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ATF3 plays a role in adipocyte hypoxia-mediated mitochondria dysfunction in obesity

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ABSTRACT

Obesity-associated adipose tissue hypoxia plays a pivotal role in insulin resistance via impaired adipocyte dysfunction including mitochondria dysfunction. In this study, we investigated the involvement of hypoxia-inducible ATF3 in adipocyte hypoxia-mediated mitochondrial dysfunction. While HIF-1α and ATF3 were increased in white adipose tissue of high fat diet (HFD) obese mice compared with control lean mice, mitochondria-related genes were significantly reduced. Treatment with hypoxia mimetics $CoCl_2$ or incubation with 2% O_2 impaired mitochondria function as demonstrated by decreases in ATP production, NADH dehydrogenase activity, mitochondrial membrane potential, and reduced expression of mitochondria-related genes including NRF-1, PGC-1α, COX1 and SOD in 3T3-L1 adipocyte cells. Furthermore, overexpression of ATF3 in 3T3-L1 cells also decreased mitochondria function as well as expression of mitochondria-related genes. ATF3 knockdown in 3T3-L1 cells partly prevented the hypoxia-mediated decrease in mitochondria function and expression of mitochondria-related genes. The mitochondria-related genes were decreased in white adipose tissue of ATF3-overexpressing mice compared with wild-type mice. These results suggest that ATF3 may play a role in adipocyte hypoxia-mediated mitochondrial dysfunction in obesity.

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1. Introduction

Obesity is a common disorder that predisposes individuals to type 2 diabetes, atherosclerosis, hypertension, and hyperlipidemia. The expansion of white adipose tissue during obesity development is caused by an increase in adipocyte size and total number of adipocytes due to preadipocyte differentiation [1]. Adipocyte size increases up to 140–180 μm in diameter during the development of obesity. However, the capacity for adipocyte hypertrophy is limited. The enlarged adipocytes endure less than adequate oxygen supply, since the diffusion distance for oxygen is utmost 100 μm. In situations where oxygen availability does not meet the demand of the surrounding tissue, hypoxia occurs. Adipocyte hypoxia was first proposed as a possible cause of inflammation in obesity in 2004 [2]. It has recently been demonstrated that white adipose tissue of obese mice is hypoxic, revealed both by pimonidazole staining and a markedly increased lactate concentration in adipose tissue [3]. These observations were also confirmed by measurements of hypoxia-inducible gene expression, which was elevated in obese animals [3].

Adipocyte hypoxia provokes adipocyte dysfunction, which plays a crucial role in the pathogenesis of obesity-related insulin resistance and type 2 diabetes [4]. Adipocyte hypoxia modulates the production of several inflammation-related adipokines, increasing interleukin 6 (IL-6), leptin and macrophage migratory inhibition factor production. However, it reduces adiponectin synthesis [5,6]. Furthermore, adipocyte hypoxia also inhibits the differentiation of preadipocytes [7] and impairs mitochondria dysfunction [8,9]. Increased glucose transport into adipocytes is observed with low O_2 incubation due to the up-regulation of GLUT-1 expression [10]. These findings suggest that cellular hypoxia may be a key factor in adipocyte physiology and the underlying cause of adipose tissue dysfunction contributing to insulin resistance associated with obesity.

Recently, the role of mitochondria in insulin sensitivity of adipocytes has been demonstrated [11,12]. Mitochondrial dysfunction caused by chemical treatment or genetic manipulation can lead to insulin resistance and decreased glucose utilization of adipocytes [12]. Mitochondrial dysfunction decreases the expression of adiponectin in adipocytes by activation of the JNK pathway [13]. The enhancement of mitochondrial biogenesis by overexpression of NRF-1 increased the expression of adiponectin in adipocytes [13]. These findings indicate that mitochondrial dysfunction not only

Abbreviations: ATF3, activating transcription factor 3; COX1, cytochrome C oxidase 1; HIF-1α, hypoxia inducible factor-1; HFD, high fat diet; MTT, 3-(4, 5 dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide; NRF-1, nuclear respiratory factor 1; SOD, superoxide dismutase; TFAM, mitochondrial transcription factor 1; TMRE, tetramethylrhodamine ethylester.

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causes insulin resistance of adipocyte but also impairs secretion of adipokines, which compromises other tissues in terms of glucose utilization. Hypoxia inducible factor 1α (HIF-1α) is a major mediator of the hypoxia signal in the inhibition of mitochondrial function [8,9]. However, the HIF-1α-independent mediator in hypoxia-mediated mitochondria dysfunction is not characterized yet. Moreover, understanding hypoxic events in adipose tissue might be helpful to better understand the pathophysiology of obesity and to target involved pathways for the treatment of obesity-related diseases.

Activating transcription factor 3 (ATF3) is a stress-inducible gene that encodes a member of the ATF/CREB family of transcription factors [14]. ATF3 is induced by signals such as hypoxia, proinflammatory cytokines, nitric oxide, high concentrations of glucose, palmitate, and ER stress. ATF3 regulates proliferation or apoptosis under stress conditions by down or upregulation of related genes [15]. We previously reported that ATF3 is increased in white adipose tissue of obese mice and negatively regulates adiponectin gene expression [16]. Furthermore, we demonstrated that ATF3 represses the expression of adiponectin receptors in adipocyte cells and liver cells [17,18]. Very recently, we also reported that ATF3 inhibits differentiation of preadipocyte 3T3-L1 cells [19].

Since ATF3 is a hypoxia-inducible transcription factor and contributes to repression of adiponectin expression and inhibition of adipocyte differentiation, which are adipocyte dysfunctions induced by adipocyte hypoxia in obesity, we studied the role of ATF3 in adipocyte hypoxia-mediated mitochondria dysfunction in 3T3-L1 cells. ATF3 overexpression in 3T3-L1 cells decreased indicators of mitochondria function including ATP production, NADH dehydrogenase activity, mitochondrial membrane potential, and reduced expression of mitochondria-related genes. ATF3 knockdown in 3T3-L1 cells partially blocked hypoxia-mediated decrease in mitochondria function and expression of mitochondria-related genes, suggesting that ATF3 may be involve in adipocyte hypoxia-mediated mitochondria dysfunction.

2. Materials and methods

2.1. Cell culture, differentiation, and treatments

3T3-L1 pre-adipocyte cells, which were purchased from American Type Culture Collection (ATCC, CL-173TM; Manassas, VA), were maintained with Dulbecco's modified Eagle's medium (DMEM; HyClone, Logan, UT) containing 10% fetal calf serum (FCS; HyClone). The cells were differentiated for 2 days with M1 [DMEM containing 10% fetal bovine serum (FBS; HyClone), 5 μM insulin, 0.5 mM 3-isobutylmethylxanthine (IBMX), and 1 μM dexamethasone (DEX)] and for 8 days with M2 (DMEM containing 10% FBS and 5 μM insulin). To see hypoxic effects on mitochondria function and expression of mitochondria-related genes, differentiated 3T3-L1 cells were treated with $CoCl_2$ (50, 100, and 200 μM) or incubated in 2% O_2 hypoxic chamber (2% O_2, 5% CO_2) (Astec, Tokyo, Japan) for 24 h. Antibodies against ATF3 and HIF-1α were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Insulin, IBMX, DEX and $CoCl_2$ were purchased from Sigma–Aldrich (St. Louis, MO).

2.2. Animals

C57BL/6 mice (male, 4 weeks old) were purchased from Jung-Ang Lab. Animal, Inc. (Seoul, South Korea) and were fed normal chow diet or high-fat (HF) diet for 60 days. The HF diet contained 30% lard compared with the AIN93M-based control diet. Adipocyte-specific ATF3 transgenic mice were produced using aP2 promoter. Overexpression of ATF3 was confirmed by RT-PCR. All animal experiments were approved by the Pusan National University Animal Experiment Ethics Committee and were conducted in accordance with the institutional guidelines for care and use of laboratory animals.

2.3. MTT assay

Mitochondrial dehydrogenase activity in 3T3-L1 cells was detected using the MTT assay kit (MTT, Sigma, CA) in a 96-well plate. The culture medium was replaced by 100 μl of medium containing 3-(4, 5 dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) solution, (5 mg/ml MTT in RPMI-1640 without phenol red) and cells were incubated for 4 h at 37 °C. The blue-colored tetrazolium crystals resulting from mitochondrial enzymatic activity on the MTT substrate were solubilized with 100 μl of 0.1 N HCl in anhydrous isopropanol. The absorbance was read at 540 nm on an ELISA microplate reader (MDS Analytical Technologies, Sunnyvale, CA).

2.4. Assay for mitochondrial membrane potential

Mitochondrial membrane potential was measured using tetramethylrhodamine ethylester (TMRE) kit (Abcam, Cambridge, MA). The 3T3-L1 cells in 96-well culture plates were incubated with 50 nM TMRE for 20 min at 37 °C in PBS containing 0.2% BSA. After the cells were washed, the TMRE fluorescence was measured with a microplate reader (Tecan, Salzburg, Austria). The excitation and emission wavelengths were set at 485 nm and 535 nm, respectively.

2.5. Assay for ATP production

Luciferase-based intracellular ATP contents were measured using the ATP determination kit (Molecular Probes, Eugene, OR) according to the manufacturer's recommendations. Briefly, 10 μl of cell lysates were mixed with 90 μl of the luciferin–luciferase reaction solution (1.25 μg/mL of firefly luciferase, 50 μM D-luciferin and 1 mM DTT in 1× Reaction Buffer). After a 15-min incubation, luminescence was measured using a Victor Light luminometer (Perkin–Elmer, Waltham, MA).

2.6. Plasmid and transfection

ATF3 expression vector (pcDNA3-ATF3) was previously described [16], and NRF-1 expression vector (pcDNA3.1-NRF-1) was a gift from Dr. Youngmi Kim Pak (College of Medicine, Kyung Hee University, South Korea). To see the effect of ATF3 on NRF-1 promoter activity, 2.5 Kb of mouse NRF-1 promoter (−2527/+17) was isolated with PCR and cloned into pGL3 (Promega, Madison, WI). Transfection of differentiated 3T3-L1 adipocytes with plasmid DNA (pCDNA, pCDNA3-ATF3, and pcDNA3.1-NRF-1) or siRNAs (ATF3siRNA or scramble) was carried out with a microporator (Digital Biotechnology, Suwon, South Korea) according to the protocol provided by the company. Briefly, differentiated 3T3-L1 were resuspended in resuspension buffer and incubated with the specified amount of plasmid DNA or siRNA. Microporation was performed using the following program: 1400 V (voltage), 30 ms (width), 1 pulse. After transfection, cells were maintained in growth medium without antibiotics for 24 h at 37 °C in a 5% CO_2 humidified atmosphere, after which the medium was replaced with complete medium. For hypoxic incubation, the transfected cells were incubated in a 2% O_2 hypoxic chamber for 24 h.

2.7. Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNAs from 3T3-L1cells or adipose tissue were extracted with 1 ml of Trizol™ (Invitrogen, Carlsbad, CA). The cDNAs were generated from 1 μg of total RNAs using Moloney Murine

Leukemia Virus reverse transcriptase (MMLV-RTase, Promega) at 42 °C for 1 h, and PCR analysis was performed with gene-specific primers.

2.8. Glucose tolerance test

The glucose tolerance test (GTT) was performed on overnight-fasted mice after 8 weeks of feeding the HFD. Glucose was injected intraperitoneally at 0.5 g/kg body weight, and blood was collected from the tail vein at 0, 30, 60, and 120 min after injection of glucose. The glucose was measured by a Glucometer (GlucoDr, Allmedicus, Anyang, South Korea).

2.9. Statistical analysis

All experiments were performed at least three times. The results are expressed as the mean ± SE. Differences among groups were determined by the Student's *t*-test. $p < 0.05$ is considered statistically significant.

3. Results

3.1. Adipose tissue hypoxia, ATF3 and mitochondria-related genes in white adipose tissue of HFD-obese mice

To assess whether hypoxia-inducible ATF3 is involved in mitochondria dysfunction in obesity, we examined the levels of HIF-1α, ATF3 and expression of mitochondria genes associated with mitochondria biogenesis and function in white adipose tissue of HFD obese mice. As shown in Supplemental Fig. S1, HIF-1α and ATF3 were increased in HFD obese mice compared with lean mice (Supplemental Fig. S1A), whereas the mitochondrial biogenesis genes such as nuclear respiratory factor 1 (NRF-1) and mitochondrial transcription factor 1 (TFAM) were decreased in HFD obese mice, and mitochondria function-related genes such as cytochrome C oxidase 1 (COX1), COX2, super oxide dismutase (SOD) were also decreased in HFD obese mice. However, tumor necrosis factor α (TNF-α) was increased in HFD obese mice as reported previously (Supplemental Fig. S1B).

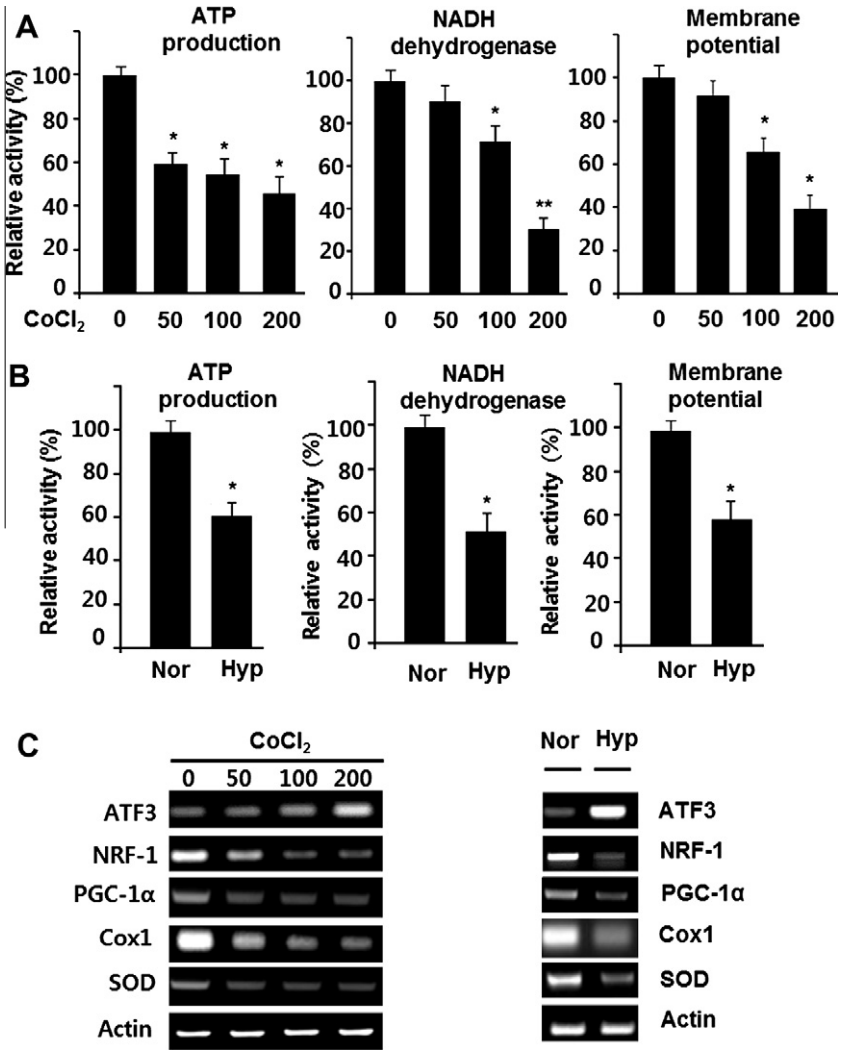

Fig. 1. Hypoxic incubation impaired mitochondria function in 3T3-L1 cells. (A) The differentiated 3T3-L1 cells were incubated with the indicated concentrations (10, 50 and 100 μg/ml) of $CoCl_2$ for 24 h and cell-based mitochondria activities were assayed. The values are expressed as the mean ± SE from three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, as compared with the vehicle. (B) The differentiated 3T3-L1 cells were incubated in 2% O_2 hypoxic chamber or normoxic conditions for 24 h, and cell-based mitochondria activities were assayed. Nor; normoxia, Hyp; hypoxia. The values are expressed as the mean ± SE from three independent experiments. $^{*}p < 0.05$, as compared with normoxia. (C) The differentiated 3T3-L1 cells were incubated with $CoCl_2$ or 2% O_2 in hypoxic chamber. The expression of mitochondria-related genes was analyzed by RT-PCR using gene specific primers.

3.2. Incubation of 3T3-L1 cells under hypoxic condition impairs mitochondria function

Next, to characterize the direct effect of hypoxia on mitochondria function of adipocytes, differentiated 3T3-L1 cells were treated with $CoCl_2$ or incubated in 2% O_2 hypoxic chamber, and then mitochondrial function was determined. Treatment with $CoCl_2$ dose-dependently decreased ATP production, NADH dehydrogenase activity and mitochondrial membrane potential, which are important indicators of mitochondria function (Fig. 1A). Incubation with 2% O_2 also significantly decreased ATP production, NADH dehydrogenase activity and mitochondrial membrane potentials in 3T3-L1 cells compared with normoxic incubation (Fig. 1B). To provide further evidence of mitochondria dysfunction, the expression levels of mitochondria-related genes were determined by RT-PCR. Consistent with impaired mitochondria function, treatment with $CoCl_2$ dose-dependently reduced mRNA levels of mitochondrial biogenesis genes such as NRF-1 and PGC-1α and of mitochondria function-related genes such as COX1 and SOD (Fig. 1C, left). A 2% oxygenic incubation also decreased the expression levels of mitochondria-related genes (Fig. 1C, right), indicating that hypoxia leads to mitochondria dysfunction in 3T3-L1 cells.

3.3. ATF3 overexpression impairs mitochondria function in 3T3-L1 cells

To determine whether ATF3 affects the mitochondria function in adipocytes, differentiated 3T3-L1 cells were transfected with ATF3 expression vector, and then mitochondria function and mitochondria-related genes were then examined in ATF3-overexpressing 3T3-L1 cells. The ATP production in ATF3-overexpressing3T3-L1 cells was less than that in vector-transfected cells. NADH dehydrogenase activity and mitochondrial membrane potential were statistically reduced by ATF3 overexpression (Fig. 2A). NRF-1 potentiates mitochondria function by increasing expression of nuclear genes providing multiple mitochondrial functions [20]. Therefore we investigated whether NRF-1 expression recovered the ATF3-mediated mitochondria dysfunction. As shown in Fig. 2A, NRF-1 expression significantly prevented ATF3-mediated decreases in ATP production, NADH dehydrogenase activity and mitochondrial membrane potential. Furthermore, mitochondria-related genes were also downregulated in ATF3-overexpressing 3T3-L1 cells (Fig. 2B). Since the levels of NRF-1 and its target genes such as COX-1 were downregulated in ATF3-overexpressing 3T3-L1 cells, we assessed whether ATF3 represses the expression of NRF-1 at the transcriptional level. To this end, −2.5 Kb of mouse NRF-1 promoter was isolated, and the effect of ATF3 on promoter activity was investigated. As shown in Fig. 2C, ATF3 significantly repressed the promoter activity of NRF-1, suggesting that ATF3 downregulates transcription of NRF-1, which represses mitochondria respiratory chain genes such as COX1.

3.4. ATF3 is implicated in hypoxia-mediated mitochondria dysfunction in 3T3-L1 cells

Next, we elucidated the involvement of ATF3 in hypoxia-mediated mitochondria dysfunction in 3T3-L1 cells by silencing ATF3

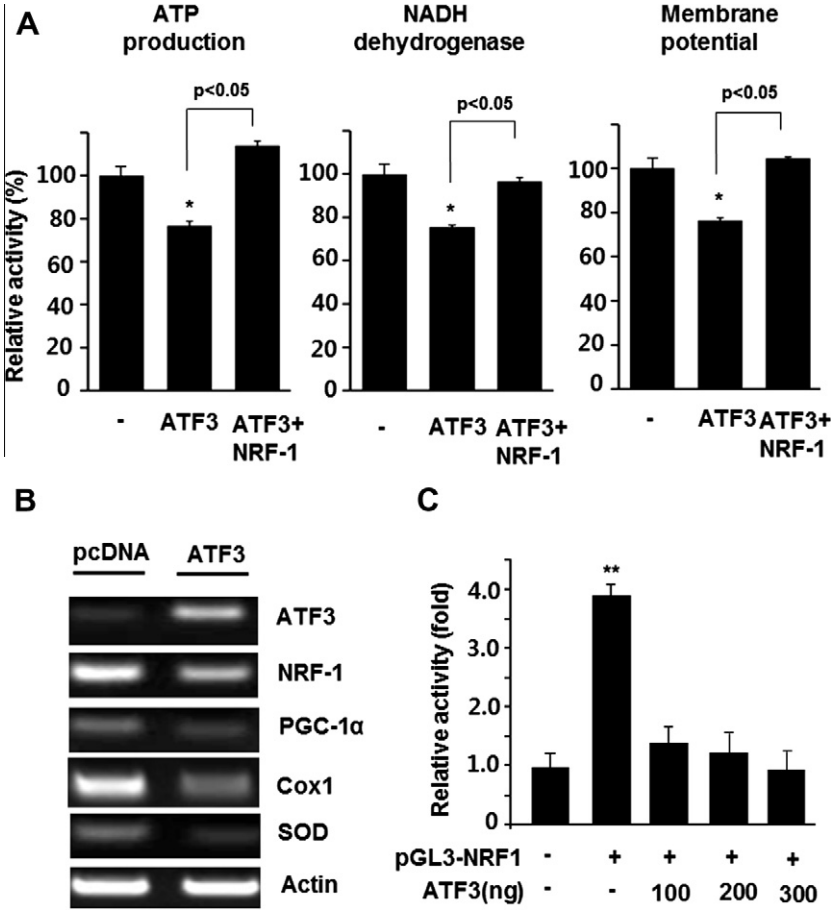

Fig. 2. ATF3 caused mitochondria dysfunction in differentiated 3T3-L1 cells. (A) The differentiated 3T3-L1 cells were transfected with 0.2 μg of pcDNA3-ATF3 and pcDNA3.1-NRF-1, respectively. Cell-based mitochondria activities were assayed. The values are expressed as the mean ± SE from three independent experiments. $^{*}p < 0.05$, as compared with control. (B) The expression of mitochondria-related genes was examined by RT-PCR using gene specific primers. (C) Preadipocyte 3T3-L1 cells were transfected with the indicated amount of pCDNA3-ATF3, and luciferase activities were measured. The values are expressed as the mean ± SE from three independent experiments. $^{**}p < 0.01$, as compared with control.

expression using small interfering RNA. Differentiated 3T3-L1 cells were transfected with scramble siRNA or ATF3 siRNA, and then incubated in 2% O_2 hypoxic chamber or normoxic chamber. ATF3 expression was decreased in ATF3siRNA-transfected 3T3-L1 cells (Fig. 3A). After that, we examined mitochondria function and expression of mitochondria-related genes. As shown in Fig. 3B, indicators of mitochondria function such as ATP production, NADH dehydrogenase activity and mitochondria membrane potential were decreased in scramble-transfected 3T3-L1 cells by incubation with 2% O_2, which is consistent with the previous result. However, the decrease was partly prevented in ATF3siRNA-transfected cells (Fig. 3B). In accordance with partial recovery of mitochondria function, the expression of mitochondria-related genes were recovered by ATF3 knockdown (Fig. 3C), suggesting that ATF3 may play a role in hypoxia-mediated mitochondria dysfunction in adipocytes.

3.5. Mitochondria-related genes are downregulated in ATF3-overexpressed mice

To further probe mitochondria impairment by ATF3, we investigated mitochondria-related genes in white adipose tissue of adipocyte specific ATF3-overexpressing transgenic mice. As shown in Fig. 4, while the level of ATF3 was significantly increased in white adipose tissue of ATF3 transgenic mice (Fig. 4A), the expression of mitochondria-related genes was decreased (Fig 4B). It has been known that mitochondria dysfunction induces insulin resistance [12]. Thus, the GTT was performed in ATF3-overexpressed mice to investigate whether ATF3 overexpression induces insulin resistance in obesity. As shown in Fig. 4C, ATF3 transgenic mice showed a significant increase in the level of blood glucose compared with wild type mice, suggesting that ATF3-overexpressed mice have impaired glucose control compared with wild type mice.

4. Discussion

Adipose tissue hypoxia may provide an important link between obesity and insulin resistance via chronic inflammation, macrophage infiltration, adiponectin reduction, leptin elevation, adipocyte death, ER stress and mitochondrial dysfunction [4]. Among them, mitochondria dysfunction in adipocytes is suggested to not only cause insulin resistance of adipocytes but also to impair secretion of adipokines [21]. HIF-1α is a key regulator in the response to alterations in hypoxia-modulated mitochondria dysfunction [8,9]. However, the HIF-1α independent mechanism is not well characterized. ATF3 is transcription factor that is increased in white adipose tissue of obesity and is inducible by hypoxia [14,15]. We previously reported that ATF3 represses expression of adiponectin and inhibits differentiation of 3T3-L1 [16,19]. Thus, we hypothesized that hypoxia-inducible ATF3 may play a role in the disturbance of mitochondria function induced by adipocyte hypoxia in obesity. Based on this hypothesis, we assessed whetherATF3 may play a role in mitochondria dysfunction, a risk factor for insulin

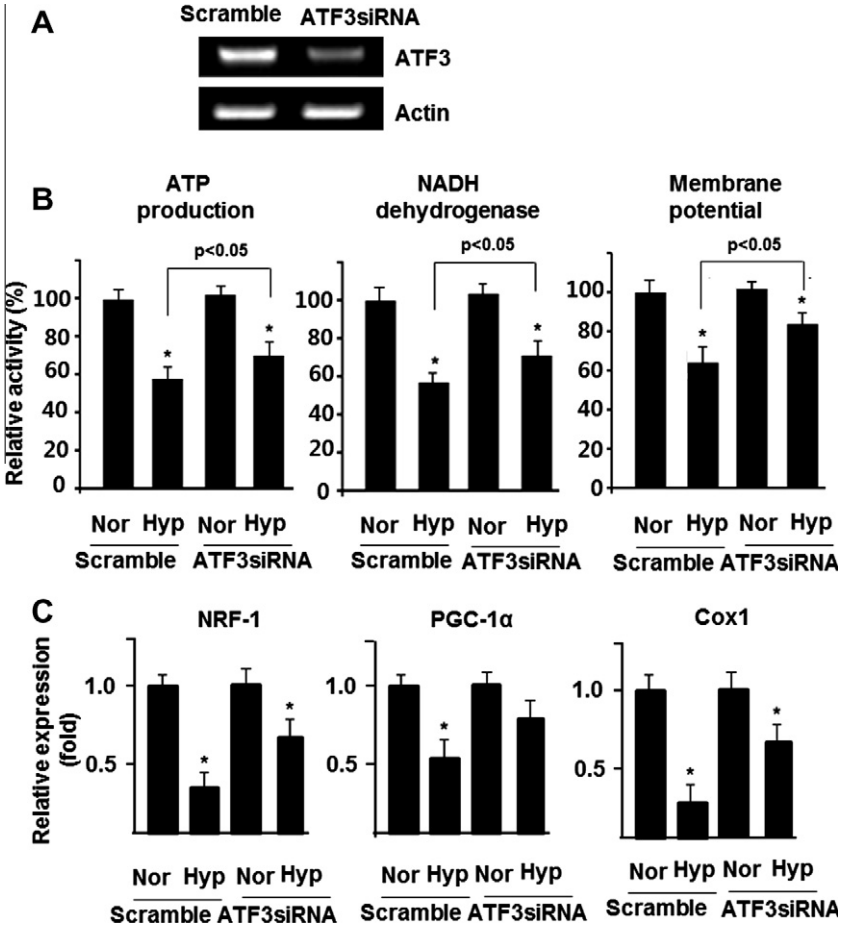

Fig. 3. ATF3 knockdown partly prevented hypoxia-mediated mitochondria dysfunction. (A) The differentiated 3T3-L1 cells were transfected with scramble siRNA or ATF3 siRNA. After 24 h, RT-PCR was performed to measure ATF3 level. (B) After transfection of differentiated 3T3-L1 cells with scramble siRNA or ATF3 siRNA for 24 h, the transfected 3T3-L1 cells were incubated in 2% O_2 hypoxic chamber or normoxic conditions, and mitochondria activities were assayed. The values are expressed as the mean value from three independent experiments. $^*p < 0.05$, compared with normoxia in scramble RNA. (C) Expression of mitochondria-related genes was examined by RT-PCR. RT-PCR data was normalized to actin as an internal control. The bars represent relative densitometric values compared with normoxia in scramble RNA. Nor, normoxia; Hyp, hypoxia. The values are expressed as the mean value from three independent experiments ($^* p < 0.05$).

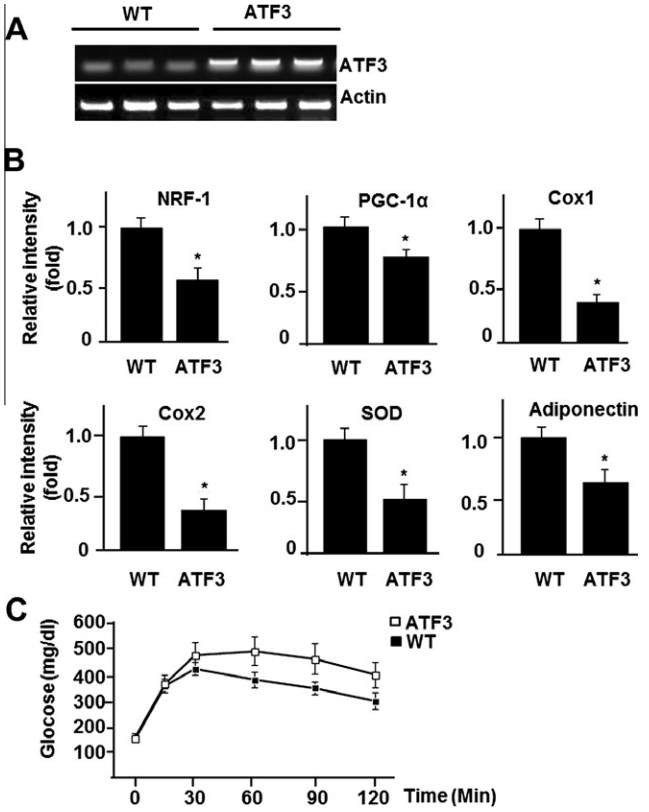

Fig. 4. Mitochondria-related genes were downregulated in ATF3-overexpressing mice. (A) Overexpression of ATF3 was confirmed in white adipose tissue of ATF3 transgenic mice by RT-PCR. (B) Expression of mitochondria-related genes was quantified by RT-PCR. RT-PCR data was normalized to actin as an internal control. The bars represent relative densitometric values compared with wild type mice. The values are expressed as the mean ± SE from three independent experiments. (*$p < 0.05$). (C) Mice feeding HFD was injected with glucose (0.5 g/kg body weight), and blood glucose levels were measured at indicated times.

resistance, caused by adipocyte tissue hypoxia. We found that ATF3 impaired mitochondria function in 3T3-L1 cells and that ATF3 knockdown in 3T3-L1 cells partly prevented the impairment of mitochondria function and decreased mitochondria-related genes expression induced by hypoxia, indicating that ATF3 may play a role in adipocyte hypoxia-mediated mitochondria dysfunction in obesity.

Recently, it was demonstrated that adipocyte tissue hypoxia in obesity is involved in the development of obesity and its associated adipocyte dysfunctions [21]. Adipocyte dysfunction plays a prominent role in the development and progression of insulin resistance [4]. Adipocyte hypoxia leads to inflammation with increased macrophage and increased expression and secretion of inflammation-related adipokines [5] such as TNFα, monocyte chemotactic protein (MCP)-1 and IL-8. Furthermore, hypoxia dysregulates the expression of several key adipocytokines such as adiponectin [6]. Expression of adiponectin and PPARγ is reduced, whereas PAI-1 and visfatin are increased in hypoxic 3T3-L1 adipocytes compared with normoxic control cells [6]. These suggest that adipose tissue hypoxia modulates, either directly or indirectly via recruitment of macrophages, adipokine expression and secretion and may therefore provide an important link between obesity and insulin resistance. Furthermore, hypoxia inhibits adipocyte differentiation [7]. Hypoxia-mediated inhibition of adipocyte differentiation is a precipitating factor in the development of type 2 diabetes [7]. Adipocyte hypoxia also induces ER stress, thereby contributing to insulin resistance [22]. Finally, adipocyte hypoxia impairs mitochondria function, which is known to induce development of insulin resistance. Mitochondrial dysfunction not only causes insulin resistance of adipocytes but also impairs secretion of adipokines, which attenuates glucose utilization in other tissues. However, the mechanisms involved in adipocyte hypoxia-induced adipocyte dysfunction including mitochondria dysfunction are not well characterized.

HIF-1α is a major mediator of the hypoxia signal involved in the adipocyte dysfunction by hypoxia. It has been demonstrated that HIF-1α functions as a negative regulator of mitochondrial biogenesis and oxygen utilization [8,9]. HIF-1α inhibits c-myc expression and proteasomal degradation [8]. Since c-myc is a positive regulator of PGC-1 α, its repression by HIF-1α results in the inhibition of mitochondria biogenesis and oxidative phosphorylation. Furthermore, HIF-1α suppresses pyruvate dehydrogenase kinase 1 and inhibition of mitochondrial pyruvate metabolism and respiration [9]. However, the HIF-1α-independent mechanism involved in adipocyte hypoxia-mediated mitochondria dysfunction has not been characterized. We previously reported that hypoxia-inducible transcriptional factor ATF3 is increased in white adipose tissue of obese mice and represses the expression of adiponectin in 3T3-L1 [16] and of adiponectin receptors in 3T3-L1 and HepG2 [18], suggesting that ATF3 may induce insulin resistance by attenuating adiponectin signaling in obesity. Also, we demonstrated that ATF3 inhibits adipocyte differentiation. From these results, we thought that ATF3 may be involved in adipocyte dysfunction induced by obesity-associated adipocyte hypoxia. Considering the possible role of ATF3 in adipocyte hypoxia-induced adipocyte dysfunction, we assessed whether ATF3 was involved in adipocyte hypoxia-mediated mitochondria dysfunction, one type of adipocyte dysfunction, in obesity. While levels of ATF3 and HIF-1α were increased in white adipose tissue of obese mice, expression of genes associated with mitochondria biogenesis and oxidative phosphorylation were decreased, suggesting that hypoxia-inducible ATF3 may play a role in mitochondrial dysfunction in adipose tissue of obese animals. We found that incubation of differentiated 3T3-L1 cells under hypoxic conditions or overexpression of ATF3 in differentiated 3T3-L1 cells impaired mitochondria function, which was revealed by decreases in ATP production, NADH dehydrogenase activity, mitochondrial membrane potential, and reduced expression of mitochondria-related genes including NRF-1α, PGC-1α, COX1 and SOD in 3T3-L1 adipocyte cells. Moreover, knockdown of ATF3 using siRNA prevented the decreased expression of mitochondria biogenesis and function-related genes and thereby partially blocked the impairment of mitochondria function, suggesting that ATF3 plays a role in mitochondria dysfunction induced by hypoxia in adipocyte tissue.

NRF-1 has now been linked to the expression of many genes required for mitochondrial respiratory function including the vast majority of nuclear genes that encode subunits of the five respiratory complexes [20]. Our results revealed that overexpression of ATF3 in differentiated 3T3-L1 cells decreased NRF-1 and its respiratory-related gene such as COX1. Furthermore, ATF3 dose-dependently repressed the promoter activity of 2.5 Kb of the NRF-1 promoter. All these results suggest that ATF3 may cause mitochondria dysfunction via repression of NRF-1 and its respiratory chain genes.

In summary, we demonstrated that hypoxia or overexpression of ATF3 impaired mitochondria function in differentiated 3T3-L1 cells. However, ATF3 deletion partially recovered the hypoxia-mediated mitochondria dysfunction, suggesting that ATF3 may play a role in adipocyte hypoxia-mediated mitochondria dysfunction in obesity.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.12.154.

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