NR4A Orphan Nuclear Receptors Modulate Insulin Action and the Glucose Transport System

POTENTIAL ROLE IN INSULIN RESISTANCE*

Received for publication, February 6, 2007, and in revised form, August 31, 2007 Published, JBC Papers in Press, September 4, 2007, DOI 10.1074/jbc.M701132200

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After observing that expression of two NR4A orphan nuclear receptors, NR4A3 and NR4A1, was altered by insulin in cDNA microarray analyses of human skeletal muscle, we studied whether these receptors could modulate insulin sensitivity. We found that both NR4A3 and NR4A1 were induced by insulin and by thiazolidinedione drugs (pioglitazone and troglitazone) in 3T3-L1 adipocytes. Furthermore, gene expression of NR4A3 and NR4A1 was reduced in skeletal muscles and adipose tissues from multiple rodent models of insulin resistance. To determine whether NR4A3 could modulate insulin sensitivity, 3T3-L1 adipocytes were stably transduced with NR4A3 or LacZ (control) lentiviral vectors. Compared with LacZ expressing cells, hyperexpression of NR4A3 increased the ability of insulin to augment glucose transport activity, and the mechanism involved increased recruitment of GLUT4 glucose transporters to the plasma membrane. NR4A3 hyperexpression also led to an increase in insulin-mediated tyrosine phosphorylation of insulin receptor substrate-1 as well as Akt phosphorylation. Suppression of NR4A3 using lentiviral short hairpin RNA constructs reduced the ability of insulin to stimulate glucose transport and phosphorylate Insulin receptor substrate-1 and Akt. Thus, NR4A3 and NR4A1 are attractive novel therapeutic targets for potential amelioration of insulin resistance, and treatment and prevention of type 2 diabetes and the metabolic syndrome.

Type 2 diabetes is a disease caused by defects in insulin secretion combined with the presence of insulin resistance in peripheral target tissues that results in dysregulation of glucose metabolism. Among non-diabetic individuals, there is a wide variation in the degree of insulin sensitivity, and relative insulin resistance is associated with a cluster of clinical and metabolic traits including central obesity, glucose intolerance, dyslipidemia, and elevated blood pressure. This trait cluster is referred to as the metabolic syndrome and confers increased risk of both type 2 diabetes and cardiovascular disease (1–3).

The primary target tissues for insulin action are skeletal muscle, adipose tissue, and liver. To better understand the molecular defects responsible for human insulin resistance, we have recently assessed differential gene expression in human skeletal muscle biopsies from insulin-sensitive and -resistant individuals using cDNA microarray technology (4). These studies identified two differentially expressed genes that are members of the NR4A (NGFI-B) family of orphan nuclear receptors within the greater steroid/thyroid receptor superfamily, namely, NR4A3 and NR4A1.

NR4A3 (also known as NOR-1, TEC, CHN, and MINOR) was originally identified as a protein induced in primary cultures of rat embryonic forebrain neurons undergoing apoptosis (5). Based on homology within the DNA binding domain, there are two additional members of the NR4A (NGFI-B) family of orphan nuclear receptors, NR4A2 (also known as TINUR, NOT, and Nurr1) and NR4A1 (also known as Nur77, NGFI-B, and TR3). NR4A/NGFI-B receptor family members share greater than 97% homology in their DNA binding domains, which consist of two zinc fingers and a domain termed the A box. NR4A3, NR4A2, and NR4A1 also display 37–53% homology in the N-terminal transactivation domains and 53–77% homology in the C-terminal ligand binding domains. In the absence of any ligand, NR4A3, NR4A2, and NR4A1 can each bind and activate the NGFI-B-responsive DNA element characterized by the nucleotide sequence AAAGGTCA (6). In the presence of retinoic acid, however, NR4A1 and NR4A2, but not NR4A3, can heterodimerize with the retinoid X receptor and regulate a DNA element composed of direct repeats separated by five nucleotides (DR5) (7–9). The NR4A/NGFI-B proteins are immediate early response gene products that are involved in neuroendocrine regulation, neural differentiation, liver regeneration, cell apoptosis, and mitogenic and inflammatory stimulation in different cell types (10–15).

Based on the observations from our human muscle microarray studies, we have now examined whether NR4A3 and NR4A1 are able to modulate insulin sensitivity in an insulin target cell. We have demonstrated that NR4A3 and NR4A1 orphan nuclear receptors are highly induced by insulin and thiazolidinedione insulin-sensitizing drugs in 3T3-L1 adipocytes, and, conversely, are suppressed in fat tissues and skeletal muscles from rodent models of insulin resistance. Moreover, increased expression of NR4A3 in 3T3-L1 adipocytes augments insulin responsiveness of the glucose transport system and recruitment of GLUT4 glucose transporters to the plasma.
membrane together with increased insulin regulated phosphorylation of insulin receptor substrate-1 (IRS-1) and Akt. Thus, NR4A3 and perhaps NR4A1 represent novel therapeutic targets for ameliorating insulin resistance and in the treatment/prevention of type 2 diabetes and the metabolic syndrome.

EXPERIMENTAL PROCEDURES

Reagents—Mouse 3T3-L1 fibroblast cells were purchased from American Type Culture Collection (Manassas, VA). Tissue culture media were purchased from Invitrogen. Insulin, dexamethasone, and isobutylmethylxanthine were purchased from Sigma. Phosphatidylcholine 3-kinase inhibitor (LY294002), p38 MAPK inhibitor (SB203580), and protein kinase C inhibitor (Ro3 18220) were purchased from Calbiochem. LacZ staining kit was purchased from Stratagene (San Diego, CA). RNA isolation solution was purchased from Biotechc Laboratory (Houston, TX). Akt and phosphor-Akt antibodies were purchased from Invitrogen. IRS-1 and phosphor-IRS-1 antibodies, GLUT1 polyclonal antibody, GLUT4 polyclonal antibody, and fluorescence- or rhodamine-conjugated second antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). 2-Deoxy-[3H]- and L-[1-3H]glucose were purchased from Amersham Biosciences. Unless otherwise specified, all other reagents were purchased from Sigma.

Cell Culture and Stimulation—3T3-L1 fibroblasts were grown and differentiated into adipocytes in 100-mm culture dishes, as described by Frost and Lane (16). Briefly, cells were grown to 100% confluence in Dulbecco’s minimal essential medium containing 25 mM glucose and 10% calf serum at 37 °C—3T3-L1 fibroblasts were grown and differentiated into adipocytes in 100-mm culture dishes, as described by Frost and Lane (16). Briefly, cells were grown to 100% confluence in Dulbecco’s minimal essential medium (DMEM) containing 25 mM glucose, 0.5 mM isobutylmethylxanthine, 1 mM dexamethasone, 10 μg/ml insulin, and 10% fetal bovine serum (FBS) for 3 days and then for 2 days in DMEM containing 25 mM glucose, 10 μg/ml insulin, and 10% FBS. Thereafter, cells were maintained in and refed every 2 or 3 days with Dulbecco’s minimal essential medium, 25 mM glucose, and 10% fetal bovine serum until used in the experiments 10–14 days after initiation of the differentiation protocol when between 80 and 90% of the cells exhibited the adipocyte phenotype. In differentiated adipocytes, various indicated periods at 37 °C.

Real Time QPCR Analysis—Five μg of total RNA was converted to first strand cDNAs in 20-μl reactions using random primers (Invitrogen). For each quantitative PCR experimental set, the target gene primers (see below) were utilized to amplify 10-fold serial diluted gene products (10−10−2) to make a linear standard curve for each individual target gene. For an endogenous standard 18 S rRNA primers (5′-AAT TTT CGC TGT TTG TGT TTC TCA GAC TAT TTT CCG AAG AAG AAA GAA TTC GTA GGA CAA ACA GC-3′, antisense 5′-AAA AGC AGT TGG TTT TCC TCA GAC TTC TTG CGG AAA GTA TCG TGA GGA CAA ACA GC-3′; sense 5′-CAC CGC TGG CAA TGT TGG GTA GCA ATT CCG AAG AAT TGT TGC ACA TGC TCA GC-3′, antisense 5′-AAA AGC TGA TGT TGC GCA ATT CTT CCT CGG AAT TGT TGC ACA TGC TCA GC-3′; sense 5′-CAC CGC TCT TGT TCC TTC TGA GAC TAT TTT CCG AAA AGC ATG TTT AAA GCT CGG ACA AGA GC-3′, antisense 5′-AAA AGC TGG TTT CCG AAG CAA TGT TTC GAA CGG ACA AGA GC-3′) that are complementary to the mouse NR4A3 gene coding sequences were synthesized by Integrated DNA Technologies (IDT, Coralville, IA) and annealed and then subcloned into the pENTR™/U6 lentiviral vectors (Invitrogen) to create the NR4A3 shRNA constructs following the

2 The abbreviations used are: MAPK, mitogen-activated protein kinase; IRS-1, insulin receptor substrate-1; QPCR, quantitative PCR; shRNA, short hairpin RNA.
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et al. (18) with minor modifications. 3T3-L1 fibroblasts, grown on glass coverslips, were differentiated into adipocytes at least 2 weeks before the experiment. Adipocytes were then incubated for 1.75 h at 37 °C in Leibovitz's L-15 medium (Invitrogen) containing 0.2% bovine serum albumin. Coverslips were incubated in the absence and presence of a maximally effective insulin concentration during the last 30 min of this incubation. Cells were then washed once with ice-cold buffer A (100 mM NaCl, 50 mM HEPES, pH 7.3) and once with ice-cold buffer B (100 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 1 mM phenylmethylsulfonyl fluoride, and 20 mM HEPES, pH 7.2) and then left in buffer B for sonification. Cells underwent sonification for 1 s using a Sonics and Materials VCX-400 Sonifier with a 1-inch diameter probe (model CV26) placed 1 cm above the cover slips (amplitude, 80%; pulse, 0.1 s on, 0.9 s off). Adherent plasma membrane sheets were washed twice in phosphate-buffered saline containing 0.88 mm Ca²⁺ and 0.49 mm Mg²⁺, fixed in 3% paraformaldehyde for 15 min, and processed for indirect immunofluorescence using polyclonal anti-GLUT4 antibody and fluorescein isothiocyanate- or rhodamine-conjugated secondary antibodies as described (19, 20). The amounts of glucose transporter GLUT4 in plasma membranes were quantified by digital image processing as described previously (20). Images of the fluorescein isothiocyanate (FITC)-stained sheets were acquired, and the regions to be quantified were marked in the image captured with the FITC filter and counted with the Image-Pro Plus software.

Animals—Adult Zucker diabetic fatty rats and their controls were purchased from Charles River Laboratories (Wilmington, MA). Fifteen-week-old ob/ob and db/db mice and their wild-type C57BL/6 mice were purchased from The Jackson Laboratory. Animals were housed in colony cages and maintained on a cycle of 12-h light/12-h dark. The animal care and procedures were approved by the Animal Resources Program of the University of Alabama at Birmingham. The tissue samples of streptozotocin-induced diabetic rats, and their controls were kindly provided by Dr. Lidia Maianu.

Statistics—Experimental results are shown as the mean ± S.E. Statistical analyses were performed by unpaired Students t test assuming unequal variance unless otherwise indicated. Significance was defined as p < 0.05.

RESULTS

To examine whether NR4A3 and NR4A1 gene expression are insulin-responsive in adipocytes, fully differentiated 3T3-L1 adipocytes were treated with 100 nM insulin for various times (0 to 8 h), and NR4A3 and NR4A1 mRNA species were measured by quantitative real-time PCR. Fig. 1A demonstrates that insulin produced a rapid increase in NR4A3 gene expression that was sustained at 2–3-fold over basal for a 4-h period. Insulin also stimulated NR4A1 gene expression in adipocytes but with a different temporal response; NR4A1 expression levels increased 5-fold at 1 h and then returned to base line by 2 h after treatment (Fig. 1B). Expression of the NR4A2 (Nurr1) gene, a third member of the NR4A nuclear receptor family, was not stimulated by insulin in fully differentiated adipocytes (data not shown).

The signal transduction pathways mediating insulin regulation of NR4A3 and NR4A1 expression were examined by treating cells with insulin for 1 h in the presence or absence of inhibitors of phosphatidylinositol 3-kinase (LY294002), p38 MAPK (SB203580), or protein kinase C (Ro3 18220). Blocking any of these pathways by administration of these chemical inhibitors partially decreased the ability of insulin to stimulate NR4A3 or NR4A1 gene expression (Fig. 1, C and D) in fully differentiated 3T3-L1 adipocytes. Although these data should be interpreted cautiously since the inhibitors are not always specific, those data suggest that the pathways regulating NR4A expression are similar to those that mediate insulin major metabolic effects in both skeletal muscle and adipocytes.

Next, two drugs in the thiazolidinedione class of insulin-sensitizing drugs, namely pioglitazone and troglitazone, were examined for their ability to induce NR4A3 and NR4A1 gene...
expression in 3T3-L1 adipocytes. The thiazolidinediones are known to improve insulin resistance and are used as a treatment for diabetes in human patients and in animal models (21). In Fig. 2, fully differentiated 3T3-L1 adipocytes were treated with 10 μM concentrations of the indicated thiazolidinediones (pioglitazone or troglitazone) from 0 to 48 h, whereas control cells received vehicle alone. Pioglitazone and troglitazone were both observed to stimulate NR4A3 and NR4A1 gene expression in adipocytes (Fig. 2, A–D). A pioglitazone effect on NR4A3 expression was not noted until 48 h, at which point mRNA levels were increased 7-fold, whereas a 3-fold increase in NR4A1 mRNA was observed at 24 h. Conversely, troglitazone led to a 3-fold increase in NR4A3 at 24 h and a gradual augmentation in NR4A1 mRNA that became significantly greater than that in controls at 48 h.

To identify the biological or functional effects of increased NR4A3 gene expression, a recombinant NR4A3 lentiviral vector was generated and used to stably transduce 3T3-L1 adipocyte cell lines using antibiotic selection (data not shown). The tests for stable recombinant NR4A3 or LacZ gene expression were performed in transformed cells by Western blot analyses (data not shown). To determine the functional effects of NR4A3 or LacZ expression in these transduced cells, we assessed basal and insulin-stimulated glucose transport activity since transport is the rate-limiting step in the ability of insulin to stimulate glucose uptake and metabolism. Insulin augments the transport of glucose into target cells by increasing the concentration of a specific glucose transporter isoform, GLUT4, at the cell surface (22). As shown in Fig. 3A, the ability of insulin to maximally stimulate glucose uptake in fully differentiated adipocytes was increased by 88% (p < 0.01) in the NR4A3-overexpressing cells when compared with LacZ-transformed control adipocytes. This was true for all five different stably transduced NR4A3-expressing cell lines that were established in these experiments (data not shown). In full-dose-response curves, glucose transport responses were markedly enhanced over the full range of insulin concentrations in NR4A3-transduced cells without changes in the insulin half-maximal effective dose (ED₅₀) for glucose transport stimulation (data not shown).

To further characterize the ability of NR4A3 to modulate glucose transport in adipocytes, we used recombinant shRNA-NR4A3 and control scramble lentiviruses to perform the NR4A3 inhibition experiments. The infection of shRNA-NR4A3 lentiviruses led to a mean 41% suppression of endogenous NR4A3 protein expression relative to control cells (data not shown) compared with controls. As shown in Fig. 3B, suppression of endogenous NR4A3 in adipocytes reduced maximally insulin-stimulated glucose transport rates by 36% (p < 0.05) when compared with the scramble controls.

To determine the mechanisms by which NR4A3 hyperexpression increases insulin responsiveness, we next assessed the
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ability of insulin to recruit GLUT4 glucose transport proteins to the cell surface using the plasma membrane “lawn” assay, as seen in Fig. 4. In these experiments, adipocytes were cultured on glass coverslips and stimulated for 30 min with or without (basal) 100 nM insulin. The adipocytes were then disrupted by sonication leaving plasma membrane sheets attached to coverslips (i.e. plasma membrane lawn assay). Plasma membrane associated GLUT4 was detected using a polyclonal anti-GLUT4 antibody and a fluorescein isothiocyanate-conjugated secondary antibody. A, basal LacZ expressing adipocytes. B, insulin-stimulated LacZ expressing adipocytes. C, basal NR4A3 overexpressing adipocytes. D, insulin-stimulated NR4A3 overexpressing adipocytes. Image analyses were performed with an Image-Pro Plus software. The Image-Pro Plus software defined the immunofluorescence staining areas and automatically calculated these defined areas when comparing with the non-staining cell areas. The experiments were performed three times, and each experiment involved triplicate samples averaged for a single data point. **, p < 0.01 for comparing insulin-stimulated control and insulin-stimulated NR4A3-overexpressing cells.

in the NR4A3-transduced adipocytes. Analogous experiments testing for effects of NR4A3 suppression were performed using shRNA-NR4A3 and scramble control lentiviral constructs (Fig. 5C). A mean 42% reduction in NR4A3 protein in the shRNA-transduced cells was associated with significant decrements in the ability of insulin to promote phosphorylation of IRS-1 and Akt (Fig. 5C) when compared with the control cells.

To determine whether NR4A3 and NR4A1 are dysregulated in states of insulin resistance and type 2 diabetes, gene expression was analyzed in muscle and adipose tissues from several rodent models. We studied streptozotocin-induced diabetic rats, Zucker diabetic fatty rats, db/db mice, and db/db mice, all of which are well characterized rodent models of insulin resistance and/or diabetes (23, 24). Skeletal muscles and adipose tissues from these diabetic and insulin-resistant rodents and respective insulin sensitive controls were obtained, and mRNA species encoding NR4A3 and NR4A1 were measured by quantitative real-time PCR. In all instances both NR4A3 and NR4A1 gene expression were decreased in skeletal muscle and adipose cells compared with that in LacZ controls. Therefore, NR4A3 gene expression increased insulin-stimulated glucose transport by enhancing the appearance of GLUT4 in the plasma membrane. These effects occurred without any significant change in the total cellular content of GLUT4 or GLUT1 proteins, as shown in Fig. 5A, comparing GLUT levels in fully differentiated adipocytes transduced with either NR4A3 or LacZ. To explore whether NR4A3 might affect adipocyte differentiation, we measured GLUT4 content (data not shown) and lipid accumulation (Fig. 5A) in 3T3-L1 cells at day 8, when the cells were less than fully mature, and did not observe any significant differences in these adipocyte phenotypes in NR4A3 hyperexpressing and LacZ control cells. This suggests that NR4A3 may not regulate the rate of adipocyte differentiation as reflected by these parameters.

We next asked whether NR4A3 affected insulin signaling in adipocytes. IRS-1 and Akt phosphorylation was assessed in fully differentiated NR4A3- and LacZ-transduced adipocytes under basal and insulin-stimulated conditions (Fig. 5B). These experiments indicated that insulin-stimulated tyrosine phosphorylation of IRS-1 (tyrosine 941) and serine phosphorylation of Akt (serine 473) were enhanced...
tissue from insulin-resistant animals. Fig. 6A showed that NR4A3 and NR4A1 gene expression was significantly decreased in the streptozotocin and Zucker diabetic fatty rat models, and Fig. 6B and C, showed similar results from skeletal muscles and adipose tissues for the ob/ob and db/db mice models. Thus, NR4A3 and NR4A1 genes were consistently suppressed as a function of insulin resistance, diabetes, and/or obesity.

**DISCUSSION**

The present results demonstrate for the first time that the NR4A3 and NR4A1 genes, both members of the NR4A family of orphan nuclear receptors, are up-regulated by insulin and thiazolidinediones and are suppressed in skeletal muscle and adipose tissue from rodent models of insulin resistance or diabetes. Moreover, NR4A3 is capable of enhancing insulin responsiveness of the glucose transport system and increasing GLUT4 translocation to the plasma membrane in adipocytes. Specifically, the current data show that 1) NR4A3 and NR4A1 genes were induced by insulin in 3T3-L1 adipocytes, and this response appeared to probably involve multiple insulin signaling pathways (phosphatidylinositol 3-kinase, p38 MAPK, and protein kinase C), 2) NR4A3 and NR4A1 mRNA levels were increased by two different thiazolidinedione insulin-sensitizing drugs in 3T3-L1 adipocytes (troglitazone and pioglitazone), 3) expression of NR4A3 and NR4A1 genes in skeletal muscle and adipose tissue was consistently decreased in multiple rodent models of insulin resistance, obesity, and type 2 diabetes (Zucker diabetic fatty rats, streptozotocin-induced diabetic rats, ob/ob mice, db/db mice), 4) in lentiviral vector stably transduced adipocyte cell lines, NR4A3 expression markedly augmented insulin responsiveness for stimulation of glucose transport activity compared with LacZ transduced controls. On the other hand, inhibition of NR4A3 expression by small interfering RNA from lentiviral shRNA-NR4A3 constructs reduced insulin-stimulated glucose transport and led to insulin resistance; 5) increased NR4A3 expression led to increased mobilization of the GLUT4 glucose transporter proteins to the cell surface in response to insulin, and 6) NR4A3 hyperexpression increased insulin-mediated phosphorylation of IRS-1 and Akt phosphorylation, and these components of insulin signal trans-
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FIGURE 6. NR4A3 and NR4A1 expression in skeletal muscle and adipose tissue of diabetic and insulin-resistant rats and mice. Skeletal muscle (gastrocnemius) and adipose tissue (white fat) from diabetic and insulin-resistant rats or mice and respective insulin sensitive controls were homogenized, and mRNAs were extracted for cDNA syntheses. Quantitative real-time PCR was used to measure the expression of NR4A3 and NR4A1 genes. A, NR4A3 and NR4A1 gene expression in skeletal muscles from streptozotocin-induced diabetic rats (STZ Rat) and in skeletal muscles and adipose tissues from Zucker diabetic fatty rats (ZDF Rat) and in respective control rats. Shown are NR4A3 and NR4A1 gene expression in skeletal muscles and adipose tissues from ob/ob mice (B) and db/db mice (C) as well as in the respective control mice. All results represent the mean ± S.E. from three separate experiments. *p < 0.05 (*) and **p < 0.01 (**) for comparing control and obese/diabetic animals.
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duction were inhibited by small interfering RNA-mediated suppression of NR4A3. The combined data implicated a role for these orphan nuclear receptors in the modulation of insulin action and in the pathogenesis of insulin resistance. Furthermore, agonists for NR4A3 receptor action would predictably increase insulin sensitivity and have application in the treatment and prevention of type 2 diabetes and metabolic syndrome.

Previous work has identified NR4A receptors as early response genes in T cells for apoptosis (25); however, the biological function of these receptors in other cell types is poorly understood. Recently, NR4A nuclear receptors have been implicated in the development of cardiovascular disease in response to diverse inflammatory stimuli in macrophages (14, 15). However, very few direct regulatory or target genes for these transcription factors have been identified. The homeotic protein, Six3, was shown in a yeast two-hybrid screen to be a putative partner of NR4A3 (26), and Six3 has been shown to bind in vitro to the DNA binding domain and to interact with NR4A3 in vivo in human chondrocyte cell lines (27). When we examined the expression of Six3 gene in 3T3-L1 adipocytes, we could only detect very low levels of this gene expression, and we did not observe that Six3 was stimulated by insulin in adipocytes (data not shown). Thus, other coactivators and target genes for NR4A3 are likely to exist in adipocytes relating to insulin signaling pathways and GLUT4 translocation. A mouse gene knock-out model revealed that NR4A3 is essential for the development of the semicircular canals of the inner ear (28); these results are consistent with the predominant expression of NR4A3 gene in the central nervous system (29–31). However, it is not clear whether these NR4A3-deficient mice exhibit metabolic defects in other tissues, such as skeletal muscle, adipose tissue, and macrophages. Interestingly, during the submission of this manuscript, Pei et al. (32) reported that NR4A orphan nuclear receptors regulate glucose metabolism in hepatocytes, although these orphan nuclear receptors expressed either very low level or undetected in liver tissue.3 It seems likely, therefore, that the target genes and physiological functions of NR4A receptors are tissue-dependent and developmentally programmed (32).

IRS-1 is a substrate of the insulin receptor-tyrosine kinase and appears to have a central role in the insulin-stimulated signal transduction pathways, and this step is highly regulated in both physiological and pathological conditions. Tyrosine phosphorylation of IRS-1 is required for downstream activation of phosphatidylinositol 3-kinase and Akt, with subsequent GLUT4 translocation and stimulation of glucose transport activity, and the current data demonstrate that overexpression of NR4A3 in adipocytes can augment all of these actions of insulin. These results demonstrated that the NR4A3 orphan nuclear receptor can exert a powerful effect to enhance insulin sensitivity in adipocytes.

Our present work also demonstrates that insulin resistance in rodent models is associated with reduced expression of NR4A3 and NR4A1 in both skeletal muscle and adipose tissues. Therefore, NR4A3 and possibly NR4A1 represent potential novel therapeutic targets for the treatment and prevention of diabetes, metabolic syndrome, and other diseases associated with insulin resistance. Such new therapeutic agents could include synthetic or endogenous ligands as agonists for NR4A3 and NR4A1 or other strategies that result in increased expression or action regarding these nuclear receptors. Because these receptors are induced by thiazolidinediones, it is possible that this effect contributes to the insulin-sensitizing action of these drugs. Thus, NR4A3 and NR4A1 could provide the basis for identifying downstream molecular events that mediate desirable therapeutic effects. For example, a search for genes regulated by NR4A3 and NR4A1 or factors that interact with these nuclear receptors could identify new drug targets. Additional research is needed to examine the precise molecular mechanisms by which these nuclear receptors could modulate insulin sensitivity and the activity of the insulin-responsive glucose transport system.

Acknowledgments—We acknowledge core facility support (Core Director, Dr. Barbara Gower) in the University of Alabama at Birmingham Clinical Nutrition Research Unit (Grant P30 DK-56336).

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