# Inhibition of preadipocyte proliferation by mitochondrial reactive oxygen species

Audrey Carrière[a], Yvette Fernandez[a], Michel Rigoulet[b], Luc Pénicaud[a], Louis Casteilla[a,*]

[a] *UMR 5018 CNRS-UPS, IFR31, Bât. L1, CHU Rangueil, 31059 Toulouse Cedex 49, France*
[b] *IBGC-CNRS, 1 rue Camille Saint-Saëns, 33077 Bordeaux, France*

Received 5 June 2003; revised 16 July 2003; accepted 23 July 2003

First published online 7 August 2003

Edited by Vladimir Skulachev

**Abstract Preadipocytes are present and can proliferate to increase fat mass throughout adult life. The importance of mitochondria in these cells has never been investigated, although we recently reported that mitochondrial oxidative metabolism is non-negligible in white preadipocytes. Mitochondrial reactive oxygen species generation is intimately associated with respiratory chain function. An increasing number of reports support their role as signalling molecules. The aim of this work was to study the effects of mitochondrial reactive oxygen species on proliferation of white preadipocytes. Rotenone and oligomycin, inhibitors of complex I and of ATP synthase respectively, increased $H_2O_2$ and inhibited cell growth of preadipocytes (without inducing necrosis or apoptosis). These effects were partly prevented by addition of radical scavengers. A chemical uncoupler had opposite effects on reactive oxygen species generation and cell growth. Propofol, which inhibits complex I but also scavenges free radicals, had effects similar to those of the uncoupler on both parameters. Thus, mitochondrial reactive oxygen species can influence development of adipose tissue by affecting the size of the white preadipocyte pool.**


*Key words:* White preadipocyte; Proliferation; Mitochondrion; Reactive oxygen species

## 1. Introduction

White fat development is critical for energy homeostasis. It depends on the preadipocyte pool and varies considerably according to physiological and pathophysiological conditions [1]. White preadipocytes were believed to have very low mitochondrial oxidative metabolism, although we and others demonstrated that these cells have a non-negligible mitochondrial apparatus and associated functions [2,3].

The mitochondrial electron transport chain accounts for 85–90% of the $O_2$ consumed in the cell and approximately 1–3% of this $O_2$ is incompletely metabolized and diverted into superoxide anion ($O_2^{\bullet -}$) [4,5]. $O_2^{\bullet -}$ and subsequently generated species, hydroxyl radicals, $^{\bullet}OH$ and hydrogen peroxide, $H_2O_2$ constitute reactive oxygen species (ROS). This release of ROS is obligatory and varies with the redox state of the electron carriers according to the mitochondrial oxidative metabolism [6,7].

Many investigations of the consequences of ROS generation on proliferation have been performed in various cell types. Opposite effects have been reported depending on the cell type or the yield of ROS production [8–10]. For instance, although $H_2O_2$ was suggested to act as a positive signal for proliferation in BALB/3T3 mouse fibroblast cells, it can act as a negative signal in mouse osteoblast cells (MC3T3) [8]. Paradoxically, antioxidants (α-tocopherol) and oxidants ($H_2O_2$) have the same effect on cell growth [9]. Even though numerous studies report strong extracellular generation of ROS (addition of $H_2O_2$ or xanthine/xanthine oxidase), most evidence suggests that the intensity and subcellular origin of ROS generation must be crucial in their effect on cell fate. The continuous release of mitochondrial oxidants has generally been considered in the context of degenerative diseases and aging [11]. However, the concept that mitochondrial ROS also function as signalling molecules is now emerging [7,12].

In this work, we hypothesized and then demonstrated that mitochondrial ROS influence white preadipocyte proliferation.

## 2. Materials and methods

### 2.1. Compounds

All chemical compounds were purchased from Sigma (L'Isle d'Abeau Chesnes, France). Ethanol was used as solvent to achieve a final ethanol concentration in cell cultures of 0.4%. Dihydrorhodamine 123 (DHR123) was purchased from Molecular Probes (Eugene, OR, USA)

### 2.2. Cell culture conditions

3T3-L1 preadipocytes were routinely cultivated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), L-glutamine (5 mM), amphotericin B (5 ng/ml), streptomycin (2 μg/ml) and penicillin (50 μU/ml), at 37°C in a 5% $CO_2$ humidified atmosphere. 3T3-L1 cells were seeded at a density of 4500 cells/cm$^2$ in DMEM supplemented with 10% FBS.

For primary culture of human stroma-vascular cells (SVC), samples of human subcutaneous adipose tissue were obtained from non-obese women and men undergoing abdominal dermolipectomy in the Department of Plastic Surgery of Toulouse Rangueil Hospital. The study was approved by the Ethical Committee of Toulouse University Hospital. SVC cells were purified and cultured as previously described [13], then seeded at 45 000 cells/cm$^2$.

For both cultures, mitochondrial inhibitors or carbonyl cyanide chlorophenylhydrazone (CCCP) were added to medium after 48 h

*Corresponding author. Fax: (33)-5-62 17 09 05.
*E-mail address:* casteil@toulouse.inserm.fr (L. Casteilla).

*Abbreviations:* ROS, reactive oxygen species; DHR123, dihydrorhodamine 123; $H_2$-DCFDA, 6-carboxy-2′,7′-dichlorodihydrofluorescein diacetate, diacetoxymethylester; DMEM, Dulbecco's modified Eagle's medium; SVC, stroma-vascular cells; FBS, fetal bovine serum; CCCP, carbonyl cyanide chlorophenylhydrazone; BHA, butylhydroxyanisole

0014-5793/03/$22.00 © 2003 Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies.
doi:10.1016/S0014-5793(03)00862-7

of culture. Radical scavengers were added 1 h before the addition of mitochondrial inhibitors.

*2.3. Assessment of cell proliferation*

Cell number was determined using a Coulter counter Z2. For the DNA synthesis assay [14], cells were pulsed with [$^3$H]thymidine (1 μCi/ml) and carrier thymidine to a final concentration of 2.5 μM for 1 h (3T3-L1) or 3 h (human SVC). The cells were washed twice with phosphate-buffered saline (PBS) and 5% trichloroacetic acid was added. After washing with 90% ethanol, the acid-insoluble fraction was dissolved in 0.5 ml of 0.1 M NaOH and then processed for scintillation counting.

*2.4. Determination of intracellular ROS generation*

Intracellular ROS generation was assessed using DHR123 or 6-carboxy-2′,7′-dichlorodihydrofluorescein diacetate ($H_2$-DCFDA) [15]. Cells were loaded with DHR123 (5 μM) or $H_2$-DCFDA (2 μM) 30 min before the end of the incubation period (2 h). After washing twice with PBS, cells were scraped and disrupted by sonication on ice (20 s). Fluorescence (excitation 520 or 493 nm, emission 543 or 527 nm for DHR123 and $H_2$-DCFDA respectively) and protein content were then measured [16]. The intensity of fluorescence was expressed as arbitrary units per mg protein.

*2.5. Determination of mitochondrial membrane potential*

The uptake of rhodamine 123 by cells, used as an index of mitochondrial membrane potential, was determined under conditions previously described [17]. Cells were incubated with 5 μM rhodamine 123 for 30 min and then treated as for determination of intracellular ROS generation. Then the fluorescence (excitation 520 nm, emission 543 nm) was measured.

*2.6. Statistical analysis*

Means ± S.E.M. were calculated and statistically significant differences between two groups were determined by Student's *t*-test at $P < 0.05$.

## 3. Results

*3.1. Reversion by radical scavengers of rotenone effects on 3T3-L1 preadipocyte proliferation and ROS generation*

3T3-L1 preadipocytes were cultured in the presence of rotenone at increasing concentrations. Fig. 1A shows that, compared with controls, rotenone reduced the cell number in a dose-dependent manner. At concentrations up to $4\times10^{-7}$ M, a reduction in the cell number occurred without affecting viability: no apoptotic or necrotic processes were observed when cells were stained with Syto 13 and propidium iodide [18] (data not shown). Similar results were observed with [$^3$H]thymidine incorporation. For example, at the concentration which gave a 50% reduction in cell number ($4\times10^{-7}$ M), rotenone significantly ($P < 0.001$) decreased [$^3$H]thymidine incorporation (Fig. 1B). On incubation of preadipocytes with this rotenone concentration, we observed a significant increase of ROS generation, assessed by measurements of $H_2$-DCFDA fluorescence (159 ± 10.6%, $P < 0.001$, Fig. 1C) as well as DHR123 fluorescence (169 ± 7.4%, $P < 0.001$, Fig. 1D).

Two radical scavengers, butylhydroxyanisole (BHA) and trolox, were tested to counteract ROS generation and rotenone inhibition of preadipocyte proliferation. For this purpose, the highest concentrations of BHA ($10^{-5}$ M) and trolox ($5\times10^{-4}$ M) which had no effect per se on [$^3$H]thymidine incorporation and cell number were determined and used (data not shown). The increase of DHR123 fluorescence induced by rotenone was significantly ($P < 0.01$) lowered when BHA or trolox was added to the culture medium (124 ± 2% and 124 ± 4% respectively) (Fig. 1E). In contrast, BHA or trolox increased [$^3$H]thymidine incorporation in supplemented

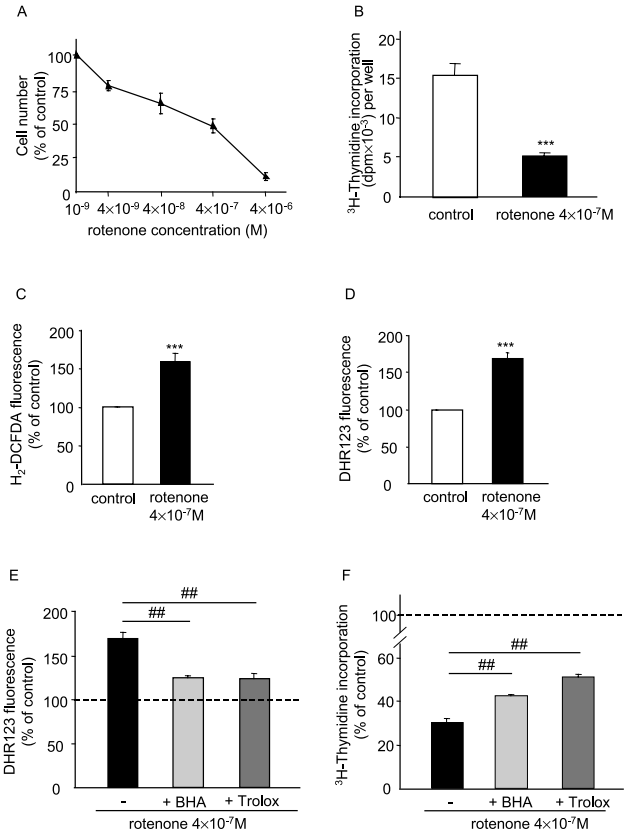


Fig. 1. Reversion by radical scavengers of rotenone effects on 3T3-L1 preadipocyte proliferation and ROS generation. A: 3T3-L1 preadipocytes were cultured with increasing concentrations of rotenone. After 24 h incubation, cells were collected by trypsinization and counted. B: 3T3-L1 preadipocytes were incubated with or without $4\times10^{-7}$ M rotenone for 24 h and then the thymidine incorporated over 1 h was measured. C,D: 3T3-L1 preadipocytes were incubated for 2 h in the presence of $4\times10^{-7}$ M rotenone. Thirty minutes before the end of the incubation period, cells were loaded with $H_2$-DCFDA (C) or DHR123 (D). Fluorescence was expressed as arbitrary units per mg protein. E: 3T3-L1 preadipocytes were preincubated for 1 h with $10^{-5}$ M BHA or $5\times10^{-4}$ M trolox and then treated as described in D. F: 3T3-L1 preadipocytes were preincubated for 1 h with $10^{-5}$ M BHA or $5\times10^{-4}$ M trolox and then the thymidine incorporated over 1 h was measured. Data are the mean ± S.E.M. of $n = 3$ (A) or $n = 5$ (B–F) independent experiments (each experiment in triplicate). $^{***}P < 0.001$ versus control, $^{\#\#}P < 0.01$ versus rotenone.

cells (42 ± 8% and 51 ± 4% respectively) compared to treatment with rotenone alone (30 ± 1%), trolox being more effective (Fig. 1F). The prevention of the rotenone effect by radical scavengers was partial but was nevertheless strongly significant ($P < 0.01$). Cell number was also significantly increased in BHA- and trolox-supplemented cells (64 ± 2% and 68 ± 1.5% respectively) compared to treatment with rotenone alone (50 ± 0.18%) ($P < 0.01$, $n = 4$, data not shown). Another radical scavenger (butylhydroxytoluene) and a thiol reductive agent (pyrrolidine dithiocarbamate) were also effective in counteracting ROS generation but they were not selected because they slightly changed [$^3$H]thymidine incorporation when they were added alone.

Similar results were obtained with human SVC. Rotenone significantly ($P < 0.001$) decreased [$^3$H]thymidine incorporation and the addition of BHA or trolox partly prevented the inhibition induced by rotenone (80.1 ± 3.6%, $P < 0.05$, and

73.7 ± 0.8%, $P < 0.05$ respectively) compared to treatment with rotenone alone (63 ± 3.8%) (data not shown).

### 3.2. Effects of oligomycin on 3T3-L1 preadipocyte proliferation and ROS generation

To validate the results obtained with rotenone, we modulated ROS generation in a way which did not involve inhibitors of respiratory chain components. In the presence of oligomycin (an ATP synthase inhibitor), the cell number was dose-dependently reduced, compared with controls. The concentration which reduced the cell number by 50% ($4 \times 10^{-9}$ M) was chosen (Fig. 2A). At this concentration, cell viability (necrosis and apoptosis) was not affected (data not shown). On incubation with oligomycin, there was a significant increase in $H_2$-DCFDA fluorescence (142 ± 7.9%, $P < 0.001$, Fig. 2C) as well as in DHR123 fluorescence (145 ± 17.8%, $P < 0.05$, Fig. 2D) and a significant ($P < 0.01$) decrease in [$^3$H]thymidine incorporation in preadipocytes (Fig. 2B). The addition of BHA or trolox partly but significantly prevented the effect of oligomycin on [$^3$H]thymidine incorporation

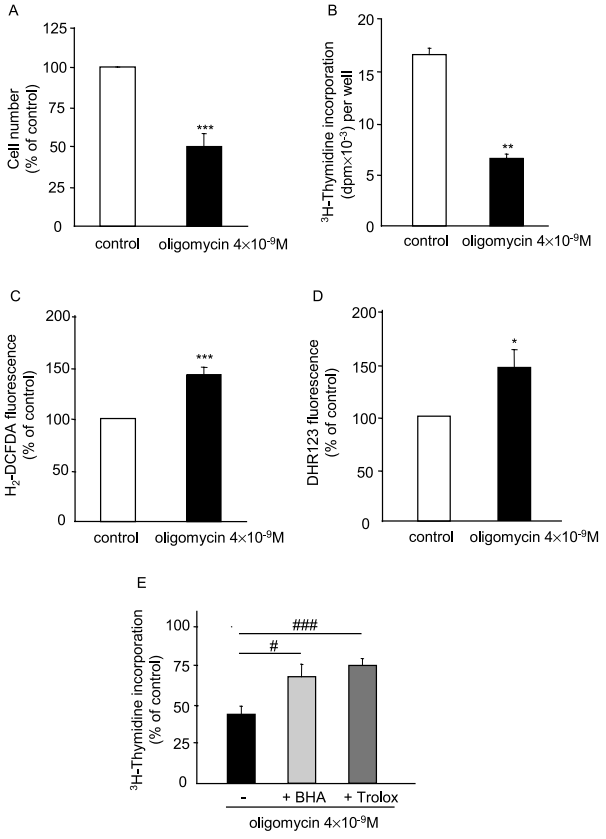


Fig. 2. Effects of oligomycin on 3T3-L1 preadipocyte proliferation and ROS generation. Reversion by radical scavengers. A: 3T3-L1 preadipocytes were incubated for 24 h with or without $4 \times 10^{-9}$ M oligomycin and then treated as described in Fig. 1A. B: 3T3-L1 preadipocytes were incubated with or without $4 \times 10^{-9}$ M oligomycin for 24 h and then the thymidine incorporated over 1 h was measured. C,D: 3T3-L1 preadipocytes were incubated for 2 h with or without $4 \times 10^{-9}$ M oligomycin and then treated as described in Fig. 1C,1D. E: 3T3-L1 preadipocytes were preincubated for 1 h with $10^{-5}$ M BHA or $5 \times 10^{-4}$ M trolox and then the thymidine incorporated over 1 h was measured. Data are the mean ± S.E.M. of $n = 5$ independent experiments (each experiment in triplicate). *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ versus control; $^{\#}P < 0.05$ and $^{\#\#\#}P < 0.001$ versus oligomycin.

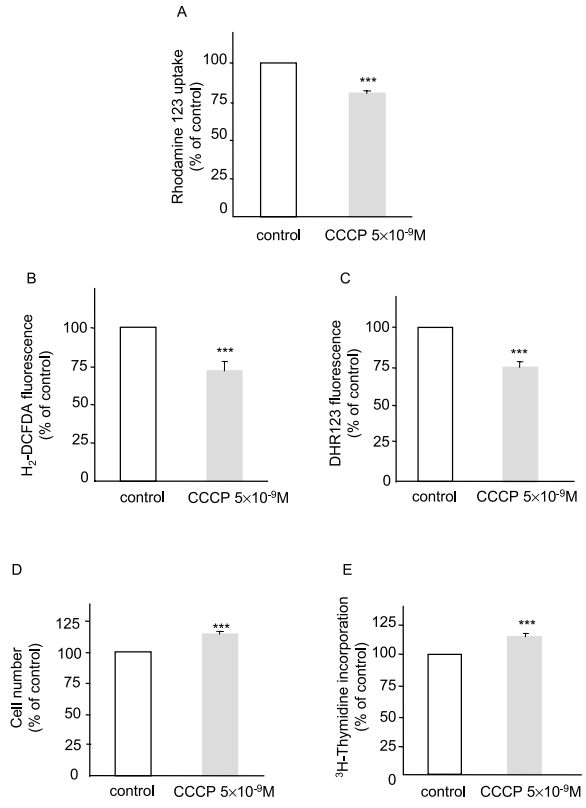


Fig. 3. Effects of CCCP on 3T3-L1 preadipocyte proliferation, rhodamine 123 uptake and ROS generation. A: 3T3-L1 preadipocytes were incubated for 30 min with rhodamine 123. Fifteen minutes before the end of the incubation period, $5 \times 10^{-9}$ M CCCP was added. Fluorescence was expressed as arbitrary units per mg protein. B,C: 3T3-L1 preadipocytes were incubated for 2 h with or without $5 \times 10^{-9}$ M CCCP and then treated as described in Fig. 1C,D. D: 3T3-L1 preadipocytes were incubated with or without $5 \times 10^{-9}$ M CCCP for 24 h and then treated as described in Fig. 1A. E: 3T3-L1 preadipocytes were incubated with or without $5 \times 10^{-9}$ M CCCP for 24 h and then the thymidine incorporated over 1 h was measured. Data are the mean ± S.E.M. of $n = 5$ independent experiments (each experiment in triplicate). ***$P < 0.001$ versus control.

(68 ± 7.8% and 75 ± 4.5%, $P < 0.05$ and $P < 0.001$ respectively) compared with treatment with oligomycin alone (44 ± 5.1%) (Fig. 2E).

### 3.3. Effects of CCCP on 3T3-L1 preadipocyte proliferation, rhodamine uptake and ROS generation

In contrast, in the presence of the chemical uncoupler CCCP, at $5 \times 10^{-9}$ M, there was a significant decrease in rhodamine 123 uptake (80.7 ± 1.2%, $P < 0.001$, Fig. 3A) associated with a decrease in $H_2$-DCFDA fluorescence (71.33 ± 6.2%, $P < 0.001$, Fig. 3B) as well as in DHR123 fluorescence (73.87 ± 3.6%, $P < 0.001$, Fig. 3C). CCCP also significantly increased cell number (114 ± 2%, $P < 0.001$, Fig. 3D) and [$^3$H]thymidine incorporation (115 ± 2.3%, $P < 0.001$, Fig. 3E).

### 3.4. Comparative effects of rotenone and propofol on 3T3-L1 preadipocyte proliferation and ROS generation

To definitively demonstrate the involvement of ROS, in a different approach, we used another inhibitor of complex I, propofol [19], which also has the ability to scavenge free radicals [20]. The effects of rotenone and propofol on cell respi-

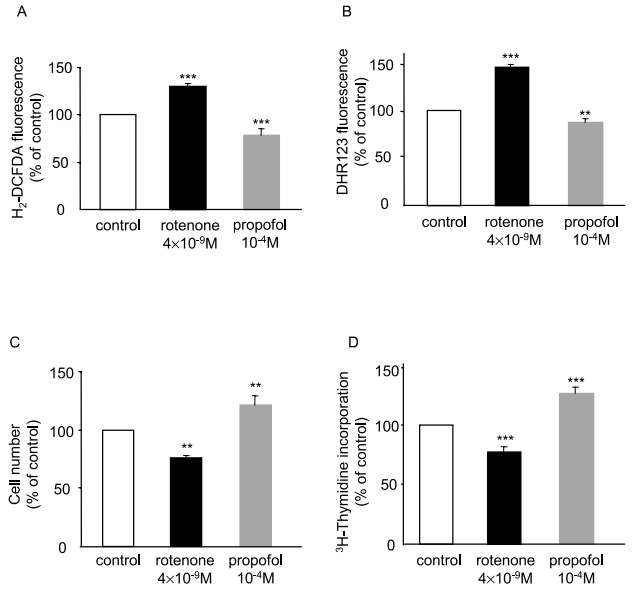


Fig. 4. Comparative effects of rotenone and propofol on 3T3-L1 preadipocyte proliferation and ROS generation. A,B: 3T3-L1 preadipocytes were incubated for 2 h with or without $4\times10^{-9}$ M rotenone or $10^{-4}$ M propofol (these concentrations are equiactive on mitochondrial respiration inhibition) and then treated as described in Fig. 1C,D. C: 3T3-L1 preadipocytes were incubated with or without $4\times10^{-9}$ M rotenone or $10^{-4}$ M propofol for 24 h and treated as described in Fig. 1A. D: 3T3-L1 preadipocytes were incubated with or without $4\times10^{-9}$ M rotenone or $10^{-4}$ M propofol for 24 h and then the thymidine incorporated over 1 h was measured. Data are the mean ± S.E.M. of $n=6$ independent experiments (each experiment in triplicate). $^{**}P<0.01$ and $^{***}P<0.001$ versus control.

ration at equiactive concentrations were compared. The oxygen consumption of 3T3-L1 preadipocytes in suspension was measured polarographically by means of a Clark-type oxygen electrode. $4\times10^{-9}$ M rotenone and $10^{-4}$ M propofol inhibited respiration of cell suspensions by 50 ± 2% (data not shown). Fig. 4 shows the opposite effects of rotenone and propofol on $H_2$-DCFDA fluorescence, DHR123 fluorescence, cell number and [$^3$H]thymidine incorporation. In these conditions, $4\times10^{-9}$ M rotenone significantly increased $H_2$-DCFDA fluorescence (130 ± 3%, $P<0.001$, Fig. 4A) and DHR123 fluorescence (146 ± 3%, $P<0.001$, Fig. 4B) but decreased cell number (76 ± 2%, $P<0.01$, Fig. 4C) and [$^3$H]thymidine incorporation (77 ± 5%, $P<0.001$, Fig. 4D). In contrast, $10^{-4}$ M propofol significantly decreased $H_2$-DCFDA (78 ± 7.3%, $P<0.001$, Fig. 4A) and DHR123 fluorescence (87.8 ± 3.2%, $P<0.01$, Fig. 4B) but increased cell number (121.8 ± 7%, $P<0.01$, Fig. 4C) and [$^3$H]thymidine incorporation (126 ± 5.2%, $P<0.001$, Fig. 4D).

## 4. Discussion

Numerous studies have investigated the role of ROS generation in the proliferation of various cells [8–10]. However, most of these studies did not take into account the subcellular origin of ROS. In white adipose tissue, the real importance of ROS as well as that of mitochondrial biology is poorly known. In this work, we attempted to demonstrate the effects of mitochondrial ROS on the proliferation of white adipocyte precursors.

Contrary to the strong extracellular generation of ROS generally used, we induced moderate changes in mitochondrial generation of ROS by different strategies. $O_2^{\bullet-}$ generation is intimately linked to electron transfer through the respiratory chain. Rotenone and oligomycin, which inhibit complex I and ATP synthase respectively, induce the accumulation of electrons inside the respiratory chain in two different ways and increase generation of $O_2^{\bullet-}$ and subsequently of other ROS ($H_2O_2$, $^{\bullet}OH$). As the use of mitochondrial inhibitors may have some effect on energetic status as well as on ROS generation, we developed several strategies to delineate the specific effect due to mitochondrial ROS. The first was to use radical scavengers in combination with rotenone and oligomycin. Second, the effects of propofol and rotenone were compared at concentrations equiactive on respiration. This is very informative because, like rotenone, propofol acts on complex I but also has the ability to powerfully scavenge free radicals at the site of $O_2^{\bullet-}$ generation. Lastly, we used a mitochondrial uncoupler alone which is very effective in decreasing mitochondrial ROS generation [12,21]. In preadipocytes, increase of ROS level by rotenone indicates ROS generation by forward and not by reverse electron transfer in the respiratory chain, complex I being responsible [22].

Treatment of 3T3-L1 cells with rotenone reduced cell number as well as [$^3$H]thymidine incorporation. This change in cell growth is due to a decrease in cell proliferation because we detected no apoptotic or necrotic events. Moreover, when rotenone was removed from the medium (data not shown) the cell number after 24 h was approximately 80% of control indicating that the inhibition of 3T3-L1 preadipocyte proliferation is a reversible phenomenon. Similar conclusions can be drawn from the results of oligomycin treatment. Treatment of 3T3-L1 cells with rotenone or oligomycin moderately enhanced generation of $H_2O_2$ (and other peroxides), which was partly reversed by radical scavengers (BHA and trolox). This is parallel to the inhibition of cell growth induced in the two ways (inhibitors of complex I or ATP synthase) and the reversion achieved by the same scavengers. Radical scavengers only brought about partial reversion of the effects of rotenone and oligomycin on cell growth, which is consistent with the less than total reversion of ROS generation by these molecules. However, these experiments strongly suggest that mitochondrial ROS are partly responsible for inhibition of 3T3-L1 preadipocyte growth. The opposite effects on ROS generation and cell growth of rotenone and propofol (at concentrations inducing similar inhibition of respiration) on the one hand, and the effects of an uncoupler, CCCP, on the other hand, are complementary demonstrations of the importance of mitochondrial ROS in the inhibition of preadipocyte growth. A similar conclusion was reached with human preadipocytes in primary culture, suggesting that this conclusion is physiologically relevant in humans as well.

Preadipocyte proliferation is a key component of adipose tissue development. Our results definitively demonstrate that the mitochondrial ROS produced, which reflect constraints on the respiratory chain and oxidative phosphorylation, act as anti-growth signals on preadipocyte signalling. Moreover, we demonstrated that mild uncoupling, by decreasing ROS generation, slightly increases preadipocyte proliferation. It is well established that for some cell types, ROS stimulate proliferation [8]. We demonstrated that mitochondrial ROS have the opposite effect on white preadipocyte proliferation. The inhibition of preadipocyte proliferation may well be a non-

specific consequence of damage to the complicated enzyme system involved in proliferation.

This work emphasizes the importance of mitochondria in the biology of white adipose tissue, which is largely neglected. It establishes a direct link between oxidative metabolism and the size of the adipocyte precursor pool which is consistent with the adipose mass changes observed in metabolic disorders such as obesity, diabetes and lipodystrophy.

*Acknowledgements:* This research was supported in part by a grant from the Agence Nationale de Recherche sur le Sida. A.C. is a fellow of the French Ministère de l'Education Nationale, de la Recherche et de la Technologie. The authors thank Drs. V. Planat, B. Prunet-Marcassus and C. Barreau for their technical help and advice. We are also grateful to P. Winterton for help in the translation of the manuscript.

## References

[1] Casteilla, L., Cousin, B., Viguerie-Bascands, N., Larrouy, D. and Pénicaud, L. (1994) Médecine/Sciences 10, 1099–1106.
[2] Kopecky, J., Clarke, G., Enerback, S., Spiegelman, B. and Kozak, L.P. (1995) J. Clin. Invest. 96, 2914–2923.
[3] Prunet-Marcassus, B., Moulin, K., Carmona, M.C., Villarroya, F., Pénicaud, L. and Casteilla, L. (1999) FEBS Lett. 464, 184–188.
[4] Boveris, A. and Chance, B. (1973) Biochem. J. 134, 707–716.
[5] Turrens, J.F., Alexandre, A. and Lehninger, A.L. (1985) Arch. Biochem. Biophys. 237, 408–414.
[6] Nishikawa, T., Edelstein, D., Du, X.L., Yamagishi, S., Matsumura, T., Kaneda, Y., Yorek, M.A., Beebe, D., Oates, P.J., Hammes, H.P., Giardino, I. and Brownlee, M. (2000) Nature 404, 787–790.
[7] Nemoto, S., Takeda, K., Yu, Z.X., Ferrans, V.J. and Finkel, T. (2000) Mol. Cell. Biol. 20, 7311–7318.
[8] Burdon, R.H. (1995) Free Radic. Biol. Med. 18, 775–794.
[9] Burdon, R.H., Gill, V. and Rice-Evans, C. (1989) Free Radic. Res. Commun. 7, 149–159.
[10] Michiels, C., Toussaint, O. and Remacle, J. (1990) J. Cell Physiol. 144, 295–302.
[11] Cadenas, E. and Davies, K.J. (2000) Free Radic. Biol. Med. 29, 222–230.
[12] Casteilla, L., Rigoulet, M. and Pénicaud, L. (2001) IUBMB Life 52, 181–188.
[13] Hazan, U., Romero, I.A., Cancello, R., Valente, S., Perrin, V., Mariot, V., Dumonceaux, J., Gerhardt, C.C., Strosberg, A.D., Couraud, P. and Pietri-Rouxel, F. (2002) FASEB J. 16, 1254–1256.
[14] Grant, M.M., Koo, H.C. and Rosenfeld, W. (1992) Am. J. Physiol. 263, L370–375.
[15] Negre-Salvayre, A., Augé, N., Duval, C., Robbesyn, F., Thiers, J.C., Nazzal, D., Benoist, H. and Salvayre, R. (2002) Methods Enzymol. 352, 62–71.
[16] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[17] Johnson, L.V., Walsh, M.L. and Chen, L.B. (1980) Proc. Natl. Acad. Sci. USA 77, 990–994.
[18] Meilhac, O., Escargueil-Blanc, I., Thiers, J.C., Salvayre, R. and Negre-Salvayre, A. (1999) FASEB J. 13, 485–494.
[19] Rigoulet, M., Devin, A., Avéret, N., Vandais, B. and Guérin, B. (1996) Eur. J. Biochem. 241, 280–285.
[20] Green, T.R., Bennett, S.R. and Nelson, V.M. (1994) Toxicol. Appl. Pharmacol. 129, 163–169.
[21] Skulachev, V. (1998) Biochim. Biophys. Acta 1363, 100–124.
[22] Korshunov, S.S., Skulatchev, V.P. and Starkov, A.A. (1997) FEBS Lett. 416, 15–18.