Fat and HIV persistence, role of fat metabolism and inflammation

Marta Giralt

Dept. Biochemistry and Molecular Biomedicine
Institute of Biomedicine of the University of Barcelona (IBUB)
Universitat de Barcelona
CIBER Fisiopatología de la Obesidad y Nutrición

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Dpt Biochemistry and Molecular Biomedicine
Institute of Biomedicine
University of Barcelona

Francesc Villarroya, *PhD*
Marta Giralt, *PhD*

Infectious Diseases Unit
Hospital de la Santa Creu i Sant Pau
Barcelona, Catalonia

Pere Domingo, *MD PhD*

JM Gallego-Escuredo, JC Domingo; J Diaz-Delfin; 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

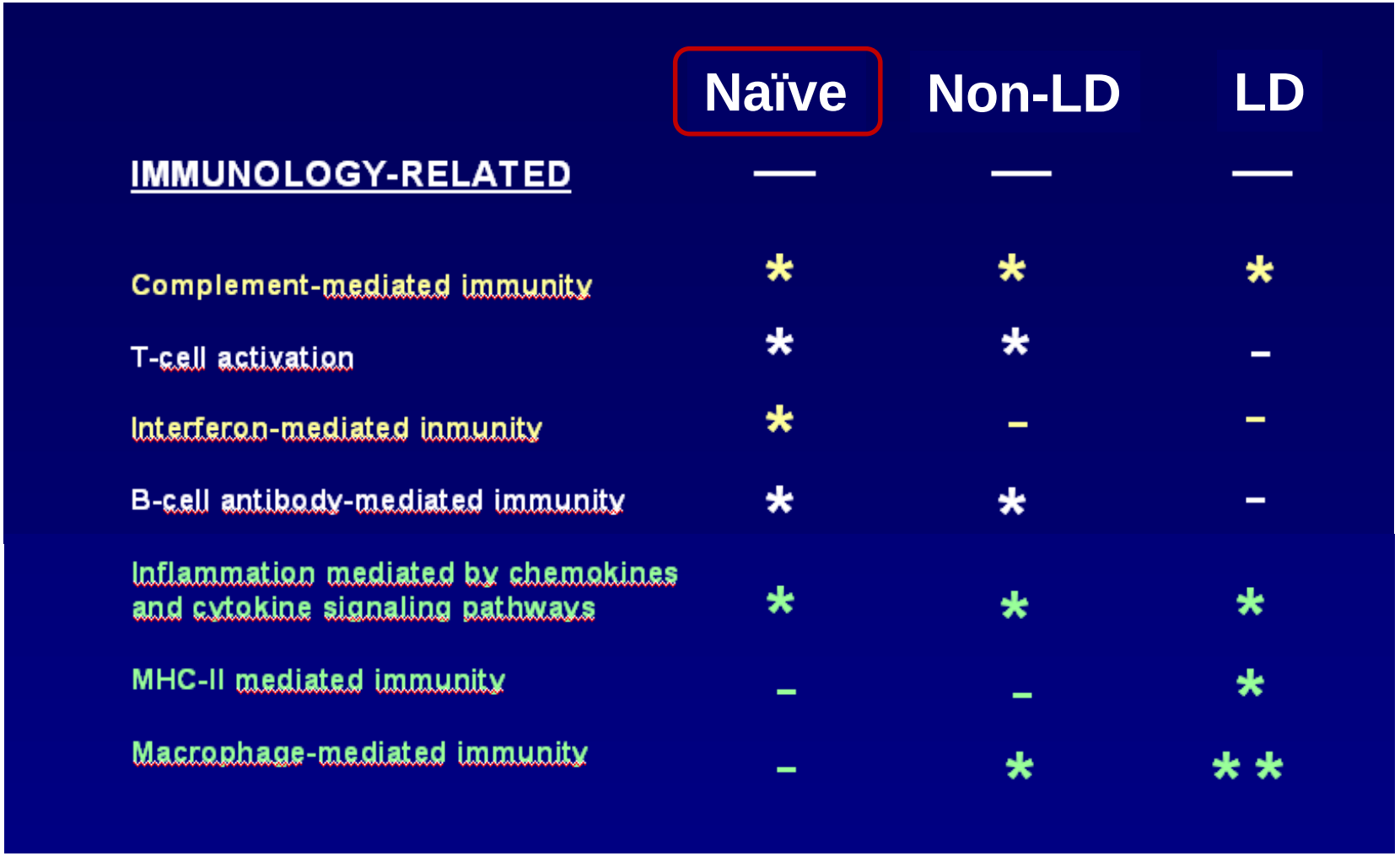
Biopsies of subcutaneous adipose tissue

RNA
DNA
protein
extraction

Applied Biosystems Human Genome Survey Arrays

Gene markers of **mitochondrial biogenesis**
adipogenesis
adipokines
inflammation

Transcriptomic analysis of subcutaneous adipose tissue from HIV-patients relative to healthy controls



* significant change (either up- or down-regulation)

PANTHER Biological Processes Analyses

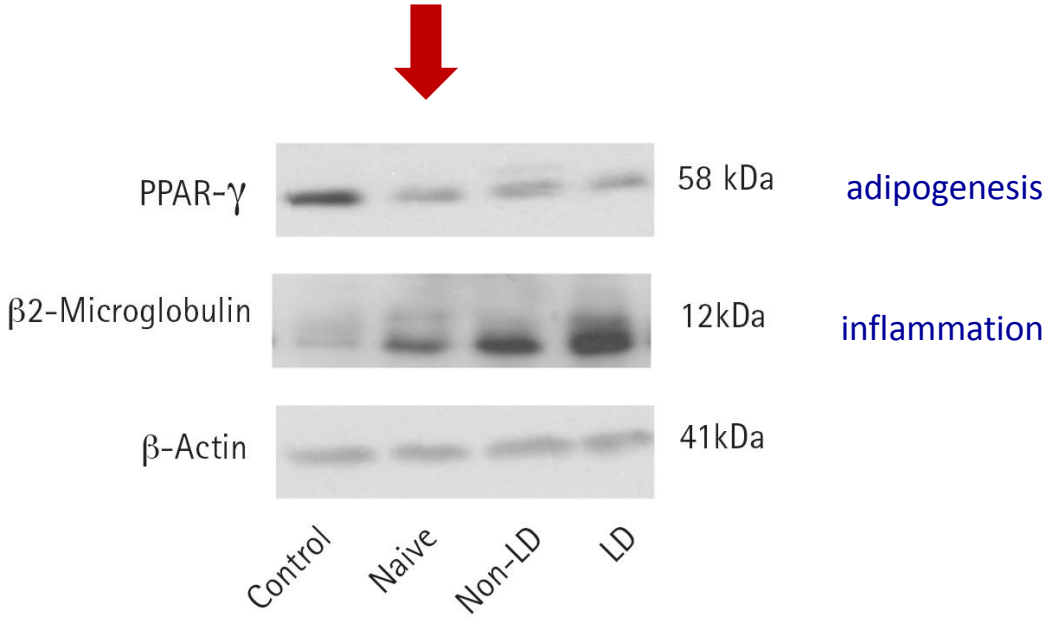
Transcriptomic analysis of subcutaneous adipose tissue from HIV-patients relative to healthy controls

	Naïve	Non-LD	LD
METABOLISM			
Lipid, fatty acid & steroid metabolism	*	* *	* *
Fatty acid metabolism	*	* *	* *
Fatty acid beta-oxidation	*	*	*
Lipid and fatty acid transport	–	–	*
Lipid and fatty acid binding	–	–	*
TCA Cycle	*	*	*
Oxidative phosphorylation	–	*	* *
Electron transport	–	*	* *
Detoxification	–	*	–
OTHER			
Apoptosis	–	–	*
Angiogenesis	*	*	*

* significant change (either up- or down-regulation)

PANTHER Biological Processes Analyses

Reciprocal repression of marker proteins of adipogenesis and induction of marker proteins of inflammation in subcutaneous adipose tissue



Giralt M, Domingo P et al. *Antivir Ther* 2006; Guallar J, et al. *AIDS* 2008; Gallego-Escuredo JM, et al. *J Acquir Immune Defic Syndr* 2013; Cereijo R, et al. *PLoS One* 2015

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

associated pro-inflammatory environment?

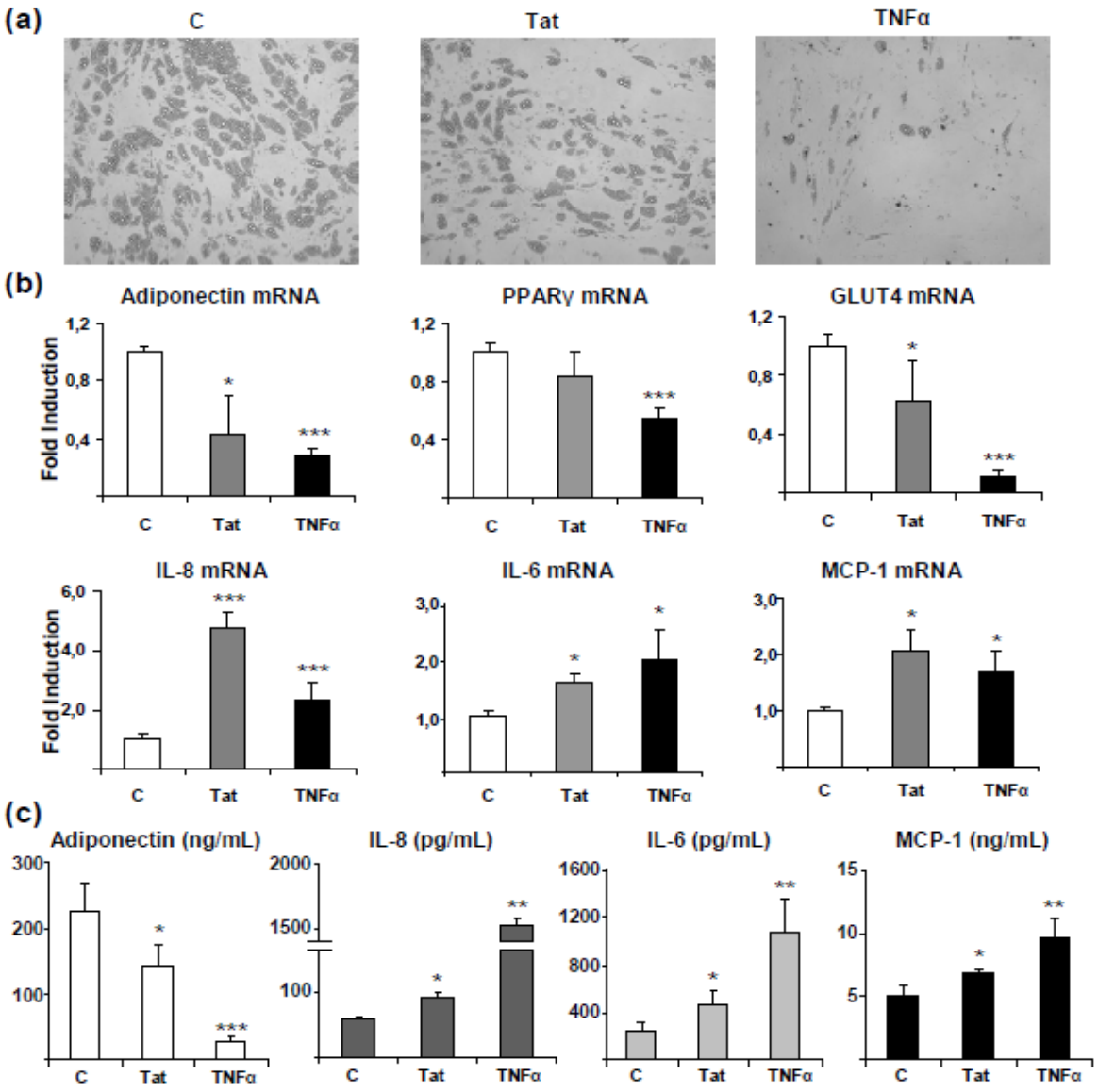
direct action of viral proteins?

HIV-Tat inhibits human adipocyte differentiation and induces expression and release of pro-inflammatory cytokines

In vitro studies

Gene expression

Protein secretion



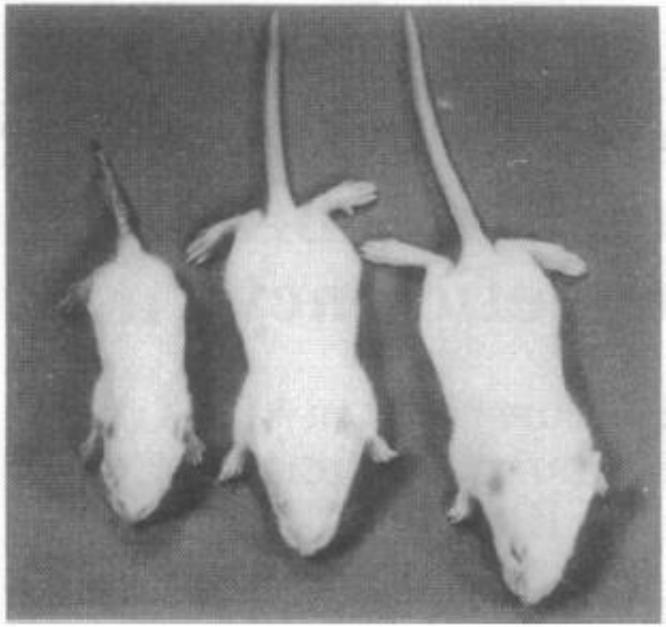
Díaz-Delfín et al.
Antivir Ther 2012

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

FVB/N-Tg(HIV)26Aln/PkltJ (Tg26) HIV-1 model

Transgenic mice expressing the HIV-1 genome devoid of the *gag-pol* genes (Tg26 mice)

Homozygous mice seldom survive to weaning



Heterozygous mice exhibit premature death (around 6 month)

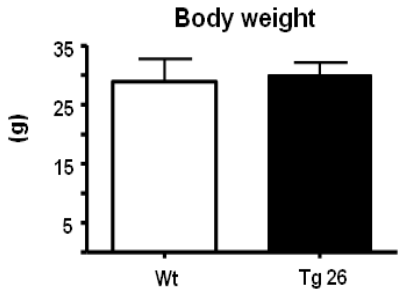
Tg26 +/+ Tg26+/- WT

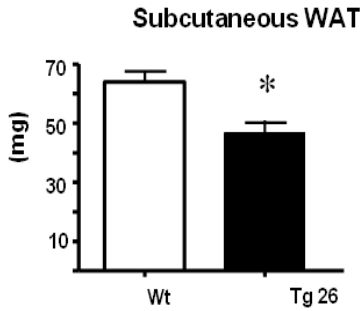
Transgenic mice containing 10-20 of a 7.4-kb proviral HIV-1 DNA lacking pol and gag genes to avoid spontaneous infection

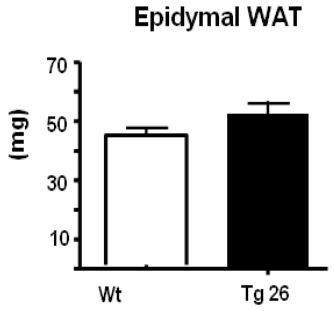
Collaboration with PE Klotman, Mount Sinai Hospital, New York (now at Baylor College of Medicine, Houston) USA

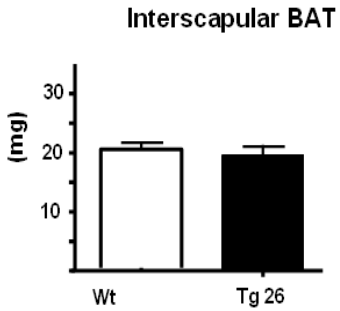
Rosenstiel et al, J Am Soc Nephrol 20: 2296–2304, 2009

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







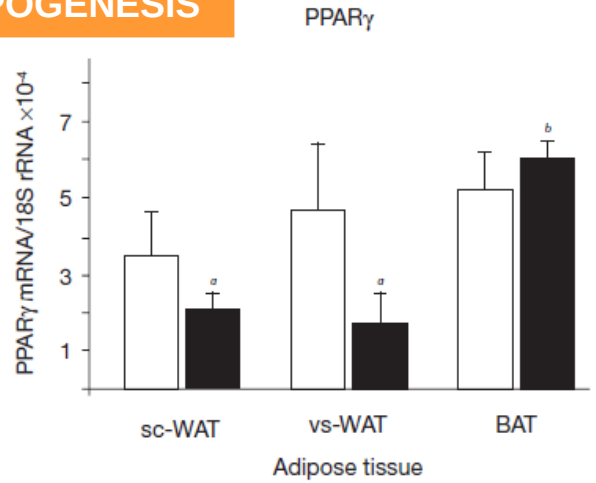


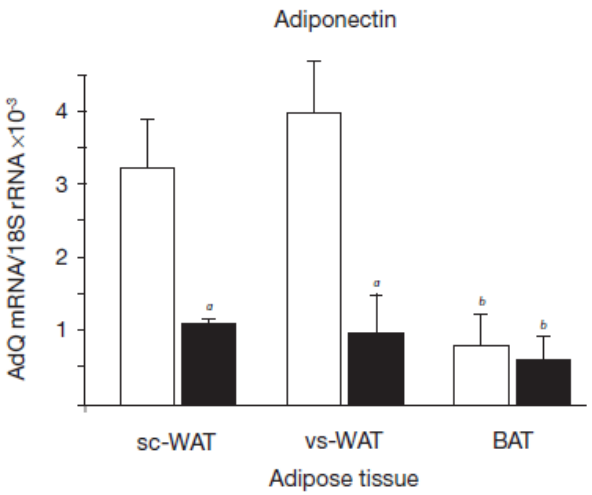
Wild-type Tg26+/-

Villarroya J, et al. *Antivir Ther* 2010

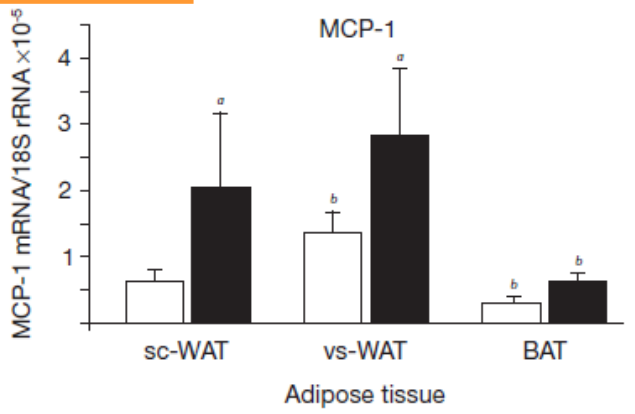
HIV-1 transgene expression in mice alters gene expression in WATs

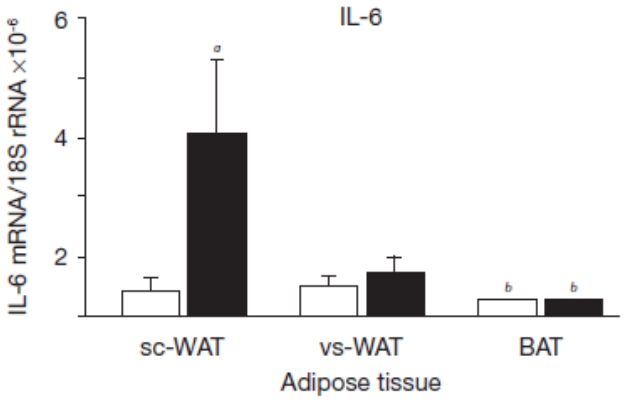
ADIPOGENESIS



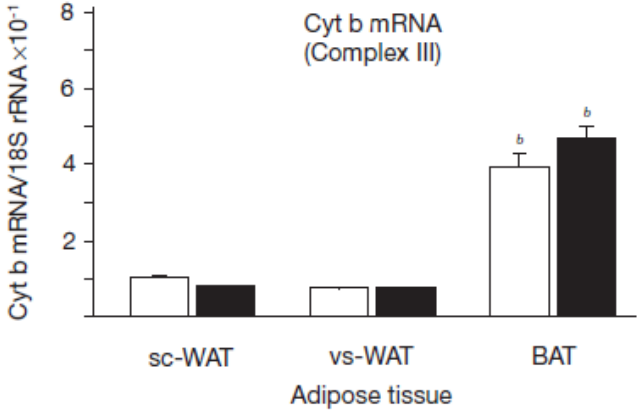


INFLAMMATION



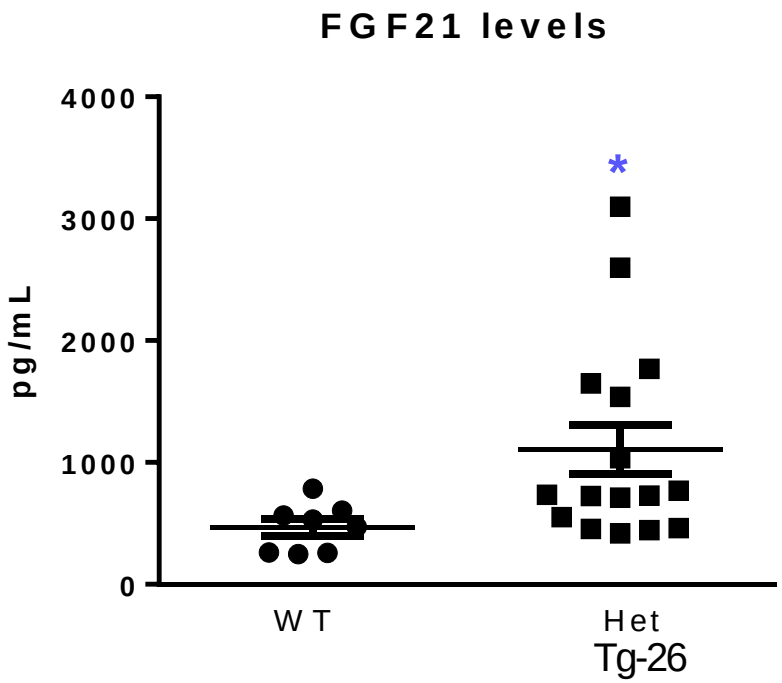


MITOCHONDRIAL FUNCTION



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*

Research article

FGF-21 as a novel metabolic regulator

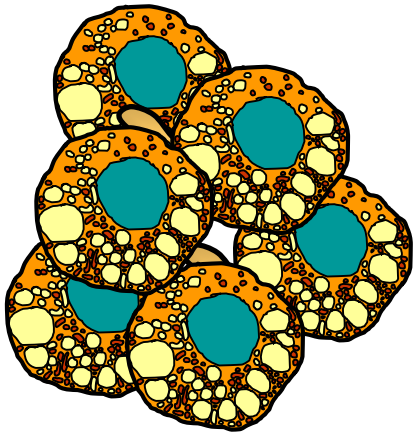
Alexei Kharitonenkov,[1] Tatiyana L. Shiyanova,[1] Anja Koester,[1]
Amy M. Ford,[1] Radmila Micanovic,[1] Elizabeth J. Galbreath,[1] George E. Sandusky,[1]
Lisa J. Hammond,[1] Julie S. Moyers,[1] Rebecca A. Owens,[1] Jesper Gromada,[2]
Joseph T. Brozinick,[1] Eric D. Hawkins,[1] Victor J. Wroblewski,[1]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

[1]Lilly Research Laboratories, Division of Eli Lilly and Co., Indianapolis, Indiana, USA. [2]Lilly 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.

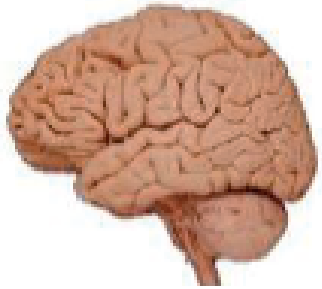
In mouse models of diabetes and obesity, FGF21 administration lowers glucose, improves insulin sensitivity and decreases body weight

JCI, 2005



CONTROL OF METABOLIC CIRCADIAN BEHAVIOR AND FEMALE REPRODUCTIVE HORMONES

Boockout et al., & Owen et al., Nature Med. 2013



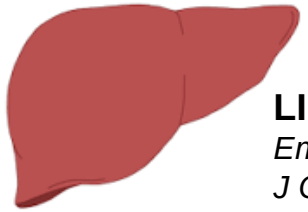
SWEET AND ALCOHOL PREFERENCE

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

THERMOGENIC ACTIVATION

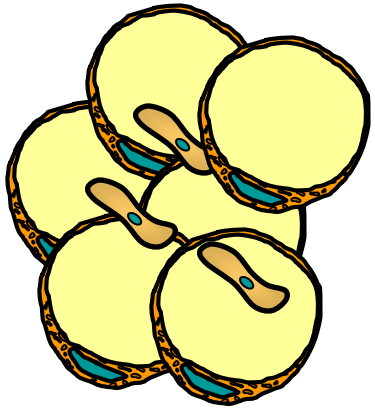
Hondares et al, Cell Metab 2010

FGF21



LIPID METABOLISM

Emanuelli et al., J Clin Invest 2014



GLUCOSE UPTAKE AND OXIDATION

Kharitonenkov et al. JCI 2008

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

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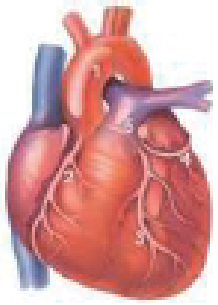
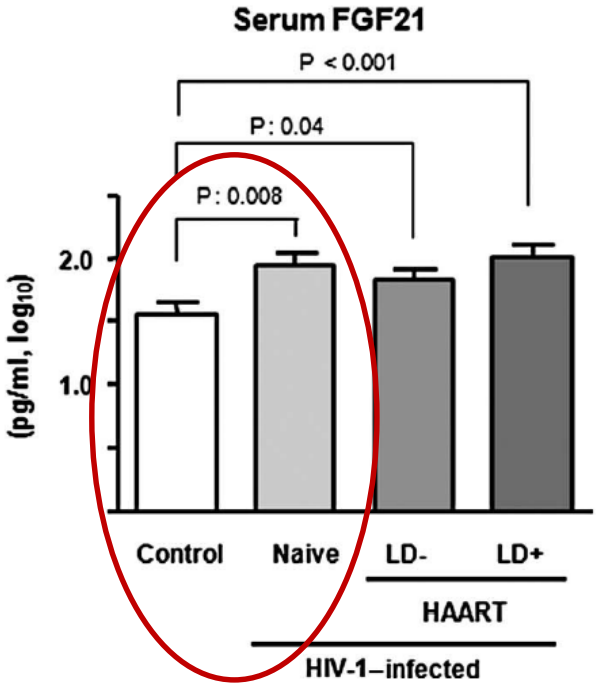
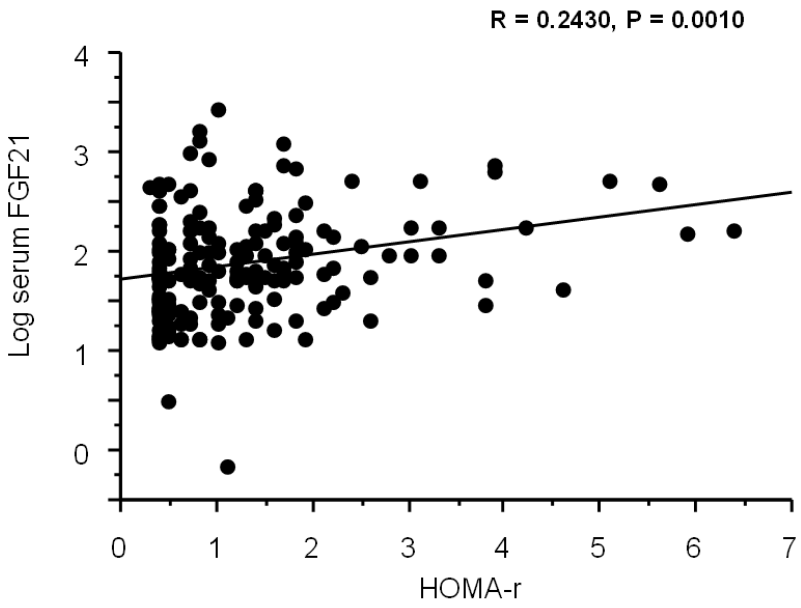
Planavila et al. Nature Comm. 2013



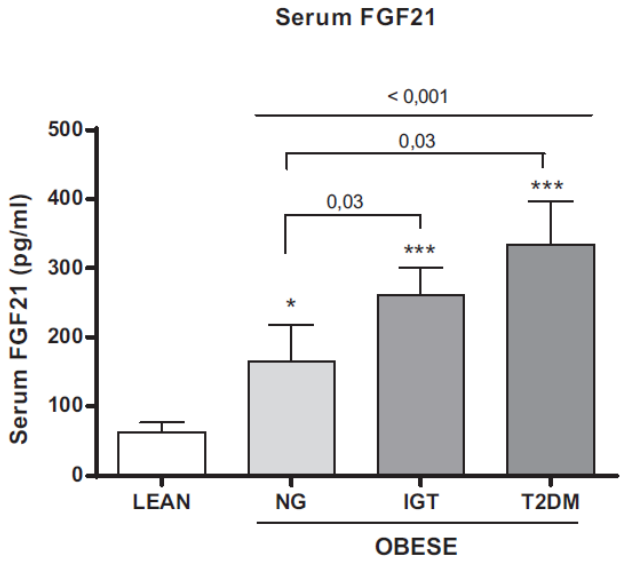
HIV-1-infected patients show increased levels of serum FGF21 associated with insulin resistance, even in the absence of cART

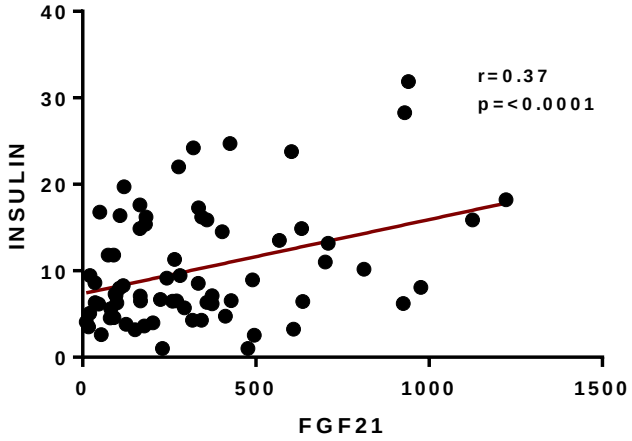




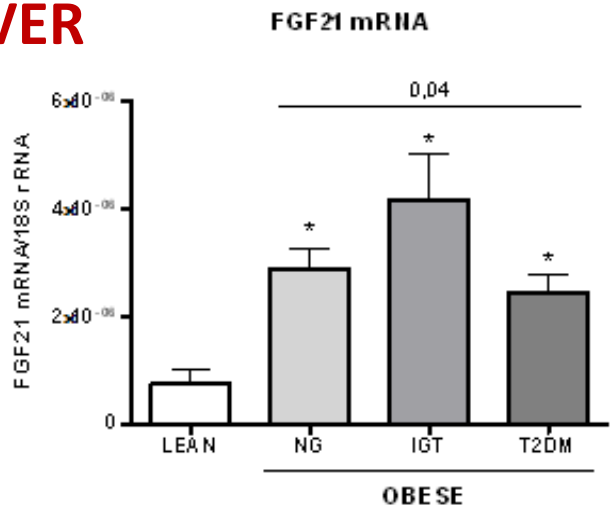
Domingo P, et al. *AIDS* 2010; Gallego-Escuredo JM, et al. *J Acquir Immune Defic Syndr* 2012

Serum FGF21 levels and FGF21 expression in liver are increased in obese and T2DM patients





LIVER



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)

Gallego-Escuredo JM, et al. *Int J Obes (Lond).* 2015 ; Gómez-Ambrosi J, et al. *Clin Nutr* 2017

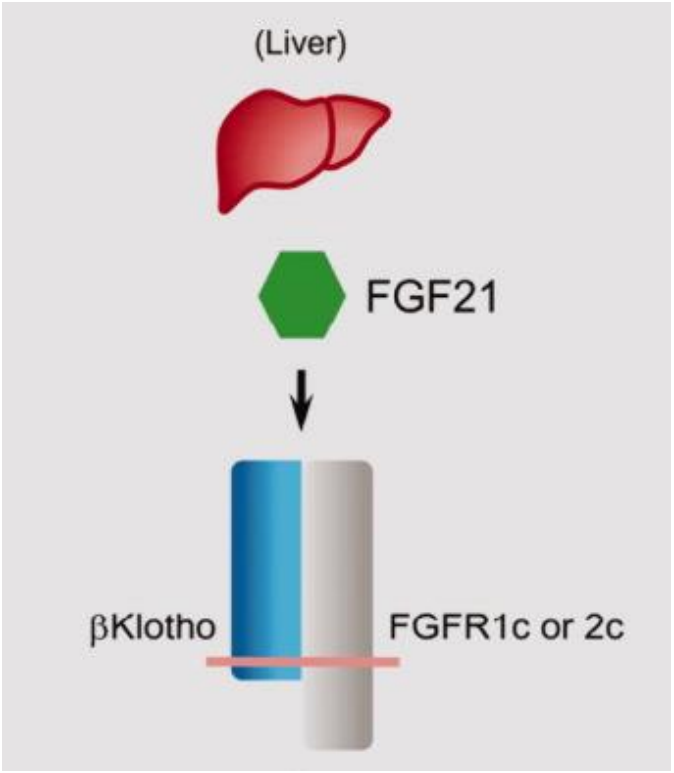
HIV-patients Obese

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?



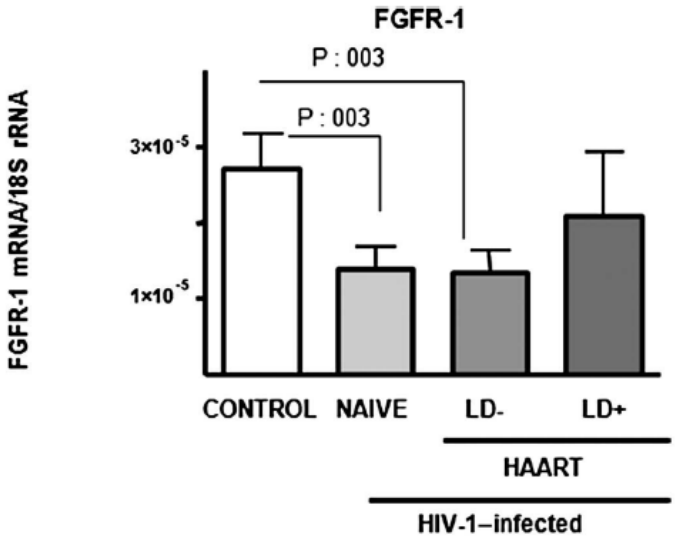
FGF21

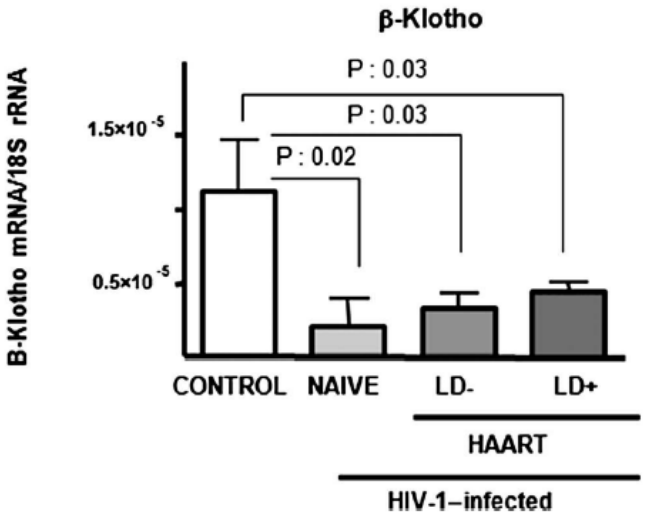
Requires the presence of a specific transmembrane protein of the klotho family, **β-klotho (KLB)** in order to bind the receptor effectively and to activate intracellular pathways

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

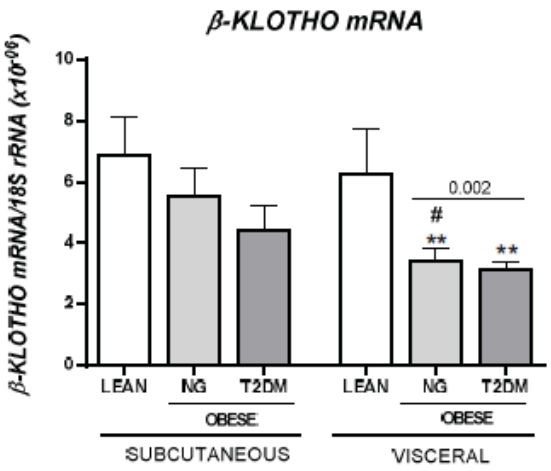


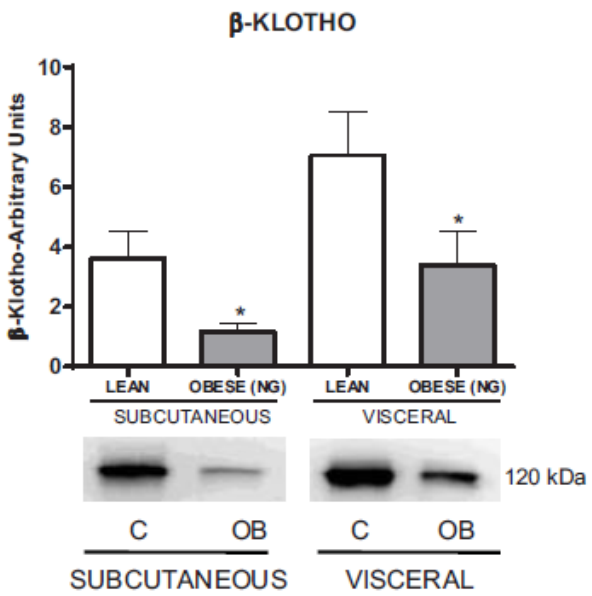


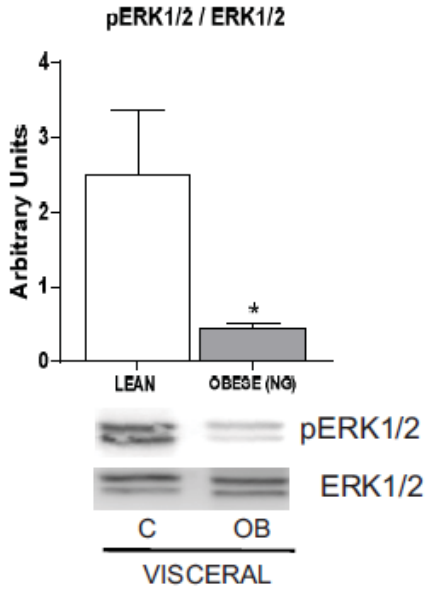
Gallego-Escuredo JM, Domingo P, *et al. J AIDS, 2012*

Obese individuals show impaired expression of β-Klotho in adipose tissue and impaired FGF21 action

ADIPOSE TISSUES







Gallego-Escuredo et al. *Int J Obes (Lond) 2015*

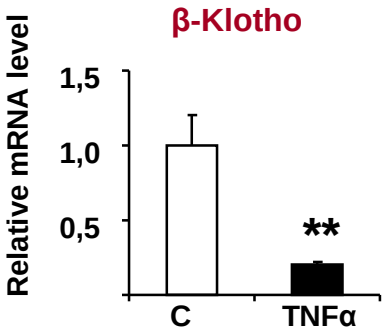
HIV-1-infected and obese patients show similar alterations in the endocrine FGF21 pathway

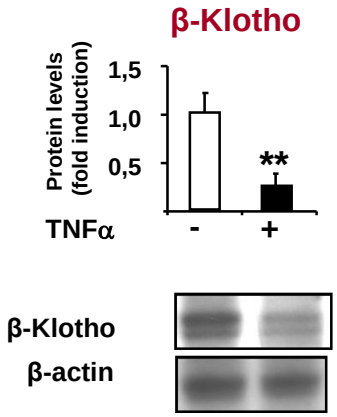
Abnormalities in the FGF21 pathway appear in HIV-1-patients, even without cART

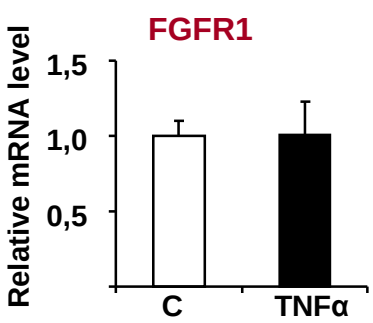
TNFα-induced repression of β-Klotho expression is associated with impaired responsiveness to FGF21 action

In vitro studies

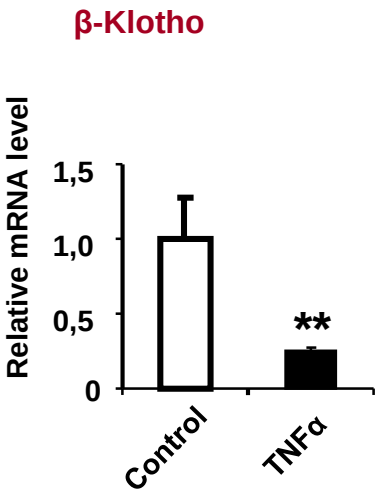
Murine white adipocytes

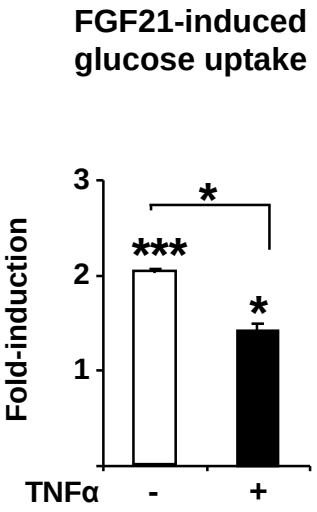


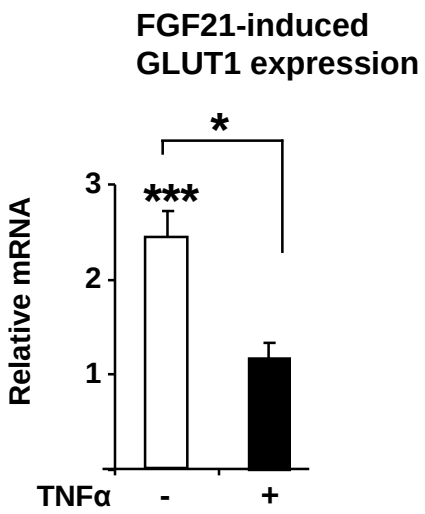




Human adipocytes

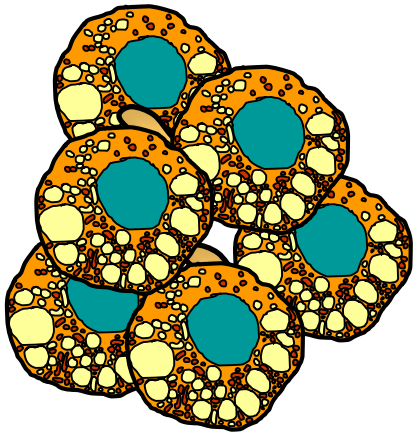






Díaz-Delfín, et al. Endocrinology, 2012

Inflammatory signaling contributes to altered FGF21 action in adipose tissue



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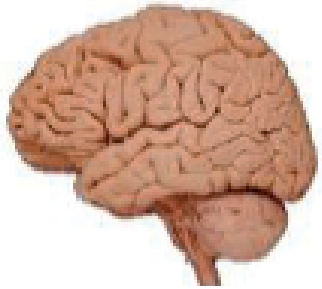
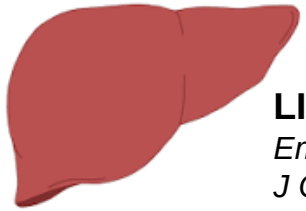
Boockout et al., & Owen et al., Nature Med. 2013



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

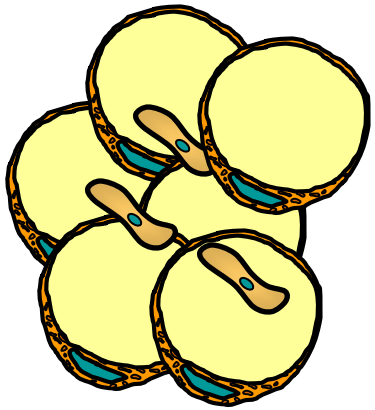
THERMOGENIC ACTIVATION
Hondares et al, Cell Metab 2010

FGF21



LIPID METABOLISM
Emanuelli et al., J Clin Invest 2014

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



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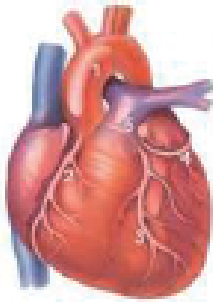
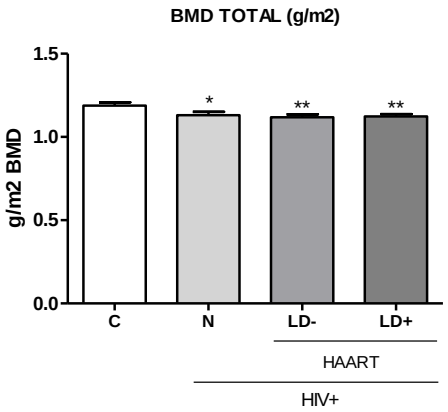
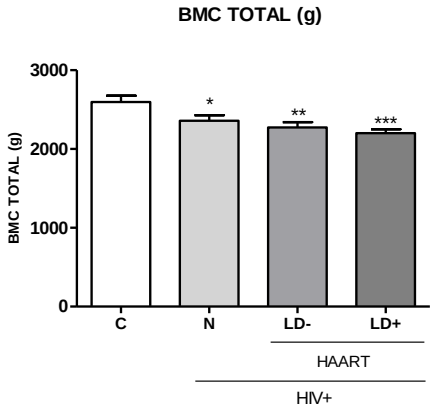
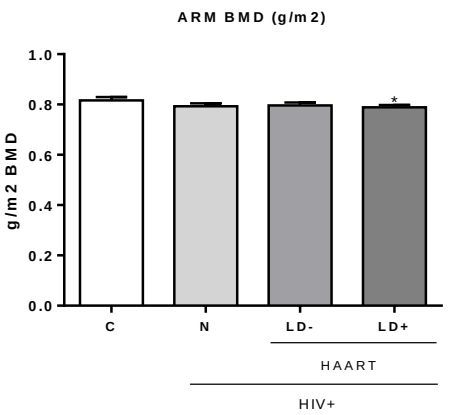
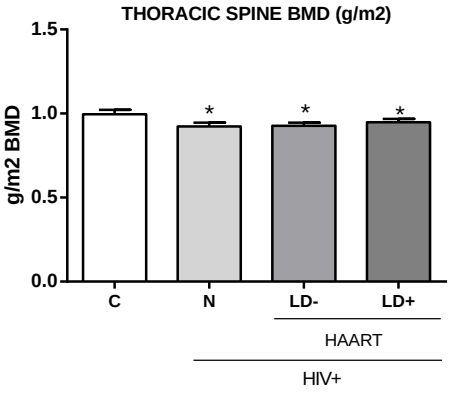


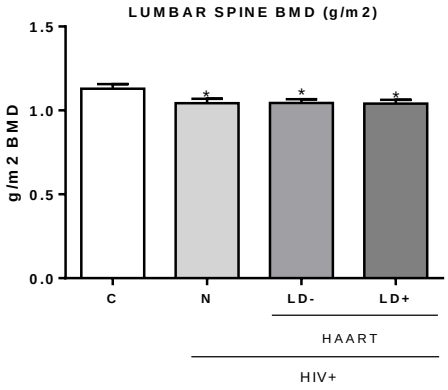
HIV-1-infected patients show altered bone parameters, even in the absence of cART

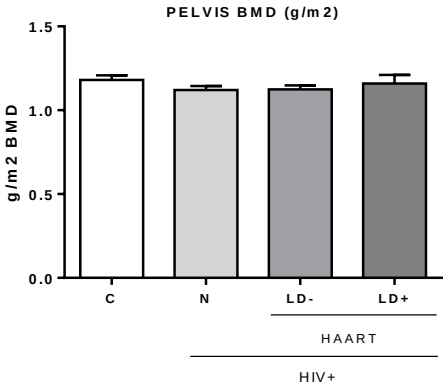






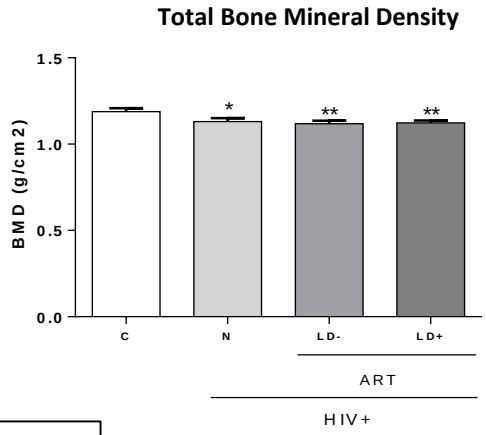


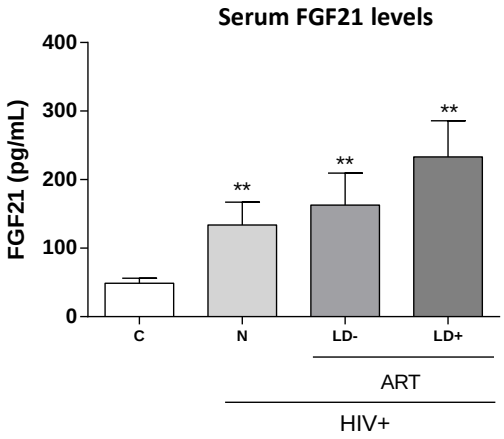


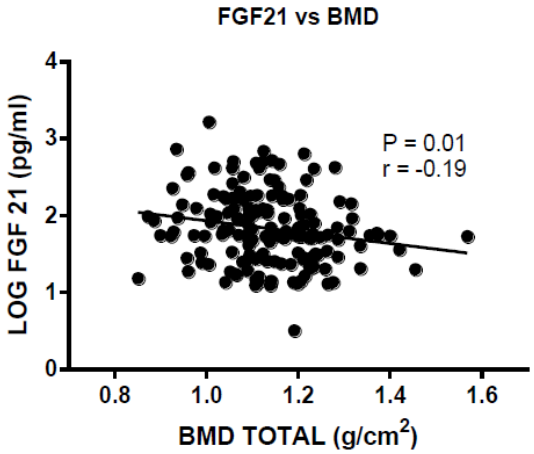


Gallego-Escuredo JM, et al. *Metabolism 2017*

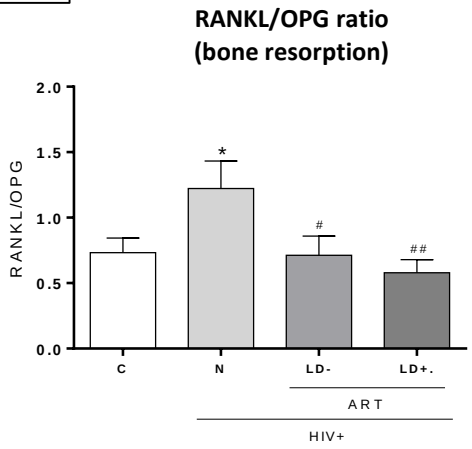
High FGF21 levels are associated with poor bone homeostasis in HIV-1-infected patients

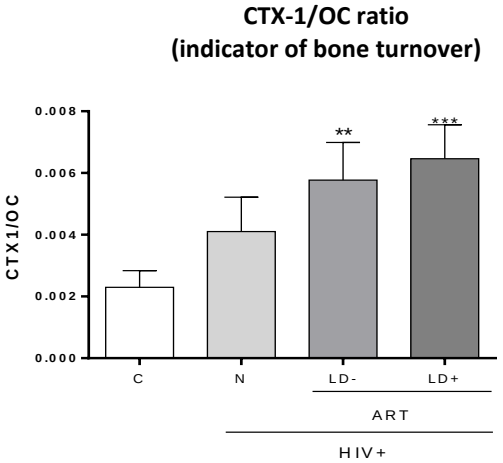


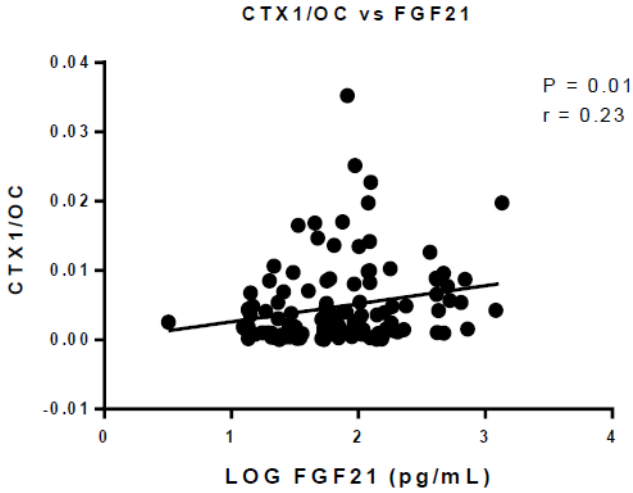




*P≤0.05 vs C
#P≤0.05 vs N





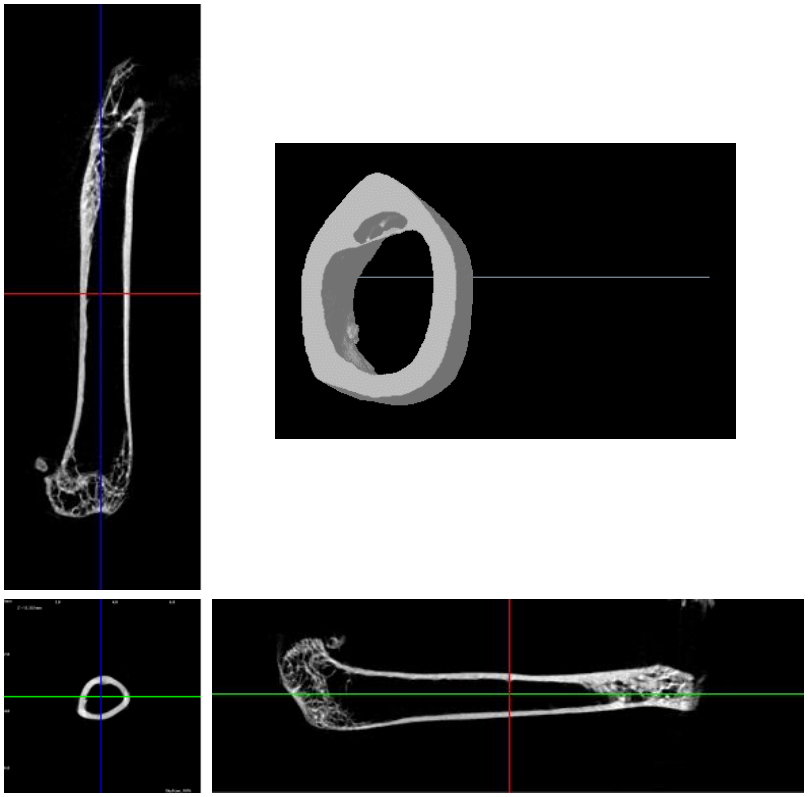


RANKL: receptor activator of NFκB ligand (osteoclastogenic regulator)
OPG: osteoprotegerin (bone turnover regulator)

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

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)

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

Wild-type
Tg26 +/-

The HIV-Tg26 mouse model largely mimics bone alterations in HIV-1 patients

J Villarroya et al. *unpublished*

TAKE HOME MESSAGES

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