The widespread use of nucleoside analog reverse transcriptase inhibitors (NRTIs) and HIV protease inhibitors (PIs) in Western countries has substantially reduced morbidity and mortality in patients with HIV infection. Concurrently, however, adverse effects associated with long-term use of these agents are becoming recognized. A growing body of literature suggests that many adverse effects associated with the use of NRTIs such as lactic acidosis, hepatic steatosis, myopathy, cardiomyopathy, peripheral neuropathy, pancreatitis and lipodystrophy syndrome are due to mitochondrial toxicity. In contrast, the adverse effects associated with the use of PIs, hyperlipidemia, lipodystrophy, and perhaps, the resulting accelerated atherosclerosis, have not been attributed to mitochondrial toxicity. In the October 2002 issue of *Atherosclerosis, Thrombosis and Vascular Biology*, Zhong et al challenge this paradigm by demonstrating PI-mediated mitochondrial dysfunction in endothelial cells and the resultant apoptosis-independent cytotoxicity and suggest that PI-induced endothelial cell toxicity contributes to accelerated atherosclerosis in HIV patients.

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Lipoprotein oxidation is a critical step in the initiation of atherosclerosis. Lipoproteins undergo oxidation by endothelial cells during transport from the plasma into the arterial wall. A clear association has been established between elevated serum LDL cholesterol levels and increased atherosclerotic disease. In addition, the extent of oxidation of LDL cholesterol impacts its atherogenic potency. Reduction of LDL cholesterol by diet, exercise, and/or pharmacologic agents reduces atherosclerotic risk. These data suggest that the accelerated atherosclerosis in HIV patients treated with PIs is due to the PI-induced hyperlipidemia.

Mechanisms of Lipodystrophy and Hyperlipidemia Associated With PIs

Lipodystrophy and hyperlipidemia can occur under conditions that affect mitochondrial integrity and function. Fatty acids are derived from hydrolysis of triglycerides and are removed from blood in mitochondria. In the mitochondrial matrix, fatty acids are degraded by oxidation at the β-carbon atom yielding ATP, in a process called beta oxidation. In damaged or dysfunctioning mitochondria, this reaction does not take place and lipid metabolism is altered (Figure), potentially leading to fat redistribution or lipodystrophy.

Despite the recognized relationship among lipodystrophy, hyperlipidemia, and PI use, the mechanisms remain to be elucidated. Two popular current theories suggest that PI therapy alters lipid metabolism by either increasing lipid release (or decreasing the storage of synthesized lipids) or by inhibiting chylomicron uptake, resulting in hypertriglyceridemia. The proposed mechanism by which PIs alter lipid release relates to sequence similarities between the catalytic region of HIV-1 protease, the region to which PIs bind, and regions of two proteins that regulate lipid metabolism. A 60% homology exists between the catalytic region of HIV-1 protease and regions of cytoplasmic retinoic acid binding protein type 1 (CRABP-1) and LDL-receptor-related protein (LRP). It was hypothesized that PIs stimulate lipid release or reduce lipid storage by inhibiting the synthesis of cis-9-retinoic acid (cis-9-RA) from retinoic acid (RA) by protease inhibitors or by binding to LRP, causing impaired chylomicron uptake and triglyceride clearance by the endothelial LRP-LPL (lipoprotein lipase complex) (Figure). The resultant hyperlipidemia can, in turn, affect mitochondrial function and alter glucose and lipid oxidation pathways.

It has also been recently reported that PIs inhibit proteasomal degradation of apolipoprotein B (Figure), a principal component of plasma lipoproteins, and increase the secretion of apolipoprotein B-lipoproteins in the presence of remnant lipoproteins or during enhanced fatty acid flux due to peripheral insulin resistance. Another possible mechanism for the pathogenesis of lipodystrophy induced by PIs is an indirect effect via secretion of tumor necrosis factor (TNF)-α (Figure). Following PI intake as part of highly-active antiretroviral therapy, TNF-α homeostasis is dysregulated. TNF-α can cause hyperlipidemia and lipodystrophy by inhibiting lipoprotein lipases, increasing hepatic triglyceride synthesis and by exerting site-specific differences on adipocyte responses, respectively.

To date, however, there remains a paucity of direct evidence regarding which, if any, of these putative mechanisms are in play in PI-treated lipodystrophic patients.
Evidence for Vascular Mitochondrial Toxicity Induced by PIs: Fact or Fancy?

Surprisingly, given the growing literature on atherosclerotic complications in HIV patients treated with PIs, virtually no data exist on the effects of PIs on vascular cells. The study by Madamanchi et al,1759 presents a new approach to this question. The authors demonstrate that ritonavir treatment diminishes endothelium-dependent vasorelaxation in monkey arteries, suggesting that ritonavir may affect mitochondrial function and alter the lipid metabolism by inhibiting the activity of LPL and LRP.

Quantitative mtDNA Assay as a Marker for Mitochondrial Toxicity Associated With Antiretroviral Therapy and Atherosclerosis

Quantitative polymerase chain reaction (QPCR) for detecting mtDNA damage used by Zhong et al8 was developed initially by Van Houten and colleagues.28 Detection of DNA damage by this method is based on the premise that any lesion in DNA template will stop a thermostable polymerase, resulting in decreased amplification of the damaged template compared with the undamaged DNA template. The sensitivity of this method relies on initial template quantity because all the samples should have equal amount of DNA.29 Precise quantification of DNA can be done using Picogreen (Molecular probes) as a sensitive marker of DNA concentration. Free Picogreen dye is nonfluorescent and yields 1000 fold fluorescence on binding to double stranded DNA with a broad linear response threshold. Quantification of QPCR product is generally achieved by using 32P-radiolabeled nucleotides, which does not seem to be the case in the assay reported by Zhang et al.8 It is important to run a 50% of studying whether the increase in mtDNA damage preceded the increase in lactate dehydrogenase levels (a marker for cell death) as was shown convincingly for the mitochondrial toxicity associated with NRTIs.22 If, in fact, mtDNA damage did not precede cell death, then it may simply reflect the cellular consequences of necrosis. The question of causal relationship between ROS and PI-induced mtDNA damage can be examined by studying these phenomena in the presence of antioxidants and ROS scavenging enzymes. Finally, the present study does not exclude the possibility that decreased mtDNA amplification was due to the effect of PIs on mtDNA replication or content rather than mtDNA damage, as has been reported for NRTIs.22

A causal relationship between ROS and mtDNA damage is interesting to entertain for the following reasons: 1) mitochondria are an abundant source of ROS in vascular cells;23 2) mutagenic lesions caused by ROS affect mtDNA polymerase and mitochondrial replication;24 3) oxidant-induced mtDNA damage is accompanied by decreased mitochondrial, RNA, and protein synthesis and defective respiratory function.25 Skepticism regarding the pathological significance of relatively low mtDNA lesion frequencies should be viewed in the background that ROS can also have direct deleterious effects on mitochondrial membranes and proteins.26 Importantly, emerging evidence indicates that mtDNA damage is an important predictor of atherosclerosis, a reported consequence of PI therapy. Recently, we reported a positive correlation between mtDNA damage and atherosclerosis in human aortic specimens and aortas from apolipoprotein E−/− (atherosclerotic) mice.27 In this study, two findings suggested a causal relationship between mtDNA damage and atherosclerosis. First, mtDNA damage preceded atherogenesis in young apolipoprotein E−/− mice. Second, apolipoprotein E−/− mice deficient in manganese superoxide dismutase, a mitochondrial antioxidant enzyme, exhibited early increases in mtDNA damage and accelerated atherogenesis phenotype at arterial branch points. Taken together with the findings of Zhong et al,8 these reports provide a plausible mechanism for PI-induced premature atherosclerosis via ROS mediated mtDNA damage that can be tested in further studies.
template control and no template control to ensure quantitative conditions and to detect contamination with spurious DNA or PCR products, respectively. In addition, it is often helpful to amplify a comparable fragment of nuclear DNA to determine whether damage is specific to mitochondrial genome. Because differences in QPCR amplification can also relate to mtDNA copy number or DNA quality (unrelated to in vivo–mediated damage), amplification of a short fragment (<300 base pairs) of mtDNA should be performed for quality control and to allow normalization of the copy number. The average estimated mtDNA lesion frequency is 1 to 3 lesions per 10 kb mtDNA under highly oxidative conditions.25–27,30 The reported mitochondrial DNA lesion frequency of 12.62 lesions/10 kb by Zhong et al8 seems out of proportion. The possibility that the mtDNA damage observed is a downstream consequence of a fatal cellular event. Validated, quantitative mitochondrial DNA damage measurements can be diagnostic in that they may indicate a causative role for mitochondrial dysfunction in diverse events, such as the response to oxidative stress, vascular lesion formation, and drug toxicities. However, the establishment of a causal role requires careful control and quantification of mtDNA lesion measurements and rigorous experimental design. The study of Zhong et al,8 while preliminary with respect to the role of mitochondrial toxicity in the vascular effects of PIs, nonetheless raises the possibility of a common mechanism linking the side effects of this class of drugs with the molecular pathogenesis of atherosclerosis, and thus provides a plausible mechanism for accelerated atherosclerosis in patients treated with these drugs.

Acknowledgment
We would like to thank Chris Horaist for assistance with graphics.

References
HIV Therapies and Atherosclerosis: Answers or Questions?
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doi: 10.1161/01.ATV.0000042080.46554.E6
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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