Mitochondrial Reactive Oxygen Species Control the Transcription Factor CHOP-10/GADD153 and Adipocyte Differentiation

A MECHANISM FOR HYPOXIA-DEPENDENT EFFECT*

Received for publication, June 29, 2004
Published, JBC Papers in Press, July 20, 2004, DOI 10.1074/jbc.M407258200

Audrey Carrière§, Maria-Carmen Carmona‡, Yvette Fernandez‡, Michel Rigoulet‡,
Roland H. Wenger‡, Luc Pénicaud‡, and Louis Casteilla†**
From the Unite Mixte de Recherche 5018 CNRS-Université Paul Sabatier, IFR31, Bât. L1, Complexe Hospitalier Universitaire Rangueil, 31059 Toulouse Cedex 9, France, ‡Institut de Biochimie et Génétique Cellulaires-CNRS, 1 rue Camille Saint-Saëns, 33077 Bordeaux, France, and †Institute of Physiology, University of Zürich-Irchel, CH-8057 Zürich, Switzerland

Recent reports emphasize the importance of mitochondria in white adipose tissue biology. In addition to their crucial role in energy homeostasis, mitochondria are the main site of reactive oxygen species generation. When moderately produced, they function as physiological signaling molecules. Thus, mitochondrial reactive oxygen species trigger hypoxia-dependent gene expression. Therefore the present study tested the implication of mitochondrial reactive oxygen species in adipocyte differentiation and their putative role in the hypoxia-dependent effect on this differentiation. Pharmacological manipulations of mitochondrial reactive oxygen species generation demonstrate a very strong and negative correlation between changes in mitochondrial reactive oxygen species and adipocyte differentiation of 3T3-F442A preadipocytes. Moreover, mitochondrial reactive oxygen species positively and specifically control expression of the adipogenic repressor CHOP-10/GADD153. Hypoxia (1% O2) strongly increased reactive oxygen species generation, hypoxia-inducible factor-1 and CHOP-10/GADD153 expression, and inhibited adipocyte differentiation. All of these hypoxia-dependent effects were partly prevented by antioxidants. By using hypoxia-inducible factor-1α (HIF-1α)-deficient mouse embryonic fibroblasts, HIF-1α was shown not to be required for hypoxia-mediated CHOP-10/GADD153 induction. Moreover, the comparison of hypoxia and CoCl2 effects on adipocyte differentiation of wild type or HIF-1α deficient mouse embryonic fibroblasts suggests the existence of at least two pathways dependent or not on the presence of HIF-1α. Together, these data demonstrate that mitochondrial reactive oxygen species control CHOP-10/GADD153 expression, are antadiopogenic signaling molecules, and trigger hypoxia-dependent inhibition of adipocyte differentiation.

White adipose tissue is the main energy store in adult mammals and displays great plasticity according to the energy needs of the organism. Adipocyte differentiation results from a subtle balance of sequential and interdependent transcription factors expression that activate or inhibit promoters of adipogenic genes. Three members of the CAAT/enhancer binding protein (C/EBP) family, C/EBPβ, C/EBPδ, and CHOP-10/GADD153 (C/EBP homologous protein also identified as growth arrest and DNA damage 153), are expressed early in the adipocyte differentiation process. By forming heterodimers with the other C/EBPs, CHOP-10/GADD153 acts as a dominant negative regulator of C/EBP (1). During the clonal expansion phase, CHOP-10/GADD153 expression falls and, simultaneously, C/EBPβ and C/EBPδ are transiently induced. This mediates the later expression of C/EBPα and peroxisome proliferation-activated receptor-γ, subsequently triggering full-blown adipocyte differentiation (2). Adipogenic hormones and nutrients are well known to stimulate adipocyte differentiation (3). However, hypoxia strongly inhibits this process (4).

The involvement of mitochondria in adipose tissue plasticity has been neglected until now. Indeed, it was only recently demonstrated that mitochondrial apparus was a fundamental aspect of white fat and adipocyte differentiation (5, 6). Thus, the concept that mitochondria could play an essential role in white adipose tissue development is now emerging. This is consistent with another study, which reports that genes encoding components of the mitochondrial respiratory chain play an essential role in lipid storage (7). Beside their key role in ATP production, mitochondria constitute the primary source of reactive oxygen species (ROS) generation in many cells. Indeed, during respiration, a small but significant proportion of O2 molecules are converted to superoxide anionic radical (O2˙−) by complex I and complex III according to their steady state reduction (8) (see Fig. 1A). This radical is subsequently diverted into secondary products such as hydrogen peroxide (H2O2) and hydroxyl radical (·OH) via O2˙− dismutation and Fenton reaction, respectively. The continuous and high generation of mitochondrial ROS has generally been considered in the context of degenerative diseases and aging (9). However, the concept that according to the yield and the time course of mitochondrial ROS generation they also function as physiological signaling

* This work was supported in part by Grant 2001/198 from the Agence Nationale de Recherche sur le Sida and Grant 4NUO5G from ATC Nutrition. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
§ Fellow of the French Ministère de l’Education Nationale, de la Recherche et de la Technologie.
** To whom correspondence should be addressed. Tel.: 033-0-5-62-17-08-91; Fax: 033-0-5-62-17-09-05; E-mail: casteil@toulouse.inserm.fr.

2 The abbreviations used are: C/EBP, CAAT/enhancer binding protein; CHOP-10/GADD153, C/EBP homologous protein/growth arrest and DNA damage 153; ROS, reactive oxygen species; HIF-1, hypoxia-inducible factor-1; PDTC, pyrrolidine dithiocarbamate; CCCP, carbonyl cyanide m-chlorophenylhydrazone; MEF, mouse embryonic fibroblasts; GPDH, glycerol-3-phosphate-dehydrogenase; MnTBAP, manganese (III) tetrakis (4-benzoic acid) porphyrin; H2-DCF, 2-carboxy-2′,7′-dichlorodihydrofluorescein; H2-DCFDA, 6-carboxy-2′,7′-dichlorodihydrofluorescein diacetate, diacetoxymethylene.
molecules is now accepted (10–12). For instance, various reports indicate that mitochondrial ROS act as a second messenger in the O2 sensing mechanism to trigger erythropoietin and vascular endothelium growth factor gene transcription via hypoxia-inducible factor-1 (HIF-1) (13).

Previously, we demonstrated that mitochondrial ROS influence the size of the white preadipocyte pool (14). This led us to postulate that mitochondrial ROS could also participate in adipocyte differentiation. Using pharmacological strategies, we confirmed this hypothesis. Second, we focused our investigation on CHOP-10/GADD153 transcription factor as a putative target of mitochondrial ROS. Indeed among the early expressed transcription factors, it is well known to be sensitive to ROS. Finally, we further tested whether hypoxia-dependent inhibition of adipocyte differentiation could be mediated by mitochondrial ROS generation.

**EXPERIMENTAL PROCEDURES**

**Chemicals**—Mitochondrial inhibitors (antimycin, rotenone, and propofol), pyrrolidine dithiocarbamate (PDTC), carboxyl cyanide m-chlorophenylhydrazone (CCCP), CoCl2, N-acetylsteine, and actinomycin D were purchased from Sigma. Manganese (III) tetrakis (4-benzoic acid) porphyrin (MnTBAP) was obtained from Calbiochem. Cell Culture Conditions—3T3-F442A preadipocytes were routinely cultivated (14) in 21% O2, 74% N2, and 5% CO2 humidified atmosphere. For the different experiments, cells were seeded at 5500 cells/cm² and were grown to confluence (day 0). At day 0, medium was changed, mitochondrial inhibitors were added as indicated, and cell cultures were stopped at different times. When cell cultures were maintained for 2 days after day 0, medium without inhibitors (except when indicated) was replaced. In hypoxia experiments, confluent cells were maintained in hypoxic conditions (1% O2, 94% N2, and 5% CO2) for 2 days after day 0, and then changed every day (4). To evaluate the hypoxia or CoCl2 effects on adipogenesis, cells were treated 2 days after confluence.

**Determination of Intracellular ROS Generation**—Intracellular ROS generation was assessed by using 6-carboxy-2',7'-dichlorodihydrofluorescein diacetate, diacetoxymethylester (H2-DCFDA) (Molecular Probes) (15). This probe enters the cells and is hydrolyzed to 6-carboxy-2',7'-dichlorodihydrofluorescein (H2-DCF). H2-O2 and other peroxides cause oxidation of H2-DCF, yielding the fluorescent product DCF. Fluorescence was excited at 493 nm, and emission was measured (527 nm) and protein content (16) then were measured. The intensity of fluorescence was expressed as arbitrary units per milligram of protein.

For ROS measurement in hypoxic conditions, cells were incubated with H2-DCFDA for 2 h while the head space was gassed with 1% O2, 94% N2, and 5% CO2 (13). Cells were immediately treated as described above. Data are normalized to values obtained from normoxic controls.

**Measurement of Glycerol-3-phosphate-dehydrogenase (GPDH) Activity and Triglyceride Content**—GPDH activity was measured as described previously (17). Enzyme activity is reflected by the disappearance of NADH and results were expressed at umol/min/g of Protein. Cellular triglyceride content was determined using a commercially available test combination (Triglycerides enzymatiques PAP 150, Biomerieux).

**Western Blot Analysis**—Cells were collected on ice in 2 mM EDTA-phosphate-buffered saline. After centrifugation at 600 x g (4°C), 10 μl of pellets were resuspended in lysis buffer (50 mM Heps, 250 mM NaCl, 0.5% Triton X-100, pH 7), containing 1 mM Na3VO4 and protease inhibitors (10 μg/ml leupeptin, 10 μg/ml aprotinin, and 1 mM phenylmethylsulfonlfyl fluoride). 40 μg of proteins were separated by 12.5% SDS-PAGE and subjected to Western blot. Antibodies used were: anti-CHOP-10/GADD153, anti-C/EBPβ (both diluted 1:300, Santa Cruz Biotechnology, Inc.), or anti-HIF-1α (diluted 1/500, Novus Biological Sciences). Immunostained proteins were visualized using the enhanced chemiluminescence detection system (Amersham Biosciences).

**RNA Extraction and Northern Blot Analysis**—Total RNA from cells was extracted using TriPure isolation reagent (Roche Applied Science). 20 μg of total RNA were denatured, electrophoresed on 1.2 formaldehydeagarose gels, and subjected to Northern blot analysis. PCR product of CHOP-10/GADD153 cDNA cloned into PGEM plasmid (primers were 5’-GGCCACCTATCTCATTCCCC and 3’-TTGGTCTACCTCAGTCTCTCCT, from position 267 to 289 of the coding phase, GenBank accession number BC03718) served as probe. Equal loading of gels was checked out by hybridization with 36B4 probe (18). The cDNA probes were labeled with [γ-32P]dCTP (Milan Panich Biomedical), using the Rediprime™ II (Amersham Biosciences) random prime labeling system. Unincorporated dNTPs were removed using Microspin S-200 HR columns (Amersham Biosciences).

**Statistical Analysis**—Means ± S.E. were calculated, and statistically significant differences between two groups were determined by Student’s t test at p < 0.05.

**RESULTS**

**Antimycin Reduces Adipocyte Differentiation**—Antimycin, a well known inhibitor of complex III (Fig. 1B), was used at a concentration of 20 nM, which induces only slight inhibition (15%, data not shown) of cell respiration. At this concentration and as expected, antimycin induced an increase in ROS generation (Fig. 2A). Chronic treatment with antimycin for 4 days greatly inhibited GPDH activity (Fig. 2B) and triglyceride content (38 ± 3% versus control cells, p < 0.001, data not shown), two indexes of adipocyte differentiation. To identify a putative critical sensitivity period, confluent cells were transiently treated with 20 nM antimycin, and GPDH activity was later assessed on day 4. Fig. 2C shows that 8 or 24 h of transient exposure from confluence was sufficient to significantly inhibit GPDH activity. 48 h of treatment had a similar effect as the chronic treatment (96 h). These data demonstrate that adipocyte differentiation is inhibited by antimycin and that transient exposure to this molecule during commitment of preadipocytes to adipocytes is sufficient for inhibition. Treatment duration of 48 h was chosen for further investigations.

**Effects of Pharmacological Modulations of Mitochondrial ROS on Adipocyte Differentiation**—As antimycin (and mitochondrial inhibitors in general) may affect not only ROS generation but also energetic status, complementary strategies (Fig. 1) were needed to demonstrate the specific effects caused by ROS.

First, the prevention of antimycin effects by the addition of antioxidants was studied. MnTBAP, a superoxide dismutase mimetic with catalase-like activity, and PDTC, a thiol-reductive and iron-chelating agent, were used to counteract ROS generation (Fig. 1B). We chose concentrations of MnTBAP (50 μM) and PDTC (5 μM), which had no effect per se on ROS generation and GPDH activity (data not shown). As expected, an increase of DCF fluorescence induced by antimycin was significantly prevented when MnTBAP or PDTC were added to the culture medium (Fig. 3A). In parallel, GPDH activities in MnTBAP- and PDTC-supplemented cells were significantly higher compared with treatment with antimycin alone (Fig. 3D). Triglyceride content in MnTBAP- and PDTC-supplemented cells was also significantly higher compared with treatment with antimycin alone (97.2 ± 3% versus 47.2 ± 5.8%, p < 0.01 and p < 0.05, respectively, data not shown).

Second, it has been demonstrated (19, 20) that an uncoupling agent, CCCP, is able to stimulate O2 generation induced by antimycin (Fig. 1C). So, we tested the addition of CCCP on antimycin effects on ROS generation and adipocyte differentiation. ROS generation in 1 μM CCCP-supplemented cells was strongly and significantly increased compared with treatment
with antimycin alone (Fig. 3B). At this concentration, CCCP alone had no effect (data not shown). This was associated with enhanced inhibition of GPDH activity when CCCP was added to the culture medium compared with treatment with antimycin alone (Fig. 3E). Moreover, triglyceride content was significantly lower in CCCP-supplemented cells compared with treatment with antimycin alone (40.2 ± 2% versus 48 ± 1.5%, \( p < 0.05 \)) (data not shown).

Last, to definitively demonstrate the involvement of mitochondrial ROS in adipocyte differentiation, we compared two inhibitors of complex I, rotenone and propofol (Fig. 1D). Propofol also has the ability to scavenge free radicals (21). As for antimycin, we used equiactive concentrations of rotenone (8 nM) and propofol (40 \( \mu \text{M} \)), which induce only a 15% decrease in cell respiration (data not shown). Fig. 3, C and F, show the opposite effects of rotenone and propofol on DCF fluorescence and GPDH activity. In these conditions, rotenone significantly increased ROS generation (Fig. 3C) and significantly decreased GPDH activity (Fig. 3F) and triglyceride content (76.7 ± 3%, \( p < 0.001 \), data not shown). On the contrary, propofol significantly decreased DCF fluorescence (Fig. 3C) and increased GPDH activity (Fig. 3F) and triglyceride content (111.4 ± 3.3%, \( p < 0.05 \), data not shown). Altogether, these three complementary strategies demonstrate a very strong and negative correlation between changes in mitochondrial ROS and adipocyte differentiation.

**Effects of Pharmacological Modulations of Mitochondrial ROS on CHOP-10/GADD153**—The inhibition of adipocyte differentiation by mitochondrial ROS generated early in the differentiation process led us to focus on CHOP-10/GADD153, a transcription factor that is expressed early and is known to be ROS-sensitive (22, 23).

Treatment of confluent cells with antimycin for 24 h strongly increased CHOP-10/GADD153 protein content. The addition of antioxidants, MnTBAP or PDTC, prevented the increase of CHOP-10/GADD153 (Fig. 4A). Moreover, CHOP-10/GADD153 protein content was greatly enhanced when cells were treated with antimycin and CCCP together compared with treatment with antimycin alone (Fig. 4B). Finally, whereas CHOP-10/GADD153 was increased after rotenone treatment, no change was observed with propofol (Fig. 4C). Thus, in all tested conditions, changes in CHOP-10/GADD153 protein content were positively correlated with mitochondrial ROS generation and inversely correlated with adipocyte differentiation.

To test the specificity of these changes, we evaluated the expression of C/EBP\( \beta \) in the same conditions. Three isoforms of C/EBP\( \beta \) are present in cells: 35- and 32-kDa proteins known as liver-enriched activating protein and a 20-kDa protein known as liver-enriched inhibitory protein. The three mitochondrial inhibitors (antimycin, rotenone, and propofol) induced a slight increase of the liver-enriched activating protein/liver-enriched inhibitory protein ratio (Fig. 4, A–C), but these modifications were not associated with those of ROS generation nor with inhibition of adipocyte differentiation. Taken together, these results demonstrate that mitochondrial ROS positively and specifically control CHOP-10/GADD153.

**Hypoxia-dependent Effects on Adipocyte Differentiation and CHOP-10/GADD153 Are Prevented by Antioxidants**—Confluent cells submitted to hypoxia demonstrated a significant increase of ROS generation (Fig. 5A), which is time-dependent (data not shown). As expected, GPDH activity was significantly inhibited by hypoxia compared with normoxia (Fig. 5B). This was associated with a strong increase of CHOP-10/GADD153 protein content (Fig. 5C). MnTBAP addition partly but significantly prevented a hypoxia-dependent increase of ROS generation (Fig. 5A) as well as inhibition of GPDH activity (Fig. 5B) and an increase in CHOP-10/GADD153 (Fig. 5C). Similar results were obtained with a glutathione precursor, N-acetyllys-
Hypoxia induced an increase in liver-enriched activating protein and a decrease in liver-enriched inhibitory protein content, which was not prevented by MnTBAP addition (data not shown). Altogether, these data suggest that mitochondrial ROS generation and CHOP-10/GADD153 induction could specifically mediate hypoxia-dependent effects on adipocyte differentiation.

Hypoxia-dependent Induction of CHOP-10/GADD153 Expression Requires a Transcriptional Mechanism—To elucidate the mechanism of hypoxia-dependent CHOP-10/GADD153 induction, Northern blotting experiments were performed. After hypoxia exposure, a very rapid increase of CHOP-10/GADD153 mRNA level was detected (Fig. 6). Addition of actinomycin D (10 μg/ml), a transcription inhibitor, prevented CHOP-10/GADD153 mRNA accumulation (Fig. 6). In these conditions, no modification of 36B4 mRNA level was observed (data not shown). These results demonstrate that hypoxia-dependent induction of CHOP-10/GADD153 requires a transcriptional mechanism.

Hypoxia-dependent Induction of CHOP-10/GADD153 Does Not Require HIF-1α Expression—HIF-1 was described as mediating numerous hypoxia transcriptional effects (24). Its activity depends on the interaction between aryl hydrocarbon nuclear translocator (ARNT/HIF-1α) constitutively expressed and HIF-1α, stabilized under hypoxic conditions (24). This led us to investigate its putative involvement in hypoxia-dependent inhibition of adipocyte differentiation. In basal conditions, HIF-1α was not detected, and hypoxia induced the increase of HIF-1α protein content in preadipocytes (Fig. 7A). The addition of MnTBAP and N-acetylcysteine partly prevented this induction (Fig. 5C) and CHOP-10/GADD153 (Fig. 5C).

Similar HIF-1α and CHOP-10/GADD153 variations under our experimental conditions suggest a putative link between both expressions. We compared hypoxia effects on MEF wild type cells (HIF-1α/+/−) and cells deficient for the HIF-1α gene (HIF-1α−/−). HIF-1α detection by Western blotting analysis confirms the identity of the HIF-1α−/− cell line (data not shown). Hypoxia-dependent induction of CHOP-10/GADD153 was observed in both cell types (Fig. 7B) demonstrating that HIF-1α is not required for hypoxia-mediated CHOP-10/GADD153 induction.

As CoCl₂ is often used as a chemical hypoxia mimic for its ability to stabilize HIF-1α protein, we investigated the effect of CoCl₂ on CHOP-10/GADD153. As expected, CoCl₂ (100 μM) strongly induced HIF-1α (data not shown). A small increase in CHOP-10/GADD153 protein content compared with that obtained after hypoxia was observed in 3T3-F442A cells (only 10% compared with hypoxia, data not shown). Moreover, CoCl₂ treatment had no effect on CHOP-10/GADD153 protein content.

Fig. 2. Antimycin reduces adipocyte differentiation. A, confluent 3T3-F442A preadipocytes were incubated for 1 h in the presence of 20 nM antimycin (A). Thirty min before the end of the incubation period, cells were loaded with H₂-DCFDA. Fluorescence was expressed as arbitrary units per milligram of protein. B, cells were incubated with 20 nM antimycin for 96 h, and GPDH specific activity was assessed. C, cells were transiently incubated with 20 nM antimycin for 8, 24, 48, or 96 h. GPDH activity was assessed 96 h after confluence. Data are the mean ± S.E. of n = 3 independent experiments (each experiment in triplicate). ***, p < 0.001 versus control (C).
in both MEF cell types (data not shown). These results show that CoCl₂ does not imitate the hypoxic conditions necessary for CHOP-10/GADD153 induction.

Comparative Effects of Hypoxia and CoCl₂ on GPDH Activities in HIF-1α+/- and HIF-1α-/- MEF—Whereas CoCl₂ (100 μM) significantly inhibited GPDH activity of HIF-1α+/+ cells (42 ± 12.7%, p < 0.01), the HIF-1α-/- cells were not significantly affected by CoCl₂ treatment (106.25 ± 6.7%) demonstrating that CoCl₂-dependent inhibition of adipocyte differentiation is totally dependent on HIF-1α expression (Fig. 8A). After hypoxia, GPDH activities of both HIF-1α+/+ and HIF-1α-/- cells types were significantly inhibited (49.5 ± 4%, p < 0.001 and 69 ± 8.4%, p < 0.01 respectively, Fig. 8B), with HIF-1α-/- cells less affected than the +/- ones. These results show that hypoxia effects on adipocyte differentiation are not completely reproduced by CoCl₂ and could be triggered by at least two signaling pathways, one HIF-1α-dependent and the other HIF-1α-independent.

DISCUSSION

In this study, we provide strong evidence that mitochondrial ROS control CHOP-10/GADD153 expression and adipocyte differentiation. Moreover, we propose that this mechanism could be involved in hypoxia-dependent inhibition of adipocyte differentiation.

Various mitochondrial inhibitors can be used to induce the accumulation of electrons inside the respiratory chain and increase ROS generation. Unfortunately, their use has side effects on energetic status. This led us to develop different complementary strategies to demonstrate the specific effects
caused by mitochondrial ROS: (i) the putative prevention of antimycin (an inhibitor of complex III) effects by antioxidants; (ii) the putative accentuation of antimycin effects by the addition of an uncoupler, CCCP, as previously demonstrated in isolated mitochondria (19) and in intact cells (20); and (iii) comparison between rotenone and propofol effects at concentrations equiactive on respiration. This last point is very informative because rotenone generates ROS by inhibition of complex I whereas propofol, which also inhibits complex I, has the ability to powerfully scavenge free radicals at this site (21). Whatever the strategies used, changes of mitochondrial ROS generation are systematically closely and inversely associated with the modulation of adipocyte differentiation. This clearly demonstrates a great sensitivity of adipocyte differentiation to mitochondrial ROS generation, which strongly participates in the control of this process. Contrary to the strong extracellular generation of ROS generally used, we induced only moderate changes in mitochondrial ROS generation by very faint alterations in mitochondria function. This is consistent with a role for mitochondrial ROS as physiological signaling molecules that control adipocyte differentiation. Moreover, we demonstrated that transient mitochondrial ROS exposure at the time of commitment is sufficient to later inhibit adipocyte differentiation. This led us to postulate that transcription factors, which play a key role early in adipocyte differentiation, i.e. C/EBP transcription factors isoforms, may be targets for mitochondrial ROS.

Among C/EBP transcription factor isoforms, CHOP-10/GADD153 expression has been shown to be regulated by various stimuli including extracellular H$_2$O$_2$ (22, 23), mitochondrial stress (25), as well as hypoxia (26). Our results are consistent with these data and demonstrate that in preadipocytes CHOP-10/GADD153 but not other C/EBP isoform expression was always strongly correlated to the yield of mitochondrial ROS generation. Furthermore, we also provide evidence for a close relationship between CHOP-10/GADD153 induction and inhibition of adipocyte differentiation. As its overexpression in such cells efficiently represses their differentiation to-
Redox Regulation of Adipocyte Differentiation

Fig. 6. Hypoxia-dependent induction of CHOP-10/GADD153 expression requires a transcriptional mechanism. Confluent 3T3-F442A preadipocytes were submitted to hypoxia (1% O₂) for 2, 4, or 8 h with or without actinomycin D (10 µg/ml), a transcription inhibitor. Total RNA was extracted and Northern blots were prepared as described under “Experimental Procedures.” The blots were hybridized with a labeled probe corresponding to CHOP-10/GADD153. Northern blots are representative of three independent experiments.

Fig. 7. Hypoxia-dependent induction of CHOP-10/GADD153 does not require HIF-1α expression. A, confluent 3T3-F442A preadipocytes were submitted to hypoxia (1% O₂) for 24 h with or without the addition of MnTBAP. Whole cell lysates were prepared and probed for the presence of HIF-1α by Western blot analysis. B, MEF wild type (HIF-1α+/+) and MEF deficient for the HIF-1α gene (HIF-1α−/−) were submitted to hypoxia for 24 h. Whole cell lysates were prepared and probed for the presence of CHOP-10/GADD153 by Western blot analysis. Western blots are representative of three independent experiments.

Fig. 8. Comparative effects of CoCl₂ and hypoxia on GPDH activities in HIF-1α+/+ and HIF-1α−/− MEF. A, MEF wild type (HIF-1α+/+) and MEF deficient for HIF-1α gene (HIF-1α−/−) were incubated with 100 µM CoCl₂ for 6 days, and GPDH-specific activity was assessed. B, MEF wild type (HIF-1α+/+) and MEF deficient for the HIF-1α gene (HIF-1α−/−) were submitted to hypoxia for 24 h, and GPDH-specific activity was assessed. Data are the mean ± S.E. of n = 3 independent experiments (each experiment in triplicate). **, p < 0.01; ***, p < 0.001 versus normoxia; #, p < 0.05 versus HIF-1α+/+.

Model. Hypoxia-dependent inhibition of adipocyte differentiation was associated with an increase of HIF-1α and CHOP-10/GADD153 protein content. All these hypoxia effects were prevented by the addition of antioxidants suggesting that mitochondrial ROS must be at the origin of hypoxia signaling pathways in adipose cells.

Concomitant HIF-1α and CHOP-10/GADD153 increases and the involvement of a transcriptional mechanism on CHOP-10/GADD153 induction under hypoxia suggest a putative causal link between both expressions. However, the use of HIF-1α-deficient mouse embryonic fibroblasts demonstrated that HIF-1α is not required for hypoxia-mediated CHOP-10/GADD153 induction. This agrees with a previous study that clearly identified a set of genes up-regulated by hypoxia in an HIF-1α-independent manner (30). However, the involvement of HIF-1α in hypoxia-dependent inhibition of adipocyte differentiation was proposed (4). The conclusion of the authors was achieved using a hypoxia chemical mimetic, i.e. CoCl₂. Our observations agree with their experimental data. Indeed, CoCl₂-mediated inhibition of adipocyte differentiation is totally dependent on the presence of HIF-1α. In such conditions, it is noteworthy that CoCl₂ does not strongly up-regulate CHOP-10/GADD153 expression as hypoxia does as described on human cancer cells (31). Furthermore, hypoxia-mediated inhibition of adipocyte differentiation is only partly dependent on the presence of HIF-1α (GPDH activity in HIF-1α−/− cells is inhibited by hypoxia in a lesser extent than in the +/+ ones).
Taken together, this clearly suggests the involvement of at least two independent pathways in the hypoxia-mediated inhibition of adipocyte differentiation, either dependent or independent of HIF-1α. This latter signaling pathway might be triggered by CHOP-10/GADD153.

This study clearly demonstrates that mitochondrial ROS must be considered as antiadipogenic signaling molecules. We also suggest that mitochondrial ROS and CHOP-10/GADD153 belong to the intracellular O2 sensing mechanism of adipose cells. This signaling pathway could be involved in limiting growth of adipose tissue as described in other tissues (32, 33). Moreover, ROS generation linked to mitochondrial dysfunction could be directly involved in adipose tissues alterations associated with metabolic disorders (34). Taken together, these data emphasize the importance of mitochondria and ROS in white adipose tissue development and function.

Acknowledgments—We thank Dr. C. Toulas and C. Delmas for work station BugBox and technical assistance and Drs. G. Mitieux and A. Gauthier for help and advice. We are also grateful to N. Crowte for help in the translation of the manuscript.

REFERENCES
Mitochondrial Reactive Oxygen Species Control the Transcription Factor CHOP-10/GADD153 and Adipocyte Differentiation: A MECHANISM FOR HYPOXIA-DEPENDENT EFFECT
Audrey Carrière, Maria-Carmen Carmona, Yvette Fernandez, Michel Rigoulet, Roland H. Wenger, Luc Pénicaud and Louis Casteilla

J. Biol. Chem. 2004, 279:40462-40469. doi: 10.1074/jbc.M407258200 originally published online July 20, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M407258200

Alerts:
  - When this article is cited
  - When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 34 references, 17 of which can be accessed free at http://www.jbc.org/content/279/39/40462.full.html#ref-list-1