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

Total: 34 – 46 million
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

1. 3'-Azido-3'-deoxythymidine
   - **NAME**: 3'-Azido-3'-deoxythymidine
   - **SYN**: AZT; Azidothymidine; Zidovudine; Retrovir; ZDV
   - **COMP**: GLAXO WELLCOME

2. cis-1-[2'-Hydroxymethyl-5'-[1,3-oxathiol-2'-yl]]cytosine
   - **NAME**: cis-1-[2'-Hydroxymethyl-5'-[1,3-oxathiol-2'-yl]]cytosine
   - **SYN**: 2'-BCH-186; dTIC; 3TC; Lamivudine; Epivir; L-(-)-S-DDC
   - **COMP**: IAF BIOCHEM INT/GLAXO WELLCOME

3. 2',3'-Dideoxyinosine
   - **NAME**: 2',3'-Dideoxyinosine
   - **SYN**: ddI; ddI; ddino; Didanosine; Videx
   - **COMP**: BRISTOL-MYERS SQUIBB

4. Thymidine, 2',3'-dideoxy-2',3'-decty.
   - **NAME**: Thymidine, 2',3'-dideoxy-2',3'-decty.
   - **SYN**: dT; dTid; dddT; dT; Stavudine; Zerit
   - **COMP**: BRISTOL-MYERS SQUIBB

5. 2',3'-Dideoxycytidine
   - **NAME**: 2',3'-Dideoxycytidine
   - **SYN**: dDC; dDCYD; Zidovudine; Hivid
   - **COMP**: HOFFMAN. LA ROCHE

6. Butanolic acid, compd. with (1S-cis)-5'-[2-amino-6-(cyclopropylamino)-9H-purine-9-y]-2-cyclopentene-4-methanol (1:1)
   - **NAME**: Butanolic acid, compd. with (1S-cis)-5'-[2-amino-6-(cyclopropylamino)-9H-purine-9-y]-2-cyclopentene-4-methanol (1:1)
   - **SYN**: Abacavir; 1592U99 succinate; Zigen
   - **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

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.


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)

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

*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

![Graph showing mtDNA depletion in skeletal muscle and liver with and without d4T treatment.](image-url)

- **Skeletal Muscle**
  - 0: 0.5 fmol mtDNA/mg mt protein
  - d4T: 0.3 fmol mtDNA/mg mt protein

- **Liver**
  - 0: 6.0 fmol mtDNA/mg mt protein
  - d4T: 1.5 fmol mtDNA/mg mt protein

*Significant difference between 0 and d4T treatments.*
OXPHOS Enzyme Specific Activities are Altered in Skeletal Muscle and Liver of Adult Patas Monkeys Given d4T

Complex I*  Complex II*  Complex IV*

*Significant (p ≤ 0.05) in comparison with unexposed monkeys.

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
- Hypertriglycerideridemia
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

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

All values are represented as mtDNA copies/cell (X ± SD) and statistical significance is p ≤ 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

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount/day (mg)</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiamine (Vitamin B&lt;sub&gt;1&lt;/sub&gt;)</td>
<td>100</td>
<td>Coenzyme of pyruvate dehydrogenase</td>
</tr>
<tr>
<td>Riboflavin (Vitamin B&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>50</td>
<td>A precursor of flavin adenine dinucleotide (FAD)</td>
</tr>
<tr>
<td>Acetyl-L-carnitine</td>
<td>1000</td>
<td>Transport fatty acids</td>
</tr>
<tr>
<td>Coenzyme Q&lt;sub&gt;10&lt;/sub&gt;</td>
<td>200</td>
<td>Cofactor for OXPHOS</td>
</tr>
<tr>
<td>Niaspan</td>
<td>1000</td>
<td>Inhibiting the release of FFA from adipose tissue and increasing lipoprotein lipase activity</td>
</tr>
<tr>
<td>Pioglitazone</td>
<td>30</td>
<td>Promotes subcutaneous adipocyte proliferation</td>
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</tbody>
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**Study Design**

**Study Regimen**

<table>
<thead>
<tr>
<th>Study Regimen</th>
<th>24 wks</th>
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<tbody>
<tr>
<td>Vitamin B1 (Thiamine) 100 mg; Vitamin B2 (Riboflavin) 50 mg; Acetyl-L-carnitine 1 gm; Coenzyme Q10 200 mg</td>
<td>qd</td>
</tr>
<tr>
<td>Pioglitazone 30 mg</td>
<td>qd</td>
</tr>
<tr>
<td>Dose Titration</td>
<td>Niaspan 500 mg qd Niaspan 1000 mg qd</td>
</tr>
<tr>
<td>Screening Visit</td>
<td>Wk 2 visit Wk 4 visit Wk 6 visit Wk 8 visit: Fasting lipids Fasting Insulin/glucose FFA Lactate Oxidative Biomarkers Wk 12 visit Wk 16 visit Wk 24 visit Fat biopsy DEXA CT Abd Fasting lipids Fasting Insulin/glucose FFA Lactate Oxidative Biomarkers</td>
</tr>
<tr>
<td>Entry Visit</td>
<td>Fat biopsy DEXA CT Abd Fasting lipids Fasting Insulin/glucose FFA Lactate Oxidative Biomarkers</td>
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<tr>
<td>Screening Visit</td>
<td>Wk 2 visit Wk 4 visit Wk 6 visit Wk 8 visit: Fasting lipids Fasting Insulin/glucose FFA Lactate Oxidative Biomarkers Wk 12 visit Wk 16 visit Wk 24 visit Fat biopsy DEXA CT Abd Fasting lipids Fasting Insulin/glucose FFA Lactate Oxidative Biomarkers</td>
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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 ± 8.6
- CD4: 420 ± 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

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

* 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

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Cecilia Shikuma, M.D.

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Kimber Cochrane
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‘Mitochondria, HIV, and Antiretrovirals’, Editor: Mariana Gerschenson