Sterol regulatory element-binding proteins (SREBFs) are transcription factors that regulate enzymes required for cholesterol and fatty acid synthesis. Expression of SREBP-1 is enhanced by insulin; however, the actual insulin-signaling cascades employed are yet unclear. We determined the roles of the phosphatidylinositol (PI) 3-kinase and mitogen-activated protein (MAP) kinase-dependent pathways in the effect of mediating insulin on SREBP-1 in L-6 skeletal muscle cells and 3T3 L1 adipocytes, using wortmannin or LY294002 to inhibit the PI 3-kinase pathway, and PD98059 to inhibit the MAP kinase-dependent pathway. In myocytes, insulin increased SREBP-1 protein in a dose-dependent manner. 1 and 10 nM insulin significantly increased expression of total cellular SREBP-1 protein at 24 and 48 h, nuclear SREBP-1 protein at 24 h, and SREBP-1a mRNA at 24 h. Although wortmannin and LY294002 had no effect on this aspect of insulin action, PD98059 completely blocked each of these responses. Transfection of a dominant negative mutant of Ras similarly blocked the insulin effect on SREBP-1. In contrast, in adipocytes, the insulin effect on SREBP-1 was mediated via the PI 3-kinase and not the MAP kinase pathway. In conclusion, although insulin increases skeletal muscle SREBP-1 expression in a dose-dependent fashion via the MAP kinase-dependent signaling pathway, insulin action on adipocyte SREBP-1 is mediated via the PI 3-kinase signaling pathway. In the state of insulin resistance, characterized by selective inhibition of the PI 3-kinase pathway, the usual stimulation of lipogenesis by insulin in adipocytes may be inhibited, whereas intramyocellular lipogenesis via the MAP kinase pathway of insulin may continue unabated.

Dyslipidemia, high levels of free fatty acids, and ectopic fat deposition are frequently associated with insulin resistance, but the mechanisms of these associations are incompletely understood. However, the pathways regulating the production of such lipids are beginning to be unraveled. Expression of the genes involved in the production and uptake of cholesterol, fatty acids, triglycerides, phospholipids, and the low density lipoprotein receptor is regulated by a group of membrane-bound transcription factors called sterol regulatory element-binding proteins (SREBFs)\(^1\). There are three isoforms of SREBP, designated SREBP-1a, SREBP-1c, and SREBP-2. Although SREBP-1a can stimulate all SREBP-responsive genes, SREBP-1c preferentially activates fatty acid synthetic genes, and SREBP-2 activates mainly cholesterol synthesis genes (2).

Expression of SREBP-1 is enhanced by insulin in all three major insulin target tissues: liver, fat, and skeletal muscle (3–6). Similarly, levels of SREBP-1 are increased in the presence of hyperinsulinemia (7–9). Conversely, insulin deficiency leads to a decrease in SREBP-1 (6). For example, in insulin-deficient streptozotocin-treated rats, hepatic SREBP-1c mRNA levels fall and can be rescued by insulin injection (10). Streptozotocin also decreases muscle SREBP-1 and specifically SREBP-1c mRNA in rats (4). Hepatic SREBP-1c is similarly inhibited by glucagon and by fasting, which lower insulin production and increase glucagon levels (11, 12). Refeeding stimulates hepatic nuclear SREBP-1c expression, presumably by restoring insulin secretion (10).

In vivo results from the human state of insulin resistance are complex. Base-line skeletal muscle SREBP-1 mRNA levels are similar in lean and obese subjects (6). In contrast, adipose tissue expression of SREBP-1 is decreased in obese versus lean subjects and further reduced in obese diabetic subjects (6, 13). Therefore, the pathways used by insulin to stimulate SREBP-1 expression may not be the same in adipocytes and skeletal muscle.

The mechanism of insulin action involves at least two pathways of intracellular signaling. The phosphatidylinositol (PI) 3-kinase-dependent pathway controls most, if not all, metabolic aspects of insulin action, whereas the mitogen-activated protein (MAP) kinase-dependent pathway controls the mitogenic effects of insulin (14, 15). Insulin resistance is accompanied by various degrees of impairment in PI 3-kinase signaling, causing impaired utilization of glucose, hyperglycemia (16), and compensatory hyperinsulinemia. In contrast, the MAP kinase pathway does not appear defective in the presence of insulin resistance (17, 18). Conceivably, compensatory hyperinsulinemia may continue to activate the MAP kinase pathway, which is left intact in insulin resistance (19–21). This could increase products downstream of the MAP kinase pathway, despite insulin resistance in the PI 3-kinase pathway.

If the mechanism of insulin action on SREBP-1 involves predominately the MAP kinase pathway in skeletal muscle, then insulin-stimulated expression of SREBP-1 would remain

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intact in the insulin-resistant state or even enhanced in the presence of compensatory hyperinsulinemia. In contrast, if insulin stimulates SREBP-1 via the PI 3-kinase pathway in adipocytes, SREBP-1 expression would not increase during insulin resistance. Together, decreased lipid deposition in adipocytes and increased lipogenesis in skeletal muscle may partly explain the ectopic lipid deposition seen in insulin resistance. We assessed this possibility in L-6 skeletal muscle cells and 3T3 L1 adipocytes grown in culture and subjected to insulin and inhibitors of the insulin signaling pathways.

EXPERIMENTAL PROCEDURES

Materials—The L-6 muscle cell line, derived from rat thigh muscle tissue, was originally obtained from ATCC (Manassas, VA). Fetal bovine serum was purchased from Gemini Bio-Products (Woodland, CA). The cell culture medium used was 1x Dulbecco’s modified Eagle’s medium from Cellgro (Herndon, VA), LY294002, phospho-p44/42 MAP kinase antibody, and p44/42 MAP kinase antibody were obtained from Cell Signaling (Beverly, MA), and PD 98059 was from Calbiochem (San Diego, CA). The spectrophotometer was the DU 640 by Beckman (Fullerton, CA). The densitometer was the Flour-S Multilamager from Bio-Rad, using the Quantity One version 4.1.1 software. Also from Bio-Rad were the 12% Tris-HCl gel, the Kaleidoscope prestained standard, the polyvinylidene difluoride membranes, the iCycler system for quantitative real time PCR, and the SYBR Green Supermix for real time PCR. The Speedvac Concentrator was from Savant (Holbrook, NY). The nuclear and cytoplasmic extraction reagent kit was from Pierce, and the protease inhibitor mixture used with the nuclear protein extraction kit was from Sigma. The MagicMark Western Standard and the SuperScript First-Strand Synthesis System for real time PCR reagents used for cDNA synthesis were from Invitrogen. The SREBP-1 antibody was a monoclonal IgG from Santa Cruz Biotechnology (Santa Cruz, CA). The ECL Western blotting analysis system was from Amer sham Biosciences. The t-α-phosphatidylinositol used in the PI 3-kinase assay was from Avanti Polar Lipids (Alabaster, AL). The PLC plates were from Fisher. RNA STAT-60 was from Tel-Test B, Inc (Friends wood, TX). The SREBP-1c primer sequence was 5’-GGACCACTTGAT TGCACATTGGCCCGAGTACACTGT-3’ (22), obtained from Integrated DNA Technologies (Corvalis, IL). The dominant negative mutant of Ras adenovirus was obtained from James De Gregori, Ph.D. (University of Colorado Health Sciences, Denver, CO). All other reagents and chemicals including insulin and Wortmannin were from Sigma.

Cell Culture—L-6 muscle cells (passes 2–6) were grown in a 75-mm flask at 37°C in medium consisting of Dulbecco’s modified Eagle’s medium with 4.5 g/liter t-glutamine, 1.5 g/liter sodium bicarbonate, and 4.5 g/liter glucose, supplemented with 112 ml/liter fetal bovine serum, 1.5 g/liter sodium bicarbonate, and 100 ml/liter inulin and penicillin, at 60% confluence, the cells were subcultured to individual plates (60 mm for total cellular protein extraction, 150 mm for nuclear protein extraction) and grown again to 60% confluence. 3T3-L1 fibroblasts were grown to confluence at 37°C in fibroblast growth medium (Dulbecco’s modified Eagle’s medium containing 5.5 mM glucose, 10% fetal calf serum, 50 µg/ml gentamicyn, 0.5 mM glutamine, and 0.5 µM fungizone). Differentiation was initiated by the addition of medium containing 10% fetal calf serum, 1 mM glutamine, 500 µM isobutylmethanamine, 1 mM deamethasone, and 1 µg/ml insulin. After 2 days, the cells were transferred to adipocyte growth medium containing 25 mM glucose, 50 µg/ml gentamycin, 0.5 mM glucose, and 1 µg/ml insulin and refed every 2 days. All of the cells were then incubated for 24 h in serum-free medium and then treated with either no additions as a control, 1 µg/ml insulin, 10 µg/ml insulin, 100 µg/ml Wortmannin (WT; a PI 3-kinase inhibitor), 50 µg LY294002 (LY; a structurally distinct inhibitor of the PI3-kinase pathway), or 20 µg PD 98059 (PD; a specific MAP kinase kinase inhibitor), in various combinations. To create the L-6 muscle extract, L-6 muscle cells were incubated for 24 h in serum-free medium and then treated for 24 h with 0, 1, 0.5, 1, 5, 10, or 100 nM insulin. To create the time course, L-6 muscle cells were incubated for 24 h in serum-free medium and then harvested for protein and mRNA immediately (time 0), at 2, 4, 6, 12, and 24 h after the addition of 10 nM insulin. In experiments using the dominant negative mutant of Ras, green fluorescent protein-labeled adenoviral dominant negative Ras construct, green fluorescent protein-labeled adenovirus, or adenovirus alone as controls were added to the L-6 cells or differentiated 3T3 L1 adipocytes, 24 h prior to serum starvation.

Preparation of Total Cellular Extracts—For the preparation of total cellular protein, the cells were incubated with the respective treatments for 24 or 48 h. The media and treatments were refreshed every 24 h. The cells were washed with phosphate-buffered saline, lysed on ice in lysis buffer (containing 50 mM HEPES, 150 mM NaCl, 5 mM MgCl2, 1 mM phenylmethylsulfonyl fluoride, 1 mM Na3HPO4, 1% Triton X-100, 1 mM dithiothreitol, 1 mM sodium vanadate, 0.05% SDS, 10 µg/ml aprotinin, 10 µg/ml leupeptin, pH 7.5), scraped, sonicated for 20 s, and then centrifuged. The supernatant was retained, and protein concentration was quantified using a bichinchonic acid standard curve. The DU 640 spectrophotometer, 100 µg of each sample was dried by the Speedvac Concentrator, reconstituted in Laemmli Buffer, and stored at ~20°C.

Preparation of Nuclear Extracts—After 24 h of treatment, the cells were washed with phosphate-buffered saline, lifted with Trypsin-EDTA, centrifuged, and washed again. Nuclear protein was prepared with the Pierce nuclear and cytoplasmic extraction kit according to the manufacturer’s recommendations. The final nuclear extract was assayed for protein concentration as above. 100 µg of each sample was dried by the Speedvac Concentrator, reconstituted in Laemmli Buffer, and stored at ~80°C.

P3-Kinase Activity Measurements—Total cellular extracts (500 ng of protein) were mixed with PI 3-kinase buffer, water, sonicated lipid (t-α-phosphatidylinositol dried and sonicated in sonication buffer), and [32P]ATP mix. After stopping the reaction with stop solution, the organic layer was extracted with chloroform and dried by Speedvac. The pellet was reconstituted in chloroform/methanol and spotted on TLC plates, which were run overnight in TLC tank with running buffer. The TLC plates were then exposed to x-ray film, and spot density was determined by densitometry.

Western Blotting—The total cellular and nuclear extracts were boiled for 10 min, and 25 µg of protein/lane were resolved by SDS-polyacrylamide gel electrophoresis (12% Tris-HCl gel). MagicMark and Kaleido scope standards were used to identify the molecular weight. The protein was then electrotransferred to polyvinylidene difluoride membranes using the standard Western blotting technique (100 V for 1 h). The membranes were prehybridized with nonfat powdered milk (or bovine serum albumin for phospho-p44/42 and p44/42 MAP kinase antibodies) and hybridized with mouse monoclonal anti-SREBP-1 antibody or rabbit polyclonal anti-phospho-p44/42 MAP kinase antibody or rabbit polyclonal anti-p44/42 MAP kinase antibody in a 1:500 dilution. The membranes were then incubated with anti-mouse or anti-rabbit antibody conjugated with horseradish peroxidase in a 1:1000 dilution, and protein was detected by chemiluminescence with the ECL Western blotting analysis system. The hybridized membranes were exposed to x-ray film, and the band intensity was measured with the densitometer.

Preparation of RNA Extracts—Cells were grown and treated as described for 24 h. Total RNA was then prepared with RNA STAT-60, a suspension of guanidinium thiocyanate and phenol. Chloroform was added to extract the RNA and isoamyl alcohol to precipitate the RNA. The resulting pellet was washed with 75% ETOH, air-dried, reconstituted with RNase-free sterile water, diluted 1:10 with sodium phosphate, and the concentration was measured on the DU 640 spectrophotometer. The samples were denatured for 5 min at 65°C and electrophoresed in a 1.2% agarose denaturing gel containing 1× MOPS, 6% formaldehyde, and diethyl pyrocarbonate-treated water (75 volts for 1–2 h) for quality assessment.

Total RNA was reverse-transcribed using the SuperScript first
strand synthesis system kit for real time PCR, according to the manufacturer’s instructions. The samples were incubated at 65°C for 5 min and then on ice for at least 1 min, followed by the addition of 10× reverse transcription buffer (200 mM Tris-HCl, 500 mM KCl, pH 8.4), 25 mM MgCl₂, 0.1 M dithiothreitol, and RNaseOUT recombinant ribonuclease inhibitor. After incubation at 25°C for 2 min, SuperScript II H/Reverse Transcriptase (50 units/μl) was added, and the samples were incubated at 25°C for 10 min, 42°C for 50 min, and 70°C for 15 min.

To remove the RNA, 1 μl of Escherichia coli RNase H (2 units/μl), was added to the cDNA product. The cDNA was then quantified using quantitative real time reverse transcriptase PCR. The PCR contained the newly generated cDNA template, iQ SYBR Green Supermix, sterile water, and forward and reverse SREBP-1c primers. Real time PCR was performed with the iCycler system (one cycle of 95°C for 90 s, 40 cycles of 95°C for 30 s, followed by 60°C for 60 s, and one cycle of 95°C for 60 s). A melt curve of 80 cycles, starting at 55°C and increasing by 0.5 degrees every 10 s, was done to determine primer specificity. Expression levels were calculated by the relative method, by comparing the target and amount of 18 S RNA subunit, according to the manufacturer.

Data Analysis—Between 7 and 18 individual experiments were performed in duplicate for the total and nuclear protein experiments, 3 individual experiments in duplicate for real time PCR, and 2 in duplicate for adenoviral transfection of the dominant negative Ras. The results are expressed as the means ± the standard error of the mean. An unpaired t test, with a p value of <0.05 was used to determine statistical significance.

RESULTS

Insulin Dose-Response Curve in L-6 Skeletal Muscle Cells—Insulin increased SREBP-1 protein in nuclear extracts in a dose-dependent fashion (Fig. 1). SREBP-1 expression was enhanced 50% of maximum at 0.5 nM insulin and 100% of maximum at 100 nM insulin. Therefore, subsequent experiments were performed at 1 and 10 nM to represent hyperinsulinemia.
Effectiveness of Inhibitors—To verify the effectiveness of the inhibitors used in this study, we measured PI 3-kinase activity and the phosphorylation of p44/42 ERK (MAP kinases) in control and insulin-treated cells incubated with WT, LY, or PD compounds. Insulin significantly stimulated PI 3-kinase activity in control and PD-treated cells. As expected, there was a 75–90% inhibition of the PI 3-kinase activity with WT and LY in L-6 myocytes (Fig. 2A), and a 70–75% inhibition with WT and LY in 3T3 L1 adipocytes (Fig. 2B).

Insulin significantly stimulated ERK phosphorylation in control and WT- and LY-treated L-6 skeletal muscle cells (Fig. 3). In contrast, inhibition of MAP kinase activity reached 80% in the presence of PD (Fig. 3). Abundance of p44/42 ERK isoforms was identical in all L-6 cells (Fig. 3). In subsequent experiments we examined the roles of the PI 3-kinase and MAP kinase-dependent signaling pathways in insulin-mediated SREBP-1 expression.

Total Cellular SREBP-1 Protein in L-6 Skeletal Muscle Cells—Insulin (1 nM) significantly increased the expression of total cellular SREBP-1 protein at 24 h (p < 0.03) (Fig. 4A) and 48 h (p = 0.02) (Fig. 4B). This response was dose-dependent, with insulin (10 nM) further increasing total cellular SREBP-1 at 24 h (p < 0.0001) and 48 h (p = 0.001) (Fig. 4). WT, blocking the PI 3-kinase pathway, had no effect on the ability of insulin to increase total cellular SREBP-1 at either 24 or 48 h (Fig. 4). LY, a structurally distinct inhibitor of the PI 3-kinase pathway, similarly had no effect on insulin stimulation of SREBP-1 at 24 h (not shown). In contrast, PD, blocking the MAP kinase pathway, completely inhibited insulin-induced increases in total cellular SREBP-1 at 24 h in response to 1 nM (p < 0.0001) and 10 nM insulin (p < 0.0001) (Fig. 4A). PD continued to block insulin-induced increases in SREBP-1 at 48 h in response to 1 nM (p < 0.01) and 10 nM insulin (p < 0.001) (Fig. 4B). Neither WT, LY, nor PD compounds alone had any effect on SREBP-1 expression at either time point (data not shown).

To confirm the role of the Ras-MAP kinase pathway in mediating insulin effect on SREBP-1 in skeletal muscle, the myocytes were transduced with adenovirus containing a dominant negative mutant of Ras. Expression of the dominant negative Ras completely blocked the ability of insulin to stimulate SREBP-1 expression in the absence or presence of WT (Fig. 5).

Nuclear SREBP-1 Protein in L-6 Skeletal Muscle Cells—SREBP exhibits its action in the cell nucleus. Therefore, we determined the effect of insulin on expression of nuclear SREBP-1, which may better reflect physiologically relevant aspects of insulin action on SREBP-1. Insulin significantly increased the expression of nuclear SREBP-1 protein at 24 h (p < 0.001 and p < 0.001 at 1 and 10 nM, respectively) (Fig. 6). WT had no effect on the ability of insulin to increase SREBP-1 nuclear protein (Fig. 6). However, PD blocked insulin-stimulated increases in nuclear SREBP-1 protein at 1 nM (p < 0.001) and 10 nM insulin (p < 0.001) (Fig. 6). As with the total cellular SREBP-1, neither WT nor PD compound alone had any effect on SREBP-1 protein expression in the nucleus (not shown).

mRNA in L-6 Skeletal Muscle Cells—We then assessed the effect of insulin on SREBP-1 expression at the mRNA level. Quantitative real time PCR analysis showed an increase in SREBP-1c mRNA expression with the addition of 10 nM insulin at 24 h (p < 0.05) (Fig. 7). The addition of WT had no effect on stimulation of SREBP-1c mRNA by insulin (Fig. 7). However, in concert with the results observed at the protein level, the addition of PD blocked the ability of insulin to stimulate SREBP-1c mRNA expression in L-6 cells (p < 0.04) (Fig. 7).

Insulin Time Course in L-6 Skeletal Muscle Cells—To examine the effect of insulin on SREBP-1 expression at earlier time points, we measured the amounts of total cellular SREBP-1 protein and SREBP-1c mRNA at 2, 4, 6, 12, and 24 h. Insulin (10 nM) increased both SREBP-1 total cellular protein (Fig. 8A) and SREBP-1c mRNA (Fig. 8B) in a time-dependent manner. Levels of SREBP-1 plateaued at 12 h and remained relatively stable and insignificantly different between 12 and 24 h in both protein and mRNA.

Insulin Effect on SREBP-1 in 3T3 L1 Adipocytes—To better understand the physiological implications of insulin action on SREBP-1, we compared the results obtained in L-6 muscle cells with those in 3T3 L1 adipocytes. 1 and 10 nM insulin
increased SREBP-1 protein expression in total cell lysates (Fig. 9). In contrast to L-6 muscle cells, both inhibitors of the PI 3-kinase pathway (WT and LY) completely blocked the effect of insulin on SREBP-1 expression (Fig. 9; \( p < 0.01 \)) in 3T3 L1 adipocytes. Blockade of the MAP kinase signaling pathway with PD did not alter the effect of insulin on SREBP-1 in these cells.

To confirm the role of the Ras-MAP kinase pathway in mediating insulin effect on SREBP-1 in adipocytes, the cells were transduced with adenovirus containing a dominant negative
mutant of Ras. Expression of the dominant negative Ras had no effect on insulin stimulation of SREBP-1 protein (Fig. 5), but the insulin effect was still blocked by WT.

**DISCUSSION**

Insulin resistance is a condition in which maintenance of normal blood glucose values requires higher than normal insulin concentrations (23), resulting in hyperinsulinemia. A critical unresolved question is whether compensatory hyperinsulinemia is merely a marker of insulin resistance or has direct detrimental effects. Insulin exerts its action through two major signaling pathways. The PI 3-kinase-dependent pathway controls most, if not all, of the metabolic actions of insulin (14), whereas the MAP kinase-dependent pathway regulates the mitogenic aspects of insulin action (15). We hypothesized that if the hyperinsulinemia of insulin resistance in skeletal muscle is due to a selective impairment of PI 3-kinase, insulin regulation of SREBP-1 by MAP kinase would increase SREBP-1. Secondarily, increased SREBP-1 would up-regulate known downstream targets, increasing lipid synthesis and deposition in skeletal muscle. In contrast, insulin regulation of SREBP-1 by PI 3-kinase in adipocytes would decrease lipid deposition in adipocytes. Thus, although the term insulin resistance implies global impairment, the existence of selective resistance would paradoxically cause a combination of decreased insulin action (hyperglycemia and decreased fat deposition in adipocytes) and
increased insulin action (ectopic lipid deposition in skeletal muscle) in one person.

The results reported here support this hypothesis, by demonstrating three critical points. First, SREBP-1 is up-regulated in a dose-dependent fashion by insulin in L-6 skeletal muscle cells (Fig. 1) and is similarly up-regulated in 3T3 L1 adipocytes (Fig. 9). Second, the dose-dependent stimulatory effect of insulin on SREBP-1 expression in myocytes remains intact in the presence of PI 3-kinase inhibition and is completely abrogated by interference with the MAP kinase pathway. Thus, the effect of insulin on SREBP-1 is MAP kinase-dependent in skeletal muscle. Third, stimulation of SREBP-1 by insulin in adipocytes is blocked by inhibition of PI 3-kinase, not the MAP kinase pathway, and thus is PI 3-kinase-dependent. Therefore, if the MAP kinase pathway remains responsive but PI 3-kinase is resistant, compensatory hyperinsulinemia of insulin resistance will increase SREBP-1 expression preferentially in skeletal muscle, as shown here.

Differential sensitivity of the two major insulin signaling pathways to the compensatory hyperinsulinemia of insulin resistance may play an important part in the pathogenesis of complications accompanying insulin resistance. PI 3-kinase activity in skeletal muscle is decreased in vastus lateralis muscle from patients with insulin resistance (17, 24), in primary muscle cell culture of vastus lateralis muscle in subjects with type 2 diabetes (25), and in quadriceps muscle from the ob/ob mouse (26, 27) and the fa/fa rat (28). In contrast, the MAP kinase signaling pathway is intact in skeletal muscle from insulin-resistant individuals (29) or even increased (25). Because the MAP kinase pathway appears critical for SREBP-1 stimulation in skeletal muscle (21), excess MAP kinase activation by compensatory hyperinsulinemia, a hallmark of insulin resistance, could result in excess SREBP-1 expression. This increase in SREBP-1 expression would consequently lead to lipid accumulation in skeletal muscle tissue.

Increased intramyocellular lipid is associated with insulin resistance in skeletal muscle (30). Overexpression of SREBP-1 may be responsible for the ectopic fat deposition seen in skeletal muscle tissue of people with insulin resistance, and the presence of these intracellular lipids may worsen insulin resistance. The further blocking PI 3-kinase signaling (31) in a vicious cycle. In contrast, the effect of insulin on SREBP-1 in adipocytes would be decreased in insulin resistance, because it appears to be mediated via the PI 3-kinase pathway. This differential regulation of SREBP-1 expression by insulin may explain shunting of lipogenesis away from adipocytes and toward muscle in insulin resistant individuals.

Insulin increases SREBP-1 expression in all three major insulin target tissues: liver, fat, and skeletal muscle (3–5). For example, insulin increases precursor and nuclear SREBP-1 mRNA in rat hepatocytes in vitro (3), and precursor and nuclear SREBP-1 mRNA in cultured adipocytes (5). In primary culture of rat skeletal muscle cells, 100 nmol/liter insulin increases SREBP-1 membrane and nuclear protein as well as SREBP-1 mRNA (4).

Although insulin appears to consistently stimulate SREBP-1 expression, the evidence regarding which insulin signaling pathways are important for SREBP-1 regulation is conflicting. For example, in primary culture of rat hepatocytes, a dominant negative mutant of PI 3-kinase and inhibition of insulin receptor subunit 1 tyrosine phosphorylation (reducing PI 3-kinase activity) both blocked insulin-induced increases in SREBP-1c (32). In addition, PI 3-kinase inhibitors decreased insulin-stimulated SREBP-1 expression in rat hepatocytes grown by primary culture (33). In contrast, studies in HepG2 cells showed that inhibition of MAP kinase with PD negated the stimulatory effect of insulin on the low density lipoprotein receptor promoter, where inhibition of PI 3-kinase with WT had no effect (34). Moreover, a forced expression of the MAP kinase activator MEK kinase 1 or MEK1 stimulated the low density lipoprotein promoter and SREBP-1a and -2 transcription (34). A direct comparison of primary hepatocytes with HepG2 cell lines is needed to identify the roles of the PI 3-kinase- and MAP-kinase-dependent pathways in the liver more clearly.

In vivo, insulin also stimulates SREBP-1 expression in adipocytes (5) and in vastus lateralis muscle of lean and type 2 diabetic humans (6). Although base-line SREBP-1 mRNA levels were similar in vastus lateralis and rectus abdominis biopsies from lean and obese subjects, adipose tissue expression of SREBP-1 was reduced in obese versus lean subjects (6, 13, 35) and further reduced in the obese diabetic subjects (6). In support, the addition of WT and LY, but not PD to adipocytes, inhibits insulin stimulation of fatty acid synthase, which is downstream of SREBP-1 (36). Our results are consistent with these findings and confirm the role of PI 3-kinase in the action of insulin on SREBP-1 in adipocytes. In contrast, insulin stimulates equally potent expression of SREBP-1 in muscle of lean, obese, and type 2 diabetic subjects (6), suggesting different pathways in skeletal muscle.

In support of this suggestion, Muller-Wieland and co-workers (34) demonstrated a strong linkage between the MAP kinase-dependent pathway and expression of SREBP-1 in muscle. Our results support an important role of the MAP kinase pathway in the mechanism of insulin-stimulated expression of SREBP-1 in muscle cell lines. Overall, the selective down-regulation of SREBP-1 expression in adipose but continued full expression of SREBP-1 in muscle as seen by Ducluzeau et al. (13) may promote partitioning of free fatty acids toward muscle, leading to an ectopic deposition of fat.

The link between increased SREBP-1 and ectopic fat deposition in skeletal muscle is further strengthened by the effect of insulin sensitizers. Although metformin decreases endogenous hepatic glucose production, lowering insulin requirements (37), the thiazolidinediones increase the triglyceride content of adipose while decreasing the triglyceride content of the liver and skeletal muscle (38, 39). The thiazolidinedione response may occur through improving aberrant insulin signaling. For example, defective skeletal muscle PI 3-kinase signaling is directly impaired by troglitazone but not metformin (40). Troglitazone also decreases SREBP-1c in rat liver and pancreatic islet cells (9), whereas rosiglitazone reduces intramyocellular lipid content (30). Thus, the fat redistribution seen with thiazolidinediones may occur by improving skeletal muscle PI 3-kinase activity, subsequently lowering insulin secretion, and thus decreasing MAP kinase activation, SREBP-1 expression, and downstream ectopic lipid deposition in skeletal muscle. This reduction in skeletal muscle lipids may then further improve insulin sensitivity. In addition, by improving PI 3-kinase signaling, lipid deposition would increase in adipocytes.

A better understanding of the aberrations in insulin signaling will help elucidate the mechanisms causing insulin resistance. In addition, it will help direct specific therapies at treating insulin resistance and its complications. By targeting therapies that improve PI 3-kinase signaling and selectively block the MAP kinase pathway or SREBP-1, it may be possible to restore normal glucose metabolism and insulin sensitivity, thus preventing complications of diabetes-related excessive MAP kinase-dependent signaling.

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