

# Mitochondrial function, morphology, and metabolic parameters improve after switching from a stavudine- to a tenofovir-containing regimen: SNAP (Switch Nucleoside Analogues Protocol)



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## ABSTRACT

**Background:** The pathogenesis of lipodystrophy in HIV-infected patients has been associated with mitochondrial changes in fat tissue associated with NRTI therapy. Substitution by the nucleoside analogue tenofovir may result in improvement in mitochondrial function, morphology, and metabolic parameters.

**Methods:** Patients from Protocol M97-720 (n=10) receiving stavudine (d4T), lamivudine (3TC), and lopinavir/ritonavir (Kaletra®) for 7 yrs were switched from d4T to tenofovir (TDF) and followed for 48 wks. Subcutaneous fat tissue biopsies, fasting metabolic tests, HIV RNA and CD4 assays, and DEXA scans of fat content were obtained at baseline and 48 wks. mtDNA copies/cell and fat tissue morphology was examined by real-time PCR and transmission electron microscopy (TEM).

**Results:** At baseline: TEM showed 1-2 mitochondria/preadipocyte and 1 mitochondria/adipocyte. Mitochondrial cristae (inner membrane) were lacking or poorly defined. Median fat mtDNA=208 copies/cell. Median HIV RNA < 50 copies/ml, median CD4 count 781cells/mm<sup>3</sup>, on examination 2 patients had lipodystrophy, 2 fat accumulation, and 6 both; 4/10 were receiving lipid-lowering drugs. Median limb fat was 4925 and trunk fat 9569 g by DEXA, total, HDL, and LDL cholesterol were 204, 46 and 116 mg/dL, respectively, triglycerides 220 mg/dL, glucose 93.5 mg/dL, lactate 0.8 mmol/L, and insulin 9.5 uIU/ml. 48 weeks after switching to TDF: TEM showed an increase in platelets and endothelial cells suggesting increased vascularization, increased collagen in the adipocytes, and less preadipocytes. Mitochondrial numbers were increased 100%. The mitochondrial inner membranes were intact and contained well-defined cristae. The median fat mtDNA increased by 1343 copies/cell (p=0.002). There was no change in limb fat by DEXA, however, trunk fat increased 14.9 % (1022 grams; p=0.006). Fasting total cholesterol decreased 33 mg/dL (p=0.04) and serum glucose decreased 4 mg/dl (p=0.005). No significant change in the other measured parameters including HIV RNA and CD4.

**Conclusions:** Fat mitochondrial function and morphology improved in adipose tissue of patients switched from d4T to TDF while remaining on Kaletra. Total cholesterol and glucose were decreased and trunk fat increased. This study suggests that switching from d4T to TDF results in improvement in fat mitochondria.

## BACKGROUND

The pathogenesis of lipodystrophy in HIV-infected patients has been associated with mitochondrial changes in fat tissue associated with nucleoside reverse transcriptase inhibitor (NRTI) therapy. Substitution by the nucleoside analogue tenofovir (TDF) may result in improvement in mitochondrial function, morphology, and metabolic parameters.

## METHODS

Patients enrolled in the Abbott M97-720 protocol at Northwestern University who were receiving a stavudine (d4T), lamivudine (3TC), and lopinavir/ritonavir (Kaletra®) regimen for at least 6 years were switched from d4T to tenofovir (TDF).

Patients (N = 10) who consented to participate in the SNAP study had the following evaluations performed before switching to tenofovir (baseline) and after 48 weeks on the tenofovir-containing regimen:

Subcutaneous adipose tissue biopsy (4-mm skin punch biopsy on proximal lateral thigh. Fat tissue frozen in RNAlater or stored in glutaraldehyde.)

Mitochondrial morphology in fat tissue was examined by transmission electron microscopy (TEM). Tissues fixed in glutaraldehyde were then post-fixed with 1% osmium tetroxide and subsequently dehydrated in a series of graded alcohol washes. Following, tissues were embedded in an epoxy resin substrate and sliced into thin micrometer cross-sections using an ultramicrotome. Sections were mounted on slot or mesh grids and images were photographed at 16000x, 25000x, and 32500x magnifications with a LEO 912EF transmission electron microscope.

Mitochondrial function was assessed by quantifying mitochondrial DNA copies/cell (fat tissue) using Real-Time PCR analysis.

Clinical Evaluations: Whole body DEXA scans of fat content  
Fasting metabolic tests (total, HDL, and LDL cholesterol, triglycerides, glucose, insulin, lactate)  
Plasma HIV-1 RNA levels, CD4 cell counts  
Evaluation of lipodystrophy of arms, legs, and face by clinician

Nonparametric signed rank test was used to assess median changes from baseline to week 48. Spearman correlation coefficient was calculated for the changes in limb fat and changes in mtDNA.

## RESULTS

	Baseline	Week 48	Median Change
Fat mtDNA (copies/mL), median	208	1567	1343 (p=0.002)
HIV RNA (copies/mL)	<50	<50	No Change
CD4 Count (cells/mm <sup>3</sup> )	781	770	No Change

✦ Fat Mitochondrial DNA copies/cell increased by 1343 copies/cell

## CLINICAL CHARACTERISTICS

N = 10 patients	Baseline	Week 48
Time on d4T, 3TC, Kaletra	358 weeks (7 years)	
Males	100%	
White	40%	
Age, mean	39 years	
	Baseline	Week 48
Clinical Lipodystrophy (% pts)		
Lipodystrophy	20	20
Fat Accumulation	20	10
Both	60	60
Taking Lipid - Lowering Drugs	40%	40% (no drug changes)
Body Weight (lbs.)	188	188 (no change)
Body Mass Index (BMI)	27.2	27.0 (no change)

## DEXA ANALYSIS

	Baseline	Week 48	Median Change
Limb Fat (g), median	4925	4930	-161 (ns)
% Change			-3.7 (ns)
Limb Fat (%), median	54.4	54.7	0.3 (ns)
Trunk Fat (g), median	9569	11273	1022 (p=0.006)
% Change			14.9 (p=0.004)
Trunk Fat (%), median	21.5	24.5	1.9 (p=0.006)

✦ NO change in limb fat by DEXA

✦ Trunk fat increased 14.9%

✦ NO correlation between change in limb fat and copies. (Spearman correlation coefficient = 0.85)

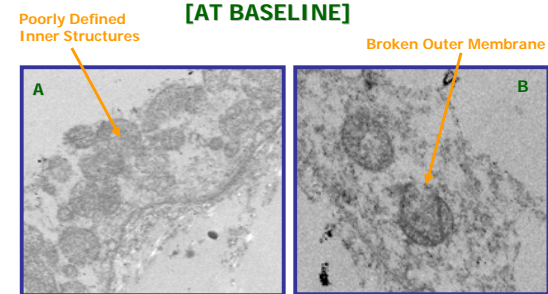
## FASTING METABOLIC TESTS

Median Values	Baseline	Week 48	Mean Change
Total Cholesterol (mg/dL)	204	173	- 33 (p=0.005)
HDL Cholesterol (mg/dL)	46	42	- 4.5 (ns)
LDL Cholesterol (mg/dL)	116	104	2.8 (ns)
Triglycerides (mg/dL)	220	178	- 25 (ns)
Lactate (mmol/L)	0.8	0.9	0.6 (ns)
Insulin (uIU/mL)	9.5	16	9.4 (ns)
Glucose (mg/dL)	93.5	91.5	- 4 (p=0.04)

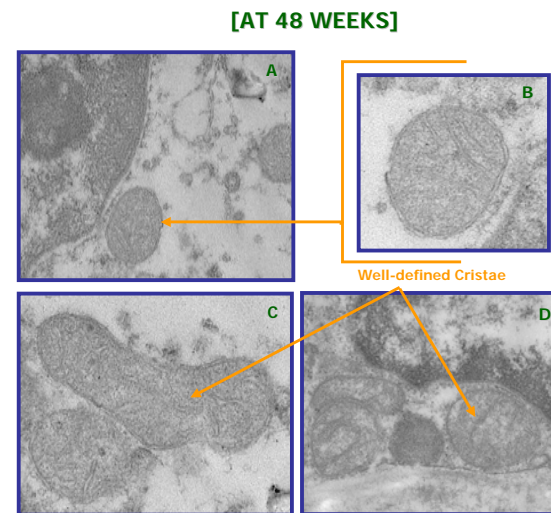
✦ Total cholesterol decreased

✦ Serum glucose decreased

## MITOCHONDRIA ELECTRON MICROSCOPY



**Fig1.** Photographs taken at (A) 16000x magnification and (B) 25000x magnification. Images show that mitochondria have poorly defined inner membrane structures and greater lysed outer membranes.



**Fig2.** Photographs taken at (A) 16000x, (B) 31500x, (C) 31500x, and (D) 25000x magnification. Images show well-defined cristae and distinct double layer outer membranes.

## CONCLUSIONS

Fat mitochondrial function and morphology improved in adipose tissue of patients switched from d4T to TDF while remaining on Kaletra. Total cholesterol and glucose were decreased and trunk fat increased.

This study suggests that switching from d4T to TDF results in improvement in fat mitochondria.

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