

Mitochondrial Effects of HIV Infection on the Peripheral Blood Mononuclear Cells of HIV-Infected Patients Who Were Never Treated with Antiretrovirals

Òscar Miró,¹ Sònia López,¹ Esteban Martínez,² Enric Pedrol,³ Ana Milinkovic,² Elisabeth Deig,³ Glòria Garrabou,¹ Jordi Casademont,¹ Josep M. Gatell,² and Francesc Cardellach¹

¹Mitochondrial Research Laboratory, Muscle Research Unit, Department of Internal Medicine, and ²Infectious Disease Unit, Hospital Clínic, August Pi i Sunyer Biomedical Research Institute, School of Medicine, University of Barcelona, Barcelona, and ³HIV-Unit, Department of Internal Medicine, Fundació Hospital-Asil de Granollers, Granollers, Catalonia, Spain

To investigate the effects of HIV infection on mitochondrial DNA (mtDNA) content and other mitochondrial parameters, we used peripheral blood mononuclear cells (PBMCs) from 25 asymptomatic antiretroviral-naive human immunodeficiency virus (HIV)-infected patients and from 25 healthy control subjects. HIV-infected patients had significant decreases in mtDNA content (decrease, 23%; $P < .05$) and in the activities of mitochondrial respiratory chain (MRC) complex II (decrease, 41%; $P < .001$), MRC complex III (decrease, 38%; $P < .001$), MRC complex IV (decrease, 19%; $P = .001$), and glycerol-3-phosphate dehydrogenase (decrease, 22%; $P < .001$), along with increased lipid peroxidation of PBMC membranes ($P = .007$). Therefore, HIV infection is associated not only with mtDNA depletion, but also with extensive MRC disturbances and increased oxidative damage.

Antiretroviral therapy that contains nucleoside reverse-transcriptase inhibitors (NRTIs) may induce adverse effects due to mitochondrial toxicity. The main pathogenic mechanism suspected involves the inhibition of mtDNA polymerase γ (mtDNA γ -pol), which is the only enzyme responsible for the replication of mtDNA (a circular, double-stranded DNA molecule of 16.5 kb), which only encodes for some components of some mitochondrial respiratory chain (MRC) complexes. Depletion of mtDNA [1–6], deletions [6, 7], and point mutations [8] have been reported to occur in some tissues as a consequence of inhibition of mtDNA γ -pol

by NRTIs. In some instances, these abnormalities may lead to an impairment of MRC function [1, 6].

Although previous studies that have assessed the mitochondrial effects of NRTIs have systematically included a group of untreated HIV-infected patients, they have essentially lacked a control group of non-HIV-infected people. Although this fact does not negate the conclusions reached by these studies regarding the harmful effects of NRTIs against mitochondria, the role (if any) of HIV in the diminishment of mtDNA content remains unclear. A recent study by Côté et al. [9] found that the mtDNA content in the buffy coats of 47 asymptomatic HIV-infected patients who had never received antiretroviral therapy was significantly reduced (56%), compared with that of 24 non-HIV-infected people (100%). This difference was not explained by the lower CD4⁺ T lymphocyte count of the HIV-infected patients, compared with the non-HIV-infected subjects. Similarly, a very recent study by Miura et al. [10] showed a significant reduction in the mtDNA content (70%) of PBMCs from 46 antiretroviral-naive HIV-infected

Received 12 December 2003; accepted 27 March 2004; electronically published 13 August 2004.

Reprints or correspondence: Dr. Óscar Miró, Muscle Research Unit, Dept. of Internal Medicine, Hospital Clínic, Villarroel 170, 08036 Barcelona, Catalonia, Spain (omiro@clinic.ub.es).

Clinical Infectious Diseases 2004;39:710–6

© 2004 by the Infectious Diseases Society of America. All rights reserved. 1058-4838/2004/3905-0016\$15.00

patients, compared with 29 healthy people (100%). In the study by Miura et al. [10], mtDNA content was positively correlated with CD4⁺ T cell count and was inversely correlated with HIV load. Nonetheless, it currently has not been ascertained whether mtDNA depletion is an isolated finding or whether it is associated with impaired MRC function or, even, with more-extensive damage of mitochondrial enzyme capacity that leads to increased oxidative damage.

METHODS

For the present study, we recruited 25 asymptomatic HIV-infected patients who had never received antiretroviral therapy, as well as 25 non-HIV-infected people who were matched by age (± 5 years) and sex and who were considered to be control subjects. Written, informed consent was obtained from all subjects before their inclusion in the study. The clinical data for individuals included in the study are presented in table 1.

PBMCs were isolated, by means of Ficoll density-gradient centrifugation (Histopaque-1077; Sigma Diagnostics), from 20 mL of peripheral venous blood collected in Vacutainer EDTA tubes (BD Vacutainers Systems). After isolation, the PBMCs were resuspended in PBS and were frozen and stored at -80°C , until their use in biochemical and genetic determinations. Protein content was measured according to Bradford's methodology [11].

An aliquot of PBMCs was used for the extraction of total DNA by means of a standard phenol-chloroform procedure. For quantification of mtDNA, the nuclear housekeeping 18S rRNA gene and the highly conserved mitochondrial ND2 gene were amplified separately by use of quantitative real-time PCR (LightCycler FastStart DNA Master SYBR Green I; Roche Molecular Biochemicals). The mtDNA content was first expressed as the ratio of mtDNA to nuclear DNA, by use of the LightCycler-based methodology (LightCycler System; Roche Diagnostics, Idaho Technology) [12], and it then was transformed to the number of mtDNA copies per cell, by use of a set of 4 "international" or "common" standards with known values of mtDNA copies/mL and nuclear DNA copies/mL (provided by E. Hammond, D. Nolan, and S. Mallal), to standardize mtDNA

assays in an international collaborative approach [13]. This approach has allowed our group of investigators to calibrate our own set of working standards (LightCycler-Control Kit DNA; Roche Diagnostics, Applied Sciences) and to report, for the present study, concordant data regarding mtDNA copies per cell.

Another aliquot of PBMCs was used for spectrophotometrical analyses. We determined glycerol-3-phosphate dehydrogenase (G3Pdh) activity, citrate synthase (CS; a mitochondrial matrix enzyme representative of the Krebs cycle) activity, and the enzyme activities of MRC complexes II–IV (the 3 complexes are representative of the MRC function; the first complex is exclusively encoded by the nuclear genome, and the latter 2 complexes are partially encoded by mtDNA). All enzyme activities were measured using standard procedures described elsewhere [14, 15] and were expressed as nanomoles of reduced or oxidized substrate per minute per milligram of total cell protein, representing the absolute activities.

Another aliquot was used to determine the degree of oxidative damage, by means of assessment of *cis*-parinaric acid to measure the lipid peroxidation of PBMC membranes. *Cis*-parinaric acid, a fatty acid that contains 4 conjugated double bonds that render it naturally fluorescent, is attacked during lipid peroxidation reactions. Accordingly, *cis*-parinaric acid fluorescence is consumed in lipid peroxidation reactions. Because *cis*-parinaric acid is readily incorporated into PBMC membranes, its loss of fluorescence is used to indirectly monitor the degree of lipid peroxidation. For this purpose, 100 μg of PBMC protein were placed into 3 mL of nitrogenized PBS that contained *cis*-parinaric acid (5 $\mu\text{mol/L}$; Molecular Probes), and they were incubated in darkness at 37°C . Afterward, fluorescence was measured at 3-min intervals, for 30 min, by use of 318-nm excitation and 410-nm emission [14, 16]. The greater the lipid peroxidation, the less fluorescence is detected.

CS activity was used to estimate the mitochondrial content, to adjust for mtDNA content and the MRC enzyme activities due to hypothetical changes in the mitochondrial amount of PBMCs. CS seems to be a rather stable mitochondrial enzyme, the activity of which is not subjected to fluctuations and path-

Table 1. Clinical characteristics of individuals included in the present study.

Characteristic	Healthy control subjects (<i>n</i> = 25)	HIV-infected patients (<i>n</i> = 25)
Age, mean years \pm SD	40 \pm 12	37 \pm 8
Male sex, % of patients	76	76
Duration of infection, mean months \pm SD	...	44 \pm 71
CD4 ⁺ T cell count, mean cells/mm ³ \pm SD	...	317 \pm 215
Viral load, ^a mean log ₁₀ HIV RNA copies/mm ³ \pm SD	...	5.0 \pm 0.9

^a Any viral load of <50 copies/mL was recorded as 49 copies/mL.

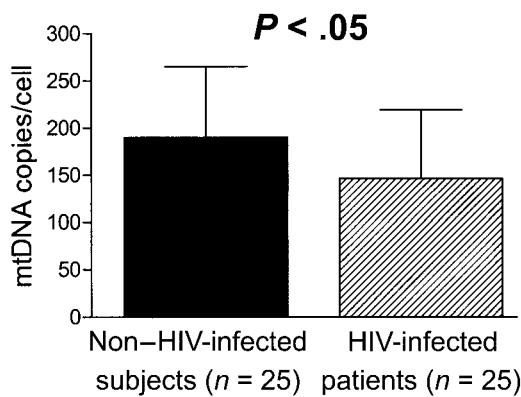


Figure 1. Quantification of mtDNA. Bars denote the results (expressed as mean values \pm SD) for each group. Antiretroviral-naive HIV-infected individuals had a significant decrease in the number of mtDNA copies per PBMC, compared with healthy (non-HIV-infected) individuals. Comparison between groups was performed using Student's *t* test. $P < .05$ denoted statistical significance.

ological changes. For this reason, when homogenates or impure mitochondrial fractions have to be used for enzymatic determinations, activities are best compared when they are divided by CS activity, to prevent artifacts caused by differences in the content of pure mitochondria. Accordingly, the mtDNA content and the MRC enzyme activities were also estimated per organelle (mitochondria)-relative values, by dividing absolute values per CS activity [17–19].

The results for HIV-infected patients and control subjects are expressed as mean values \pm SD, for quantitative variables, and they were compared using an unpaired Student's *t* test. Results are also expressed as percentages, for qualitative variables, and they were compared using the χ^2 test. Differences between groups are expressed as percentages with a 95% CI. A 2-way analysis of variance was used when measurements were repeated to compare *cis*-parinaric curves, and linear regression was used to search for any association between quantitative variables. For all cases, $P < .05$ was considered to be statistically significant.

RESULTS

The number of mtDNA copies per cell was significantly decreased in HIV-infected patients (by 23%; 95% CI, 4%–42%; $P < .05$), compared with healthy control subjects (figure 1). HIV-infected patients also exhibited a significant decrease in the MRC enzyme activities of complexes partially encoded by mtDNA. Specifically, complex III activity decreased by 38% (95% CI, 31%–51%; $P < .001$), and complex IV activity decreased by 19% (95% CI, 9%–29%; $P = .001$) (figure 2). On the other hand, the enzymatic activity of complex II, which is exclusively encoded by the nuclear DNA, was also found to be

decreased in HIV-infected patients (decrease, 41%; 95% CI, 24%–58%; $P < .001$) (figure 2). When the activities of the representative enzymes of other metabolic pathways were determined, no differences in CS activity were found when HIV-infected patients were compared with healthy control subjects (mean CS activity [\pm SD], 123 ± 15 nmol/min/mg protein and 131 ± 24 nmol/min/mg protein, respectively; P is not significant). However, a significant decrease in G3Pdh activity was noted in HIV-infected patients, compared with healthy control subjects (decrease, 22%; 95% CI, 11%–33%; $P < .001$) (figure 2). All the differences in mitochondrial parameters found between HIV-infected individuals and healthy people remained significant, even when they were estimated per mitochondria (by dividing mtDNA content and enzyme activities per CS activity; data not shown).

The oxidative damage to the PBMC membranes was significantly increased in HIV-infected patients, compared with healthy control subjects. As shown in figure 3, a greater loss of *cis*-parinaric acid fluorescence over time was noted among HIV-infected patients, a finding that indicates increased lipid peroxidation ($P = .007$).

Correlation of the mitochondrial parameters with immunological status (as assessed by the CD4⁺ T lymphocyte count) was performed for the HIV-infected patients. The mitochondrial parameters were also correlated with the severity of the

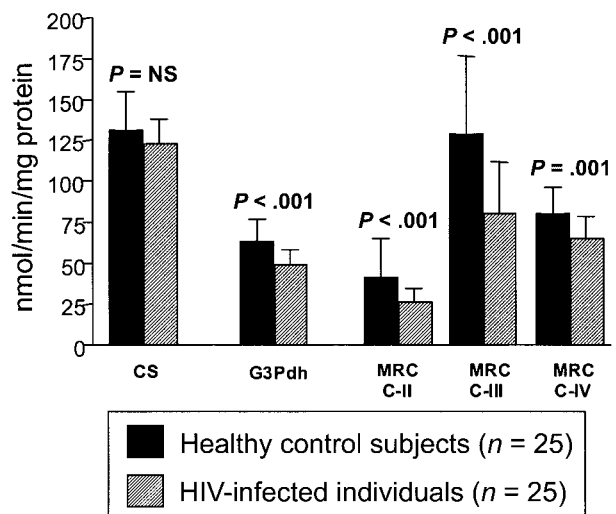


Figure 2. Enzyme activities, expressed as nanomoles of reduced or oxidized substrate per minute per milligram of total cell protein, of citrate synthase (CS), glycerol-3-phosphate dehydrogenase (G3Pdh), and mitochondrial respiratory chain (MRC) complex II (C-II), MRC complex III (C-III), and MRC complex IV (C-IV). Bars denote the results (expressed as mean values \pm SD) for each group. PBMCs from untreated HIV-infected individuals showed a significant decrease in all enzyme activities (with the exception of CS activity), compared with PBMCs from healthy (non-HIV-infected) individuals. Student's *t* test was used for comparison between groups. $P < .05$ denoted statistical significance. NS, not significant.

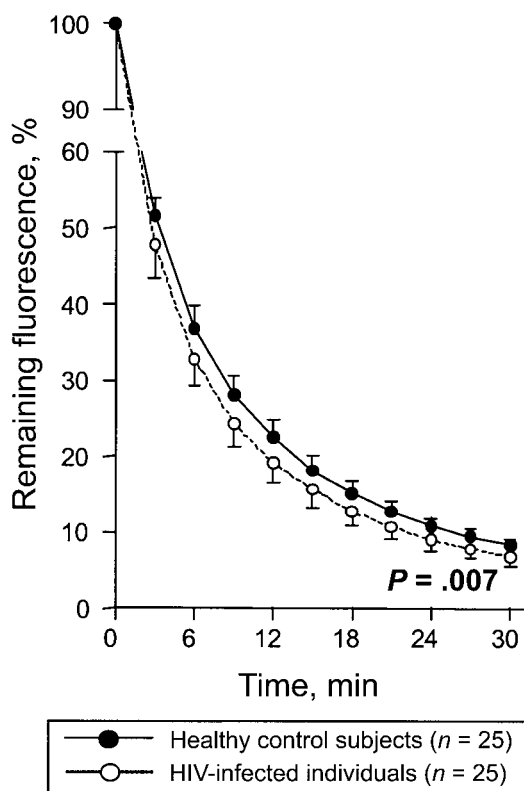


Figure 3. Studies of lipid peroxidation measured as loss of *cis*-parinaric acid fluorescence over time. Results are expressed as the percentage of the remaining fluorescence (\pm SD) at 3-min intervals. Untreated HIV-infected individuals had greater and faster loss of fluorescence, compared with healthy (non-HIV-infected) individuals, denoting an increase in lipid peroxidation of PBMC membranes. Comparison of *cis*-parinaric acid curves was performed using 2-way analysis of variance. $P < .05$ denoted statistical significance.

HIV infection (as assessed by plasma HIV load). We did not find any statistically significant association between either the CD4⁺ T lymphocyte count or the viral load in HIV-infected patients, with respect to any of the altered mitochondrial parameters. However, although not significant, a trend was noted toward a decrease in MRC complex IV activity, along with an increase in CS activity, in association with viral load (figure 4).

DISCUSSION

Previous studies have noted that some degree of mtDNA depletion is present in the PBMCs [10, 20, 21], skeletal muscle [2], adipose tissue [3], liver [22, 23], or placenta [24] of HIV-infected patients. However, these data were indirectly obtained in studies with main objectives that did not focus on the effects of HIV on mitochondria. In addition, to date, no studies demonstrating mitochondrial dysfunction associated with mtDNA depletion in human PBMCs have been reported. Therefore, the results of the present study show that HIV-infected patients

who have never been treated with antiretrovirals have decreased mtDNA levels, along with decreased enzyme activity of the MRC complexes and other metabolic pathways, as well as increased oxidative damage of the PBMC membranes. This is the first direct evidence that HIV is associated with extensive functional mitochondrial damage, which does not seem to only be limited to MRC complexes encoded by mtDNA.

The mechanism by which HIV causes this mtDNA depletion is currently elusive. Nonetheless, the coexistence of a generalized impairment that affects both mtDNA- and nuclear DNA-encoded MRC complexes, as well as G3Pdh activity, indicates that mtDNA depletion may be better interpreted as resulting from generalized mitochondrial damage rather than from a specific mechanism of the mtDNA lesion induced by HIV. This hypothesis agrees with the findings of recent studies that have reported signs of mitochondrial necrosis in HIV-infected cells [25]. Moreover, a main feature of HIV pathogenesis is cell death of CD4⁺ T lymphocytes as a result of apoptosis, and, currently, it is well known that several HIV-1-encoded proteins (Env, Vpr, Tat, and PR) are directly or indirectly associated with the dissipation of mitochondrial membrane potential, thereby causing apoptotic cell death [26–32]. In fact, the appearance of morphologic mitochondrial abnormalities, along with increased apoptosis, has been demonstrated in both ex vivo studies of individuals with seroconversion [33] and in vitro experiments involving acutely infected monocytoïd and lymphoblastoid cells [25]. Therefore, it is conceivable that, in addition to contributing to CD4⁺ T lymphocyte depletion, proapoptotic effects of virion proteins also have a role in the mtDNA depletion and the mitochondrial functional disturbances observed in the present study. This hypothesis, which probably is better addressed to cultured HIV-infected cells, revolves around the adverse effects of the viral gene products against mitochondria.

Although some authors have suggested that the intensity of HIV infection could correlate with the degree of mitochondrial damage, other authors have reported discordant data. In this sense, although Miura et al. [10] reported that mtDNA levels in HIV-infected individuals have a direct correlation with the CD4⁺ T cell count and an inverse correlation with the number of HIV RNA copies, Côté et al. [9] did not identify such an association. In our series, no significant association was found between markers of severity of infection and mitochondrial function; only a tendency toward an increase in the mitochondrial content in patients with a higher number of circulating HIV copies was remarkable. This fact could reflect that the classically known mitochondrial proliferation observed in the skeletal muscle in primary MRC defects is a form that responds to toxic insults.

The combined effects of HIV and antiretrovirals on mitochondria should be considered, because mitochondriotoxicity

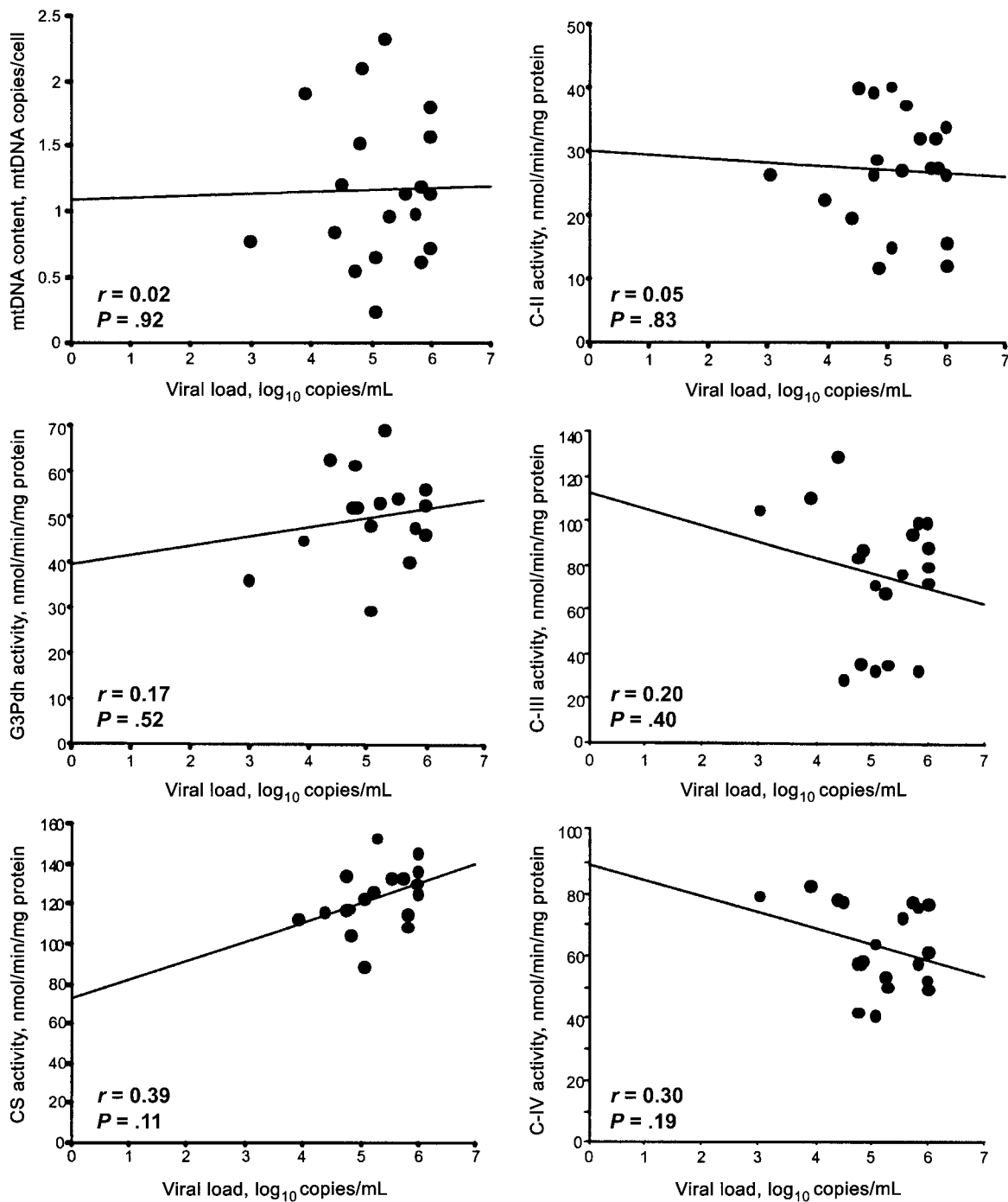


Figure 4. Association between viral load and mitochondrial parameters in antiretroviral-naive HIV-infected individuals, as established by a linear regression model. There was no significant association with any of the parameters assessed (mtDNA; glycerol-3-phosphate dehydrogenase [G3Pdh] activity; citrate synthase [CS] activity; and mitochondrial respiratory chain [MRC] complex II [C-II], MRC complex III [C-III], and MRC complex IV [C-IV] activity). $P < .05$ denoted statistical significance.

is a well known side effect of antiretrovirals. On one hand, the negative effects of HIV infection, per se, on mtDNA could render HIV-infected patients more susceptible to the mitochondrial toxicities of NRTIs, compared with the general population, because HIV-infected patients can reach the “thresh-

old” for clinically relevant adverse effects faster. As an example, we have found that untreated HIV-infected patients with greater viremia showed less complex IV activity; this finding, although not statistically significant, suggests that HIV-infected patients may be more susceptible than non-HIV-infected individuals to

the mitochondrial toxic effects of antiretrovirals. In addition, NRTIs also induce apoptotic death in several cell types [34]. Conversely, protease inhibitors exert antiapoptotic effects, which seem to be relevant for their clinical benefit, in a way different from that achieved by means of their antiviral activity [35, 36]. Thus, the net effect of HIV and antiretrovirals on mitochondrial function may differ from one patient to another, and it may explain, at least in part, the existence of discordant results of studies of the mitochondrial toxicity of antiretrovirals.

Whatever the mechanism involved in the effects of HIV on mitochondria, we believe that the findings of the present study support the hypothesis that the effects of HIV on mtDNA content are nonspecific and would be better reflected in a scenario of more diffuse mitochondrial damage, probably in association with apoptotic changes caused by HIV. Moreover, our results should also be taken into account in the design of further studies evaluating the mitochondrial toxic effects of antiretrovirals, because untreated individuals with HIV infection should be included in the control group of such studies.

Acknowledgments

We thank Anna Beato for her technical laboratory support; Emma Hammond, from the Centre for Clinical Immunology and Biomedical Statistics, Royal Perth Hospital, and Murdoch University (Perth, Western Australia), for providing us with the set of "international" standards for standardization of mtDNA quantification; and Pierre Rustin, from the Unité de Recherches sur les Handicaps Génétiques de l'Enfant, Hospital Necker des Enfants Malades (Paris, France), for his helpful counseling.

Financial support. Fundación para la Investigación y la Prevención del SIDA en España (FIPSE; grant 3102-00), Fundació La Marató de TV3 (grant 02/0210), Redes de Investigación en Mitocondrias (grant V2003-REDC06E-0) y SIDA (grant Redg173), and Suport a Grups de Recerca Consolidats de la Generalitat de Catalunya (grant 2001/SRG/00379).

Conflict of interest. All authors: No conflict.

References

- Lewis W, Dalakas MC. Mitochondrial toxicity of antiviral drugs. *Nat Med* **1995**; 1:417–22.
- Casademont J, Barrientos A, Grau JM, et al. The effect of zidovudine on skeletal muscle mtDNA in HIV-1 infected patients with mild or no muscle dysfunction. *Brain* **1996**; 119:1357–64.
- Shikuma CM, Hu N, Milne C, et al. Mitochondrial DNA decrease in subcutaneous adipose tissue of HIV-infected individuals with peripheral lipodystrophy. *AIDS* **2001**; 15:1801–9.
- Walker UA, Bickel M, Lutke Volksbeck SI, et al. 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* **2002**; 29:117–21.
- Cherry CL, Gahan ME, McArthur JC, Lewin SR, Hoy JF, Wesselingh SL. Exposure to dideoxynucleosides is reflected in lowered mitochondrial DNA in subcutaneous fat. *J Acquir Immune Defic Syndr* **2002**; 30:271–7.
- Vittecoq D, Jardel C, Barthelemy C, et al. Mitochondrial damage associated with long-term antiretroviral treatment: associated alteration or causal disorder? *J Acquir Immune Defic Syndr* **2002**; 31:299–308.
- Gómez-Zaera M, Miró O, Pedrol E, et al. Mitochondrial involvement in antiretroviral therapy-related lipodystrophy. *AIDS* **2001**; 15:1643–51.

- Martin AM, Hammond E, Nolan D, et al. Accumulation of mitochondrial DNA mutations in human immunodeficiency virus-infected patients treated with nucleoside-analogue reverse-transcriptase inhibitors. *Am J Hum Genet* **2003**; 72:549–60.
- Côté HC, Brumme ZL, Craib KJ, et al. Changes in mitochondrial DNA as a marker of nucleoside toxicity in HIV-infected patients. *N Engl J Med* **2002**; 346:811–20.
- Miura T, Goto M, Hosoya N, et al. Depletion of mitochondrial DNA in HIV-1-infected patients and its amelioration by antiretroviral therapy. *J Med Virol* **2003**; 70:497–505.
- Bradford MM. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **1976**; 72:248–54.
- Miró O, López S, Pedrol E, et al. Mitochondrial DNA depletion and respiratory chain enzyme deficiencies are present in peripheral blood mononuclear cells of HIV-infected patients with HAART-related lipodystrophy. *Antivir Ther* **2003**; 8:333–8.
- Hammond EL, Sayer D, Nolan D, et al. Assessment of precision and concordance of quantitative mitochondrial DNA assays: a collaborative international quality assurance study. *J Clin Virol* **2003**; 27:97–110.
- Miró O, Alonso JR, Jarreta D, et al. Smoking disturbs mitochondrial respiratory chain function and enhances lipid peroxidation on human circulating lymphocytes. *Carcinogenesis* **1999**; 20:1331–6.
- Barrientos A. In vivo and in organello assessment of OXPPOS activities. *Methods* **2002**; 26:307–16.
- Hedley D, Chow S. Flow cytometric measurement of lipid peroxidation in vital cells using parinaric acid. *Cytometry* **1992**; 13:686–92.
- Pallotti F, Lenaz G. Isolation and subfractionation of mitochondria from animal cells and tissue culture lines. In: Pon LA, Schon EA, eds. *Methods in cell biology*. San Diego: Academic Press, **2001**:1–35.
- Zeviani M, Gellera C, Antozzi C, et al. Maternally inherited myopathy and cardiomyopathy: association with a mutation in mitochondrial DNA tRNA^{Leu(UUR)}. *Lancet* **1991**; 338:143–7.
- Miró O, López S, Martínez E, et al. Short communication: reversible mitochondrial respiratory chain impairment during symptomatic hyperlactatemia associated with antiretroviral therapy. *AIDS Res Hum Retroviruses* **2003**; 19:1027–32.
- Cherry CL, McArthur JC, Hoy JF, Wesselingh SL. Nucleoside analogues and neuropathy in the era of HAART. *J Clin Virol* **2003**; 26:195–207.
- Poirier MC, Divi RL, Al-Harthi L, et al. Long-term mitochondrial toxicity in HIV-uninfected infants born to HIV-infected mothers. *J Acquir Immune Defic Syndr* **2003**; 33:175–83.
- Chariot P, Drogou I, de Lacroix-Szmania I, et al. Zidovudine-induced mitochondrial disorder with massive liver steatosis, myopathy, lactic acidosis, and mitochondrial DNA depletion. *J Hepatol* **1999**; 30:156–60.
- Walker UA, Bauerle J, Laguno M, et al. Depletion of mitochondrial DNA in liver under antiretroviral therapy with didanosine, stavudine, or zalcitabine. *Hepatology* **2004**; 39:311–7.
- Shiramizu B, Shikuma KM, Kamemoto L, et al. Brief report: placenta and cord blood mitochondrial DNA toxicity in HIV-infected women receiving nucleoside reverse transcriptase inhibitors during pregnancy. *J Acquir Immune Defic Syndr* **2003**; 32:370–4.
- Plymale DR, Tang DS, Comardelle AM, Fermin CD, Lewis DE, Garry RF. Both necrosis and apoptosis contribute to HIV-1-induced killing of CD4 cells. *AIDS* **1999**; 13:1827–39.
- Chen D, Wang M, Zhou S, Zhou Q. HIV-1 Tat targets microtubules to induce apoptosis, a process promoted by the pro-apoptotic Bcl-2 relative Bim. *EMBO J* **2002**; 21:6801–10.
- Muthumani K, Hwang DS, Desai BM, et al. HIV-1 Vpr induces apoptosis through caspase 9 in T cells and peripheral blood mononuclear cells. *J Biol Chem* **2002**; 277:37820–31.
- Jacotot E, Ravagnan L, Loeffler M, et al. The HIV-1 viral protein R induces apoptosis via a direct effect on the mitochondrial permeability transition pore. *J Exp Med* **2000**; 191:33–46.
- Strack PR, Frey MW, Rizzo CJ, et al. Apoptosis mediated by HIV protease is preceded by cleavage of Bcl-2. *Proc Natl Acad Sci U S A* **1996**; 93:9571–6.

30. Creaven M, Hans F, Mutskov V, et al. Control of the histone-acetyltransferase activity of Tip60 by the HIV-1 transactivator protein, Tat. *Biochemistry* **1999**; 38:8826–30.
31. Williams MD, Van Remmen H, Conrad CC, Huang TT, Epstein CJ, Richardson A. Increased oxidative damage is correlated to altered mitochondrial function in heterozygous manganese superoxide dismutase knockout mice. *J Biol Chem* **1998**; 273:28510–5.
32. Macho A, Calzado MA, Jimenez-Reina L, Ceballos E, Leon J, Munoz E. Susceptibility of HIV-1–TAT transfected cells to undergo apoptosis: biochemical mechanisms. *Oncogene* **1999**; 18:7543–51.
33. Cossarizza A, Mussini C, Mongiardo N, et al. Mitochondrial alterations and dramatic tendency to undergo apoptosis in peripheral blood lymphocytes during acute HIV syndrome. *AIDS* **1997**; 11:19–26.
34. Badley AD, Roumier T, Lum JJ, Kroemer G. Mitochondrion-mediated apoptosis in HIV-1 infection. *Trends Pharmacol Sci* **2003**; 24:298–305.
35. Piketty C, Weiss L, Thomas F, Mohamed AS, Belec L, Kazatchkine MD. Long-term clinical outcome of human immunodeficiency virus–infected patients with discordant immunologic and virologic responses to a protease inhibitor–containing regimen. *J Infect Dis* **2001**; 183: 1328–35.
36. Wood E, Hogg RS, Yip B, et al. “Discordant” increases in CD4 cell count relative to plasma viral load in a closely followed cohort of patients initiating antiretroviral therapy. *J Acquir Immune Defic Syndr* **2002**; 30:159–66.