To the Editor:

In a previous issue of Blood, Macho et al.1 described an abnormality in mitochondrial respiration in lymphocytes from human immunodeficiency virus-positive (HIV+)* subjects. These subjects were predominantly asymptomatic with CD4+ counts between 200 and 300/mL. Increased permeability of the inner mitochondrial membrane was shown by oxidation of the lipophilic dye hydroethidine as detectable by fluorescence-activated cell sorting analysis. Macho et al.1 postulated that this may be a direct effect of viral proteins or RNA on T cells. However, the proportion of peripheral T cells infected by HIV, even in the acquired immunodeficiency syndrome (AIDS) population, is too small to account for the abnormality observed.2 We suggest instead that the abnormality found by Macho et al. represents a mitochondrial abnormality that affects multiple organs in the HIV+ population and interacts with other mitochondrial toxins such as nucleoside analogs to produce a wide variety of adverse events.

Other investigators have shown metabolic abnormalities in the HIV+ population.3 In particular, elevated free fatty acids (FFA) and plasma lipoproteins and decreased use of citrate have been observed.4 These abnormalities imply an error in the mitochondrial metabolism. Also, elevated levels of cytokines, which are observed in HIV, are a potential etiology for the mitochondrial abnormality in HIV. Whether directly (tumor necrosis factor [TNF]) or indirectly through the elevation of FFA,3 because both TNF and FFA have been shown to be mitochondrial toxins in cell culture4,5 and both produce respiratory chain abnormalities similar to those seen by Macho et al.1

We have been interested in the time- and dose-dependent toxicities of nucleoside analogues, in particular the fatal hepatic steatosis associated with zidovudine (ZDV). Freiman et al.6 described this syndrome of hepatic steatonecrosis, lactic acidosis, and ultimately fatal liver failure in obese women who had received ZDV for at least 6 months. These women were in generally good health and without AIDS-defining diseases. The hepatic failure associated with chronic ZDV use was similar to that found in a trial with fialuridine (FIAU)7 and included hepatic steatosis and lactic acidosis. These abnormalities suggest that the syndrome is caused by mitochondrial dysfunction that is produced or unmasked by antiviral drugs. Other clinical syndromes with similar fatty infiltration of the liver produced by mitochondrial dysfunction include Reye's syndrome6 and inborn errors of β-oxidation of fatty acids.8 In addition, in vitro studies show that ZDV inhibits mitochondrial DNA biosynthesis9 and clinically abnormal mitochondria, "ragged red fibers," and fatty deposits are reported in the muscles of HIV+ patients with ZDV-associated myopathy.10

Because congenital abnormalities of β-oxidation have been associated with steatosis, we postulated that β-oxidation of free fatty acids by mitochondria would be a key mitochondrial function to investigate. The standard screen for β-oxidation defects is examination of the urine by gas chromatography-mass spectroscopy for abnormal organic acids in the urine (organic aciduria).11 Therefore, we investigated whether subjects on ZDV for a prolonged period of time showed organic aciduria. We conducted a single-center, pilot study in HIV+ subjects to determine whether examination of the urine for organic aciduria would be a suitable screen for mitochondrial toxicity of ZDV. Entry criteria for the group believed to be at risk for β-oxidation defects (the at-risk group) were continuous medication with at least 300 mg/d of ZDV for at least 6 months; the control group had no exposure to any antiretroviral nucleoside analog for at least 3 months before the study. Both groups consisted of HIV+ patients more than 18 years of age not receiving medications, not experiencing conditions known to alter fat metabolism, and not experiencing any other known metabolic disease. Patients meeting entry criteria fasted overnight, after which a morning urine was obtained, immediately frozen, and stored at −20°C until analyzed. Subjects were interviewed and their medical records were reviewed by one of the investigators. Because the ZDV-associated hepatic steatosis has been found to a disproportionate extent in obese, female HIV+ patients, we required at least 50% of our subjects to be female, with the at-risk group consisting of 35 subjects (18 female) and a control group of 15 subjects (8 female).

The at-risk group differed from the control group by having lower CD4 counts (248.8 ± 23.3 vs 558.2 ± 76.2; P < .001), a higher incidence of AIDS (54% in the ZDV group vs 20% in the control group; P < .01), lower weight (147.0 ± 3.5 pounds in the ZDV group vs 179.8 ± 10.1 lb in the control group; P < .05, and more were treated with Trimethoprim-Sulfamethoxazole (49% in the ZDV group vs 13% in the comparison group; P < .05. These differences between the groups probably reflect a selection bias, because the at-risk population on chronic ZDV is likely to have more advanced disease because it is currently recommended to withhold nucleoside therapy until CD4 counts are less than 500.

We designed the trial to have a 95% power to detect a prevalence of 20%, with a type I error of 0.05% to find at least 1 patient with organic aciduria. We anticipated a 20% frequency of subclinical abnormality of mitochondrial metabolism in the group believed to be at risk for steatosis (group A) to have a 95% chance of seeing at least one patient with an abnormality. Although the study had more than a 92% power of detecting an abnormality if the prevalence was 7% or greater in the group of 35 subjects, we failed to find abnormal organic aciduria in any subject in the trial.
Negative results are difficult to interpret and extrapolate to other studies. Our study population was similar to that of Macho et al with respect to age, disease status, and CD4 count. Although we did not detect a defect in mitochondrial β-oxidation, we now believe that the defect in respiratory function shown by Macho et al is a more plausible etiology for hepatic steatosis than a defect in β-oxidation. Given that GC-MS is the standard screen for abnormalities of β-oxidation, it seems unlikely that clinically significant abnormalities of β-oxidation were present and not detected. It is possible that, even if this test could detect all subclinical mitochondrial toxicities, the low prevalence of obesity in the study and comparison groups prevented us from finding any abnormalities. Although the study had greater than 95% power to detect a 20% prevalence of a subclinical abnormality, even if it occurred solely in women, if the ZDV effect on β-oxidation occurred only in obese people, we would not have had sufficient power to detect it reliably. However, this result differs from that of Macho et al.

Although the pathophysiology of hepatic steatosis may not be due to a mitochondrial abnormality, this seems unlikely given the growing evidence both in vitro and in prospective clinical trials that ZDV affects mitochondrial function. An alternative explanation is that there is indeed a mitochondrial effect from ZDV, but involving a respiratory defect like that described by Macho et al and affecting β-oxidation only indirectly. This is not unreasonable given the plethora of metabolic activities that interact within the mitochondrion. An abnormality with mitochondrial respiration such as that described by Macho et al has been shown in an in vitro system capable of interfering with mitochondrial β-oxidation. The abnormality of mitochondrial respiration shown by Macho et al in blood cells of HIV+ patients may also be operating in other organs and predisposing to toxicity by other mitochondrial toxins.

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**REFERENCES**


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Lack of beta-oxidation defects in human immunodeficiency virus-positive subjects with and without chronic zidovudine exposure [letter; comment]

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