster (15), and their regulation is currently being studied. Despite the fact that both mouse *SCD* genes are structurally similar, sharing ~87% nucleotide sequence identity in the coding regions, their 5′flanking regions differ resulting in divergent tissue-specific gene expression. Under normal dietary conditions, mouse and rat *SCD1* mRNAs are expressed constitutively in adipose tissue and are markedly induced in liver upon feeding fasted mice or rats a fat-free high carbohydrate diet, correlating pos-

Abbreviations used: SCD, stearoyl-CoA Δ9 desaturase, MDI, differentiation cocktail consisting of methylisobutylxanthine, dexamethasone, and insulin; PPARγ, peroxisome proliferator-activated receptor; aP2, adipocyte lipid binding protein.

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0006-291X/99 $30.00

itively with *SCD1* gene transcription (8, 9, 16). Similar to *SCD2*, *SCD1* mRNA is expressed to a lesser extent in kidney, spleen, heart, and lung in response to a high carbohydrate diet (6, 17, 18). The *SCD2* mRNA is expressed in B cells (19), whereas mature T cells do not express either isoform (17, 19). The *SCD2* gene is developmentally induced in brain during the neonatal myelinating period (20) and downregulated during lymphocyte development (21). The physiological significance of having two isoforms and their different tissue distribution is not currently known, but could be related to the substrate specificity of each SCD isoforms and their regulation in a tissue-specific manner for determining and maintaining cellular lipid composition.

Over the past several years we have studied the genetic regulation of the mouse stearoyl-CoA desaturase by hormonal and dietary factors, especially in response to dietary polyunsaturated fatty acids and cholesterol (7, 22). This review discusses advances made in the understanding of the role of SCD in cellular metabolism with emphasis and our recent findings on the differential regulation of *SCD* genes by thiazolidinediones during preadipocyte differentiation.

ROLE OF SCD IN FATTY ACID BIOSYNTHESIS AND CELLULAR METABOLISM

Lipids play important biological roles in the living cell serving primarily as constituents of lipid membranes and as a storage form of energy in adipose tissue. The major role of the adipose tissue is to store excess calories in the form of triacylglycerides that are mobilized and utilized during the periods of energy deprivation. When dietary intake of fat is insufficient for daily requirements, animals derive fatty acids mainly from *de novo* synthesis. This is accomplished by the fatty acid synthase complex (FAS) which converts acetyl-CoA and malonyl-CoA to palmitate, which is the end product of the pathway (23). Palmitate can then serve as a substrate for the microsomal maloyl-CoA dependent elongase to produce stearate (C18:0). Because stearate and to a lesser extent palmitate are insoluble to be stored (24, 25), the end product of this pathway is usually oleate (C18:1Δ9) and palmitoleate (Fig. 1).

Regardless of diet, country of origin, sex, and age, the dominant storage form of fatty acids in human adipose tissue have long been recognized as palmitoleate and oleate (25). The composition of monounsaturated fatty acids in adipose tissue tends to reflect the average fatty acid composition of dietary fat (26, 27), but it is not an exact mirror suggesting the need for *de novo* biosynthesis of oleate and palmitoleate from stearate and palmitate, respectively. This raises interesting questions regarding the physiological and metabolic role of

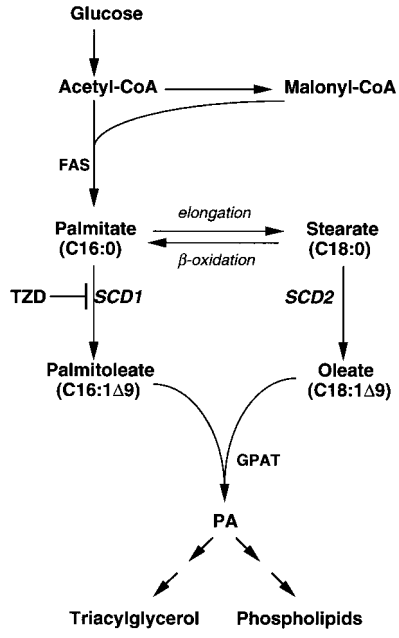

FIG. 1. A simplified pathway for synthesis of monounsaturated fatty acids and their incorporation into triacylglycerols and phospholipids. The proposed sites for the action of SCD1 and SCD2 isoforms are depicted. The site for TZD action is also shown. Stearate can be reconverted to palmitate by β-oxidation. SCD1 and SCD2, stearoyl-CoA desaturase 1 and 2; FAS, fatty acid synthase; PA: phosphatidic acid; GPAT: glycerol-3-phosphate acyltransferase.

SCD gene expression in preadipocyte differentiation and metabolism. Thus it is important to know how the monounsaturated fatty acids from the diet or *de novo*-synthesized by SCD are partitioned within the cell. A key question is whether the products of the SCD form a distinct lipid pool in the cell that can be separated from fatty acids derived from exogenous sources. Perhaps the compartmentalized pool of monounsaturated fatty acids would then have an important regulatory function, signaling the energy state of adipocytes. Furthermore, in order to regulate the SCD in response to a variety of metabolic and physiological stimuli, cells should be able to distinguish between saturated and unsaturated fatty acids in the pool. Thus, the *SCD* genes provide a useful model for studying the link between fatty acids and genetic control.

REGULATION OF MOUSE SCD GENES DURING PREADIPOCYTE DIFFERENTIATION

The mouse embryo-derived 3T3-L1 preadipocyte cell line is of mesodermal origin and represents an excellent model system for studying the mechanisms of cellular differentiation and development (28–30). Under the appropriate adipogenic stimuli, MDI; methylisobutylxanthine (MIX: a phosphodiesterase inhibitor), dexamethasone (DEX: a synthetic glucocorticoid), insulin, and fetal bovine serum, these cells differentiate

in culture into cells possessing the morphological and biochemical characteristics of *in vivo* mature adipocytes (31). Accompanying the acquisition of the adipocyte phenotype is a dramatic rise in almost all of the cellular enzymes required for *de novo* synthesis of fatty acids and triacylglycerols (28, 32, 33). Among the enzymes induced is the stearoyl-CoA desaturase whose activity increases 20-100 due primarily to increased transcription of the *SCD* genes (34).

The thiazolidinediones (TZDs) are ligands for the adipocyte-specific nuclear receptor PPARγ and exert potent antidiabetic effects by lowering hyperglycemia, hyperinsulinemia, and hyperlipidemia in both human and animal models of non-insulin dependent diabetes mellitus (19, 35). When added to the hormonal cocktail, TZD-activated PPARγ activates expression of various adipocyte-specific genes and elicits more complete adipocyte conversion both *in vivo* and *in vitro* (36, 37). Furthermore PPARγ is expressed predominantly in adipose tissue (38, 39), and ectopic expression of PAPRγ enhances the conversion of NIH 3T3 fibroblasts into adipocytes, particularly in the presence of a ligand (38). PPARγ is also shown to be expressed very early in preadipocyte differentiation, and upregulates adipocyte-specific genes including the fatty acid binding protein P2 (*aP2*) gene (40) and phosphoenolpyruvate carboxykinase (41). The effects of TZDs on differentiation and adipose-specific genes were mostly studied in adipocyte precursor cells, leaving a question on their effects in mature adipocytes. Using the mRNA differential display method, Kurebayashi *et al.* recently reported that TZDs inhibited the SCD enzyme activity by repressing the *SCD1* gene expression in fully differentiated 3T3-L1 preadipocytes (42). This repression was correlated with an increase in saturated fatty acids and a decrease in monounsaturated fatty acids. However comparative information on *SCD2* expression was not obtained, and deconvolution of the individual effects of MDI and TZDs was not possible due to the use of mature MDI-differentiated adipocytes.

Currently the physiological reasons for the requirement of two SCD isoforms to maintain fatty acid composition in the adipocyte have not been elucidated. To determine whether TZDs differentially regulate the *SCD* genes, we examined the expression of *SCD* mRNA isoforms induced by MDI and MDI plus troglitazone in differentiating 3T3-L1 preadipocytes. We found that enhanced differentiation by MDI plus TZDs resulted in selective repression of the *SCD1* mRNA expression (43). The repression of *SCD1* gene expression also resulted in a paralleled decrease in the level of SCD protein. In contrast, troglitazone was with no significant effect on *SCD2* mRNA and another marker gene, *aP2*. This selective inhibitory effect of troglitazone on *SCD1* leads to a unique decrease in the desaturation of C16:0 to C16:1Δ9, but not the conversion of C18:0 to C18:1Δ9 (Kim *et al.*, unpublished results). This strongly suggests a selective desaturation of each *SCD* isoforms in determining fatty acid composition and controlling a fine-tuning for each isoform during preadipocyte differentiation. We extended our observation to animals *in vivo* using 6-week old B10.PL(73NS)/Sn mice from Jackson Laboratory (Bar Harbor, ME). Mice were starved for 24 hrs and refed a high carbohydrate fat-free diet to induce the SCD as we previously described (16). The animals were then treated by oral gavage with troglitazone (200 mg/kg/day) for 48 hrs. The *SCD1* mRNA expression was repressed in troglitazone-treated mice in epididymal fat, while aP2 as expected was induced in a short term feeding in adipose tissue (unpublished data). These results indicate that TZDs target the *SCD1* gene not only during preadipocyte differentiation *in vitro* but also in fat tissues *in vivo.* The fact that ligands for PPARγ known to promote adipocyte differentiation and to exert antidiabetic actions repress the *SCD1* gene expression may suggest that the major differences between MDI-mediated and TZD-PPARγ-mediated differentiation lie in phenotypic characteristics of mature adipocytes. In spite of the fact that TZD enhanced the adipocyte conversion, it caused a dramatic decrease in cell size and lipid droplets compared with MDI differentiation as observed by Oil red O staining (43). This observation is consistent with recent reports made by other investigators (19, 36, 37, 44), where adipocytes from TZD-treated animals have been shown to be smaller in size with smaller fat droplets. At the moment, we do not know how the *SCD* genes contribute to the phenotypes of preadipocytes treated with TZDs and the ability to exhibit increased insulin sensitivity. However, the selective repression of the *SCD1* gene expression by TZD that accompanied changes in fatty acid composition suggests important roles that each *SCD* isoform plays in antidiabetic actions of the TZD as well as in adipogenesis.

CONCLUSIONS

Since SCD is directly responsible for the synthesis of monounsaturated fatty acids and its activity is reflected in membrane phospholipid and triglyceride composition of the adipocyte, the regulation of SCD isoforms can influence a variety of physiological variables including adiposity. Figure 1 summarizes the major enzymatic steps involved in the interconversions of C16 and C18 fatty acids. We hypothesize that "normal" lipid composition arises by the complementary activity of two SCD isoforms with differing catalytic selectivities. Thus *SCD1* is regulated to produce a high metabolic flux of *de novo* fatty acid biosynthesis into C16:1Δ9 for storage lipid. By contrast, *SCD2* appears to be constitutively expressed primarily to maintain the C18:1Δ9 pool in both storage lipid and cellular membranes. The other possibility is that both SCD

isoforms may desaturate C16:0 and C18:0 into C16:1Δ9 and C18:1Δ9, which may then partition in different lipid pools. The differential regulation of SCD isoforms by troglitazone might be associated with the morphological changes (smaller fat cell size and lipid droplets) and its pharmacological effect of insulin sensitivity on adipocytes.

ACKNOWLEDGMENTS

We thank Dr. Brian Fox and Enerique Gomez for useful discussions. Research reported in this work was supported by USDA Hatch Grant 3784 and support from the Graduate School of the College of Agriculture and Life Sciences of the University of Wisconsin–Madison and the Department of Biochemistry of the University of Wisconsin–Madison.

REFERENCES

1. Shimakata, T., Mihara, K., and Sato, R. (1972) *J. Biochem. (Tokyo)* **72,** 1163–1174.
2. Stittmatter, P., Spatz, L., Corcoran, D., Rogers, M. J., Setlow, B., and Redline, R. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 4565–4569.
3. Enoch, H. G., Catala, A. and Strittmatter, P. (1976) *J. Biol. Chem*. **251,** 5095–5103.
4. Griinari, J. M., and Bauman, D. E. (1999) Advances in Conjugated Linoleic Acid Research, Vol. 1, pp. 180–200, AOCS Press, Champaign, IL.
5. Kasturi, R., and Joshi, V. C.. (1982) *J. Biol. Chem*. **257,** 12224–12230.
6. Ntambi, J. M. (1995) *Prog. Lipid Res.* **34,** 139–150.
7. Ntambi J. M. (1999) *J. Lipid Res.* **40,** 1549–1558.
8. Waters, K. M., and Ntambi, J. M. (1994) *J. Biol. Chem*. **269,** 27773–27777.
9. Ntambi, J. M., Buhrow, S. A., Kaestner, K. H., Christy, R. J., Sibley, E., Kelly, T. J., Jr., and Lane, M. D. (1988) *J. Biol. Chem*. **263,** 17291–17300.
10. Kaestner, K. H., Ntambi, J. M., Kelly, T. J., Jr., and Lane, M. D. (1989) *J. Biol. Chem*. **264,** 14755–14761.
11. Mihara K. (1990) *J. Biochem*. **108,** 1022–1029.
12. Zhang, L., Ge, L., Parimoo, S., Stenn, K., and Prouty, S. M. (1999) *Biochem. J*. **340,** 255–264.
13. Stukey, J. E., McDonough, V. M., and Martin, C. E. (1990) *J. Biol. Chem*. **265,** 20144–20149.
14. Ward, R. J., Travers, M. T., Richards, S. E., Vernon, R. G., Salter, A. M., Buttery, P. J., and Barber, M. C. (1998) *Biochim. Biophys. Acta* **1391,** 145–156.
15. Ideta, R., Seki, T., and Adachi, K. (1995) *J. Dermatol. Sci.* **9,** 94–102.
16. Ntambi, J. M. (1992) *J Biol Chem* **267**(15), 10925–10930
17. Tebbey, B. W., VanCleave, S., and Buttke, T. M. (1994) *Biochem. Mol. Biol. Int.* **33,** 991–1000.
18. Ntambi, J. M., Sessler, A. M., and Takova, T. A. (1996) *Biochem. Biophys. Res. Commun.* **220,** 990–995.
19. Saltiel, A. R., and Olefsky, J. M. (1996) *Diabetes* **45,** 1965–1969.
20. DeWillie, J. W., and Farmer S. J. (1992) *Dev. Neurosci.* **14**(1), 61–68.
21. Tebbey, P. W., and Buttke T. M (1992) *Biochem. Biophys. Acta* **1171**(1), 27–34.
22. Sessler, A. M., and Ntambi J. M. (1998) *J. Nutr.* **128,** 923–926.
23. Bloch, K., and Vance, D. (1977) *Annu. Rev. Biochem*. **46,** 263–298.
24. McGilvery, R. W. (1979) A Functional Approach, 2nd ed. Saunders, Philadelphia.
25. Berry, E. M. (1997) Biochemistry, Physiology and Behavioral Neurobiology, Chap. 4, pp. 89–101.
26. Insull, W., Jr., and Bartsch, G. E. (1967) *Am. J. Clin. Nutr.* **20,** 13–23.
27. Beynen, A. C., Hermus, R. J. J., and Hautvast, J. G. A. J. (1980) *Am. J. Clin. Nutr.* **33,** 81–85.
28. MacDougald, O. A., and Lane, M. D. (1995 *Annu. Rev. Biochem*. **64,** 345–73.
29. Green, H., and Kehinde, O. (1974) *Cell* **1,** 113–116.
30. Green, H., and Kehinde, O. (1976) *Cell* **7,** 105–113.
31. Rubin, C. S., Hirsch, A., Fung, C., and Rosen, O. M. (1978) *J. Biol. Chem*. **253,** 7570–7578.
32. Tontonoz, P., Hu, E., and Spiegelman, B. M. (1994) *Cell* **79,** 1147–1156.
33. Christy, R. J., Yang, V. W., Ntambi, J. M., Geiman, D. E., Kelly, T. J., and Lane, M. D. (1989) *Genes Dev.* **8**(10), 1224–1335.
34. Bernlohr, D. A., Balanowski, M. A., Kelly, T. J., Jr., and Lane, M. D. (1985) *J. Biol. Chem*. **260,** 5563–5567.
35. Hofmann, C. A., and Colca, J. R. (1992) *Diabetes Care* **15,** 1075–1078.
36. Kletzien, R., Clarke, S., and Ulrich, R. (1991) *Mol. Pharmacol.* **41,** 393–398.
37. Hallakou, S., Doare, L., Foufelle, F., Kergoat, M, Guerre-Millo, M., Berthault, M., Dugali, I., Morin, J., Auwerx, J., and Ferre, P. (1997) *Diatetes* **46,** 1393–1399.
38. Tontonoz, P., Hu, E., and Spiegelman, B. M. (1994) *Cell* **79,** 1147–1156.
39. Braissant, O., Foufelle, F., Scotto, C., Dauca, M., and Whali, W. (1996) *Endocrinology* **137,** 354–366.
40. Tontonoz, P., Hu, E., Graves, R. A., Budavari, A. I., and Spiegelman, B. M. (1994) *Genes Dev.* **8,** 1224–1234
41. Tontonoz, P., Hu, E., Devine, J., Beale, E. G., and Spiegelman, B. M. (1995*) Mol. Cell. Biol.* **15,** 351–357.
42. Kurebayashi, S., Hirose, T., Miyashita, Y., Kasayamu, S., and Kishimoto, T. (1998) *Diabetes* **46,** 2115–2118.
43. Kim, Y-C, Choi, Y., and Ntambi, J. M. (1999) *FASEB J.* **13**(5), A874. [Abstract Part II (661.4)]
44. Okuno, A., Tamemoto, H., Tobe, K., Ueki, K. Mori, Y., Iwamoto, K., Umesono, K., Akanuma, Y., Fujiwara, T., Horikoshi, H., Yazaki, Y., and Kadowaki, T. (1998) *J. Clin. Invest.* **101,** 1354–1361.