John A. Burns School of Medicine
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Antiretroviral Therapy and Mitochondria Dysfunction: A Role for Carnitine

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Adults and Children Estimated to be Living with HIV/AIDS in 2003

World Health Organization

UNAIDS

2003 Global HIV/AIDS estimates for adults and children

- **People living with HIV/AIDS** — **40 million (34-46 million)**
- **New HIV infections in 2003** — **5 million (4.2-5.8 million)**
- **Deaths due to HIV/AIDS in 2003** — **3 million (2.5-3.5 million)**

Anti-HIV Drugs

NRTI

Zidovudine (AZT), Lamivudine (3TC), Didanosine (DDI),Stavudine (D4T), Zalcitabine (DDC)

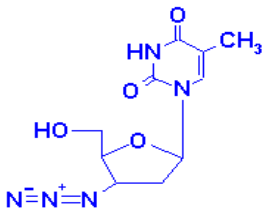
NNRTI

Nevirapine, Delavirdine, Efavirenz

PI

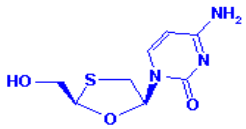
Saquinavir, Ritonavir, Indinavir, Nelfinavir

NRTIs used to treat HIV–Infected Patients



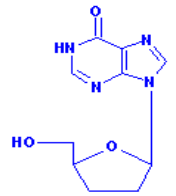
MW 267.24

NAME	3'-Azido-3'-deoxythymidine
SYN	AZT; Azidothymidine; Zidovudine; Retrovir; ZDV
COMP	GLAXO WELLCOME



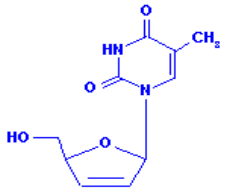
MW 229.26

NAME	cis-1-[2'-Hydroxymethyl-5'-(1,3-oxathiolanyl)] cytosine
SYN	(-)-BCH-189; DTHC; 3TC; Lamivudine; Epivir; L-(-)-S-ddC
COMP	IAF BIOCHEM INT/GLAXO WELLCOME



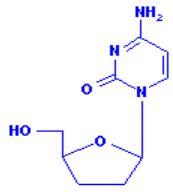
MW 236.23

NAME	2',3'-Dideoxyinosine
SYN	DDI; D2I; ddIno; Didanosine; Videx
COMP	BRISTOL-MYERS SQUIBB



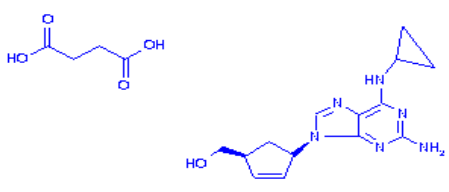
MW

NAME	Thymidine, 2',3'-didehydro-,3'-deoxy-
SYN	D4T; ddeThd; ddeTyd; DHT; Stavudine; Zerit
COMP	BRISTOL-MYERS SQUIBB



MW 211.22

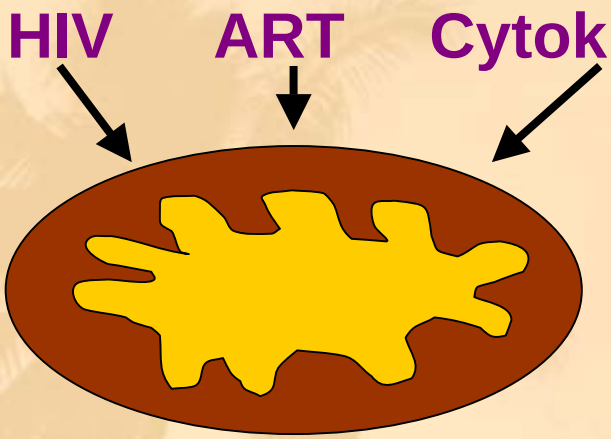
NAME	2',3'-Dideoxycytidine
SYN	ddC; ddCyd; D2C; Zalcitabine; Hivid
COMP	HOFFMAN- LA ROCHE



MW 404.42

NAME	Butanedioic acid, compd. with (1S-cis)-4-[2-amino-6-(cyclopropylamino)-9H-purine-9-yl]-2-cyclopentene-1-methanol (1:1)
SYN	Abacavir ; 1592U89 succinate; Ziagen
COMP	GLAXO WELLCOME

Multi-Hit Effects of HIV, ART, and Cytokines On Mitochondria



DNA polymerase-γ

Uncoupling

Transport

Oxidative Stress

Apoptosis

Phosphorylation

Proteolytic Processing

Glycosylation

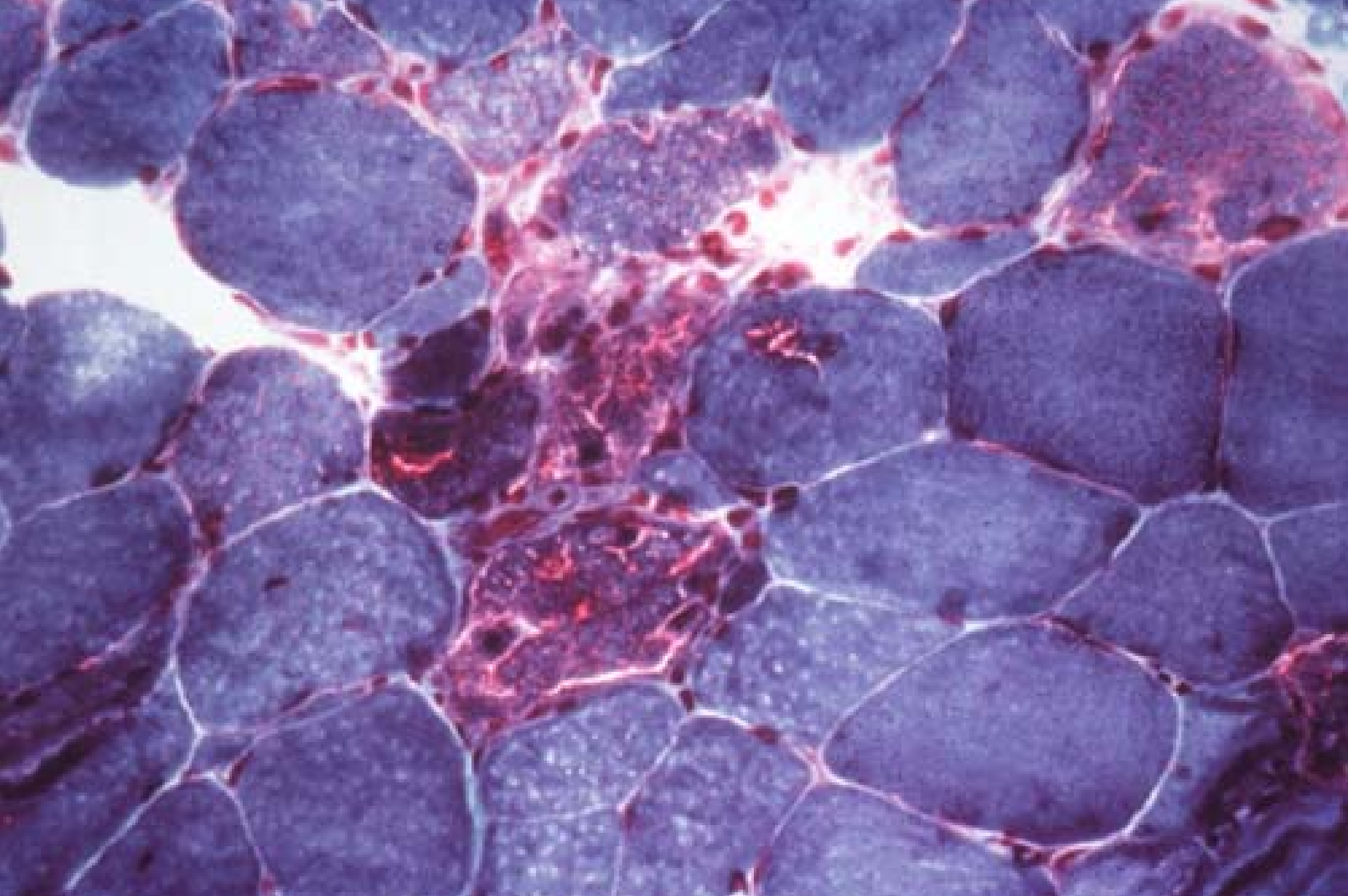
Gerschenson, M. and Brinkman, K. *Mitochondrion*, in press, 2004.

Antiretroviral Drugs Cause Mitochondrial Dysfunction in HIV Patients

- Lipodystrophy
- Neuropathies
- Hepatic Steatosis
- Myopathy
- Pancreatitis
- Lactic Acidosis

Long Term AZT Exposure Leads to Skeletal Myopathies

- The myopathy presents with fatigue, myalgia, muscle weakness, wasting, elevated serum creatine kinase, and high lactate/pyruvate ratio in the blood.
- In skeletal muscle biopsies, there are ‘ragged red fibres’ and an accumulation of fat intracellularly.
- Biochemical studies have shown decreases in Complex IV activity, carnitine levels, and mtDNA.



Mitochondrial Genotoxic and Functional Consequences of Antiretroviral Drug Therapy

GENOTOXICITY

- **The antiviral nucleoside analog is phosphorylated and incorporated into mtDNA.**
- **MtDNA replication is truncated.**

FUNCTIONAL CONSEQUENCES

- **Altered mitochondrial morphology**
- **OXPHOS enzymology is affected**
- **MtDNA Depletion/ Degradation**

Non-human primate transplacental studies with antiretrovirals

- NRTIs are incorporated into fetal mtDNA.
- Fetal heart, skeletal muscle, cerebellum, cerebrum, and placental mtDNA depletion and degradation.
- All organs have decreases in Complex I and IV activities and increases in Complex II.
- Mitochondrial DNA morphology by electron microscopy is aberrant.

Gerschenson, M. et al. (2004) Mitochondrial toxicity in fetal Erythrocebus patas monkey exposed transplacentally to Zidovudine and Lamivudine, *AIDS Res. and Human Retroviruses, 20:* 91-101.

Ewings, E.L. et al. (2000) The genotoxic and functional consequences of transplacental zidovudine exposure in fetal monkey brain mitochondria, *J. of AIDS*, 24: 100-105.

Gerschenson, M. et al. (2000) Fetal mitochondrial heart and skeletal muscle damage in Erythrocebus patas monkeys exposed in utero to 3'-azido-3'-deoxythymidine (AZT). *AIDS Res. and Human Retroviruses*, 16: 635-644.

Adult *Erythrocebus patas* Monkeys Given Oral Stavudine (D4T)

3 mg D4T twice daily for 80 days (about 1.2 mg D4T/kg bw/day = human equivalent dose).

Liver and Quadricep Muscle

↓

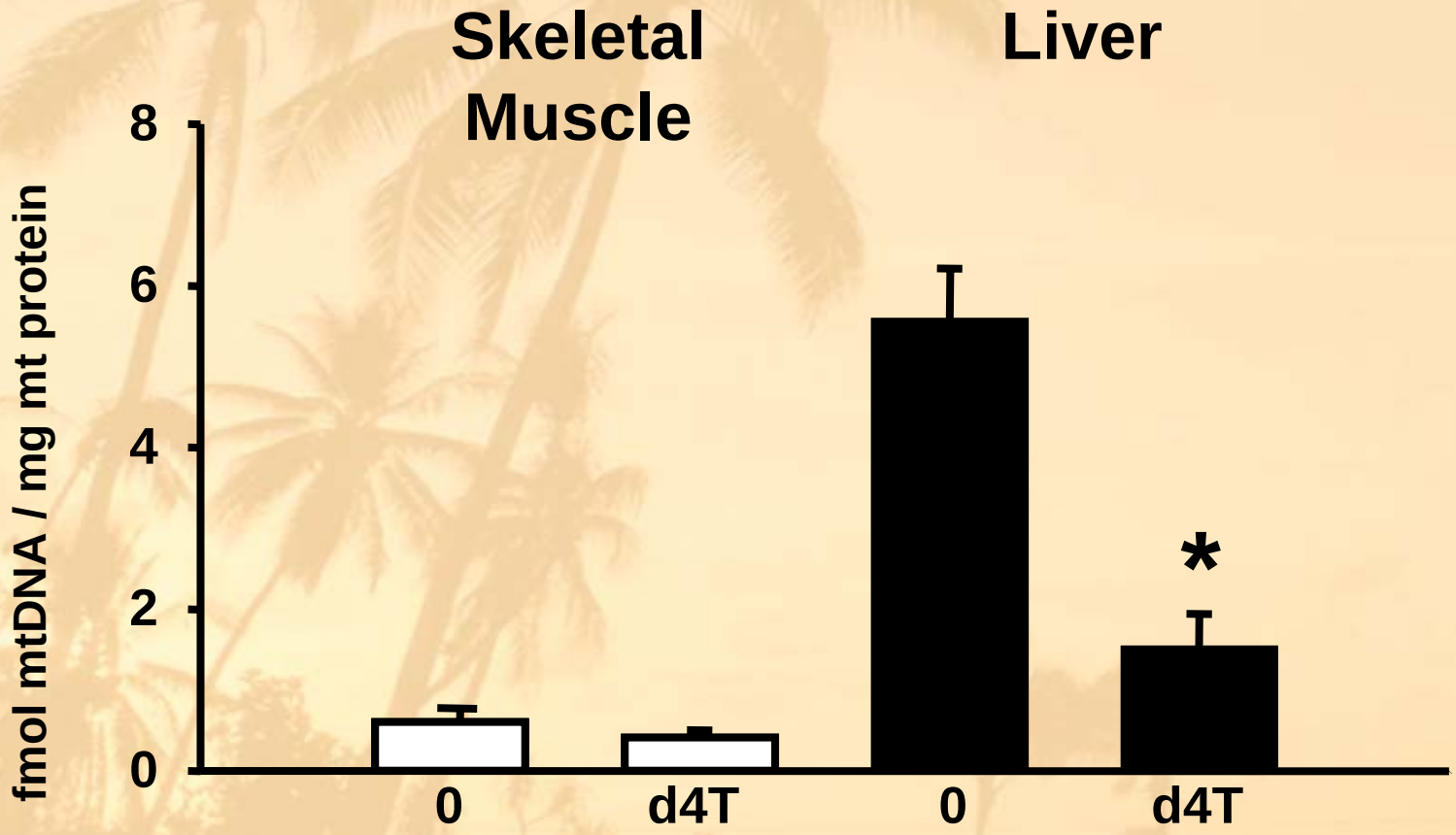
- **Isolate Mitochondria**
- **Analyze OXPHOS Enzyme Activities**
- **Southern and Slot Blot Analysis of MtDNA**

Blood clinical chemistry values* for unexposed and pre- and post-d4T exposed patas monkeys (n=3 per group)

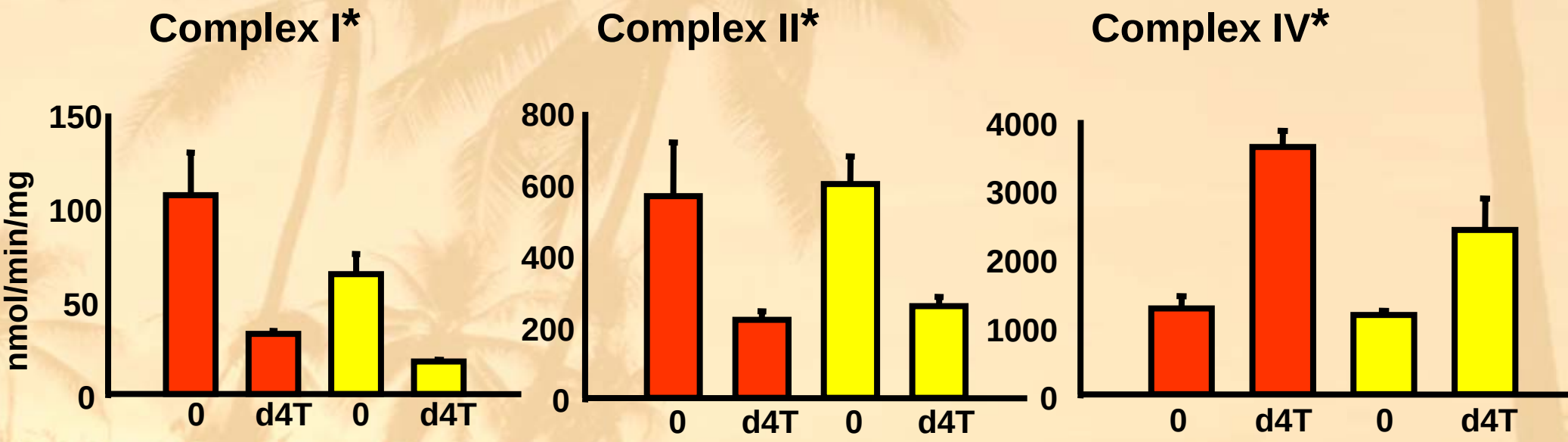
	Control	Pre-D4T	Post-D4T
Lactic Acid (mmol/l)	2.24 (1.35-3.73)	2.75 (1.31-4.67)	**3.91 (1.55-8.02)**
Alkaline Phosphatase U/l	165 (119-207)	121 (87-168)	**109 (80-126)**
Phosphorus mg/dl	3.8 (2.7-5.3)	4.9 (4.1-5.4)	**2.1 (1.8-2.3)**
Creatine Phosphokinase U/l	435 (351-479)	399 (270-608)	**310 (231-430)**
Lipase U/l	108 (68-130)	72 (34-141)	**62 (49-82)**
Cholesterol mg/d	121 (103-138)	101 (85-116)	**89 (80-94)**

***Values are represented as the mean (range in parenthesis) for 3 animals. Statistical significance between d4T-exposed and unexposed animals is indicated by bold text.**

Stavudine Causes MtDNA Depletion in the Liver



OXPHOS Enzyme Specific Activities are Altered in Skeletal Muscle and Liver of Adult Patas Monkeys Given d4T



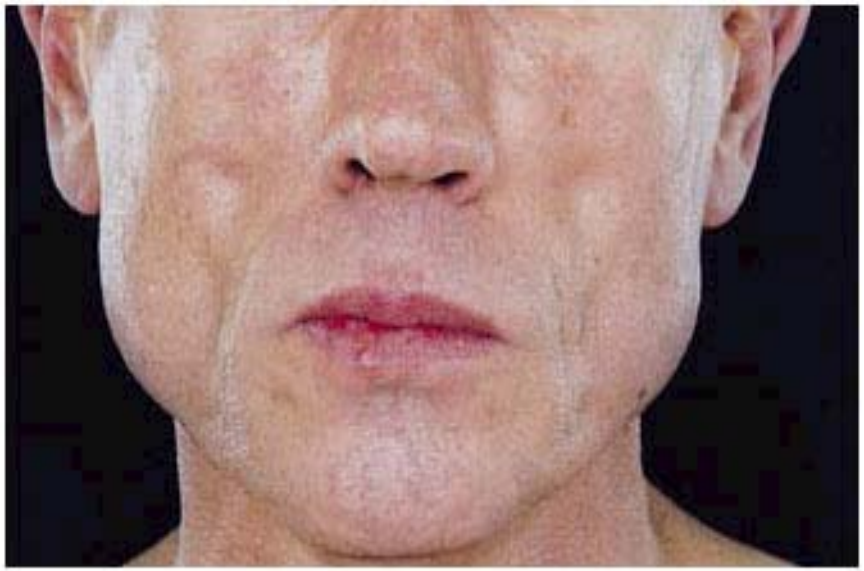
*Significant ($p \leq 0.05$) in comparison with unexposed monkeys.

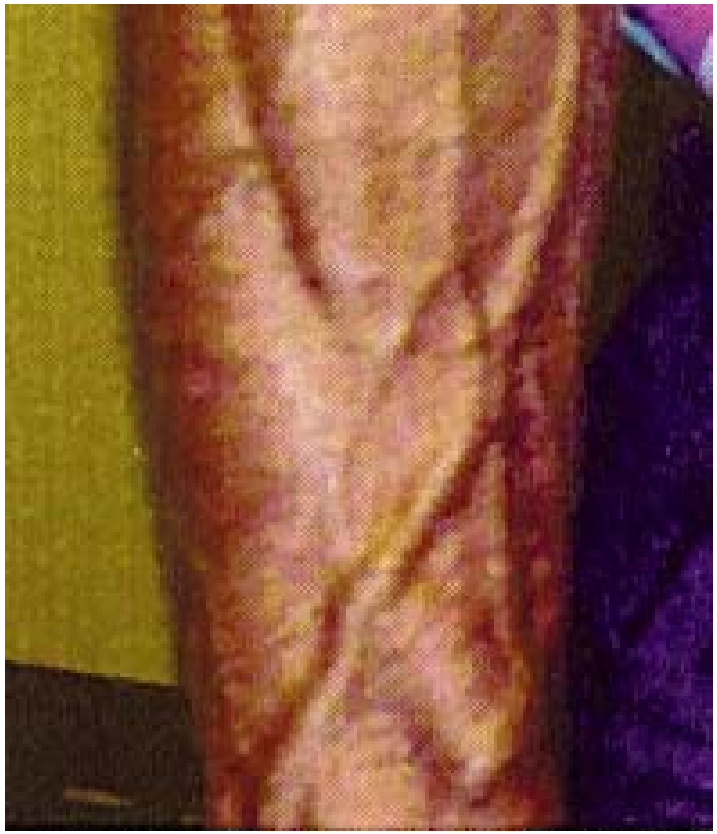
Gerschenson, M. et al. (2001) Chronic Stavudine Induces Hepatic Mitochondrial Toxicity in Adult Erythrocebus patas Monkeys, *J. of Human Virology*, 4: 335-42.

HIV-Lipodystrophy

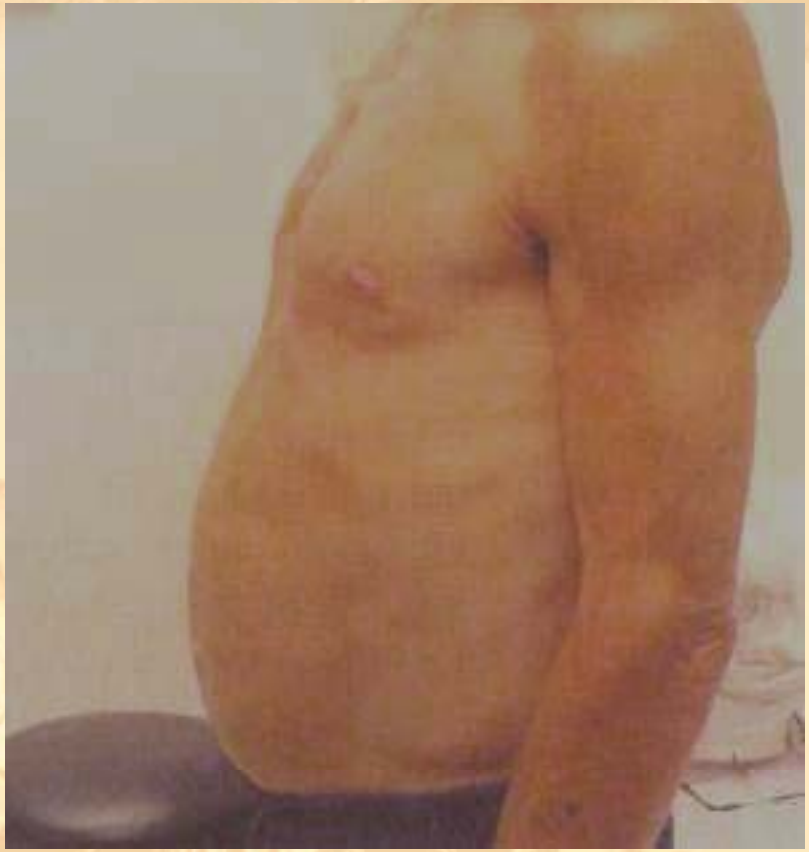
- **20-50% of HIV-Patients taking NRTIs +/- PI develop the phenotype within the first year**
- **Accumulation of visceral fat and loss of subcutaneous fat**
- **Insulin resistance**
- **Hypertriglyceridemia**

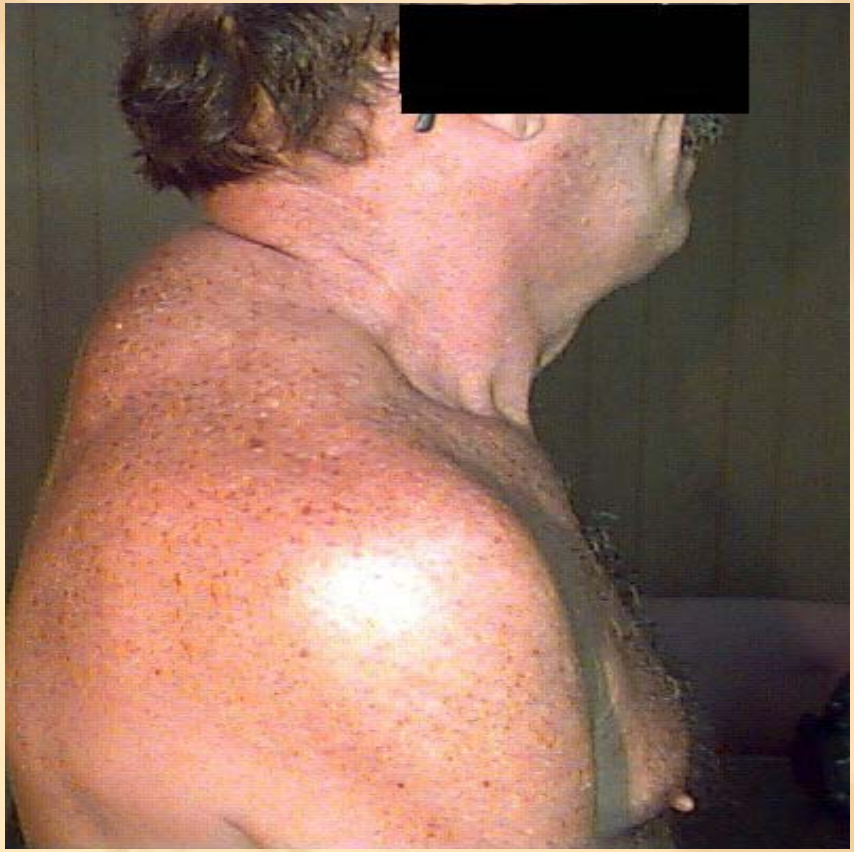
Examples of Lipoatrophy





Examples of Fat Accumulation





How do we diagnose it?

- Self report
- More objective?
  - Anthropometry
  - Bioelectrical impedance analysis (BIA)
  - CT scan, MRI, DEXA
- No easy and reliable method
- Reasonable to look at old photos and log of anthropometric measures

HIV-Lipoatrophy and Mitochondria

- **Human subcutaneous adipocytes from HIV-infected patients taking antiretroviral therapy have:**
  - **decreased mtDNA**
  - **increased UCP1, fatty acid transport and binding protein, IL-6, and CD45**
  - **decreased UCP2 and 3, PPAR-γ, PGC-1, lipoprotein lipase, acyl coenzyme A synthase, and glucose transport protein 4**
  - **increased apoptosis**

HIV Lipoatrophy is Associated with Mitochondrial DNA Depletion in Subcutaneous Fat

	HIV (-)	HIV(+) Naive	HIV (+), No Lipodystrophy	HIV (+) Lipoatrophic
Thigh	**435 ± 63** **N=4**	489 ± 100 N=5	**267 ± 136** N=6	**255 ± 124** N=6
Abdomen	790 ± 292 N=5	545 ± 190 N=5	**335 ± 158** N=6	**244 ± 148** N=7
Neck	976 ± 292 N=6	676 ± 271 N=5	**396 ± 249** N=6	**205 ± 78** N=7
PBMC	201 ± 62 N=10	105 ± 48 N=3	157 ± 49 N=7	148 ± 53 N=7

All values are represented as mtDNA copies/cell (X ± SD) and statistical significance is $p \leq 0.05$. Bold green text indicates statistical significance compared to HIV (-) and HIV (+) Naïve. Bold blue text indicates statistical significance against HIV(-). Thigh fat mtDNA copies/cell (red) is statistically decreased compared to abdomen and neck (Gerschenson et al. 11th Conference on Retroviruses and Opportunistic Infections, pg. 328, 2004).

Conclusions

- **HIV lipoatrophy is associated with mitochondrial DNA depletion in different subcutaneous fat depots.**
- **Neck and abdomen fat has increased mtDNA copies/cell compared to the thigh.**
- **PBMC mtDNA copies/cell did not correlate with lipoatrophy.**

This research was supported by the National Institutes of Health (MD-000173, RR-14607, RR-03061), USA.

Potential Therapies for Lipodystrophy

- Testosterone – increases lean muscle mass (? Fat), may be beneficial to patients with visceral adiposity and hypogonadism
- Metformin – appears safe, but improvements in peripheral fat loss not seen
- Thiazolidinediones – inconsistent results from different studies
- Diet / exercise
- Niaspan – our local study did not show any obvious trends
- Switch
- Acetyl-L-carnitine

Acetyl-L-Carnitine Studies for HIV-Lipodystrophy

- **1000 mg/day for 3 months in 12 patients resulted in a decrease in serum cholesterol, S. Mauss, *HIV Medicine* (2001), 2: 59-60**
- **3000 mg/day for 9 months in 16 patients decreased serum triglycerides, M. Loignon, *AIDS*, 15:1194-5**

Pioglitazone in combination with Vitamin and Mitochondrial Co-factors for the Treatment of HAART-associated Lipoatrophy

University of Hawaii IRB Approval for Version 2 on 01/09/04
DSMB met on 02/17/04

Objectives for Intervention

Primary Objective:

- **Efficacy is defined as 60%or more of subjects on an intervention for 24 weeks show 7% or greater increase in total peripheral subcutaneous fat as assessed by DEXA.**

Objectives for Intervention

Secondary Objectives:

- **To correlate changes in visceral fat with changes in peripheral fat content**
- **To correlate changes in hepatocellular fat with changes in peripheral fat content**
- **To correlate changes in blood metabolic parameters with changes in peripheral fat content**
- **To explore the pathophysiologic mechanisms underlying lipoatrophy in subcutaneous adipose tissue**

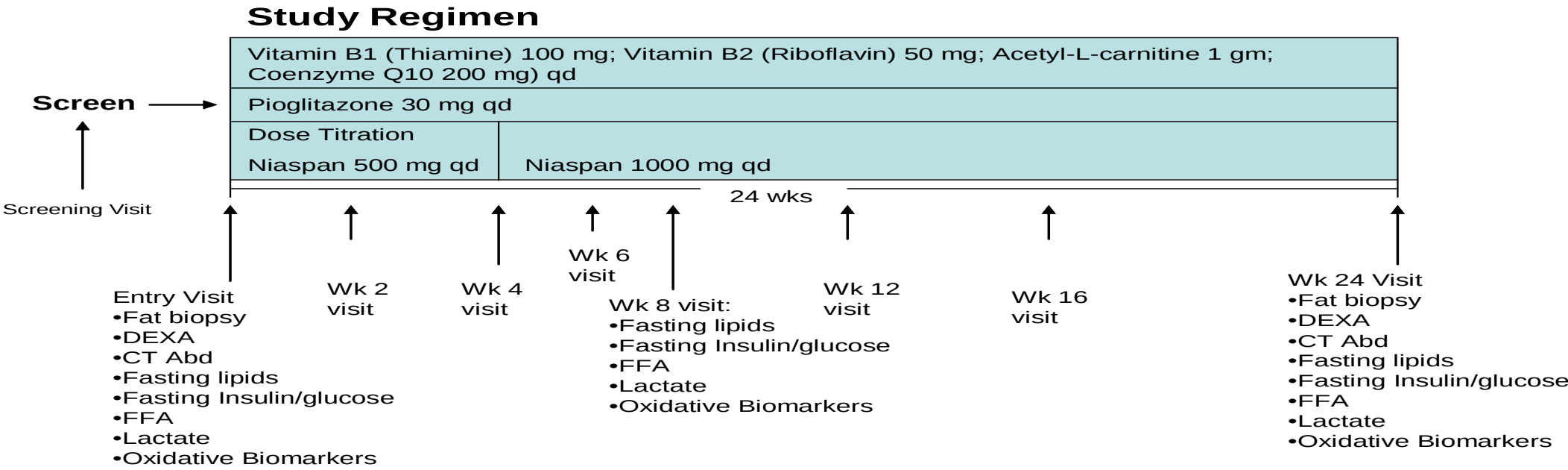
Assessment and Procedures in Study

- Whole body DEXA for the assessment of peripheral (arms and legs) of subcutaneous fat content
- Abdominal 8-slice CT scan for the assessment of visceral fat and hepatocellular fat contents
- Thigh skin punch biopsy for subcutaneous fat to assess mitochondrial and lipid metabolism in the tissue of interest
- Fasting blood analysis of various metabolic parameters

Drugs Used in Study

Drug	Amount/day (mg)	Purpose
Thiamine (Vitamin B_1)	100	Coenzyme of pyruvate dehydrogenase
Riboflavin (Vitamin B_2)	50	A precursor of flavin adenine dinucleotide (FAD)
Acetyl-L-carnitine	1000	Transport fatty acids
Coenzyme Q_{10}	200	Cofactor for OXPHOS
Niaspan	1000	Inhibiting the release of FFA from adipose tissue and increasing lipoprotein lipase activity
Pioglitazone	30	Promotes subcutaneous adipocyte proliferation

Study Design



Enrollment Status

- **Began enrolling in April, 2003.**
- **10 subjects on drug arm.**
- **3 subjects completed study and four will finish in April, 2004.**
- **3 subjects off study due to adverse event not related to medications, change in antiretroviral therapy, difficulty with tolerating flushing secondary to Niaspan.**

Patient Characteristics

- All males
- Self-reported peripheral fat wasting following initiation of NRTI-containing HAART (ZDV, D4T, or DDI)
- 2 Asian Pacific Islanders, 1 Hispanic, and 4 Caucasian
- Mean age: 52.6 $\pm$ 8.6
- CD4: 420 $\pm$ 252

Preliminary Data

- There are no changes from baseline to week 8 or week 24 in:
  - Peripheral fat in arm, legs, and trunk by DEXA analysis
  - BMI
  - Creatinine
  - Glucose
  - Insulin
  - Triglyceride

Mitochondrial Interventions Decrease ALT and Lactic acid

Week	0 (n=7)	8 (n=7)	12 (n=3)	16 (n=3)	24 (n=3)
ALT (IU/L)	32 ± 18	28 ± 20	21 ± 11* P= 0.03	50 ± 53	27 ± 11 P= 0.16
Lactic acid (mmol/L)	2.2 + 1.5	1.5 + 0.7	N/A	N/A	1.6 + 0.6 P= 0.05

* Statistical significance as measured by paired t-test.

Conclusions

- **The preliminary clinical chemistry data suggests that this intervention may be affecting mitochondrial metabolism**
- **Future research will include gene expression studies of mitochondrial and nuclear genes**

Future Clinical Acetyl-L-Carnitine Studies

- Acetyl-L-Carnitine for the Treatment of HAART-associated Lipoatrophy
- An Open-Label, Dose Escalation Pilot Study of Acetyl-L-Carnitine for the Treatment of Dideoxynucleoside-Associated Distal Symmetric Peripheral Neuropathy

Acknowledgements

Hawaii AIDS Clinical Research Program Patients, Volunteers, and Staff

Acknowledgements to NIH for Extramural Funding

- P20 MD000173 - M. Gerschenson and C. M. Shikuma
- U54 NS43049 - M. Gerschenson and C. M. Shikuma
- U01 AI34853 - M. Gerschenson and C. M. Shikuma
- U01 AA013566 - M. Gerschenson
- R01 NS40302 - M. Gerschenson
- G12 RR-14607- C. M. Shikuma
- G12 RR-03061- C. M. Shikuma

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In press:

Special Journal Issues in *Mitochondrion* on:

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