Fat and HIV persistence, role of fat metabolism and inflammation

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JM Gallego-Escureso, JC Domingo; J Diaz-Delfín; JP Guallar
GENE EXPRESSION ANALYSIS

- Healthy controls

- Naïve: HIV-1-infected, untreated patients

- Non-LD: HIV-1-infected patients, on cART without clinical signs of lipoatrophy

- LD: HIV-1-infected patients, on cART with clinical lipoatrophy

Applied Biosystems Human Genome Survey Arrays

Gene markers of mitochondrial biogenesis, adipogenesis, adipokines, inflammation.

Biopsies of subcutaneous adipose tissue

RNA DNA protein extraction
Transcriptomic analysis of subcutaneous adipose tissue from HIV-patients relative to healthy controls

<table>
<thead>
<tr>
<th>IMMUNOLOGY-RELATED</th>
<th>Naïve</th>
<th>Non-LD</th>
<th>LD</th>
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<tbody>
<tr>
<td>Complement-mediated immunity</td>
<td>*</td>
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<tr>
<td>T-cell activation</td>
<td>*</td>
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<tr>
<td>Interferon-mediated immunity</td>
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<tr>
<td>B-cell antibody-mediated immunity</td>
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<tr>
<td>Inflammation mediated by chemokines and cytokine signaling pathways</td>
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<tr>
<td>MHC-II mediated immunity</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Macrophage-mediated immunity</td>
<td>–</td>
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* significant change (either up- or down-regulation)
Transcriptomic analysis of subcutaneous adipose tissue from HIV-patients relative to healthy controls

<table>
<thead>
<tr>
<th>METABOLISM</th>
<th>Naïve</th>
<th>Non-LD</th>
<th>LD</th>
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<tr>
<td>Lipid, fatty acid &amp; steroid metabolism</td>
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<td>Fatty acid metabolism</td>
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<td>Fatty acid beta-oxidation</td>
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<tr>
<td>Lipid and fatty acid transport</td>
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<tr>
<td>Lipid and fatty acid binding</td>
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<tr>
<td>TCA Cycle</td>
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<tr>
<td>Oxidative phosphorylation</td>
<td>-</td>
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<tr>
<td>Electron transport</td>
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<tr>
<td>Detoxification</td>
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<tr>
<th>OTHER</th>
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<tr>
<td>Apoptosis</td>
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<tr>
<td>Angiogenesis</td>
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* significant change (either up- or down-regulation)
Reciprocal repression of marker proteins of adipogenesis and induction of marker proteins of inflammation in subcutaneous adipose tissue

Viral infection, in the absence of cART, impacts on adipose tissue biology:

associated pro-inflammatory environment?

direct action of viral proteins?
**HIV-Tat** inhibits human adipocyte differentiation and induces expression and release of pro-inflammatory cytokines

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**Gene expression**

- **Adiponectin mRNA**
- **PPARγ mRNA**
- **GLUT4 mRNA**

**Protein secretion**

- **Adiponectin (ng/mL)**
- **IL-8 (pg/mL)**
- **IL-6 (pg/mL)**
- **MCP-1 (ng/mL)**

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*Diaz-Delfín et al. Antivir Ther 2012*
HIV-1 secreted proteins (e.g., Tat) may play a role in the adipose tissue alterations that ultimately lead to adipose tissue and systemic metabolic disturbances observed in HIV-1-infected patients.
Transgenic mice expressing the HIV-1 genome devoid of the \textit{gag-pol} genes (Tg26 mice)

Transgenic mice containing 10-20 of a 7.4-kb proviral HIV-1 DNA lacking pol and gag genes to avoid spontaneous infection

Collaboration with PE Klotman, Mount Sinai Hospital, New York (now at Baylor College of Medicine, Houston) USA

HIV-1 transgene expression in mice alters the amount of scWAT

Villarroya J, et al. Antivir Ther 2010
HIV-1 transgene expression in mice alters gene expression in WATs

**Adipogenesis**

- **PPARγ**
  - sc-WAT
  - vs-WAT
  - BAT

- **Adiponectin**
  - sc-WAT
  - vs-WAT
  - BAT

**Inflammation**

- **MCP-1**
  - sc-WAT
  - vs-WAT
  - BAT

- **IL-6**
  - sc-WAT
  - vs-WAT
  - BAT

**Mitochondrial Function**

- **Cyt b mRNA**
  - sc-WAT
  - vs-WAT
  - BAT

- **Complex III**
  - sc-WAT
  - vs-WAT
  - BAT
Transgenic HIV expression in mice recapitulates partially several features of the adipose tissue and adipokine alterations in HIV-1-infected patients associated with lipodystrophy, specially mild alterations not attributable to antiretroviral treatment.
HIV-1 transgene expression in mice increases serum FGF21 levels

J Villarroya et al. unpublished
FGF-21 as a novel metabolic regulator

Alexei Kharitonenkov,¹ Tatiyana L. Shiyanova,¹ Anja Koester,¹
Amy M. Ford,¹ Radmilja Micanovic,¹ Elizabeth J. Galbreath,¹ George E. Sandusky,¹
Lisa J. Hammond,¹ Julie S. Moyers,¹ Rebecca A. Owens,¹ Jesper Gromada,²
Joseph T. Brozinick,¹ Eric D. Hawkins,¹ Victor J. Wroblewski,¹ De-Shan Li,¹
Farrokh Mehrbod,¹ S. Richard Jaskunas,¹ and Armen B. Shanafelt¹

¹Lilly Research Laboratories, Division of Eli Lilly and Co., Indianapolis, Indiana, USA. ²Lilly Research Laboratories, Hamburg, Germany.

Diabetes mellitus is a major health concern, affecting more than 5% of the population. Here we describe a potential novel therapeutic agent for this disease, FGF-21, which was discovered to be a potent regulator of glucose uptake in mouse 3T3-L1 and primary human adipocytes. FGF-21–transgenic mice were viable and resistant to diet-induced obesity. Therapeutic administration of FGF-21 reduced plasma glucose and triglycerides to near normal levels in both ob/ob and db/db mice. These effects persisted for at least 24 hours following the cessation of FGF-21 administration. Importantly, FGF-21 did not induce mitogenicity, hypoglycemia, or weight gain at any dose tested in diabetic or healthy animals or when overexpressed in transgenic mice. Thus, we conclude that FGF-21, which we have identified as a novel metabolic factor, exhibits the therapeutic characteristics necessary for an effective treatment of diabetes.
THERMOGENIC ACTIVATION
Hondares et al, Cell Metab 2010

CONTROL OF METABOLIC CIRCADIAN BEHAVIOR AND FEMALE REPRODUCTIVE HORMONES
Boockout et al., & Owen et al., Nature Med. 2013

SWEET AND ALCOHOL PREFERENCE
Talukdar et al., von Holstein Rathlou et al., and Soberg et al. Cell Metab 2016, 2017

LIPID METABOLISM
Emanuelli et al., J Clin Invest 2014

FGF21

GLUCOSE UPTAKE AND OXIDATION
Kharitonenkov et al. JCI 2008

«BROWNING»
Fisher et al, Genes Dev. 2012

ADIPONECTIN
Holland et al.; Li et al. Cell Metab, 2013

PROTECTION AGAINST PATHOLOGICAL HYPERTROPHY
HIV-1-infected patients show increased levels of serum FGF21 associated with insulin resistance, even in the absence of cART.
Serum FGF21 levels and FGF21 expression in liver are increased in obese and T2DM patients

WAT

FGF21 mRNA was undetectable in scAT and vAT from lean and obese individuals

In collaboration with Gema Frühbeck, Clínica Universidad de Navarra (Pamplona, Spain)

In a Mexican population with a high prevalence of obese HIV-infected patients, no additive effects on abnormally high FGF21 levels are observed.

In collaboration with Francisco J. López, Universidad Autónoma de Coahuila, México
Increased serum FGF21 levels in obese and HIV-patients despite altered metabolism, ... a paradox?

FGF21-resistance in these patients?
**FGF21**
Requires the presence of a specific transmembrane protein of the klotho family, **β-klotho (KLB)** in order to bind the receptor effectively and to activate intracellular pathways.

Kurosu H, Kuro OM, *Endocrine fibroblast growth factors as regulators of metabolic homeostasis*. Biofactors, 2009
HIV-1-infected patients show impaired expression of β-Klotho and FGFR1 in adipose tissue, consistent with impaired FGF21 action, even in the absence of cART.

**SC ADIPOSE TISSUE**

![Graphs showing expression levels of FGFR1 and β-Klotho in control, naive, LD-, and LD+ conditions under HIV-1 infection and HAART treatment.](image)

Obese individuals show impaired expression of $\beta$-Klotho in adipose tissue and impaired FGF21 action

**ADIPOSE TISSUES**

**$\beta$-KLOTHO mRNA**

- Lean
- Obesity (NG)
- Obesity (T2DM)

**$\beta$-KLOTHO**

- Lean
- Obesity (NG)
- Obesity (VISCERAL)

**pERK1/2 / ERK1/2**

- Lean
- Obesity (NG)
- Obesity (VISCERAL)

Gallego-Escuredo et al. *Int J Obes (Lond)* 2015
HIV-1-infected and obese patients show similar alterations in the endocrine FGF21 pathway

Abnormalities in the FGF21 pathway appear in HIV-1-patients, even without cART
TNFα-induced repression of β-Klotho expression is associated with impaired responsiveness to FGF21 action

Murine white adipocytes

**β-Klotho**

- Relative mRNA level
- Protein levels (fold induction)

**FGFR1**

- Relative mRNA level

Human adipocytes

**β-Klotho**

- Relative mRNA level

FGF21-induced glucose uptake

- Fold induction

FGF21-induced GLUT1 expression

- Relative mRNA level

Inflammatory signaling contributes to altered FGF21 action in adipose tissue
CONTROL OF METABOLIC CIRCADIAN BEHAVIOR AND FEMALE REPRODUCTIVE HORMONES
Boockout et al., & Owen et al., Nature Med. 2013

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Talukdar et al., von Holstein Rathlou et al., and Soberg et al. Cell Metab 2016, 2017

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Emanuelli et al., J Clin Invest 2014

BONE LOSS
Wei et al. PNAS, 2012; Wang et al, Cell Metab. 2015

LIPID METABOLISM
Emanuelli et al., J Clin Invest 2014

BONE LOSS
Wei et al. PNAS, 2012; Wang et al, Cell Metab. 2015

PROTECTION AGAINST PATHOLOGICAL HYPERTROPHY
HIV-1-infected patients show altered bone parameters, even in the absence of cART

Gallego-Escuredo JM, et al. Metabolism 2017
High FGF21 levels are associated with poor bone homeostasis in HIV-1-infected patients.

**Total Bone Mineral Density**

- **BMD (g/cm²)**
  - C
  - N
  - LD-
  - LD+

**Serum FGF21 levels**

- **FGF21 (pg/mL)**
  - C
  - N
  - LD-
  - LD+

**RANKL/OPG ratio** (bone resorption)

- **RANKL/OPG**
  - C
  - N
  - LD-
  - LD+

**CTX-1/OC ratio** (indicator of bone turnover)

- **CTX1/OC**
  - C
  - N
  - LD-
  - LD+

**FGF21 vs BMD**

- **P = 0.01**
  - r = -0.19

**CTX1/OC vs FGF21**

- **P = 0.01**
  - r = 0.23

RANKL: receptor activator of NFκB ligand (osteoclastogenic regulator)

OPG: osteoprotegerin (bone turnover regulator)

CTX-1: c-terminal telopeptide of type-1 collagen (soluble marker of bone resorption)

OC: osteocalcin (soluble marker of bone formation)

*P≤0.05 vs C

#P≤0.05 vs N

Gallego-Escuredo JM, et al. Metabolism 2017
Femur bone morphometric parameters

Micro-Computed Tomography Scans

- Bone volume (BV)
- Total cross-sectional area (B.Ar)
- Total cross-sectional perimeter (B.Pm)
- Cross-sectional thickness (Cs.Th)
- Trabecular thickness (Tb.Th)
- Trabecular separation (Tb.Sp)
- Trabecular number (Tb.N)
- Bone volume fraction (BV/TV)

Tg26 HIV-1 mouse model
HIV-1 transgene expression in mice alters bone homeostasis

Normal bone development

Bone malformation

Bone loss

HIV-1 transgene expression in mice increases markers of bone turnover

Increased gene expression and circulating levels of osteoclastogenic markers

The HIV-Tg26 mouse model largely mimics bone alterations in HIV-1 patients
Alterations in HIV-patients without treatment (naïve) evidence the pathogenic capacity of infection-related events on adipose tissue, bone and metabolic homeostasis.

*In vitro* studies reveal that adipocytes are targets of HIV-proteins and inflammatory signals, as occurring in HIV-patients.

The HIV-Tg26 mouse may be useful as a model to study alterations in patients non-attributable to cART.