An Update on LIPOATROPHY in the Treatment of HIV

Dr Roger LeBlanc FRCP C
Associate Professor McGill University
Internal Medicine and Infectious Diseases
Immunodeficiency Unit Montreal Chest Hospital
Clinical Director of Projet LORI
24th May 2006
Metabolic Complications

- Dysregulation of glucose metabolism
- Lipid abnormalities
- Body Fat Gain/Loss
- Mitochondrial Toxicity
- Bone

Questions:

- What are the symptoms of metabolic complications?
- How do dysregulated glucose metabolism affect lipid abnormalities?
- What is the relationship between body fat gain/loss and mitochondrial toxicity?
- How do metabolic complications impact bone health?
Treatment Responses in 1st Year of HAART Improving Over Time

- 4143 subjects from 5 clinic cohorts in Europe and Canada
- Treatment-naïve; started HAART from 1996–2002
- ↓ risk of virologic failure, ↑ median CD4 increase in later years
  - In recent years, most “failure” due to loss to follow-up or treatment discontinuation

RECOVER Study: Identification of NRTI Treatment-Limiting Toxicities

Toxicities causing NRTI withdrawal (≥2%); total N=1350

- Hypertriglyceridemia: 2.7%
- Anemia: 5.5%
- Hypersensitivity: 2%
- Fat accumulation*: 2%
- Nausea: 3%
- Vomiting: 3%
- Lipodystrophy*: 10%
- Peripheral neuropathy: 12%
- Lipoatrophy*: 40%

* Investigator-defined

Palacios R, et al. 9th EACS; 2003; Warsaw, Poland. Poster 9.1/4.
LIPOATROPHY RISK FACTORS

- **Host/ demographic effects**
  - Age ✓
  - Race ✓
  - Sex ✓
  - IR?
  - BMI?

HIV effects?

- CD4 ?
- HIV ?
- mtDNA depletion by HIV ?

**HAART**

- **ART effects**
  - Duration of D4t > AZT ✓ ✓
  - Abacavir / 3TC / Tenofovir?

Reversal ?
LIPOATROPHY

♦ Loss of fat
  - Face/Cheeks
  - Limbs
  - “Arm cabling”
  - Buttocks

“Lipodystrophy Associated with an HIV-Protease Inhibitor”
MRI: Image Reconstruction

- All tissue
- Subcutaneous adipose tissue
- Skeletal muscle
- Visceral and intermuscular adipose tissue
- Bone and organ

Lipo Dublin 2005 Plenary 1 Gallagher
RAVE (Facial Substudy): Cheek Fat and Limb Fat

- 3-D laser surface assessment scans at baseline and Week 48 superimposed using bony landmarks
- Areas for comparison: forehead, left cheek, right cheek
- Variation between scans calculated using the net volume difference (mm$^3$) of these areas
- Associations between facial imaging parameters and other measures of body composition at 48 weeks evaluated
Adherence to HAART Declines With the Development of Morphological Alterations

Adherence rate according to time since self-reported morphological alterations

N=83 pts  Time since self-reported morphological alterations

Adherence rate (%)

- 0-6 months: 100 Adherent, 0 Non adherent
- 6-12 months: 92 Adherent, 8 Non adherent
- 12-24 months: 82 Adherent, 18 Non adherent
- >24 months: 75 Adherent, 25 Non adherent

Adherence rate declining by 7% to 9% per year

Definition of treatment adherence: Having missed one or more doses of antiretroviral drugs during the preceding week

LIPOATROPHY: Insights into Pathogenesis and Risk Factors
LIPOATROPHY RISK

- Low prevalence in the general population

"Anthropometrics and examiner-reported body habitus abnormalities in the Multicenter AIDS Cohort Study"

Compared 384 HIV+ men receiving HAART with 314 HIV- men

<table>
<thead>
<tr>
<th></th>
<th>HIV-</th>
<th>HIV+ART- (78)</th>
<th>HIV+HAART+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipoatrophy:</td>
<td>1%</td>
<td>3%</td>
<td>20%</td>
</tr>
<tr>
<td>Abdominal fat accumulation:</td>
<td>16%</td>
<td>9%</td>
<td>28%</td>
</tr>
</tbody>
</table>


Rare = Stigmatising
Total Fat in HIV+ and Control Men and Women

Median (CI).

- Control
- All HIV
- LA +
- LA -

Men:
- p < 0.001
- p < 0.001
- p < 0.001

Women:
- p < 0.001
- p = 0.004
- p < 0.001
- p = 0.38
Differential effects of NRTI regimens on adipocyte mitochondrial DNA depletion in HIV-infected patients

D Nolan et al. 6th Lipodystrophy, 2004, Abstr. 16
An Explanation for ↑ Risk of Lipoatrophy in Women and With More Advanced HIV Disease?

Triphosphate Concentrations in PBMC and Baseline CD4 Cell Count

- ZDV-TP (2.3 x ↑ in women)
- 3TC-TP (1.6 x ↑ in women)

Mechanisms by Which TA-NRTIs May Be Detrimental To Adipocytes

- Apoptosis
- Scavenging of ROS
- Mitochondrial permeability
- Lipogenesis

nRTI

mtDNA mutations

mtDNA

mtRNA

PGC-1

HIV

Mitochondrial biogenesis

↑

Mitochondrial RC dysfunction

ATP production

TG biosynthesis

Apoptosis

Lipogenesis
Measures of Adipose Tissue

- Tissue morphology
- Mitochondrial Parameters
  - DNA depletion
  - Protein expression
  - Enzyme activity
- Cytokine production
- %leg fat / BMI
NRTI-induced Lipoatrophy

3-12 months
MtDNA content
Pathophysiological effects:
Cellular Toxicity
Clinical lipoatrophy

ABC/TDF
1707 copies/cell
p = NS*

AZT
537 copies/cell
p = 0.001*

d4T
234 copies/cell
p < 0.0001*

* Compared to HIV+ ARV naïve Patients.
Sub study: 26 patients who had initiated/switched NRTIs. One healthy control was also included (n= 56 longitudinal biopsies).

Immunohistochemistry and Confocal Microscopy

**Mitochondrial Protein Expression**

Mitochondrial Protein Expression

- mtDNA encoded COX-I
- nDNA encoded COX-IV

**Analysis**

Independently assessed by a histopathologist blinded to the clinical status and treatment history of the subjects.

1 Hammond et al, 10th CROI, Boston, USA, 2003. Abstract 759
No evidence of adipose tissue pathology: Abacavir.

ART naive
mtDNA content = 1436 copies/adipocyte
30% leg fat

5 months ABC/3TC/Efav
mtDNA content = 2997 copies/adipocyte
30% leg fat

COX I (mitochondrial encoded) COX IV (nuclear encoded) Nucleus
LIPOATROPHY has a distinctive pathological signature.

<table>
<thead>
<tr>
<th></th>
<th>ART naive</th>
<th>7 months stavudine</th>
<th>13 months stavudine</th>
</tr>
</thead>
<tbody>
<tr>
<td>mtDNA (log_{10})</td>
<td>2.84</td>
<td>2.41</td>
<td>2.43</td>
</tr>
<tr>
<td>macrophage count</td>
<td>10</td>
<td>25</td>
<td>NA</td>
</tr>
<tr>
<td>av cytokine score</td>
<td>0.03</td>
<td>0.97</td>
<td>NA</td>
</tr>
<tr>
<td>%leg fat</td>
<td>20</td>
<td>19</td>
<td>9.4</td>
</tr>
<tr>
<td>BMI</td>
<td>26</td>
<td>26</td>
<td>25</td>
</tr>
</tbody>
</table>

Pathological changes:
- loss of tissue architecture
- adipocyte pleomorphism
- mitochondrial toxicity
- cell loss
- ↑ macrophages
- ↑ all pro-inflammatory cytokines

Images showing changes in tissue architecture over time.
Adipocyte apoptosis in lipoatrophy improves within 48 weeks of switching (TARHEEL study)

Indinavir: dose-dependant inhibitory effect on adipocyte differentiation => 50% inhibition at 15 μm

Nelfinavir: dose-dependant inhibitory effect on adipocyte differentiation => 50% inhibition at 20 μm

Amprenavir: minimal effect on adipocyte differentiation

Multiple Mechanisms by Which PIs May Be Detrimental to Adipocytes

- Insulin
- HSLipase
- GLUT4
- Glucose uptake
- Lipolysis
- Lipogenesis
- Glycerol
- TG
- Apoptosis
- Differentiation
- SREBP-1
- TNFα, IL-6
Median change in limb fat from baseline (%)

Weeks

EFV
NFV

* \( p < 0.05 \) between groups.
† \( p < 0.05 \) within groups.

Role of Nucleosides: Body Composition Survey* Results at Year 5

* Self-reported body composition abnormality confirmed by physical exam

- Flatting of buttocks
- Thinning of cheeks
- Thinning/decr of arm muscles
- Thinning/decr of leg muscles
- Thinning of temples
- Increase in waist size
- Hump on back of neck
- Enlargement of breasts

Cameron DW et al. 9th CROI, Seattle, 2002, #550
The Hypothalamus Plays a Key Role in Maintaining the Body’s Energy Balance and Metabolism

Different Discrete Sets of Parasympathetic and Sympathetic Hypothalamic Neurons Project to Either Subcutaneous or Visceral Fat Depots

Parasympathetic activity: Build up of fat
Sympathetic activity: Breakdown of fat

Selective Modulation by ARVs of Autonomic Neurons Projecting to Different Adipose Tissue Compartments?

Subcutaneous Fat Loss
-Δ gene expression
-Δ lipolysis/glucose uptake

Visceral Fat Increase

Mitochondria and Macrophages in HIV Lipoatrophy Model

- BLOOD
  - Monocytes
  - HIV virus
  - HIV-infected and activated monocytes
  - Infiltrating activated macrophages

- FAT
  - NRTIs

Mitochondrial and cellular dysfunction induced by NRTIs

Macrophage-induced amplification of inflammation and cellular damage

Lipo Dublin 2005 Oral 1 Shikuma
Macrophage recruitment is associated with early pathological changes

**ART naive**
- mtDNA ($\log_{10}$) = 2.9
- Average cytokine score = 0.03
- **Macrophage count = 3**
- % Leg fat = 22
- BMI = 24

**7 months d4T/3TC/rit/lop**
- mtDNA ($\log_{10}$) = 2.3
- Average cytokine score = 1.86
- **Macrophage count = 25**
- % Leg fat = 24
- BMI = 25

Hammond et al, 2005. Lipodystrophy Conference Dublin
Changes from ART-naïve baseline in macrophage count and cytokine expression according to changes from baseline at 6 months of therapy.

All P values < 0.05 for AZT&D4T, all P values >0.05 for abacavir, except for negative association with IL18 (P=0.039). Values are based on longitudinal changes in macrophage count and IL 6, IL-12, TNF, IL-8, IL18, (and INF gamma, mtDNA).
Choosing First-line Regimens to Avoid Lipoatrophy
DHHS Guidelines for the Use of ARV Agents in HIV-1-Infected Adults and Adolescents; October 2005.

### Preferred Regimens

| EFV + (3TC or FTC) + (AZT or TDF) | LPV/r + (3TC or FTC) + AZT |

### Alternative Regimens

#### NNRTI-based
- EFV + (3TC or FTC) + (ABC, ddl or d4T)
- NVP + (3TC or FTC) + (AZT, d4T, ddl, ABC or TDF)

#### PI-based
- ATV + (3TC or FTC) + (AZT, d4T, ABC or ddl) or (TDF+RTV 100 mg/d)
- LPV/r + (3TC or FTC) + (d4T, ABC, TDF or ddl)
- FPV or FPV/r or IDV/r or NFV or SQV/r + (3TC or FTC) + (AZT, d4T, ABC, TDF or ddl)

#### 3 NRTI-based
- ABC + AZT + 3TC – only when a preferred or an alternative NNRTI- or PI-based regimen cannot or should not be used
Initiating Therapy at Higher CD4 Counts May Reduce the Incidence of Lipoatrophy

<table>
<thead>
<tr>
<th>Min CD4</th>
<th>Max CD4</th>
<th>Incidence of lipoatrophy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;350</td>
<td>&gt;350</td>
<td>3.3%</td>
</tr>
<tr>
<td>200–349</td>
<td>&gt;200</td>
<td>12%</td>
</tr>
<tr>
<td>&lt;200</td>
<td>&gt;500</td>
<td>13.2%</td>
</tr>
<tr>
<td>&lt;200</td>
<td>350–499</td>
<td>17%</td>
</tr>
<tr>
<td>&lt;200</td>
<td>200–349</td>
<td>18.2%</td>
</tr>
<tr>
<td>&lt;200</td>
<td>&lt;200</td>
<td>30.8%</td>
</tr>
</tbody>
</table>

Differential effects of NRTI therapy on lipoatrophy risk: Initiating Abacavir

Mean total limb fat
ABC +1.75 kg vs d4T -1.15 kg

11th CROI, 2004 Abstract 716
Study 903
Significantly More Patients (%) With LIPOATROPHY on the d4T Arm

Investigator-defined.
†p value < 0.001.

903E (TDF Continuation vs d4T to TDF Switch)
Total Limb Fat by DEXA Through Week 192 (Mean)

Adapted from Madruga, et al. Presented at: 3rd IAS Conference; Rio de Janiero, Brazil. 2005; Poster No. TUPe2.2B12.
Adapted from Cassetti, et al. Presented at: 3rd IAS Conference; Rio de Janiero, Brazil. 2005; Poster No. WePe6.3C05.
Study GS 934
Median Total Limb Fat - Preliminary 96 Week Results for Subgroup with Week 48 Data

Data on file, Gilead Sciences.
Antiretroviral Switch Studies
Protease Inhibitor Withdrawal Does Not Improve Body Fat Changes

NEFA Study
Lipoatrophy


Nevirapine (n=155)  Efavirenz (n=156)  Abacavir (n=149)

$p=ns$
RAVE
Median Changes at Week 48 in Limb Fat by DEXA by Baseline Characteristics

Median Baseline Limb Fat

<table>
<thead>
<tr>
<th>Group</th>
<th>Median Fat (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Subjects</td>
<td>3.0kg 2.9kg</td>
</tr>
<tr>
<td>AZT at Baseline</td>
<td>5.12kg 2.97kg</td>
</tr>
<tr>
<td>d4T at Baseline</td>
<td>2.91kg 2.74kg</td>
</tr>
</tbody>
</table>

Change in fat mass (g) by DEXA

- TDF
- ABC

<table>
<thead>
<tr>
<th>Group</th>
<th>Change in Fat Mass (g)</th>
<th>n</th>
<th>AZT</th>
<th>d4T</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Subjects</td>
<td>393</td>
<td>49</td>
<td>12</td>
<td>37</td>
</tr>
<tr>
<td>AZT at Baseline</td>
<td>316</td>
<td>44</td>
<td>16</td>
<td>28</td>
</tr>
<tr>
<td>d4T at Baseline</td>
<td>66</td>
<td>66</td>
<td>210</td>
<td>529</td>
</tr>
<tr>
<td>No PI</td>
<td>374</td>
<td>31</td>
<td>32</td>
<td>357</td>
</tr>
<tr>
<td>On PI</td>
<td>432</td>
<td>18</td>
<td>12</td>
<td>247</td>
</tr>
</tbody>
</table>

*p = 0.97

Moyle G. Presented at: 12th CROI; 2005. No. 44LB.
RAVE Facial Sub-Study: Mean Volume (mm³) Change at 48 Weeks

RAVE Mean Change in Metabolic Outcomes to Week 48

<table>
<thead>
<tr>
<th></th>
<th>Lactate</th>
<th>Total Cholesterol</th>
<th>HDL Cholesterol</th>
<th>LDL Cholesterol</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDF</td>
<td>-0.3</td>
<td>-0.5</td>
<td>-0.1</td>
<td>-0.3</td>
<td>-0.3</td>
</tr>
<tr>
<td>ABC</td>
<td>-0.2</td>
<td>0.2</td>
<td>0</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

*P values by paired t-test.

All individuals included.
Lipid-lowering therapy commenced during study for TDF n=1, at 273 days, ABC n=8, at median 91.5 days.
Includes fasting and nonfasting samples. Observations are similar when only fasting samples are included.

Hepatic Steatosis

- Hepatic fat associated with hepatic insulin resistance and insulin requirements in Type 2 DM, general population and to some extent in HIV-LD

- Adipoctyes in liver recruit macrophages → secrete cytokines, PA1-1 and other inflammatory markers ↑ CV risk

- Hepatic adipocytes increased FFA and reduced adiponectin

- Unsolved mystery- why do some people accumulate fat in liver and others not?

- What is role of hepatic fat in HIV?
Hepatic Steatosis And Insulin Resistance In HIV

- Cross sectional study of 28 HIV+ pts (7 women)
- Measured HOMA-IR and MRS to look for fatty liver
- Fatty liver (defined > 5% fat by MRS) found in 46% subjects
- Factors associated with fatty liver:
  - Older age, higher HOMA, higher ALT and VAT
  - % liver fat strongly correlated with VAT and HOMA-IR
  - MV model- VAT strongest predictor liver fat
- Common problem- deserves further study in HIV

Lipo Dublin 2005 Poster 47 Grinspoon
Lifestyle changes
- Diet
- Exercise

Switching or discontinuing
- PIs
- PI → NNRTI
- NRTIs

Surgical interventions
- Surgical removal/liposuction
- Facial implants
- Fat transfer techniques

Drugs
- Growth hormone
- Anabolic steroids
- Uridine supplements
- Dietary supplements

Hypoglycemic agents
- Thiazolidinediones
- Biguanides

Lipid-lowering agents
- Fibric acid derivatives
- Statins
Patients Taking Metformin Lost Weight and Had Decreased Waist Circumference

Rosiglitazone Was No Different Than Placebo in Recovery of Limb Fat (kg)

- Rosiglitazone: 53 52 52
- Placebo: 55 53 53

STARs Study: Human Growth Hormone (hGH) for Fat Accumulation

**VAT (on CT L4-5)**

- Placebo: -4 cm²
- 4 mg AD: 0 cm², p=0.052
- 4 mg DD: 0 cm², p<0.001

**Trunk:limb fat ratio (on DEXA)**

- Placebo: -0.1
- 4 mg AD: 0, p<0.001
- 4 mg DD: 0, p<0.001

*p values for change from baseline to week 12 compared to placebo group*

AD=alternative days
DD=daily dosage

Uridine Supplementation as Potential Treatment for Lipoatrophy

Pravastatin Restores Fat During PI Treatment

Use of pravastatin 40mg daily for 12 weeks in this population resulted in significant increases in limb fat.

Polylactate (PLA) Injections for Facial Lipoatrophy

Changes in Patient Assessment of Visual Changes and Level of Anxiety at Week 12

- Depression score at week 24 fell from 6 at baseline to 2 (immediate) and 3 (delayed)

*PLA given on week 0, 2, and 4; †PLA given on week 12, 14, and 16.

Conclusions

- No evidence of HIV on adipose tissue
- No evidence of abacavir / tenofovir toxicity
- Major risk factors for adipose pathology - duration of D4T>AZT
- Minor influences on adipose tissue – age, BMI, pre-switch CD4 count?
- Lipoatrophy involves mtDNA depletion & mitochondrial toxicity with distinctive signature of pathology involving macrophages, cytokine-mediated pro-inflammation, and fat loss.
- Future studies: implications of pro-inflammatory adipose pathology
BACK UP SLIDES
Does lipoatrophy contribute to metabolic complications?

NRTIs
- d4T>ZDV

PI

Mitochondrial toxicity
Adipocyte loss and/or ↓ function
Subcutaneous fat wasting

Insulin resistance
Dyslipidemia
Visceral fat accumulation

Lipodystrophy

White, ↑ age, TNF
Host factors
Therapy

Non-white, Sedentary, Diet

Does lipoatrophy contribute to metabolic complications?
Differential Effects of NRTI Therapy on Lipoatrophy Risk: d4T vs AZT at 30 Months

- Lipoatrophy in OZCOMBO (n=84): 57% d4T/3TC, 46% d4T/ddI, 19% ZDV/3TC
- Lipoatrophy in ALBI (n=120): 54% d4T/3TC, 12% d4T/ddI, 44% ZDV/3TC
- Lipoatrophy in NOVAVIR (IDV) (n=100): 18% d4T/3TC, 44% d4T/ddI, 57% ZDV/3TC

Differential Effects of NRTI Therapy on Lipoatrophy Risk: d4T vs AZT

A5005s Metabolic Substudy of ACTG 384:
Median % change in limb and trunk fat estimated by DEXA scans

![Graph showing median % change in limb and trunk fat over weeks for ZDV+3TC and ddI+d4T.](image)

- **Limb Fat**: MMANOVA $p<0.001$
- **Trunk Fat**: MMANOVA $p=0.02$

Subset of individuals (9 d4T, 13 AZT; 103 DEXA scans)

concurrent longitudinal leg fat and biopsy data

Mixed effects analysis

fat loss (%leg fat/BMI) associated with

- severity of mtDNA depletion ($p = 0.01$)
- duration of NRTI therapy ($p = 0.001$)
- choice of NRTI ($p = 0.03$ univariate) not significant after adjusting for effect mtDNA depletion ($p = 0.3$)
Uridine Supplementation May Restore Fat Loss in Patients on TA-NRTIs

- Intra-abdominal fat was also restored in the NucleomaxX arm at significant values vs both baseline and at 3 months within the study group

* p<0.05 for change between baseline and 3 months within each study group for all values

RAVE Methods: Laser scan

Benn et al
Macrophages contribute to pro-inflammation

• macrophage count & cytokine expression from adipocytes was highly correlated (p<0.0001)

• macrophages themselves appear to be potent producers of cytokines

ART naive

7 months d4T/3TC/rit/lop

TNF alpha

Mac 387
Time to Fat Loss From Start of PI Therapy: Concurrent d4T vs AZT

Median 18.5 months for d4T, PI

Median 26 months for AZT, PI

$p=0.003$

A5148: Impact of Niacin Supplementation on Lipids

- N = 37 HIV-infected men
  - 4 wks dietary advice
  - 44 wks niacin (↑ dose)
- No significant change in transaminases observed
- Fasting insulin & HOMA-IR persistently elevated

P ≤ .01 for all values