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Respiratory chain dysfunction associated with multiple mitochondrial DNA deletions in antiretroviral therapy-related lipodystrophy [Research Letters]

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Highly-active antiretroviral therapy (HAART) can induce a characteristic lipodystrophy syndrome characterized by peripheral fat wasting and central adiposity, usually associated with hyperlipidaemia and insulin resistance [1,2]. Indirect data have led some authors to propose that mitochondrial dysfunction could play a role in this syndrome [3,4].To date, as recently outlined by Kakuda *et al*. [5] in this journal, HIV-infected patients developing lipodystrophy have not been studied for mitochondrial changes or respiratory chain capacity.

We studied a 67-year-old woman with an unremarkable past history except for HIV infection diagnosed *5* years earlier, without previous opportunistic infections. During the 4 months before the current evaluation, she developed central and peripheral lipodystrophy associated with raised serum triglyceride levels (from 130 to 543 mg/dl) and no changes in cholesterolaemia or glycaemia. The total CD4 lymphocyte count was 401/mm^3, and viral load was 790 copies/mm^3. She had previously received zidovudine (total cumulative dose; TCD 200 g) and didenosine (TCD 216 g) and, when lipodystrophy appeared, she was receiving saquinavir (TCD 1512 g), lamivudine (TCD 288 g) and stavudine (TCD 58 g). After obtaining informed consent, a muscle biopsy was performed. Some subcutaneous fat lobules were removed during the same surgical procedure. Thirty mL of venous blood was extracted for the isolation of lymphocytes.

A fragment of skeletal muscle was used for histological and ultrastructural studies. The biochemical function of the mitochondrial respiratory chain (MRC) was analysed on fresh skeletal muscle mitochondria and lymphocyte suspensions using a double method. First, state III respiratory rates were pollarographically determined using pyruvate-malate, succinate, glycerol-3-phosphate and ascorbate as substrates that transfer electrons at complexes I, II, III and IV of MRC, respectively. Intact cell respiration was also assessed for lymphocytes. Second, the individual enzyme activity for each complex of the MRC was spectrophotometrically quantified. For molecular studies, whole DNA of skeletal muscle, lymphocytes and adipocytes were extracted and analysed by Southern blotting and polymerase chain reaction (PCR), to look for rearrangements and to quantify mitochondrial DNA (mtDNA) abundance. The methodology has been reported elsewhere [6-8].

We found abundant lipid storage inside both type I and II myocytes on a histological study of skeletal muscle (Fig. 1); the rest of the morphological examination was otherwise irrelevant. With respect to biochemical analyses, clearly decreased complex III and IV activities were found in both skeletal muscle mitochondria and lymphocytes, which in turn caused a decay in state III respiratory rates for nearly all substrates. When mtDNA was analysed, multiple deletions were found in skeletal muscle and adipocytes, but not in lymphocytes (Fig. 2), whereas the total amount of mtDNA was preserved in all tissues.

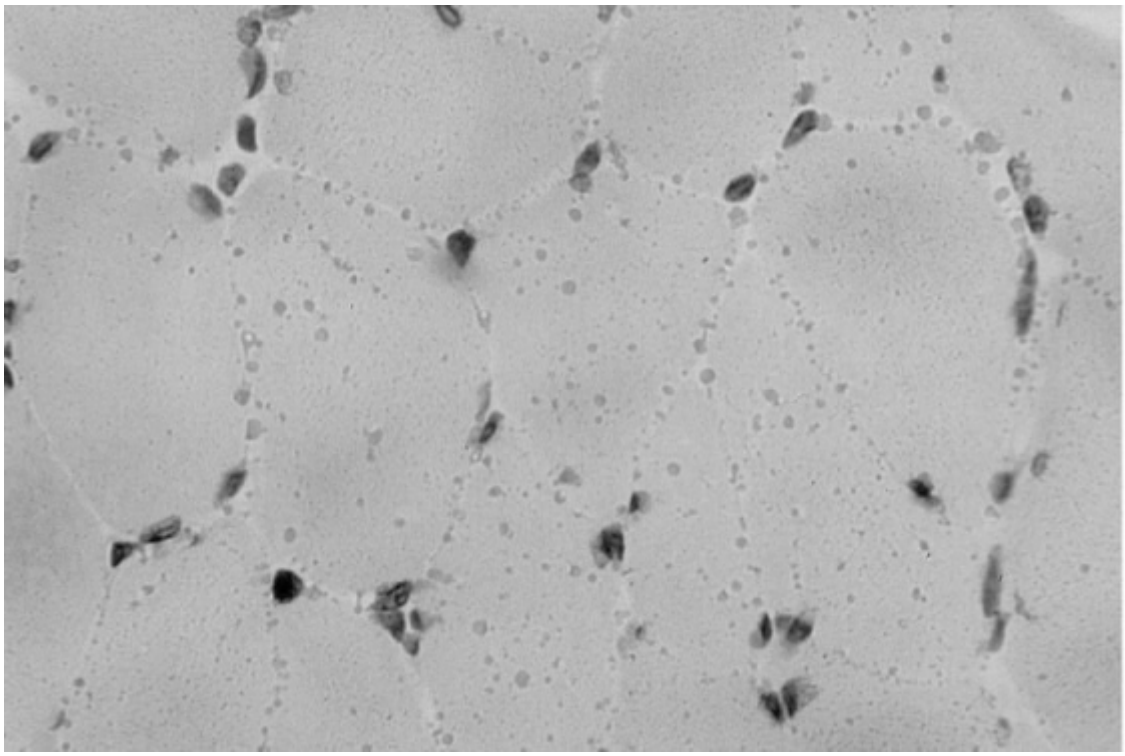

Fig. 1. Skeletal muscle from HIV patient with highly-active antiretroviral therapy-related lipodystrophy. Oil-red 0 staining shows abundant neutral lipids (up; original magnification: 200×). Lipid droplets are also evident in semithin sections (down, original magnification: 800×).

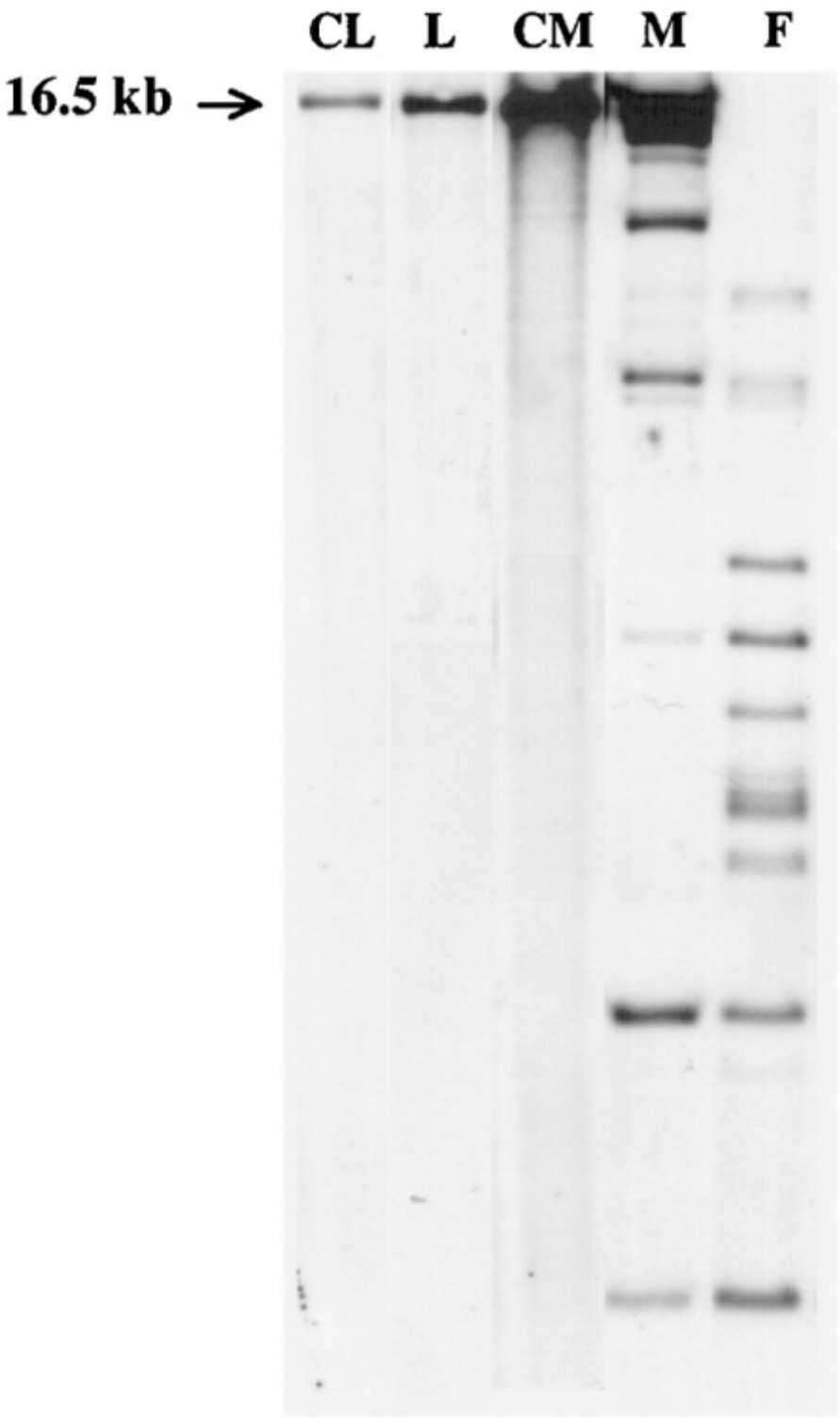

Fig. 2. Left: Southern blot. Samples were digested with *Pvu* II, electrophoresed in a 0.8% agarose gel, blotted onto nylon membrane, and hybridized with total mitochondrial DNA (mtDNA). The arrow indicates the normal 16.5 kb mtDNA bands. CL, Control lymphocytes; CM, control muscle. L, M, and F correspond to lymphocytes, muscle and fat from the patient. Muscle and fat from the patient presented abundant multiple deletions. Right: Enzyme and oxidative activities from lymphocytes and muscle were decreased (bold numbers) for most of the parameters and were evaluated with respect to control values (italic numbers between brackets).

We believe this is the first case of an HIV-infected patient with HAART-related lipodystrophy in whom mitochondrial dysfunction was demonstrated. Protease inhibitors have been invoked as the main cause of the syndrome through disturbing adipocyte metabolism and causing apoptosis by means of altered retinoid signalling [1,9]. In addition, it has also been proposed that the well-known mitochondrial toxicity of nucleoside analogue reverse transcriptase inhibitors [8] could contribute to the development of HAART-related lipodystrophy, on the basis of its occasional appearance in patients on protease-sparing regimens, and on its similarity with multiple symmetrical lipomatosis, a disorder sometimes associated with single and multiple mtDNA deletions and decreased complex IV activity [10]. Why the effects of adding protease inhibitors to nucleoside analogue reverse transcriptase would produce a lipodystrophy syndrome instead of the more classical phenotype resembling an inherited mitochondrial disease is not known. We hypothesized that protease inhibitors could interfere with certain patient proteases in a selective way in different tissues. In this sense, some proteases are essential for mitochondrial biogenesis and function, such as mitochondrial processing of peptidases involved in the import and activation of mitochondrial protein precursors (including diverse DNA-encoded MRC subunits) synthesized in cytoplasmic ribosomes.

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References

1. Carr A, Samaras K, Chisholm DJ, Cooper DA. **Pathogenesis of HIV-1-protease inhibitor-associated peripheral lipodystrophy, hyperlipidaemia, and insulin resistance.** Lancet 1998, 351: 1881 -1883.
[Context Link]
2. Carr A, Samaras K, Burton S, Law M, Freund J, Chisholm DJ, Cooper DA. **A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors.** AIDS 1998, 12: F51 -F58.
[Fulltext Link] [CrossRef] [Context Link]
3. Brinkman K, Smeitink JA, Romijn JA, Reiss P. **Mitochondrial toxicity induced by nucleoside-analogue reverse-transcriptase inhibitors is a key factor in the pathogenesis of antiretroviral-therapy-related lipodystrophy.** Lancet 1999, 354: 1112 -1115.
[Medline Link] [CrossRef] [Context Link]
4. Teplitsky V, Halabe A. **Fat distribution in AIDS.** N Engl J Med 1999, 340: 969 -970.
[CrossRef] [Context Link]
5. Kakuda TN, Brundage RC, Anderson PL, Fletcher CV. **Nucleoside reverse transcriptase inhibitor-induced mitochondrial toxicity as an etiology for lipodystrophy.** AIDS 1999, 13: 2311 -2312.
[Fulltext Link] [CrossRef] [Context Link]
6. Miró O, Casademont J, Grau JM, Jarreta D, Urbano-Márquez A, Cardellach F. **Histological and biochemical assessment of mitochondrial function in dermatomyositis.** Br J Rheumatol 1998, 37: 1047 -1053.
[Context Link]
7. Masands F, Barrientos A, Cebrián M. et al. **Clinical, histological and molecular reversibility of zidovudine myopathy.** J Neurol Sci 1998, 159: 226 -228.
[Medline Link] [CrossRef] [Context Link]
8. 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 -1364.
[CrossRef] [Context Link]

9. Domingo P, Matías-Guiu X, Pujol RM. et al. **Subcutaneous adipocyte apoptosis in HIV-1 protease inhibitor-associated lipodystrophy.** AIDS 1999, 13: 2261 -2267.
[Fulltext Link] [CrossRef] [Context Link]
10. Morris AAM, Can A. **HIV nucleoside analogues: new adverse effects on mitochondria?** Lancet 1999, 354: 1046 -1047.
[Medline Link] [CrossRef] [Context Link]