# Essential Role of Mitochondrial Function in Adiponectin Synthesis in Adipocytes

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**OBJECTIVE**—Adiponectin is an important adipocytokine that improves insulin action and reduces atherosclerotic processes. The plasma adiponectin level is paradoxically reduced in obese individuals, but the underlying mechanism is unknown. This study was undertaken to test the hypothesis that mitochondrial function is linked to adiponectin synthesis in adipocytes.

**RESEARCH DESIGN AND METHODS**—We examined the effects of rosiglitazone and the measures that increase or decrease mitochondrial function on adiponectin synthesis. We also examined the molecular mechanism by which changes in mitochondrial function affect adiponectin synthesis.

**RESULTS**—Adiponectin expression and mitochondrial content in adipose tissue were reduced in obese *db/db* mice, and these changes were reversed by the administration of rosiglitazone. In cultured adipocytes, induction of increased mitochondrial biogenesis (via adenoviral overexpression of nuclear respiratory factor-1) increased adiponectin synthesis, whereas impairment in mitochondrial function decreased it. Impaired mitochondrial function increased endoplasmic reticulum (ER) stress, and agents causing mitochondrial or ER stress reduced adiponectin transcription via activation of c-Jun $NH_2$-terminal kinase (JNK) and consequent induction of activating transcription factor (ATF)3. Increased mitochondrial biogenesis reversed all of these changes.

**CONCLUSIONS**—Mitochondrial function is linked to adiponectin synthesis in adipocytes, and mitochondrial dysfunction in adipose tissue may explain decreased plasma adiponectin levels in obesity. Impaired mitochondrial function activates a series of mechanisms involving ER stress, JNK, and ATF3 to decrease adiponectin synthesis. ***Diabetes*** **56:2973–2981, 2007**

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Ad-NRF-1, adenovirus-mediated overexpression of nuclear respiratory factor 1; ATF, activating transcription factor; ATP, adenosine triphosphate; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; ER, endoplasmic reticulum; JNK, c-Jun $NH_2$-terminal kinase; mtDNA, mitochondrial DNA; mtTFA, mitochondrial transcription factor A; NRF, nuclear respiratory factor; ORP150, 150-kDa oxygen-regulated protein; PPAR, peroxisome proliferator–activated receptor; ROS, reactive oxygen species; siRNA, small interfering RNA; TNF, tumor necrosis factor; TZD, thiazolidinedione; XBP, X-box binding protein.



It is now clear that the adipocyte is not a simple energy store but rather an endocrine gland playing an important role in fuel metabolism (1,2). Adiponectin is one of the adipocyte hormones (adipocytokines) that have many favorable effects on metabolism, including improvement of insulin action and reduction of atherosclerotic processes (3,4). Unlike other adipocytokines, the plasma level of adiponectin is reduced in obese individuals (1,5), presumably due to selective suppression of its synthesis in adipocytes (5). However, the mechanism responsible for reduced adiponectin synthesis in obesity is presently unknown.

Adipocytes undergo two stages of maturation: differentiation and hypertrophy (6). During the early stage of maturation (differentiation), adipocytes have high levels of metabolic activities and increased fuel consumption. These young cells are relatively small and insulin sensitive and show increased adiponectin expression. By contrast, older cells increase in size (hypertrophy) and lose most of their functional activities, including adiponectin synthesis (6). Recent studies have demonstrated that mitochondrial biogenesis is increased during adipocyte differentiation (7), but the number of mitochondria is reduced in adipocytes of obese *db/db* mice (8). These data indicate that mitochondrial biogenesis may be critically required for adipocyte differentiation (and metabolic activities) and that a reduction in mitochondrial mass is responsible for adipocyte hypertrophy (and loss of metabolic activities).

Two widely used classes of drug are known to affect plasma adiponectin levels: peroxisome proliferator–activated receptor (PPAR)γ agonists (thiazolidinediones [TZDs]) increase plasma adiponectin levels (1), whereas anti-HIV drugs, including protease inhibitors, decrease adiponectin levels (9). We noted that the actions of these drugs are associated with changes in mitochondrial mass or function in adipocytes. TZDs, which stimulate adipocyte differentiation but prevent hypertrophy (10), increase the mitochondrial content in adipocytes (8). On the other hand, anti-HIV drugs induce a characteristic lipodystrophy syndrome of peripheral fat wasting and central adiposity, the latter characterized by mitochondrial defects (11). Thus, the increased adiponectin synthesis with TZDs is associated with increased mitochondrial content in adipocytes, whereas the reduced adiponectin synthesis with anti-HIV drugs is associated with impaired mitochondrial function. Based on these findings, we hypothesized that mitochondrial mass or function is linked to adiponectin synthesis in adipocytes and performed a series of studies both in vivo and in vitro to test this hypothesis.

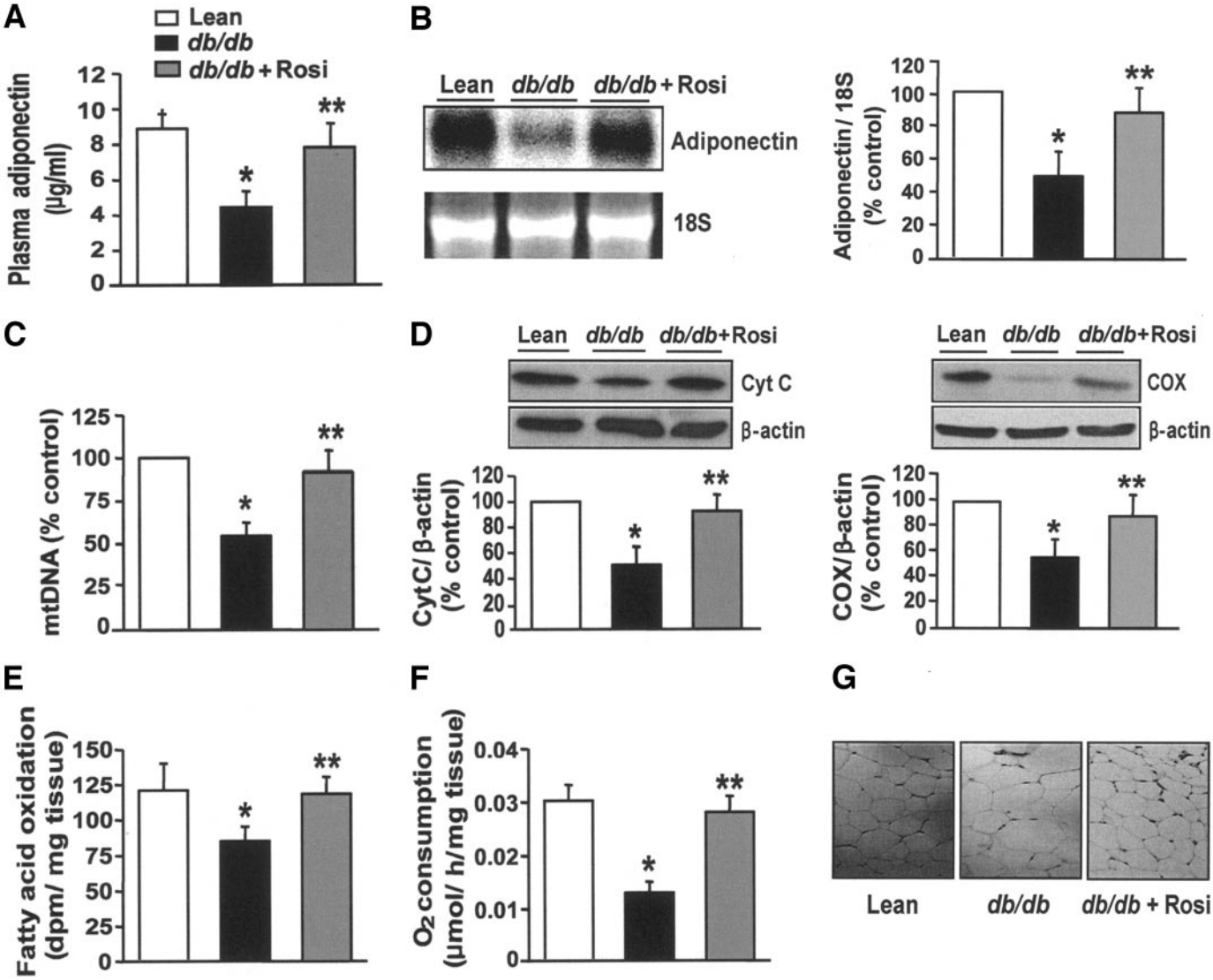


**FIG. 1. Rosiglitazone increases adiponectin and mitochondria in adipocytes of *db/db* mice. Data are means ± SEM. *A* and *B*: Plasma levels of adiponectin (*A*) and adiponectin mRNA expression in adipose tissue (*B*) of *db/+* (lean) and *db/db* mice at age 12 weeks treated with or without rosiglitazone (Rosi) ($n = 7$). *C*: Quantification of mtDNA content in adipose tissues ($n = 7$). *D*: Western blot analysis of mitochondrial proteins in adipose tissues ($n = 5$). Cyt C, cytochrome *c*; COX, cytochrome *c* oxidase subunit II. *E*: Fatty acid oxidation in isolated adipose tissues ($n = 5$). *F*: Oxygen consumption in adipose tissues ($n = 5$). *G*: Histologic examination of adipose tissues. Each field is a representative hematoxylin-and-eosin–stained image. Original amplification: ×400. *$P < 0.05$ vs. lean mice; **$P < 0.05$ vs. untreated *db/db* mice.**

## RESEARCH DESIGN AND METHODS

Male *db/db* mice aged 8 weeks and their lean littermates (*db/+*) were housed in cages containing four mice per cage and allowed ad libitum access to water and food. The *db/db* mice were treated for 4 weeks with 20 mg · kg$^{-1}$ · day$^{-1}$ rosiglitazone (GlaxoSmithKline, Middlesex, U.K.), which was added to powered, standard mouse chow. Control *db/+* and *db/db* mice were fed on standard mouse chow ad libitum. The experiments were approved by the institutional animal care and use committee at the Asan Institute for Life Sciences, Seoul, Korea.

**Cell culture.** 3T3L1 fibroblasts were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum in an incubator with 5% $CO_2$/95% $O_2$ at 37°C. 3T3L1 preadipocytes were differentiated to mature adipocytes by a conventional method (7) and were maintained as subconfluent cultures.

**Measurement of adiponectin concentration.** Adiponectin concentrations in culture media and mouse plasma were measured by radioimmunoassay (Linco Research, St. Charles, MO).

**Determination of mRNA and protein expression.** mRNA expressions of adiponectin were measured by Northern blot analysis. Mitochondrial transcription factor A (mtTFA) mRNA levels were analyzed by real-time PCR. For details of Northern blot and real-time PCR analysis, refer to the online appendix (available at http://dx.doi.org/10.2337/db07-0510). Protein expression in cells and tissues was measured by Western blot analysis as previously described (12).

**Quantification of mitochondrial DNA content and measurement of mitochondrial mass.** Quantification of mitochondrial DNA (mtDNA) was performed using real-time PCR analysis. Mitochondrial mass was measured by cytometric analysis using Mito Tracker Green FM (Molecular Probes, Eugene, OR). Details for both can be found in the online appendix.

**Fatty acid oxidation and oxygen consumption.** Fatty acid oxidation was determined by measuring $^{14}CO_2$ generation from [$^{14}$C] palmitate (NEN Life Sciences, Boston, MA) as previously described (13). Oxygen comsumption in epididymal adipose tissue was measured using a 782 single-dual-channel oxygen meter (Strathkelvin Instruments, Glasgow, U.K.). Isolated mitochondria were washed in Tris-based, $Mg^{2+}$- and $Ca^{2+}$-deficient buffer (0.137 mol/l NaCl, 5 mmol/l KCl, 0.7 mmol/l $Na_2HPO_4$, and 2.5 mmol/l Tris-HCl, pH 7.4) at 37°C and resuspended in the same buffer. Oxygen consumption was monitored continuously for 6 min.

**Histology.** Epididymal adipose tissues from *db/+* and *db/db* mice were fixed in 10% formalin, dehydrated, embedded in paraffin, and sectioned for hematoxylin/eosin staining. Images were captured using an Olympus BX60 camera (Olympus, Tokyo, Japan) and processed in Adobe Photoshop (Adobe Systems).

**Analysis of splicing of X-box binding protein-1 mRNA.** The target of X-box binding protein (XBP)-1 cDNA was amplified by PCR. This fragment

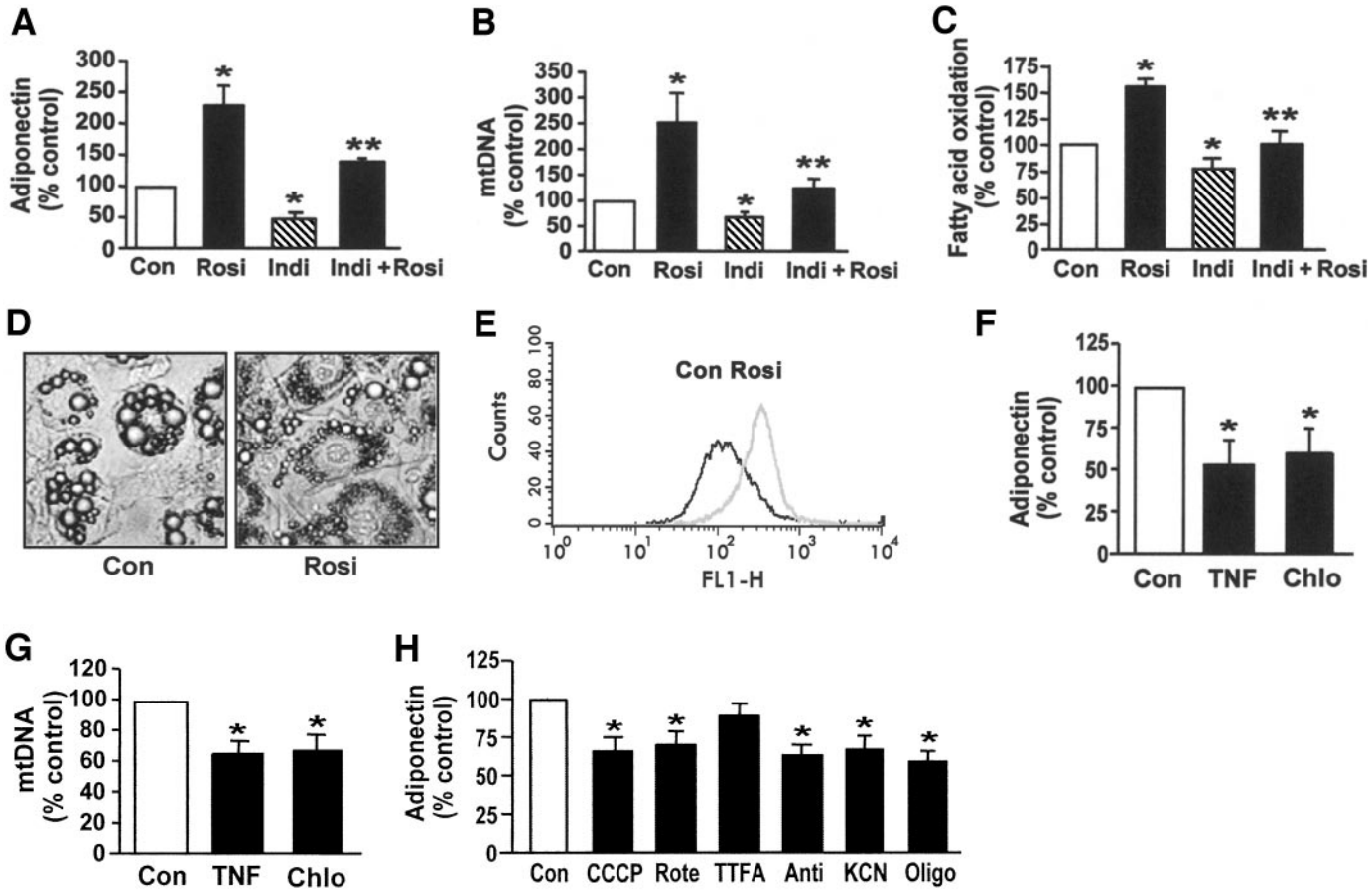


**FIG. 2. Mitochondrial function is related to adiponectin synthesis in cultured adipocytes. *A–C*: Effects of rosiglitazone and indinavir on adiponectin levels in the media (*A*), mtDNA content (*B*), and fatty acid oxidation (*C*) in differentiated 3T3L1 adipocytes. 3T3L1 adipocytes were cultured with 15 μmol/l rosiglitazone (Rosi) or 100 μmol/l indinavir (Indi) for 48 h ($n = 6$). *$P < 0.05$ vs. untreated (Con) cells; **$P < 0.05$ vs. rosiglitazone-treated cells. Data in *A–C* are means ± SEM. *D*: Confocal laser scanning of differentiated 3T3L1 adipocytes with or without rosiglitazone treatment (15 μmol/l for 48 h). The magnification was 800-fold. *E*: Flow cytometric analysis of untreated and rosiglitazone-treated cells stained with Mito Tracker Green FM. *F–H*: Effects of chemicals that impair mitochondrial function on adiponectin synthesis. Adiponectin levels in the media (*F*) and mtDNA contents (*G*) of untreated, TNF-α–treated (588 pmol/l), and chloramphenicol-treated (309 mmol/l) (Chlo) cells for 8 h ($n = 6$). *H*: Adiponectin levels in the media from untreated, CCCP-treated (1.95 μmol/l), rotenone-treated (100 μmol/l) (Rote), thenoyltrifluoroacetone-treated (10 μmol/l) (TTFA), antimycin A–treated (18.2 μmol/l) (Anti), potassium cyanide–treated (1 mmol/l) (KCN), or oligomycin-treated (12. 9 μmol/l) (Oligo) cells for 8 h ($n = 5$). *$P < 0.05$ vs. untreated cells. Data in *F–H* are means ± SEM.**

was further digested by restriction endonulcease *Pst*I for 2 h at 37°C to reveal a restriction site that is lost after inositol-requiring enzyme-1–mediated cleavage and splicing of the mRNA (14). After separating the restriction digests, the cDNA fragments were resolved on 2% agarose gel. The sequences of XBP-1 primers were as follows: forward 3S, 5′-AAACAGAGTAGCAGCG CAGACTGC-3′; reverse 2AS, 5′-GGATCTCTAAAACTAGAGGCTTGGTG-3′.

**Recombinant adenovirus.** Nuclear respiratory factor (NRF)-1 and 150-kDa oxygen-regulated protein (ORP150) adenoviruses were kind gifts from Prof. Y. K. Pak (Kyung Hee University, Seoul, Korea) and Prof. S. Ogawa (Kanazawa University School of Medicine, Kanazawa City, Japan), respectively.

**Adiponectin promoter and measurement of promoter activity.** Profiles of transcription factors are different between adipocytes and preadipocytes. However, since differentiated adipocytes are resistant to transfection by lipofectamine, we performed the luciferase assay in preadipocytes, as previously described (15). The adiponectin promoter constructs containing the nucleotide sequence from −984 to +15 of the mouse adiponectin gene were a kind gift from Prof. J.B. Kim (Seoul National University, Seoul, Korea) (16). Site-mutated plasmid where the putative binding site for activating transcription factor (ATF)3 was mutated was constructed by the two-step PCR method (17). The pAdiponectin-984-Luc reporters (1 μg) and p-cytomegalovirus-β-gal (0.1 μg) were used as internal controls of transfection/expression and were transfected using lipofectamine. The luciferase activity was normalized to the β-galactosidase activity.

**Small interfering RNA.** The murine mtTFA sequence 5′-GUA CAU UUU GGG AAA GUA A-3′ was targeted for RNA interference. We also made control small interfering RNAs (siRNAs), which have the same GC content as the target sequences and do not affect silencing of gene expression (Bioneer, Daejeon, Korea). ATF3-specific siRNA (M-058604-01-0010) and nontargeting control siRNA (D-001210-01-20) were designed and purchased from Dharmacon (Lafayette, CO). Using lipofectamine (Invitrogen, Carlsbad, CA), $1.5 \times 10^5$ cells were seeded onto sixwell plates and transfected with 100 pmol siRNA according to the manufacturer's instructions.

**Statistics.** All values are given as means ± SE. Differences between two groups were assessed using unpaired two-tailed *t* tests. Data from more than two groups were assessed by ANOVA followed by a post hoc least-significant difference test. Repeated ANOVA was used for the studies of adiponectin promoter activity and the effect of adenovirus-expressing ORP150 on adiponectin synthesis. Statistical analyses were performed using SPSS-PC13 (SPSS, Chicago, IL).

## RESULTS

**Mitochondrial function is linked to adiponectin synthesis in adipocytes.** As previously reported (16), plasma adiponectin levels and adiponectin expression in adipose tissue were lower in obese *db/db* mice than in lean control mice (Fig. 1*A* and *B*). Treatment of the obese animals with rosiglitazone, a widely used TZD drug, increased both plasma adiponectin levels and adiponectin expression in adipose tissue. In contrast, plasma levels of other adipocytokines, such as leptin, tumor necrosis factor (TNF)-α, plasminogen activator inhibitor 1, and interleukin-6 were higher in *db/db* mice and were not affected by rosiglitazone (supplementary Fig. 1). mtDNA content, expression of

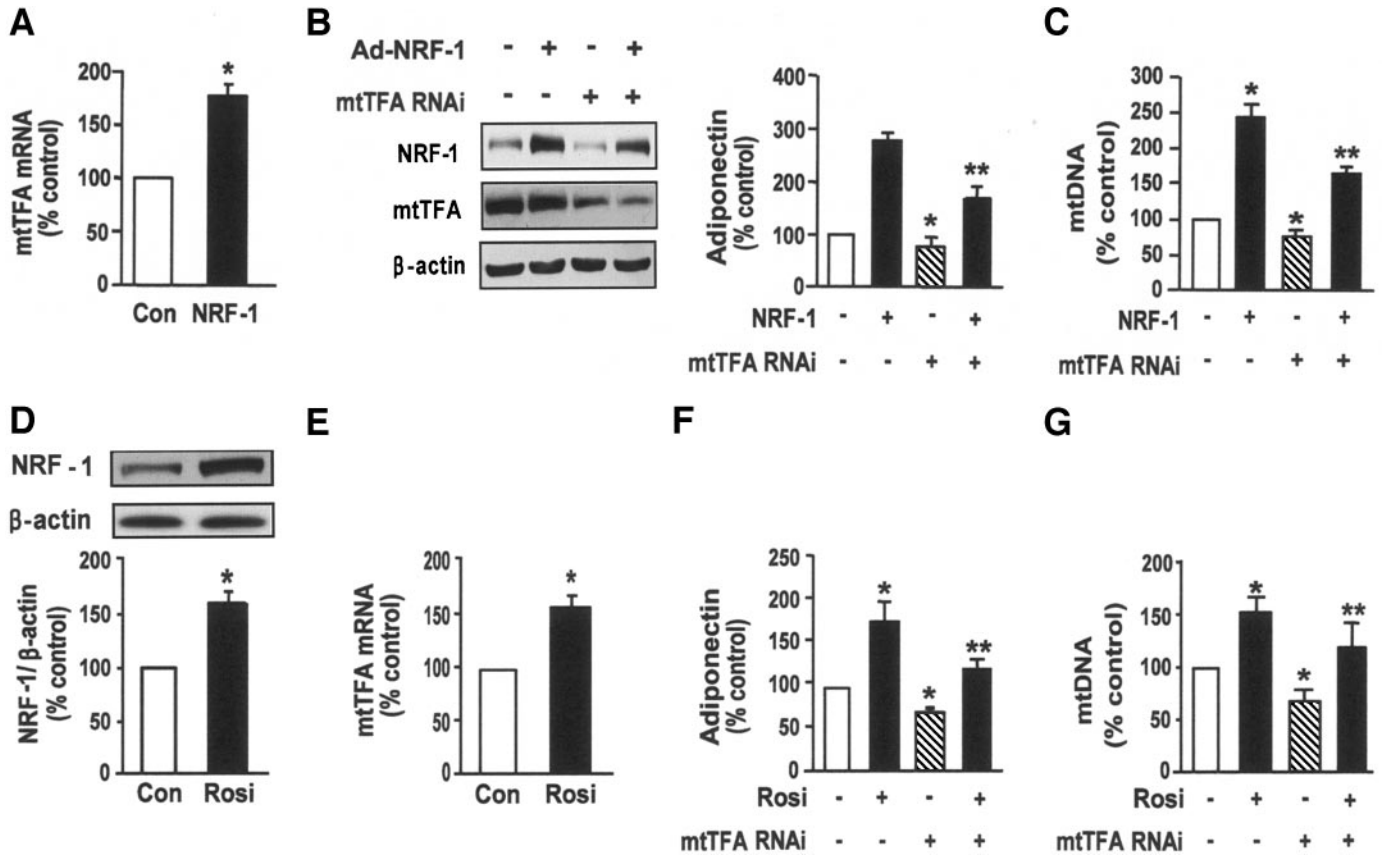


**FIG. 3. Increased mitochondrial biogenesis increases adiponectin synthesis. *A*: Effects of Ad-NRF-1 on mtTFA mRNA levels. Cells treated with $1 \times 10^7$ plaque-forming units of Ad-NRF-1 (NRF-1) or adenovirus-expressing β-gal (Con) were cultured for 48 h ($n = 6$). *$P < 0.05$ vs. adenovirus-expressing β-gal. *B* and *C*: Effects of Ad-NRF-1 and mtTFA siRNA on mtDNA content and adiponectin synthesis. Adiponectin levels in the media (*B*) and mtDNA content (*C*) in the differentiated 3T3L1 cells overexpressing Ad-NRF-1, with or without mtTFA-siRNA transfection. Cells were cotransfected with 100 pmol mtTFA siRNA (mtTFA RNAi) or control siRNA. *$P < 0.05$ vs. adenovirus-expressing β-gal and control siRNA-transfected cells; **$P < 0.05$ vs. Ad-NRF-1 and control siRNA transfected cells. *D* and *E*: Effects of rosiglitazone treatment on NRF-1 and mtTFA expression. NRF-1 protein expression (*D*) and mtTFA mRNA levels (*E*) in the differentiated 3T3L1 adipocytes cultured with or without 15 μmol/l rosiglitazone (Rosi) for 48 h ($n = 6$). *$P < 0.05$ vs. untreated cells. *F* and *G*: Effect of mtTFA siRNA on adiponectin synthesis from the cells. Adiponectin levels in the media (*F*) and mtDNA content in the cells (*G*). Cells were transfected with 100 pmol mtTFA siRNA (mtTFA RNAi +) or control siRNA (mtTFA RNAi −) in the absence or presence of 15 μmol/l rosiglitazone for 48 h ($n = 5$). *$P < 0.05$ vs. untreated cells; **$P < 0.05$ vs. rosiglitazone-treated cells. Data are means ± SEM. □, lean *db*/+ mice; ■, *db/db* mice; ▨, db/db mice treated with rosiglitazone.**

mitochondrial proteins, fatty acid oxidation, and oxygen consumption in adipose tissues were reduced in the obese mice but increased with rosiglitazone treatment (Fig. 1*C–F*). Individual adipocytes were larger in the obese animals but became smaller in rosiglitazone-treated mice (Fig. 1*G*). Electron microscopy examination confirmed that adipocytes of the obese animals contained fewer mitochondria than those of the lean animals; however, the numbers of mitochondria increased with rosiglitazone treatment (8) (supplementary Fig. 2).

In accordance with these in vivo data, rosiglitazone increased the levels of adiponectin synthesis in cultured 3T3L1 adipocytes, which was associated with increases in mtDNA content and fatty acid oxidation (Fig. 2*A–C*). Rosiglitazone reduced the sizes of individual adipocytes and intracellular fat droplets (Fig. 2*D*) and increased mitochondrial mass (Fig. 2*E*). Treatment with indinavir, an anti-HIV protease inhibitor, reversed rosiglitazone-induced increases in adiponectin synthesis, mtDNA content, and fatty acid oxidation (Fig. 2*A–C*). Both TNF-α (a cytokine shown to reduce mitochondrial biogenesis and increase susceptibility to mitochondrial injury [18,19]) and chloramphenicol (a specific inhibitor of mitochondrial protein synthesis [20]) reduced adiponectin synthesis and mtDNA content (Fig. 2*F* and *G*). Furthermore, most of the inhibitors for individual respiratory complexes and the mitochondrial uncoupler carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) decreased adiponectin synthesis (Fig. 2*H*) at concentrations that did not affect cell viability (supplementary Fig. 3). In contrast, these chemicals did not affect or increase the synthesis of other adipocytokines (supplementary Fig. 4). Additionally, rosiglitazone did not affect or decrease the synthesis of other adipocytokines. Collectively, these results indicate that mitochondrial function is selectively linked to adiponectin synthesis in adipocytes.

**Increased mitochondrial biogenesis increases adiponectin synthesis.** NRF-1 is a transcription factor that regulates the expression of nuclear-encoded mitochondrial genes (21), including mtTFA, a mitochondrial matrix protein essential for the replication and transcription of mtDNA (22). Adenovirus-mediated overexpression of NRF-1 (Ad-NRF-1) (Fig. 3*B*) increased mtTFA expression and mtDNA content in cultured 3T3L1 adipocytes, and these changes were accompanied by increased levels of adiponectin synthesis (Fig. 3*A–C*). Inhibition of mtTFA expression using an mtTFA-specific siRNA (Fig. 3*B*) reversed the Ad-NRF-1–induced increases in adiponectin synthesis and mtDNA content (Fig. 3*B* and *C*). Interestingly, rosiglitazone increased the expression levels of

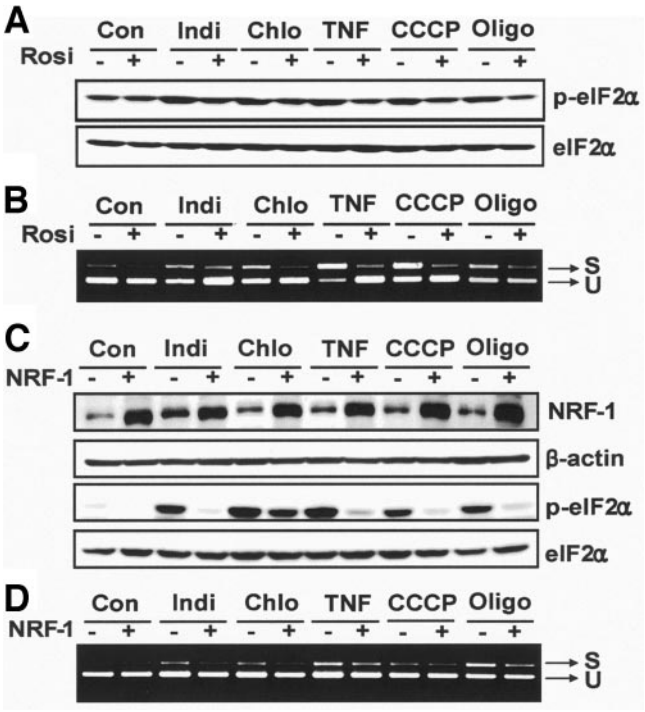


**FIG. 4. Impaired mitochondrial function induces ER stress in adipocytes. *A* and *B*: Effects of chemicals that impair mitochondrial function and rosiglitazone on ER stress responses. *A*: Representative Western blots of eukaryotic initiation factor-2α phosphorylation (p-eIF2α) in differentiated 3T3L1 cells treated with 100 μmol/l indinavir (Indi), 309 μmol/l chloramphenicol (Chlo), 588 pmol/l TNF-α, 1.95 μmol/l CCCP, or 12.9 μmol/l oligomycin (Oligo) for 6 h or without rosiglitazone (Rosi). *B*: Representative figures of RT-PCR analysis of spliced (S) and unspliced (U) XBP-1 mRNA under the experimental conditions described in (*A*). *C* and *D*: Effects of Ad-NRF-1 on ER stress responses. Quantification of eukaryotic initiation factor-2α phosphorylation (p-eIF2α) (*C*) and spliced (S) and unspliced (U) XBP-1 mRNA (*D*) under the experimental conditions described in *A*.**

NRF-1 and mtTFA in 3T3L1 adipocytes (Fig. 3*D* and *E*), and mtTFA-specific siRNA reversed the rosiglitazone-induced increases in adiponectin synthesis and mtDNA content (Fig. 3*F* and *G*). Collectively, these results indicate that mitochondrial biogenesis is an important regulator of adiponectin synthesis in adipocytes.

**Mitochondrial dysfunction induces endoplasmic reticulum stress in adipocytes.** Protein folding in the endoplasmic reticulum (ER) requires large amounts of adenosine triphosphate (ATP), and the ER has close physical and functional contact with mitochondria. Glucose deprivation and hypoxia, both of which deplete cellular ATP levels, are well-known stimuli of ER stress (23). In addition, a recent study has shown that nitric oxide–induced disruption of mitochondrial respiratory chain induced ER stress responses (24). We therefore examined the possibility that mitochondrial dysfunction induces ER stress. Chemicals that have been shown to reduce mitochondrial function and adiponectin synthesis (such as indinavir, chloramphenicol, TNF-α, CCCP, and oligomycin) increased the levels of ER stress markers such as eukaryotic initiation factor-2α phosphorylations and XBP-1 splicing (14,25) (Fig. 4*A* and *B*), indicating that mitochondrial dysfunction induces ER stress. Rosiglitazone treatment or Ad-NRF-1 reduced the mitochondrial dysfunction–induced increases in ER stress markers (Fig. 4*A–D*).

**Mitochondrial dysfunction/ER stress reduces transcription of adiponectin through activation of JNK and ATF3.** Rosiglitazone has been shown to increase the promoter activity of adiponectin (26) (Fig. 5*A*). Ad-NRF-1 also increased the adiponectin promoter activity (Fig. 5*B*). mtTFA-specific siRNA and chemicals that impair mitochondrial function reversed the rosiglitazone-induced increase in adiponectin promoter activity (Fig. 5*A* and *C*). Adenoviral overexpression of ORP150 (Fig. 5*D*), a molecular ER chaperone shown to reduce insulin resistance (27), restored mitochondrial dysfunction–induced decreases in promoter activity and synthesis of adiponectin (Fig. 5*D* and *E*). These data indicate that changes in mitochondrial or ER function affect adiponectin synthesis, at least in part, by affecting the transcription of adiponectin.

Agents that impair mitochondrial function or promote ER stress increased the expression of ATF3, a protein shown to negatively regulate adiponectin gene expression (17) (Fig. 6*A*). Ad-NRF-1 reduced the increase in ATF3 expression seen with chemicals that impair mitochondrial function (Fig. 6*A*). ATF3-specific siRNA (Fig. 6*B*) reversed the mitochondrial dysfunction–induced reduction in adiponectin promoter activity and synthesis (Fig. 6*B* and *C*). Also, mitochondrial dysfunction did not decrease adiponectin promoter activity when the putative ATF3 binding site in the adiponectin promoter was mutated (Fig. 6*D*). Taken together, these results indicate that mitochondrial dysfunction reduces adiponectin transcription by inducing ATF3.

ER stress activates the JNK pathway to induce insulin resistance (28,29). In the present study, chemicals that impair mitochondrial function increased phosphorylation of JNK (Fig. 7*A*), which was reversed by Ad-NRF-1 (Fig. 7*A*). Inhibition of JNK activity with the synthetic JNK inhibitor SP600125 (30) reversed mitochondrial dysfunction–induced increases in ATF3 expression (Fig. 7*B*) and reductions in adiponectin promoter activity and synthesis (Fig. 7*C* and *D*). Taken together, activation of JNK and the consequent induction of ATF3 are involved in the inhibitory effects of mitochondrial dysfuction/ER stress on adiponectin synthesis.

## DISCUSSION

The present study demonstrates that mitochondrial function is essential for the production of adiponectin in adipocytes. Treatment with rosiglitazone and measures that increase mitochondrial biogenesis (Ad-NRF-1) increased adiponectin synthesis. By contrast, chemicals that impair mitochondrial function and measures that reduce mitochondrial biogenesis (mtTFA-specific siRNA) reduced adiponectin synthesis. These effects of rosiglitazone or mitochondrial activity were specific to adiponectin, as other adipocytokines either were not affected or were changed in the opposite direction by the treatments. Our data also demonstrate that impaired mitochondrial function induces ER stress, which in turn activates a series of reactions to decrease adiponectin transcription.

As discussed earlier, adipocytes undergo maturation by two steps: differentiation and hypertrophy (6). It is conceivable that increased mitochondrial function is necessary for adipocyte differentiation, as it requires much energy. Newly differentiated cells with increased mitochondrial content would be small in size because increased fatty acid oxidation would reduce intracellular triglyceride accumulation. It is well known that levels of adiponectin synthesis are increased in small fat cells (6), and the present data indicate that this is due to increased

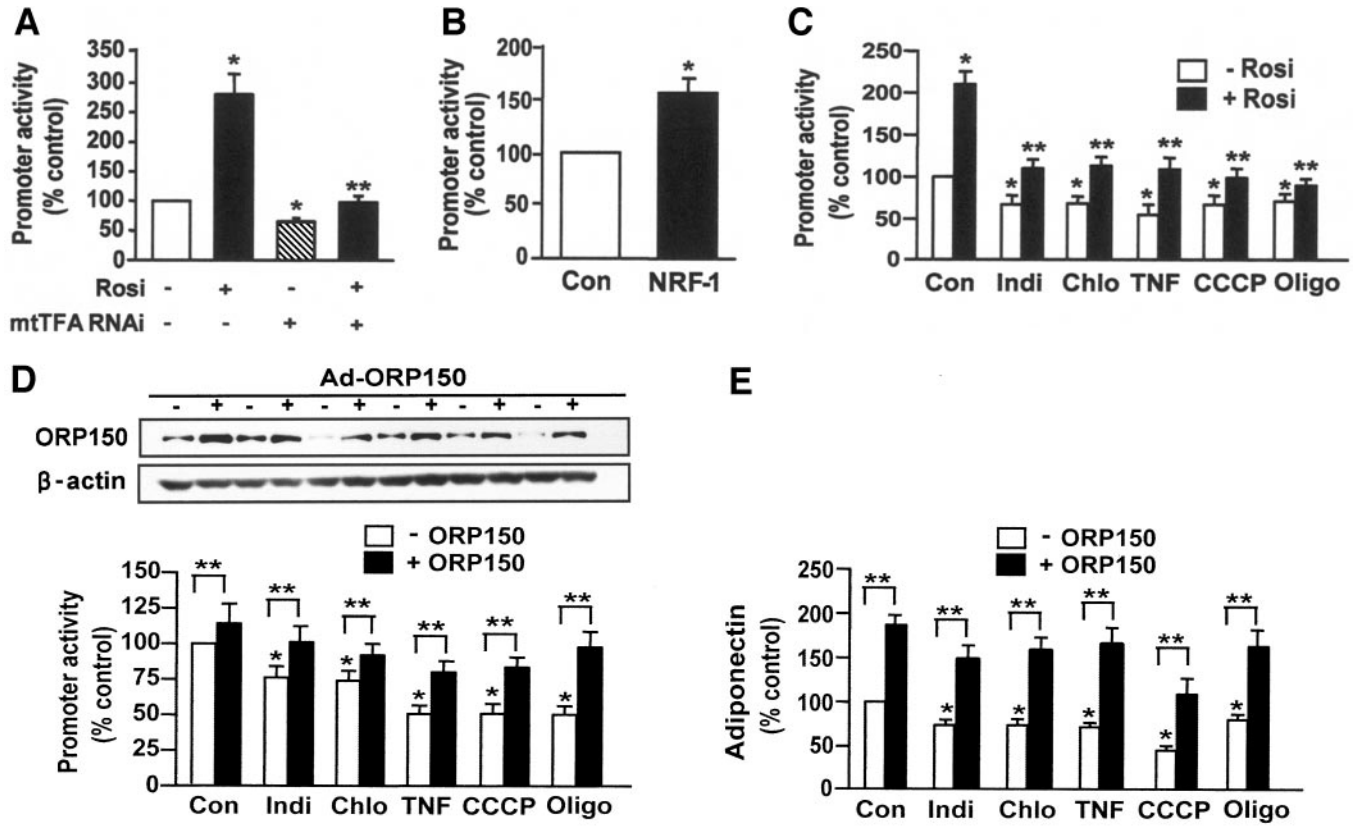


**FIG. 5. Impaired mitochondrial function/ER stress reduces transcription of adiponectin. Data are means ± SEM. *A*: Reversal of rosiglitazone-induced increases in adiponectin promoter activity by mtTFA siRNA ($n = 6$). *$P < 0.05$ vs. untreated cells; **$P < 0.05$ vs. rosiglitazone-treated cells. □, lean *db*/+ mice; ■, *db/db* mice; ▨, *db/db* mice treated with rosiglitazone. *B*: Adiponectin promoter activity in the cells overexpressing Ad-NRF-1 ($n = 6$). *$P < 0.05$ vs. adenovirus-expressing β-gal–transfected cells (Con). *C–E*: Effects of chemicals that impair mitochondrial function and adenovirus-expressing ORP150 on adiponectin promoter activity. *C*: Effect of chemicals that impair mitochondrial function on adiponectin promoter activity. 3T3L1 preadipocytes were treated with the chemicals in the presence or absence of rosiglitazone (Rosi) for 8 h as described for Fig. 4*A* ($n = 6$). *$P < 0.05$ vs. untreated cells. **$P < 0.05$ vs. rosiglitazone-treated, chemicals-untreated cells. Adiponectin promoter activity (*D*) and adiponectin levels of the media (*E*) in the cells overexpressing adenovirus-expressing ORP150, treated with or without chemicals ($n = 6$). *$P < 0.05$ vs. untreated cells; **$P < 0.05$ vs. adenovirus-expressing β-gal–transfected cells.**

mitochondrial function. By contrast, in hypertrophied fat cells, adiponectin synthesis is reduced because of impaired mitochondrial function. The number of hypertrophied fat cells is increased in obese individuals, explaining the reductions in plasma adiponectin levels. As yet, it is unclear why adiponectin production is specifically regulated by mitochondrial function. However, because adipocytes produce huge quantities of adiponectin compared with other proteins (31), adiponectin synthesis could be an important energy-consuming process in adipocytes. If so, it is logical that adiponectin synthesis is tightly regulated by the cellular energy-production system (i.e., mitochondrial function).

Calcium-handling and protein-folding processes in the ER require large amounts of ATP, and glucose deprivation and hypoxia—conditions that deplete cellular ATP—are well-known stimuli that elicit ER stress (23). In this regard, it is surprising that little is known about how changes in mitochondrial function affect ER function. In our study, impaired mitochondrial function induced ER stress, and this was reversed by increasing mitochondrial biogenesis. Impaired mitochondrial function sequentially activated JNK and ATF3, both of which have been shown to be activated by ER stress (28,32). Inhibition of JNK and ATF3 reversed the mitochondrial dysfunction–induced reductions in adiponectin transcription, demonstrating that mitochondrial dysfunction reduces adiponectin transcription by a series of molecular mechanisms, including ER stress and activation of JNK and ATF3.

Chevillotte et al. (33) recently reported that oligomycin and antimycin A decreased adiponectin expression by increasing reactive oxygen species (ROS) production and CHOP10 expression, which inhibited adiponectin gene expression by interfering with the CCAAT/enhancer-binding protein–binding region of the adiponectin gene promoter. Since the mitochondria is one of the major organelles of ROS production, especially when its function is impaired (34), there is the possibility that mitochondrial dysfunction decreases adiponectin synthesis by increasing ROS production (33,35). The significance of this potential mechanism in linking mitochondrial function to adiponectin synthesis needs to be directly addressed in future studies.

Previous studies have identified numerous hormones and transcription factors involved in adiponectin synthesis (16,26,36). Among them, TNF-α and interleukin-6 are well known to decrease adiponectin synthesis (36), and elevated levels of these adipocytokines have been thought to contribute to reduced adiponectin levels in obesity. In the present study, we used TNF-α as a cytokine that reduces mitochondrial biogenesis and increases susceptibility to mitochondrial injury (18,19), but we cannot exclude the possibility that some of its effects were independent of its effect on mitochondria. Similarly, the effects of rosiglita-

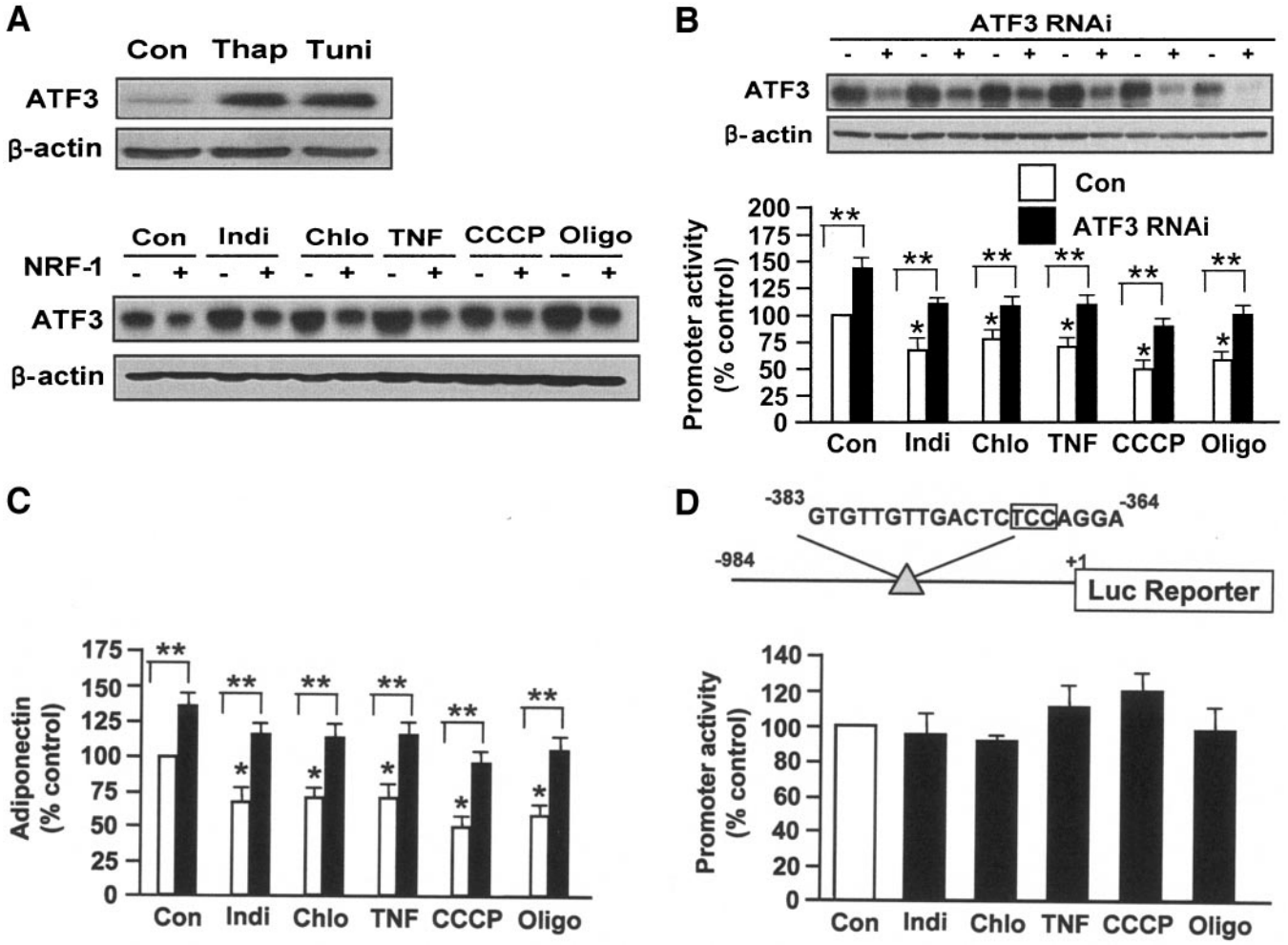


**FIG. 6. Impaired mitochondrial function/ER stress reduces transcription of adiponectin through activation of ATF3. *A*: Induction of ATF3 expression by ER stressors or chemicals that impair mitochondrial function in cultured cells. *Upper panel* shows ATF3 expression under ER stress conditions. ER stress was induced by 8-h treatment with 0.3 μmol/l thapsigargin (Thap) or 236 nmol/l tunicamycin (Tuni). *Lower panel* shows a representative Western blot of ATF3 expression under conditions of impaired mitochondrial function with or without Ad-NRF-1 transfection. Cells were treated with 100 μmol/l indinavir (Indi), 309 μmol/l chloramphenicol (Chlo), 588 pmol/l TNF-α (TNF), 1.95 μmol/l CCCP, or 12.9 μmol/l oligomycin (Oligo) for 8 h. *B–D*: Reversal of the mitochondrial dysfunction–induced decreases in adiponectin promoter activity and adiponectin synthesis by ATF3 inhibition. 3T3L1 preadipocytes were transfected with ATF3 siRNA (ATF3 RNAi) or control siRNA (Con), followed by treatment with chemicals (as described for Fig. 6*A*). Adiponectin promoter activity in the cells transfected with ATF3 siRNA (*B*) and adiponectin levels (*C*) in the media ($n = 6$). *$P < 0.05$ vs. untreated cells. **$P < 0.05$ vs. control siRNA-transfected cells. *D*: No effect of chemicals that impair mitochondrial function on promoter activity in adiponectin promoter where putative ATF3 binding site was mutated. 3T3L1 preadipocytes were transfected with mutated promoter, followed by treatment with chemicals (as described for *A*). Data in *B–D* are means ± SEM. □, control; ■, ATF3 RNAi.**

zone on adiponectin synthesis may involve, at least in part, mechanisms independent of mitochondria: PPARγ/retinoid X receptors have been shown to directly bind to a functional PPAR-responsive element in the human adiponectin promoter and to increase the promoter activity (26).

Mitochondrial function in skeletal muscle is regarded as an important determinant of whole-body insulin sensitivity (37,38). A recent study showed that adiponectin increases mitochondrial number and function in skeletal muscle by activating 5-AMP–activated protein kinase (39), which is also known to stimulate skeletal muscle mitochondrial biogenesis in response to chronic energy deprivation or endurance training (40). If so, it is very interesting to find that the synthesis of adiponectin is regulated by mitochondrial function in adipocytes. Thus, there is the possibility that mitochondrial (or metabolic) activity in adipocytes regulates mitochondrial (or metabolic) activity and insulin action in skeletal muscle via adiponectin. This concept is in line with previous studies demonstrating that metabolic alterations in adipose tissue affect insulin action in skeletal muscle (41).

In conclusion, mitochondrial function is linked to adiponectin synthesis in adipocytes, and impaired mitochondrial function in adipose tissue may explain decreased plasma adiponectin levels in obesity. Impaired mitochondrial function activates a series of mechanisms involving ER stress, JNK, and ATF3 to decrease adiponectin synthesis. Impaired mitochondrial function (37,38,42,43) and ER stress (27–29,44) have separately been proposed to be important determinants of insulin resistance in various tissues of obese subjects. In view of the present finding of sequential changes in mitochondrial function and ER stress in adipose tissue, further studies are warranted to examine whether these individual mechanisms also occur

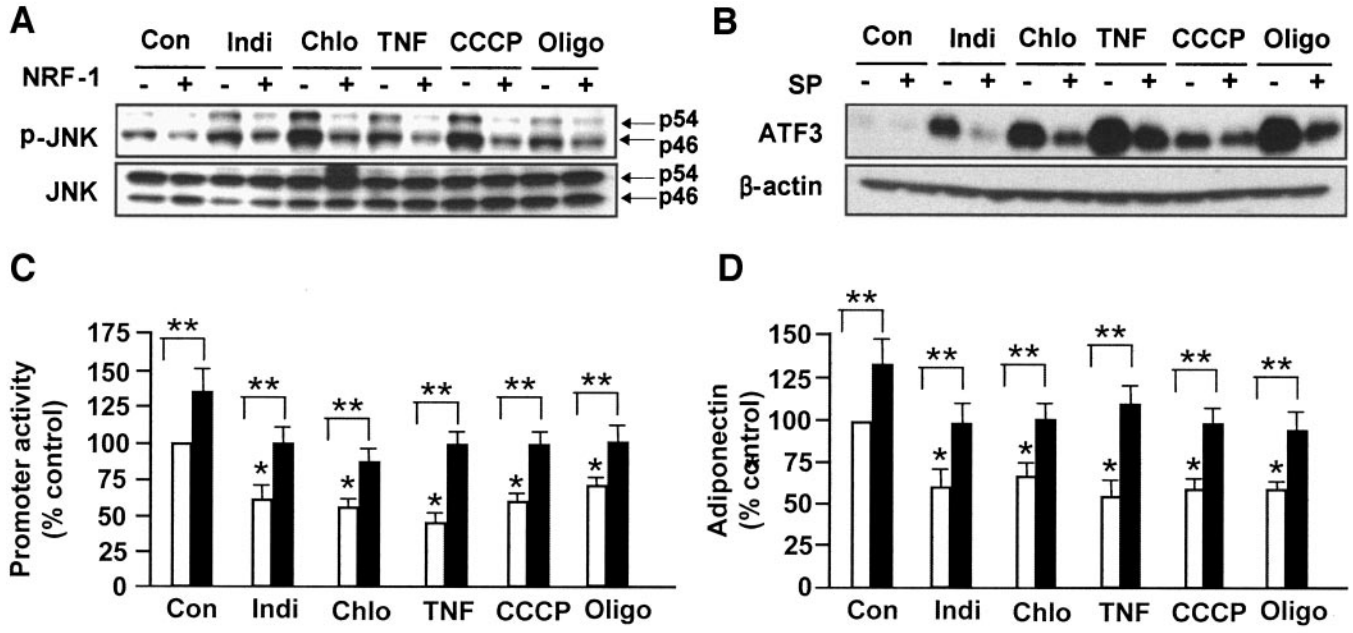


**FIG. 7. Impaired mitochondrial function/ER stress reduces adiponectin transcription through JNK activation. *A*: Reversal of impaired mitochondrial function–induced increases in JNK phosphorylation by Ad-NRF-1 (concentrations same as described for Fig. 6*A*, 2-h treatment). *B*: Inhibition of impaired mitochondrial function–induced ATF3 expression by JNK inhibition. Cells were cultured in the absence or presence of a JNK inhibitor SP600125 (25 μmol/l) (SP) for 8 h. Treatments with chemicals were the same as described for Fig. 6*A*. *C* and *D*: Effect of JNK inhibition on the mitochondrial dysfunction–induced decreases in adiponectin promoter activity and adiponectin synthesis ($n = 6$). *$P < 0.05$ vs. control cells; **$P < 0.05$ vs. cells not treated with JNK inhibitors. Data in *C* and *D* are means ± SEM. □, −SP; ■, +SP.**

sequentially in other tissues involved in the pathogenesis of insulin resistance.


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