Insulin-induced gene 1 (INSIG-1) is a key regulator in the processing of the sterol regulatory element-binding proteins (SREBPs). We demonstrated that INSIG-1 is regulated by peroxisome proliferator-activated receptor γ (PPARγ) providing a link between insulin sensitization/glucose homeostasis and lipid homeostasis. INSIG-1 was identified as a PPARγ target gene using microarray analysis of mRNA from the white adipose tissue of diabetic (db/db) animals treated with PPARγ agonists. INSIG-1 was induced in subcutaneous (9-fold) and epididymal (4-fold) fat pads from db/db mice treated for 8 days with the PPARγ agonist rosiglitazone (30 mg/kg/day). This in vivo response was confirmed in differentiated C3H10T1/2 adipocytes treated with rosiglitazone. To elucidate the molecular mechanisms regulating INSIG-1 expression, we cloned and characterized the human INSIG-1 promoter. Co-expression of PPARγ and RXRα transactivated the INSIG-1 promoter in the presence of PPARγ agonists. This induction was attenuated when a dominant negative PPARγ construct was transfected into cells. Furthermore, a PPARγ antagonist repressed the transactivation of the INSIG-1 promoter-reporter construct. Truncations of the promoter resulted in the identification of a PPAR response element that mediated the regulation of the promoter. We demonstrated with recombinant proteins that the PPARγ/RXRα heterodimer binds directly to this PPAR response element. In addition to regulation by PPARγ/RXRα, we demonstrated that the INSIG-1 promoter is regulated by transcriptionally active SREBP. The sterol response element was identified 380 base pairs upstream of the transcriptional start site. These findings suggest that the regulation of INSIG-1 by PPARγ agonists could in turn regulate SREBP processing and thus couple insulin sensitizers with the regulation of lipid homeostasis.

Ligands of the peroxisome proliferator-activated receptor γ (PPARγ) promote adipogenesis, stimulate glucose disposal in skeletal muscle, and depress glucose production from the liver (1–9). The molecular mechanisms and target tissues through which these ligands (such as the thiazolidinediones) exert their antidiabetic activity are not entirely clear, but this activity is thought to have a direct effect in adipose tissue, where PPARγ is abundant, and an indirect effect in muscle and liver, where levels of PPARγ are much lower. The synthetic PPARγ ligands clinically used to treat type 2 diabetes have the problematic side effects of inducing weight gain and edema, with the potential for congestive heart failure (4, 5). Safer PPARγ modulators that retain the insulin sensitization properties while minimizing side effects are desirable. However, it is not clear whether the adipogenic activity (by lowering circulating free fatty acids) is also responsible for insulin sensitization in other tissues.

PPREs have been identified in many genes encoding enzymes involved in fatty acid oxidation and synthesis (6, 7). Microarray and related technologies have allowed identification of a number of other genes in which expression is modulated by PPARγ ligands (8–10). We have identified insulin-induced gene 1 (Insig-1) as one of the genes highly induced in the adipose tissue of db/db mice treated with rosiglitazone. Insig-1/C/EBPδ was originally isolated as the most abundant immediate early transcript in both insulin-treated hepatoma cells and in regenerating rat liver (11, 12). It was also found to be induced both in epididymal fat in a diet-induced obesity model (13) and late in adipocyte differentiation of 3T3-L1 fibroblasts treated with dexamethasone, insulin, and isobutylmethylxanthine (14, 15). Recently, the co-immunoprecipitation of INSIG-1 with SREBP cleavage-activating protein (SCAP) implicated involvement of INSIG-1 in cholesterol and lipid homeostasis (16).

SREBPs are transcription factors whose activity is modulated by intracellular sterol levels. Full-length SREBP is a membrane-bound protein located in the endoplasmic reticulum where it is associated with SCAP. When intracellular sterol levels are depleted, SCAP and SREBP migrate to the Golgi where SREBP is cleaved twice, releasing the transcriptionally activated receptor γ; AOX, acyl-CoA oxidase; SRE, sterol regulatory element; SREBP, sterol regulatory element-binding protein; nSREBP, amino-terminal polypeptide SREBP; SCAP, SREBP cleavage-activating protein; INSIG-1, insulin-induced gene 1; LDL, low density lipoprotein; PPAR, PPAR response element; CEBP, CCAAT enhancer-binding protein; LG268, LG100268 (RXR-specific agonists); RXR, retinoid X receptor.
active amino-terminal polypeptide (nSREBP). nSREBP translocates to the nucleus where it activates the transcription of a number of genes involved in cholesterol metabolism (notably LDL receptor and hydroxymethylglutaryl-CoA reductase) and fatty acid and triglyceride synthesis (e.g. fatty-acid synthase and phosphoglycerol acyltransferase) (17). Two genes code for SREBP-1 and SREBP-2, and there are alternate splice forms as well (17). The major forms in liver are SREBP-1c (primarily influencing fatty acid metabolism and lipogenesis) and SREBP-2 (central to cholesterol homeostatic mechanisms). A third form, SREBP-1a, differs from SREBP-1c by containing additional acidic amino acids at the amino terminus, which confer greater transactivational activity. When the INSIG-1 protein is overexpressed in cultured cells, it tethers the SCAP-SREBP complex in the endoplasmic reticulum and prevents translocation to the Golgi. This blocks SREBP matura-

### Table I

| Target | Encoded protein | Probe | Primers | Description |
|--------|----------------|-------|---------|-------------|
| 36B4   | 60S acidic ribosomal protein PO | f-ATAAACATCTGAGAGGACTGAGGAGG-3' | f-GCCACCATGAGATGCTGTTGAG-3' |
| C/EBPα | CCAAT enhancer binding protein α | f-CCGGAGCCACAAACAATGGCGG-t | f-GACCGGATGACCGGGTACAG-t |
| INSIG-1| Insulin-induced gene 1 | f-TGGGTCATGTGATGGTGCCCAAGAG-3' | f-GAGCCCATCTTCTTGAGTTG |
| PGAR   | PPARγ angiopoietin-related protein, fasting induced adipose factor, angiopoietin-like 4 | f-TGGGACCAAGAGACATGACCTCTGAG-3' | f-GTGGTACGAGTGGTACCTGTC |
| PPARγ1| Peroxisome proliferator-activated receptor γ, isoform 1 | f-CCCTGCCCATGAGGACTGATACTTGAGT-t | f-GCCCGTGAAGAACATCTGTC |
| PPARγ2| Peroxisome proliferator-activated receptor γ, isoform 2 | f-CCAGGACATGGTGGCTGCCCTTGAT-t | f-GTAAGACTCGGGAAGTGGTCTG |

*Fluorescent labels in probes are: f, FAM (6-carboxyfluorescein); t, TAMRA (6-carboxytetramethylrhodamine). Forward primers are designated by f and reverse primers by r.*
RESULTS

INSIG-1 Is Induced by a PPARγ Agonist in Adipose Tissue of db/db Mice—We originally identified INSIG-1 mRNA in microarray experiments as being increased compared with vehicle-treated animals in epididymal fat from db/db mice 6 h following a single dose of either the RXR agonist LG268 or the PPARγ agonist rosiglitazone (Incyte Genomics). The signal remained elevated 6 h after a third daily dose of either compound. These results were confirmed by real-time PCR using cDNA prepared from pooled RNAs from the same experiment (Fig. 1A). Rosiglitazone treatment was more effective at inducing Insig-1 mRNA compared with LG268 or the PPARγ agonist fenofibrate. Induction by rosiglitazone was confirmed in two independent in vivo experiments at time points up to 14 days (Fig. 1, B and C). Fig. 1C shows that rosiglitazone-treated animals had significantly higher levels of Insig-1 mRNA than vehicle-treated controls at days 3 and 8 (n = 5, p < 0.05) in both epididymal (4-fold) and subcutaneous (9-fold) fat depots. For comparison, in subcutaneous fat, CEBPα was induced 2.6-fold (p < 0.05), and fatty-acid–CoA ligase, long-chain 2 (FACL2) was induced 5.2-fold (p < 0.05) at day 8 (data not shown). Compared with vehicle-treated db/db mice, Insig-1 mRNA levels in the fat of lean littermates were 1.6-fold higher (p < 0.05) in subcutaneous fat and not significantly different in epididymal fat (data not shown). Insig-1 was also induced in liver following rosiglitazone treatment but to a lesser extent than in fat.

INSIG-1 Regulation by PPARγ and SREBP

Fig. 1. Insig-1 expression in epididymal and subcutaneous fat. A, epididymal fat was removed from male db/db mice fasted for 6 h following 1 or 3 daily doses of 100 mg/kg/day fenofibrate (Feno), 30 mg/kg/day rosiglitazone (or BRL49653 (BRL)), 30 mg/kg/day LG268, or Vehicle. Relative levels of Insig-1 mRNA were determined by real-time PCR of cDNAs prepared from RNA pooled from each treatment group (n = 10). Levels of Insig-1 mRNA were normalized to 36B4 and then expressed relative to vehicle-treated animals at the same time point. B, epididymal fat was removed from male db/db mice fasted for 6 h following a naïve or 14th daily dose of 300 mg/kg/day fenofibrate, 10 mg/kg/day rosiglitazone, or 30 mg/kg/day LG268. Relative levels of Insig-1 mRNA were determined by real-time PCR of cDNAs prepared from RNA pooled from each treatment group (n = 7) as in A. C, epididymal and subcutaneous fat were removed from male db/db mice fasted for 6 h following 1, 3, or 8 daily doses of 30 mg/kg/day rosiglitazone. cDNAs were prepared from individual animal RNAs, and relative levels of Insig-1 mRNA were determined as in A. Data are presented as mean ± S.E. and were analyzed by unpaired Student’s t test. A p value of <0.05 was considered statistically significant. *, p < 0.05 (n = 5).

Insig-1 Is Induced during Adipogenesis in Vitro—Next we tested for induction of Insig-1 mRNA in cultured preadipocytes. C3H10T1/2 preadipocytes were plated and grown to confluence, at which time insulin was added in the presence or absence of rosiglitazone. Insulin alone did not induce Insig-1 at any time point. By 5 days, rosiglitazone in the presence of

Reporter Genes—The human LDL receptor promoter-luciferase construct has been described previously (22). The human INSIG-1–1233 promoter (−1233 to +23, relative to the transcription start site) was amplified from human genomic DNA using a 5′ primer that contained an NheI restriction site followed by the nucleotides corresponding to −1233 to −1214 (5′-gagagctgaaatgcgtgacagtcta-3′) and the 3′ primer that contained a BglII site and the nucleotides corresponding to −946 to −925 (5′-gagagctgaaatgcgtgacagtcta-3′) of the published genomic sequence (14). The PCR product was cloned into NheI/BglII sites of the pGL3 basic vector (Promega) to generate hINSIG-1–1233 (−1233 to +23). The hINSIG-1–1233 promoter-truncation constructs were generated using the following 5′ primer and the common 3′ primer as mentioned above. The hINSIG-1–1233 construct was generated using a 5′ primer with nucleotides corresponding to −861 to −841 (5′-gagagctgaaatgcgtgacagtcta-3′). The hINSIG-1–752 construct was generated using a 5′ primer corresponding to nucleotides −752 to −738 (5′-gagagctgaaatgcgtgacagtcta-3′). The hINSIG-1–375 (−375 to +23) construct was generated using a 5′ primer corresponding to nucleotides −375 to −362 (5′-gagagctgaaatgcgtgacagtcta-3′).

Cell Culture, Transient Transfections, and Reporter Gene Assays—HepG2 cells were maintained in minimum Eagle’s medium with 10% fetal bovine serum and were transiently transfected in 96-well plates using the FuGENE 6 transfection reagent (Roche Applied Science). Unless otherwise indicated, the cells were transfected with reporter plasmid (40 ng/well) and pCMX-hPPARγ (15 ng/well) with pCMX-RXRα (1.5 ng/well) and pCMV-β-galactosidase (30 ng/well). Each well received a total of 75 ng of DNA. The cells were transfected in minimum Eagle’s medium with 10% charcoal-stripped fetal bovine serum for 12 h and treated with ligands for an additional 24 h before harvesting and assaying for luciferase and β-galactosidase activity. G969622 (23), an irreversible PPARγ-antagonist was purchased from VWR International.

Electrophoretic Mobility Shift Assays—Complementary single-stranded DNAs were annealed. The double-stranded DNA was isolated and labeled with 32P. DNA binding reactions were carried out with double-stranded 32P-labeled DNA, and recombinant PPARγ and RXRα were incubated in the binding buffer (10 mM Hepes, 0.5 mM MgCl2, 1 mM dithiothreitol, 80 mM KCl, and 10% glycerol) for 10 min at 4 °C. Complexes were resolved in 6% nondenaturing polyacrylamide gels at 4 °C for 2 h and analyzed on a PhosphorImager. The oligonucleotides used were AOs PPRF, 5′-gcatactgaactggcctgaggtgacctgtcctggccggagctagccaagcccgccattg-3′; hINSIG-1 PPRE1, 5′-gtggctttgccttccagagtctcgccttgctgcttacgctgctggcctg-3′; and hINSIG-1 PPRE2, 5′-gtggctttgccttccagagtctcgccttgctgcttacgctgctggcctg-3′. The DR-1 sites are boldface and underlined.

FIG. 1. Insig-1 expression in epididymal and subcutaneous fat. A, epididymal fat was removed from male db/db mice fasted for 6 h following 1 or 3 daily doses of 100 mg/kg/day fenofibrate (Feno), 30 mg/kg/day rosiglitazone (or BRL49653 (BRL)), 30 mg/kg/day LG268, or Vehicle. Relative levels of Insig-1 mRNA were determined by real-time PCR of cDNAs prepared from RNA pooled from each treatment group (n = 10). Levels of Insig-1 mRNA were normalized to 36B4 and then expressed relative to vehicle-treated animals at the same time point. B, epididymal fat was removed from male db/db mice fasted for 6 h following a naïve or 14th daily dose of 300 mg/kg/day fenofibrate, 10 mg/kg/day rosiglitazone, or 30 mg/kg/day LG268. Relative levels of Insig-1 mRNA were determined by real-time PCR of cDNAs prepared from RNA pooled from each treatment group (n = 7) as in A. C, epididymal and subcutaneous fat were removed from male db/db mice fasted for 6 h following 1, 3, or 8 daily doses of 30 mg/kg/day rosiglitazone. cDNAs were prepared from individual animal RNAs, and relative levels of Insig-1 mRNA were determined as in A. Data are presented as mean ± S.E. and were analyzed by unpaired Student’s t test. A p value of <0.05 was considered statistically significant. *, p < 0.05 (n = 5).

Insig-1 Is Induced during Adipogenesis in Vitro—Next we tested for induction of Insig-1 mRNA in cultured preadipocytes. C3H10T1/2 preadipocytes were plated and grown to confluence, at which time insulin was added in the presence or absence of rosiglitazone. Insulin alone did not induce Insig-1 at any time point. By 5 days, rosiglitazone in the presence of
insulin had induced Insig-1 mRNA nearly 10-fold (Fig. 2). We also measured transcripts for other markers of adipogenesis. At 24 h, PPARγ/PPARα was induced 14-fold by rosiglitazone. PPAR, SREBP-1a, and SREBP-1c transcripts were maximally induced by 3 days, whereas C/EBPα and Insig-1 continued to rise from day 3 to day 5. PPARγ2 mRNA appears to approach a plateau at day 5. Although Insig-1 was originally identified as a target of insulin signaling in liver, it is apparently not regulated by the insulin treatment of preadipocytes. Insig-1 is induced by a PPARγ agonist relatively late in the adipogenesis program. Therefore, we wanted to determine whether Insig-1 is directly regulated by PPARγ or indirectly through other transcriptional activities of PPARγ.

Regulation of INSIG-1 Promoter-Reporter Genes in Response to Co-expression of PPARγ—To determine the molecular mechanisms involved in the regulation of INSIG-1 gene expression, we transfected cells with a luciferase reporter gene under the control of the proximal promoter of the human INSIG-1 gene. Co-transfection of the hINSIG-1 reporter construct with PPARγ1 or PPARγ2 and RXRα into HepG2 cells stimulated the transcriptional activity 3.5-fold in the absence of ligand (Fig. 3B). There was a further increase (2-fold) in expression upon the addition of the PPARγ ligand rosiglitazone (7-fold over basal). The agonist effect of rosiglitazone (but not LG268) was attenuated when the co-transfection was repeated with a dominant negative form of PPARγ (Fig. 3C). In addition, PPARγ/RXRα induction of the hINSIG-1 promoter in response to rosiglitazone is repressed in a dose-dependent manner in the presence of an irreversible PPARγ antagonist, GW9662 (Fig. 3D). This repression was also observed in a reporter gene under the control of three copies of the PPRE from the acyl-CoA oxidase gene (3x-AOx). The high basal activity of these reporter genes in the presence of PPARγ (but in the absence of exogenous ligand) likely results from an endogenous agonist.

We analyzed the human proximal promoter of INSIG-1 to determine the cis-acting elements necessary for the induction of INSIG-1 in response to PPARγ ligands. We identified two putative PPARγ/RXRα binding sites, designated PPRE1 and PPRE2 (Fig. 3A). A series of truncation reporter constructs were designed that systematically deleted the PPREs. Deletion of PPRE2 had no effect on the transactivation of hINSIG-1 by PPARγ and RXRα (Fig. 3E). However, deletion of PPRE1 resulted in loss of activation by PPARγ/RXRα. Therefore, we conclude that PPRE1 (but not PPRE2) is necessary for PPARγ regulation of hINSIG-1.
PPRE1 appears to be substantially weaker than to the AOX PPRE.

**DISCUSSION**

We demonstrated that Insig-1 mRNA levels are induced by PPARγ agonists in a diabetic mouse model (db/db). This induction was observed in white adipose tissue (epididymal fat and subcutaneous fat) by day 3 of agonist treatment, and activation was maintained for as long as 14 days (Fig. 1). Similarly, Way et al. (8) demonstrate that Insig-1 mRNA expression increases in adipose tissue (epididymal white adipose tissue and interscapular brown adipose tissue) of Zucker diabetic fatty rats treated with the PPARγ agonist GW1929 for 7 days. In this rat model, they observe a greater induction of INSIG-1 mRNA levels in brown adipose tissue compared with white adipose tissue (8). In support of our in vivo observations, we showed that Insig-1 is induced by PPARγ agonists in C3H10T1/2 cells following 3 days of treatment (Fig. 2).
along with a plasmid encoding hINSIG-1 reporter construct (pGL3-hINSIG-1 -1233 to +23), the LDL receptor reporter construct, or pGL3 were transfected into HepG2 cells in triplicate along with a plasmid encoding β-galactosidase. The cells were co-transfected with plasmids encoding SREBP-1a, SREBP-2, or dominant negative SREBP-1a. After 24 h, the cells were lysed, and the normalized luciferase values were determined. All transfections are representative of three separate experiments. B, HepG2 cells in a 96-well dish were co-transfected with hINSIG-1 (−1233 to +23) and increasing concentrations of the nuclear form of SREBP-1a and SREBP-2 as indicated. The cells were lysed 24 h later and were normalized for luciferase activity. C, HepG2 cells were transiently transfected with hINSIG-1 (−1233 to +23), hINSIG-1 (−861 to +23), hINSIG-1 (−752 to +23), hINSIG-1 (−375 to +23), and the LDL receptor. The cells were transfected for 24 h with or without SREBP-1a and a plasmid encoding β-galactosidase. On the following day, the cells were lysed, and the luciferase activities were determined and normalized for β-galactosidase activity. RLUs, relative light units. D, schematic illustration of the human LDLr and human INSIG-1 proximal promoters.

Fig. 4. Regulation of hINSIG-1 promoter-reporter genes by co-expression of SREBP-1a or SREBP-2. A, the hINSIG-1 promoter-reporter construct (pGL3-hINSIG-1 -1233 to +23), the LDL receptor reporter construct, or pGL3 were transfected into HepG2 cells in triplicate along with a plasmid encoding β-galactosidase. The cells were co-transfected with plasmids encoding SREBP-1a, SREBP-2, or dominant negative SREBP-1a. After 24 h, the cells were lysed, and the normalized luciferase values were determined. All transfections are representative of three separate experiments. B, HepG2 cells in a 96-well dish were co-transfected with hINSIG-1 (−1233 to +23) and increasing concentrations of the nuclear form of SREBP-1a and SREBP-2 as indicated. The cells were lysed 24 h later and were normalized for luciferase activity. C, HepG2 cells were transiently transfected with hINSIG-1 (−1233 to +23), hINSIG-1 (−861 to +23), hINSIG-1 (−752 to +23), hINSIG-1 (−375 to +23), and the LDL receptor. The cells were transfected for 24 h with or without SREBP-1a and a plasmid encoding β-galactosidase. On the following day, the cells were lysed, and the luciferase activities were determined and normalized for β-galactosidase activity. RLUs, relative light units. D, schematic illustration of the human LDLr and human INSIG-1 proximal promoters.

studies, Li et al. (15) establish, using 3T3-L1 cells, that Insig-1 expression is induced by day 9 of adipocyte differentiation. We demonstrated that in the presence of rosiglitazone, this induction occurs much earlier (day 3) and is more robust when compared with insulin-treated cells (10-fold, Fig. 2). Fig. 2 illustrates that prior to the activation of Insig-1 (observed at day 5) both PPARγ1 and SREBP-1c are maximally induced as early as 72 h. This time course, when taken together with our transfection data (which demonstrated that both transcription factors directly activate the INSIG-1 promoter), suggests that they are involved in the activation of Insig-1 transcription during adipocyte differentiation.

We established that PPARγ directly transactivates the human INSIG-1 promoter (Figs. 3 and 5). A dominant negative form of PPARγ was completely inactive, and the INSIG-1 transactivation was repressed when a PPARγ antagonist was added (Fig. 3D). These data indicate that activation of the INSIG-1 promoter is mediated by the ligand-gated transactivation function (AF-2) in the ligand-binding domain of PPARγ. Truncation reporter constructs localized the region through which PPARγ/RXRα mediated this induction to ~90 base pairs in the proximal promoter (Fig. 3E). Analysis of this region revealed a PPRE, to which the PPARγ/RXRα heterodimer binds directly in vitro (Fig. 5B).

INSIG-1 has recently been identified as a modulator of SREBP activity (16). INSIG-1 appears to tether the SCAP-SREBP complex in the endoplasmic reticulum in the presence of sterols. Interestingly, Insig-1 mRNA is induced in the livers of nSREBP-1a and nSREBP-2 transgenic animals when compared with wild-type littermate controls (26). In addition, Janowski (24) determined that INSIG-1 mRNA expression is inhibited by oxysterols, which also inhibit the expression of SREBP. When the oxysterol-mediated effect is reversed (using a hypocholesterolemic agent), SREBP processing increases, and the INSIG-1 gene is induced (24). Utilizing the hINSIG-1 promoter-reporter construct (−1233 to +23), we were able to determine that SREBP-1a potently transactivates the promoter (Fig. 4). Through the use of truncation constructs, the sterol response element was localized to a 400-base pair region in the proximal promoter of INSIG-1. A putative SRE identified within the region is similar to that identified for the LDL receptor, differing by only two nucleotides.

The current studies, as well as previous work, have demonstrated that there are at least three mechanisms by which INSIG-1 expression is altered. Insig-1 mRNA levels can be induced in the liver by increasing insulin levels (12, 28), in adipose and cultured cells by activated PPARγ/RXRα (Figs. 1–3 and Ref. 8), as well as by SREBPs in the cultured liver cells (Fig. 4 and Refs. 24 and 26). Although we defined the PPRE and SRE, the region of the INSIG-1 promoter necessary for insulin responsiveness has not been examined. Future studies using
the truncation-reporter constructs will be useful in analyzing
the mechanism by which insulin confers this regulation within
the context of the liver. One possibility is that insulin mediates
this effect on INSIG-1 via SREBP. Using 3T3L1 cells, Le Lay
et al. (29) identifies a subset of genes (such as fatty-acid synthase
and the LDL receptor) within the adipocyte that are regulated
by insulin via SREBP.

What becomes apparent is a complex network of coordi-
nated regulatory events in which SREBP, PPARγ, and
INSIG-1 modify the expression and/or activity of one another.
We demonstrated that transcriptionally active SREBP and
ligand-activated PPARγ/RXRα increase INSIG-1 at the
mRNA level (Fig. 6). In turn, INSIG-1 inhibits the processing
of SREBPs, thus providing a feedback mechanism by which
INSIG-1 regulates lipid homeostasis as well as indirectly
modifying its own expression. Li et al. (15) describe the
reduction of both SREBP-1c and PPARγ2 transcripts in 3T3L1
adipocytes, which overexpressed INSIG-1. Similarly, we ob-
served reciprocal mRNA expression between Insig-1
and SREBP-1c in C3H10T1/2 cells (Fig. 2). A more rigorous time
course might also reveal further changes in PPARγ2 levels.
Finally, within the adipocyte, SREBP in the presence
of C/EBP is thought to generate PPARγ agonists (29, 30).
Therefore, what we describe is a mechanism that links the
insulin sensitization observed with the PPARγ ligands to
lipid metabolism that is observed as a result of altering the
processing of SREBP.

These observations suggest a complex network with multiple
checkpoints to couple insulin signaling with lipid homeostasis.
Although insulin signaling via SREBP and Insig-1 is compro-
mised in diabetic animal models, treatment with rosiglitazone
activates Insig-1 via PPARγ. This convergence of the PPARγ
pathway reestablishes lipid homeostasis and insulin sensitiv-
ity within the adipose tissue. In future studies, it will be
important to determine whether the observations made in
white adipose tissue are also true in brown adipose tissue. In
addition, it will be of interest to understand the impact of
PPARα and PPARγ ligands on the hepatic expression of
Insig-1 and its role in modifying the processing of SREBPs. A
thorough understanding of the interplay between INSIG-1,
SREBPs, and PPARγ will be valuable in understanding the
full spectrum of thiazolidinedione-induced antidiabetic activ-
ities. This will facilitate attempts to design improved insulin
sensitizers without the negative side affects (such as edema
and weight gain) that often affect individuals treated with
thiazolidinediones.
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