ELSEVIER

Mitochondrion 5 (2005) 154–161

Mitochondrion

www.elsevier.com/locate/mito

Oxygen consumption by cultured human cells is impaired by a nucleoside analogue cocktail that inhibits mitochondrial DNA synthesis

Caroline Petit[a], France Piétri-Rouxel[a], Annick Lesne[b], Thierry Leste-Lasserre[a], Dominique Mathez[c], Robert K. Naviaux[d], Pierre Sonigo[a,*], Frédéric Bouillaud[e], Jacques Leibowitch[c]

[a]*Département Maladies Infectieuses, Institut Cochin, INSERM U567, CNRS UMR 8104, Université René Descartes, Laboratoire Génétique des Virus, 22 rue Méchain, 75014 Paris, France*
[b]*Laboratoire de Physique Théorique des Liquides, Université Pierre et Marie Curie, Paris, France*
[c]*Unité d'Immuno-Virologie, Hôpital Raymond Poincaré, 92380 Garches, France*
[d]*INSERM UR523, Institut de Myologie, 75651 Paris, France*
[e]*The Mitochondrial and Metabolic Disease Center, Biochemical Genetics and Metabolism, UCSD School of Medicine, 214 Dickinson St., CTF C-103, San Diego, CA 92103-8467, USA*
[f]*CNRS UPR-9078, Faculté de Médecine Necker-Enfants Malades 75015 Paris, France*

Received 27 July 2004; received in revised form 21 September 2004; accepted 29 September 2004

Abstract

We evaluated oxygen consumption rates in human cells cultured in the presence of a nucleoside analog reverse transcriptase inhibitor (NRTI) cocktail that inhibits mitochondrial DNA synthesis. We treated a proliferating human lymphocyte cell line and a primary culture of human adipose cells with antiretroviral drugs (AZT + ddC + d4T). The effects of these drugs on mitochondrial DNA (mtDNA) levels and oxygen consumption rates were evaluated using semi-quantitative real-time PCR and an on-line monitoring Clark electrode system.

We found that the NRTI treatment lowered oxygen consumption rates and inhibited mitochondrial DNA replication in human cell cultures. Inhibition of oxygen consumption was linearly proportional to inhibition of mtDNA replication. These results show for the first time that mitochondrial respiration is impaired in NRTI sensitive cells. The linear relationship between NRTI inhibition of respiration and NRTI inhibition of mtDNA replication indicates that small decreases in mtDNA levels can lead to respiratory deficits in the tissues of patients treated with anti-HIV drugs. We propose a model that takes into account the small differences in metabolic dynamics between peripheral and axial/visceral fat tissues. This model explains how NRTI-related respiratory deficits may lead to the presentation of opposing lipodystrophic syndromes in same patient.

Keywords: Nucleoside reverse transcriptase inhibitors; Mitochondrial DNA; Oxygen consumption; Human cells

* Corresponding author. Tel.: +33 1 40 51 64 13; fax: +33 1 40 51 64 07.
E-mail address: sonigo@cochin.inserm.fr (P. Sonigo).

1567-7249/$ - see front matter © 2005 Elsevier B.V. and Mitochondria Research Society. All rights reserved.
doi:10.1016/j.mito.2004.09.004

1. Introduction

Highly active antiretroviral therapies (HAART) dramatically reduce the risk of progressive immune deficiency in HIV infected patients by controlling viral replication. Nucleoside analog reverse transcriptase inhibitors (NRTIs) (Johnson et al., 2001; Kakuda, 2000; Martin et al., 1994; Squires, 2001) are part of most HAART regimens and are generally taken with protease inhibitors or as triple/quadruple reverse transcriptase inhibitor combinations. Several of these antiviral drug regimens alter the activity of peripheral and cardiac muscles, nerves and the pancreas. These treatments also lead to abnormal fatty acid metabolism in the liver, a side effect that can occur with or without concomitant abnormalities in lactate production. These symptoms show histopathological, histochemical, and metabolic similarities to those occurring in individuals with congenital or sporadic mitochondrial cytopathies. These iatrogenic syndroms may be linked to NRTI-mediated inhibition of mitochondrial DNA synthesis (Brinkman et al., 1998; Fromenty and Pessayre, 1995; Lewis and Dalakas, 1995).

NRTIs inhibit DNA synthesis by competing with endogenous nucleic acids for incorporation into the DNA chain. They cause premature termination of DNA chain elongation when incorporated. NRTIs function by preventing HIV reverse transcriptase-mediated viral DNA synthesis (Kakuda, 2000). mtDNA polymerase gamma, which is essential for mtDNA replication, is particularly prone to NRTI inhibition because it cannot discriminate between nucleoside analogues and endogenous nucleotides (Brinkman et al., 1999; Longley et al., 1998).

Lipodystrophy (LDP) is the most frequently reported side effect of anti-HIV therapies (Brinkman et al., 1999; Carr et al., 1999; Longley et al., 1998). This condition is characterized by visceral/axial over-adiposity and fat wasting in the limbs, buttocks and face, resulting in an increase in the ratio of visceral to subcutaneous abdominal fat (Brinkman et al., 1999; Carr et al., 1999; Longley et al., 1998). LDP was originally thought to be associated with the protease inhibitor (PI) component of the antiviral cocktails, however, more recent studies have shown that patients receiving NRTI combinations (without previous or concurrent PI treatment) also present this symptom (Chene et al., 2002; Molina et al., 1999; personnal unpublished results).

Epidemiological studies have identified a link between NRTI-treatment and LDP. These data suggest that the symptoms of this syndrome result from a form of mitochondrial cytopathy in the adipose tissues of these patients. A functional point mutation at nucleotide 8344 of the mitochondrial tRNA-lysine gene has been identified in some individuals with familial multiple symmetric lipomatosis (MSL) type 1 (Munoz-Malaga et al., 2000), a syndrome that shares distant similarities with rare localized forms of LDP in patients taking anti-HIV drugs (Brinkman et al., 1999). In-house real-time PCR estimates of mitochondrial DNA copy numbers in NRTI-treated patients, and analyses of ultrastructural mitochondrial abnormalities in subcutaneous fat tissue biopsies from NRTI-treated patients with and without out LDP, support the hypothesis that mitochondrial defects lead to lipoatrophy in patients with LDP (Nolan et al., 2003; Shikuma et al., 2001; Walker et al., 2002). The results from cross-sectional studies have provided no statistical proof to support this hypothesis. However, these studies generally involve only small numbers of patients and detect large inter-patient variations.

In a previous study (Petit et al., 2003), we analyzed mtDNA levels in blood lymphocytes from a series of HIV patients before and after treatment with AZT, ddC and d4T. This previous study provided no evidence to support the hypothesis that there is a link between LPD and NRTI-treatment. Here, we describe the effects of nucleoside analogues on mtDNA replication and oxygen consumption in a primary culture of normal human preadipocytes/adipocytes. We found that NRTIs altered both oxygen fixation and mtDNA replication without a threshold effect. These results led us to propose a metabolic model for LDP in which NRTI-related respiratory cell defects lead to either fat atrophy or excess adipogenesis depending on the metabolic status of the fat tissues involved.

2. Experimental procedures

2.1. Cell culture, drug treatments and quantification of lipid accumulation

Samples of human subcutaneous adipose tissue were obtained from healthy subjects undergoing

aesthetic surgery. Tissue samples were cut into small pieces and processed as previously described (Hazan et al., 2002). Preadipocytes from the stromal-vascular fraction were cultured in medium (1:1 mixture of Hams F12 and Dulbecco's Modified Eagle's Medium (Invitrogen)) supplemented with 20 mM HEPES and 10% decomplemented fetal calf serum (FCS). A mixture (Mix) containing 8.5 nM insulin (Sigma Aldrich), 1 μM dexamethasone (Sigma Aldrich), 250 nM isobutyl methyl xanthine (IBMX; Sigma Aldrich,) and 1 μM pioglitazone (gift from V. Zilberfarb, institut Cochin Inserm U567, CNRS UMR8104 France) was added to the cell cultures at confluence. Cells were allowed to differentiate for 12–15 days. MT4 cells were cultured in RPMI medium supplemented with standard FCS (15%).

MtDNA replication was inhibited by adding a nucleoside analogue cocktail (NRTI: AZT, ddC and d4T) to the culture medium every 2 days. Cells treated with a combination of NRTIs and protease inhibitors (PI) were given the nucleoside analogue cocktail plus a PI cocktail (containing 1 μM concentrations of saquinavir, ritonavir and nelfinavir) every two days.

2.2. Quantification of mitochondrial DNA levels and respiration measurements

Total DNA (nuclear and mitochondrial) was extracted from drug-treated and untreated cells using the QIAamp DNA Blood Mini Kit (QIAGEN). Extracts were collected in a final volume of 100 μl of elution buffer. MtDNA levels were quantified by real-time PCR as described previously (Petit et al., 2003). The nuclear gene encoding human β-globin and the mitochondrial gene encoding human 12S rRNA were quantified separately for each DNA extract. Data were analyzed using the LightCycler Software version 3.5. The β-globin gene was quantified using the LightCycler-Control Kit DNA (Roche).

Cellular oxygen consumption was measured using a Clark-type electrode connected to an Oxytherm unit (Hansatech, Norfolk, England). Cells were suspended at a concentration of 2×10^6 cells per ml of culture medium. Respiration rates were monitored before (basal rate) and after adding 0.5 μg/ml of oligomycin (ATP synthase inhibitor), and before and after adding increasing concentrations (from 2 to 6 μM) of the uncoupler, CCCP (Carbonyl cyanide m-chlorophenylhydrazone). To check that oxygen consumption was cyanide-sensitive, and thus related to mitochondrial activity, we monitored respiration rates in cells exposed to 1 mM cyanide.

3. Results and discussion

3.1. Effect of antiviral drugs on adipose cells

Most studies of LDP have involved cell culture experiments using established rodent cell lines. In this study we have assessed the effects of antiviral drugs on primary human adipose cells. Preadipocytes were isolated from normal subcutaneous fat depots and either exposed immediately to a drug cocktail (NRTI, PI or both) or stimulated to differentiate into adipocytes and then exposed to these drugs. We found that in vitro differentiation of primary preadipocytes led to accumulation of triglycerides in the cytoplasm of 60–100% of the adipose cells (Fig. 1A).

We treated both preadipocytes and adipocytes with the following NRTI cocktail: AZT, ddC and d4T. Our previous study (Petit et al., 2003) showed that these nucleoside analogues dramatically reduced MtDNA levels in vitro. For both cell types, mtDNA content was lower for NRTI-treated cultures than for untreated cultures (Fig. 1B). MtDNA levels began to decline after 4 days of NRTI treatment. This decline continued over time; mtDNA levels had decreased by 75% after 13 days of treatment (data not shown). These results show that mtDNA replication in human adipose cells is sensitive to nucleoside analogues and that this sensitivity is independent of differentiation state. We obtained similar results with subcutaneous adipose cells from several locations (data not shown). These data are consistent with those obtained with non-proliferating normal human T cells cultured in the presence of NRTIs (Petit et al., 2003).

No differences in mtDNA content were found between preadipocytes or adipocytes cultured in presence of the HIV protease inhibitor saquinavir, and untreated cultures (Fig. 1B). We then investigated whether saquinavir acted in synergy with

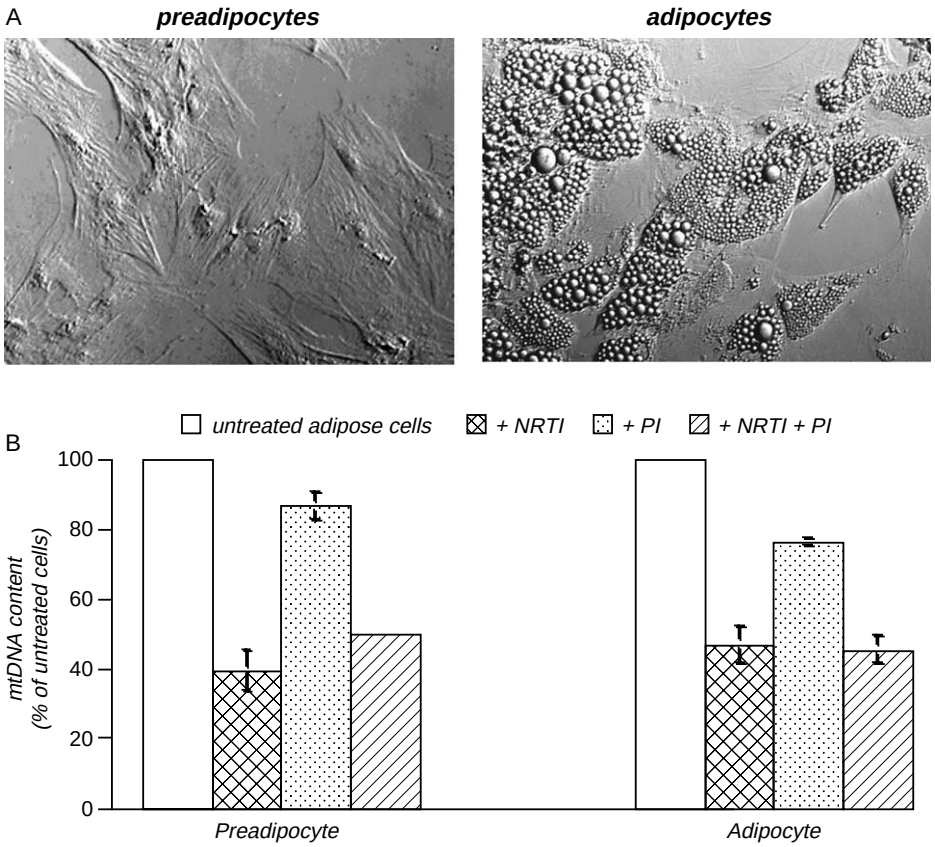

Fig. 1. (A) Preadipocytes were isolated from a sample of mammary adipose tissue taken from a woman undergoing esthetic surgery as previously described (Hazan et al., 2002). Untreated preadipocytes were cultured for 13 days in the presence (right panel) or absence (left panel) of a mixture promoting differentiation. 20× magnification. (B) mtDNA levels were analyzed in preadipocyte and adipocyte cells cultured in the presence of various drugs. Cells were treated with a cocktail containing AZT, ddC and D4T (+NRTI; 1 μM of each), or with a protease inhibitor cocktail containing saquinavir, ritonavir and nelfinavir (+PI; 1 μM of each), or with both compounds (+NRTI+PI). MtDNA levels in treated cells are expressed as a percentage of those in untreated cells (controls). The data presented were obtained from preadipocytes after a 10-day drug treatment and from adipocytes after a 6-day treatment.

the nucleoside analogues. No significant differences in mtDNA content were found between mature adipose cells treated with both saquinavir and the NRTI cocktail and those treated with the NRTI cocktail alone (Fig. 1B). Similar results were observed for preadipocyte cell cultures (Fig. 1B). We also found that the kinetics and dose-response curves of NRTI-mediated inhibition of mtDNA synthesis in cultured human adipose tissue cells (data not presented) were similar to those determined for PHA-stimulated or quiescent lymphocytes, and for the MT4 human T-lymphocyte cell line (Petit et al., 2003).

3.2. NRTI-induced inhibition of mtDNA synthesis affects mitochondrial respiration

We investigated whether NRTI-induced inhibition of mtDNA synthesis altered cell culture oxygen consumption rates. We determined the respiration rates of NRTI-treated MT-4 and preadipocyte cell cultures before and after treatment with oligomycin (ATP synthase inhibitor), CCCP (uncoupler) and cyanide (inhibitor of mitochondrial respiration). The CCCP treatment was used to determine maximal O_2 consumption rates for the NRTI-treated cultures.

We found that basal respiration rates were lower for NRTI-pretreated cells than for controls. In addition, respiration rates were lower for NRTI-CCCP-treated cells than for controls (Fig. 2A).

NRTI-induced alterations in respiration rates were compared to changes in mtDNA levels as follows: we calculated the ratio of mtDNA levels in untreated cells to those in NRTI-treated cells (values ranged from 1 to 33); mtDNA ratios were then plotted against the ratio of control versus treated culture cell respiration rates (respiration rate of control cultures: respiration rate of NRTI-treated cultures). The relationships between

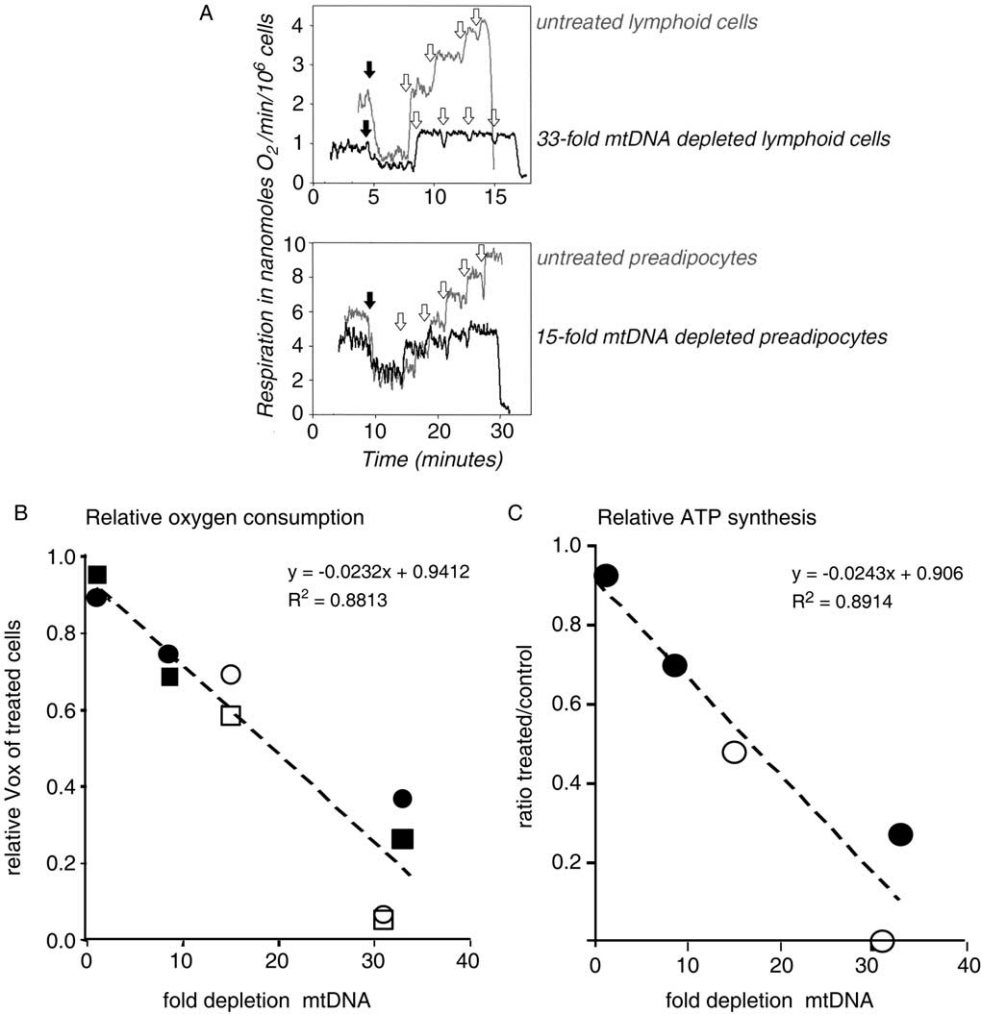

Fig. 2. (A) Respiratory rates of MT4 cells (top) and of human preadipocytes (bottom). Grey line: respiratory rate of control (untreated cells). Black line: respiratory rate of NRTI-treated cells. The mtDNA ratio for the NRTI-treated MT4 cells was 33 and that for the NRTI-treated preadipocytes was 15. Black arrows: addition of oligomycin. White arrows: successive addition of the uncoupler CCCP. Cyanide (KCN) was added at the end of each experiment. (B) *X*-axis: ratio of mtDNA levels in control cultures to those in NRTI-treated cultures. *Y*-axis: ratio of oxygen consumption in NRTI-treated cells to that in control cells. Squares: basal state (no additional treatment). Circles: maximal respiration rate in the presence of an uncoupler (1–5 μM CCCP). Black symbols represent data obtained with the MT4 cell line (three independent experiments), and white symbols represent data obtained with the primary human preadipocyte culture (two independent experiments). Dashed line: regression curve. (C) ATP production was estimated by calculating the difference between the basal respiratory rate and that after oligomycin addition. *Y*-axis: ratio of ATP synthesis in NRTI-treated cells to that in untreated cells. *X*-axis: mtDNA ratio. Black circles: MT4 cells; white circles: primary human preadipocyte culture.

the basal and maximal respiration ratios and the mtDNA ratio were linear for the MT4 cell line (black symbols, Fig. 2B). A linear relationship was also observed for primary human preadipocytes (white symbols; Fig. 2B), demonstrating that the linear relationship between mtDNA content and respiratory activity is not restricted to a single cell type.

Inhibition of mtDNA production may lead to different responses: when the inhibition involves the mutation of a single gene encoding an enzyme in the respiratory pathway, alterations in the overall flux through this pathway are not proportional to the decreases in activity of the relevant enzyme. This lack of proportionality is known as the 'threshold effect' (for review, see Rossignol et al., 2003). In cases of mitochondrial genome deletions where wild type and partially deleted genome co-exist and lead to various levels of heteroplasmy, there is a linear relationship between the degree of heteroplasmy and the activity of the respiratory enzymes (Gellerich et al., 2002). In our study, changes in respiration rates were proportional to changes in mtDNA content suggesting that there was no competition between partially deleted and complete mitochondrial genomes and no imbalance in the levels of transcription of the genes encoded by these genomes in our system. Hundreds of thousands copies of mtDNA are present in normal cells. Thus, the residual mtDNA levels detected the NRTI-treated cells actually constituted a large number of mitochondrial genome copies. However, this residual mtDNA was not sufficient for normal levels of cell respiration. Our data show that even slight reductions in mtDNA levels result in detectable alterations in mitochondrial respiration rates. Thus, mtDNA copy number is a limiting factor in cell respiration. Inhibition of mtDNA synthesis will reduce the availability of the mitochondrially encoded enzyme subunits that make up the respiratory and ATP synthase complexes. This results in a 'pan-mitochondrial' respiration defect and explains the absence of the threshold effect.

The link between mitochondrial oxygen consumption and ATP production (i.e. respiratory coupling) was investigated using oligomycin, a inhibitor of mitochondrial ATP synthase. The addition of this inhibitor to MT4 and preadipocyte cultures led to a decrease in respiratory rates, showing that oxygen consumption was coupled to mitochondrial ATP synthesis in these cells (Fig. 2A). Thus, respiration rates can be used to calculate the rate (ou: the level) of mitochondrial ATP production in this system. Our analysis showed that the relationship between NRTI-induced inhibition of ATP production and NRTI-induced inhibition of mtDNA replication was linear in our cell cultures (Fig. 2C).

However, NRTI-induced inhibition of mitochondrial ATP synthase activity did not prevent growth or differentiation of the treated cells, suggesting that ATP was produced by anaerobic glycolysis in the cytosol in these cell cultures. The relationship between mitochondrial activity and anaerobic glycolysis and lipogenesis in NRTI-treated cells in vitro has yet to be investigated.

3.3. A model for NRTI-induced lipodystrophy

Comprehensive models of the biological process(es) that lead to lipodystrophy in patients treated with NRTI drugs need to take into account the fact that both the hyper- (central/axial) and hypotrophic (peripheral) forms of the syndrome occur simultaneously in the same patient. We propose that the opposing effects of NRTI-induced inhibition of mitochondrial respiration on lipogenesis occur because of underlying differences in metabolic dynamics between axial/visceral and peripheral fat tissues.

Our modelization is based on the relationships between the respiratory (R) and glycolytic/fermentative fluxes (F). We assume that lipids are synthesized from fermentation substrates that are not consumed by the respiratory pathway (Fig. 3). The resulting complex function is termed $F(R)$ and its stationary state is defined as $F(R)=R$. Multiple enzyme regulation mechanisms link the respiration and fermentation fluxes (for example, the Pasteur effect) and thus the simplest generic relationship between F and R is represented by a sigmoid curve (see Fig. 3). This curve contains concave and convex regions where decreases in R leading to values that fall below that required to maintain the stationery state ($F(R)=R$) have opposing effects on lipid metabolism. The predominating metabolic status (glycolytic/fermentative versus oxidative) in the three sections (Fig. 3: (1), (2) or (3)) of the $F(R)$ curve determines the direction of the lipogenic pathway: adipogenesis increases when the flux through the fermentation

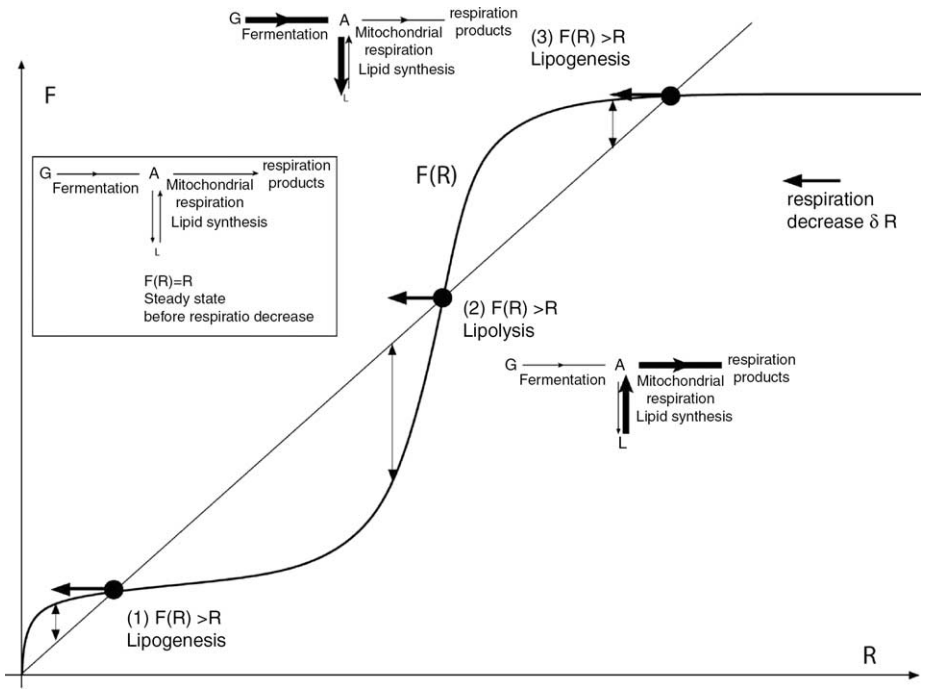

Fig. 3. A model of the qualitative response of lipid synthesis and a respiration decreased. The relationship between respiratory flux (F) and fermentative flux (F) can be represented by a sigmoid curve. Three metabolic states are possible (black circles). Decreases in respiration (bold arrows) have opposing effects on lipid synthesis (value of $F(R)-R$) depending on metabolic status. In tissues in states 1 and 3, decreases in respiration lead to $F(R)>R$, and thus an increase in lipid content. In contrast, for tissues in state 2, decreases in respiration lead to $F(R)<R$ and therefore induce lipolysis. Thus, regions of the body where lipid synthesis is taking place are characterized by a convex relationship between R and F, whereas regions where lipolysis is occurring are characterized by a concave relationship.

pathway is higher than that through the respiration pathway ($F>R$), whereas it decreases when $F<R$. According to this model, gross phenotypic differences lipoatrophy or lipohypertrophy would result from the small differences in the metabolic status of regions of the body where fat deposits normally accumulate[1].

In conclusion, our data and modelisation are consistent with the hypothesis that mitochondrial respiration inhibition plays a central role in the pathogenesis of anti-HIV drug-related lipodystrophy in vivo. Our in vitro testing system may be a useful tool for a systematic dose/response studies of the effect of each individual anti-HIV drug and various drug combinations on mitochondrial respiration and mtDNA replication. Such in vitro studies are underway in our laboratory and the results obtained so far are consistent those obtained from the epidemiological studies that provided evidence supporting an association between LDP prevalence and certain anti-HIV drug regimens. These studies may help to further increase our understanding of the mechanisms involved in the association between NRTI-related respiratory defects and lipodystrophy.

[1] We assumed that in the normal situation, the lipid content of fat tissue depot in a given body location does not vary significantly. Conceivably, the basal metabolic equilibrium of a given fat depot could be influenced by the dominant metabolic status in adjoining muscles, rather glycolytic in troncular static muscles, and more oxidative in slow-twitch stretching muscles.

Acknowledgements

This work was supported by the Ensemble Contre le Sida-SIDACTION Foundation. We would like to thank Dr Richard Léandri for providing the fat samples and Christel Gentil for excellent technical assistance.

References

Brinkman, K., ter Hofstede, H.J., Burger, D.M., Smeitink, J.A., Koopmans, P.P., 1998. Adverse effects of reverse transcriptase inhibitors: mitochondrial toxicity as common pathway. Aids 12, 1735–1744.

Brinkman, K., Smeitink, J.A., Romijn, J.A., Reiss, P., 1999. Mitochondrial toxicity induced by nucleoside-analogue reverse-transcriptase inhibitors is a key factor in the pathogenesis of antiretroviral-therapy-related lipodystrophy. Lancet 354, 1112–1115.

Carr, A., Samaras, K., Thorisdottir, A., Kaufmann, G.R., Chisholm, D.J., Cooper, D.A., 1999. Diagnosis, prediction, and natural course of HIV-1 protease-inhibitor- associated lipodystrophy, hyperlipidaemia, and diabetes mellitus: a cohort study. Lancet 353, 2093–2099.

Chene, G., Angelini, E., Cotte, L., Lang, J.M., Morlat, P., Rancinan, C., May, T., Journot, V., Raffi, F., Jarrousse, B., Grappin, M., Lepeu, G., Molina, J.M., 2002. Role of long-term nucleoside-analogue therapy in lipodystrophy and metabolic disorders in human immunodeficiency virus-infected patients. Clin. Infect Dis. 34, 649–657.

Fromenty, B., Pessayre, D., 1995. Inhibition of mitochondrial beta-oxidation as a mechanism of hepatotoxicity. Pharmacol. Ther. 67, 101–154.

Gellerich, F.N., Deschauer, M., Chen, Y., Muller, T., Neudecker, S., Zierz, S., 2002. Mitochondrial respiratory rates and activities of respiratory chain complexes correlate linearly with heteroplasmy of deleted mtDNA without threshold and independently of deletion size. Biochim. Biophys. Acta. 1556, 41–52.

Hazan, U., Romero, I.A., Cancello, R., Valente, S., Perrin, V., Mariot, V., Dumonceaux, J., Gerhardt, C.C., Strosberg, A.D., Couraud, P.O., Pietri-Rouxel, F., 2002. Human adipose cells express CD4, CXCR4, and CCR5 [corrected] receptors: a new target cell type for the immunodeficiency virus-1?. Faseb. J. 16, 1254–1256.

Johnson, A.A., Ray, A.S., Hanes, J., Suo, Z., Colacino, J.M., Anderson, K.S., Johnson, K.A., 2001. Toxicity of antiviral nucleoside analogs and the human mitochondrial DNA polymerase. J. Biol. Chem. 276, 40847–40857.

Kakuda, T.N., 2000. Pharmacology of nucleoside and nucleotide reverse transcriptase inhibitor-induced mitochondrial toxicity. Clin. Ther. 22, 685–708.

Lewis, W., Dalakas, M.C., 1995. Mitochondrial toxicity of antiviral drugs. Nat. Med. 1, 417–422.

Longley, M.J., Ropp, P.A., Lim, S.E., Copeland, W.C., 1998. Characterization of the native and recombinant catalytic subunit of human DNA polymerase gamma: identification of residues critical for exonuclease activity and dideoxynucleotide sensitivity. Biochemistry 37, 10529–10539.

Martin, J.L., Brown, C.E., Matthews-Davis, N., Reardon, J.E., 1994. Effects of antiviral nucleoside analogs on human DNA polymerases and mitochondrial DNA synthesis. Antimicrob. Agents Chemother. 38, 2743–2749.

Molina, J.M., Chene, G., Ferchal, F., Journot, V., Pellegrin, I., Sombardier, M.N., Rancinan, C., Cotte, L., Madelaine, I., Debord, T., Decazes, J.M., 1999. The ALBI trial: a randomized controlled trial comparing stavudine plus didanosine with zidovudine plus lamivudine and a regimen alternating both combinations in previously untreated patients infected with human immunodeficiency virus. J. Infect. Dis. 180, 351–358.

Munoz-Malaga, A., Bautista, J., Salazar, J.A., Aguilera, I., Garcia, R., Chinchon, I., Segura, M.D., Campos, Y., Arenas, J., 2000. Lipomatosis, proximal myopathy, and the mitochondrial 8344 mutation. A lipid storage myopathy?. Muscle Nerve 23, 538–542.

Nolan, D., Hammond, E., Martin, A., Taylor, L., Herrmann, S., McKinnon, E., Metcalf, C., Latham, B., Mallal, S., 2003. Mitochondrial DNA depletion and morphologic changes in adipocytes associated with nucleoside reverse transcriptase inhibitor therapy. Aids 17, 1329–1338.

Petit, C., Mathez, D., Barthelemy, C., Leste-Lasserre, T., Naviaux, R.K., Sonigo, P., Leibowitch, J., 2003. Quantitation of blood lymphocyte mitochondrial DNA for the monitoring of antiretroviral drug-induced mitochondrial DNA depletion. J. Acquir. Immune Defic. Syndr. 33, 461–469.

Rossignol, R., Faustin, B., Rocher, C., Malgat, M., Mazat, J.P., Letellier, T., 2003. Mitochondrial threshold effects. Biochem. J. 370, 751–762.

Shikuma, C.M., Hu, N., Milne, C., Yost, F., Waslien, C., Shimizu, S., Shiramizu, B., 2001. Mitochondrial DNA decrease in subcutaneous adipose tissue of HIV- infected individuals with peripheral lipoatrophy. Aids 15, 1801–1809.

Squires, K.E., 2001. An introduction to nucleoside and nucleotide analogues. Antivir. Ther. 6 (Suppl. 3), 1–14.

Walker, U.A., Bickel, M., Lutke Volksbeck, S.I., Ketelsen, U.P., Schofer, H., Setzer, B., Venhoff, N., Rickerts, V., Staszewski, S., 2002. Evidence of nucleoside analogue reverse transcriptase inhibitor– associated genetic and structural defects of mitochondria in adipose tissue of HIV-infected patients. J. Acquir. Immune Defic. Syndr. 29, 117–121.