miRNA, dicer and adipose tissue in HIV: novel pathways of fat regulation and organ specific cross-talk in HIV

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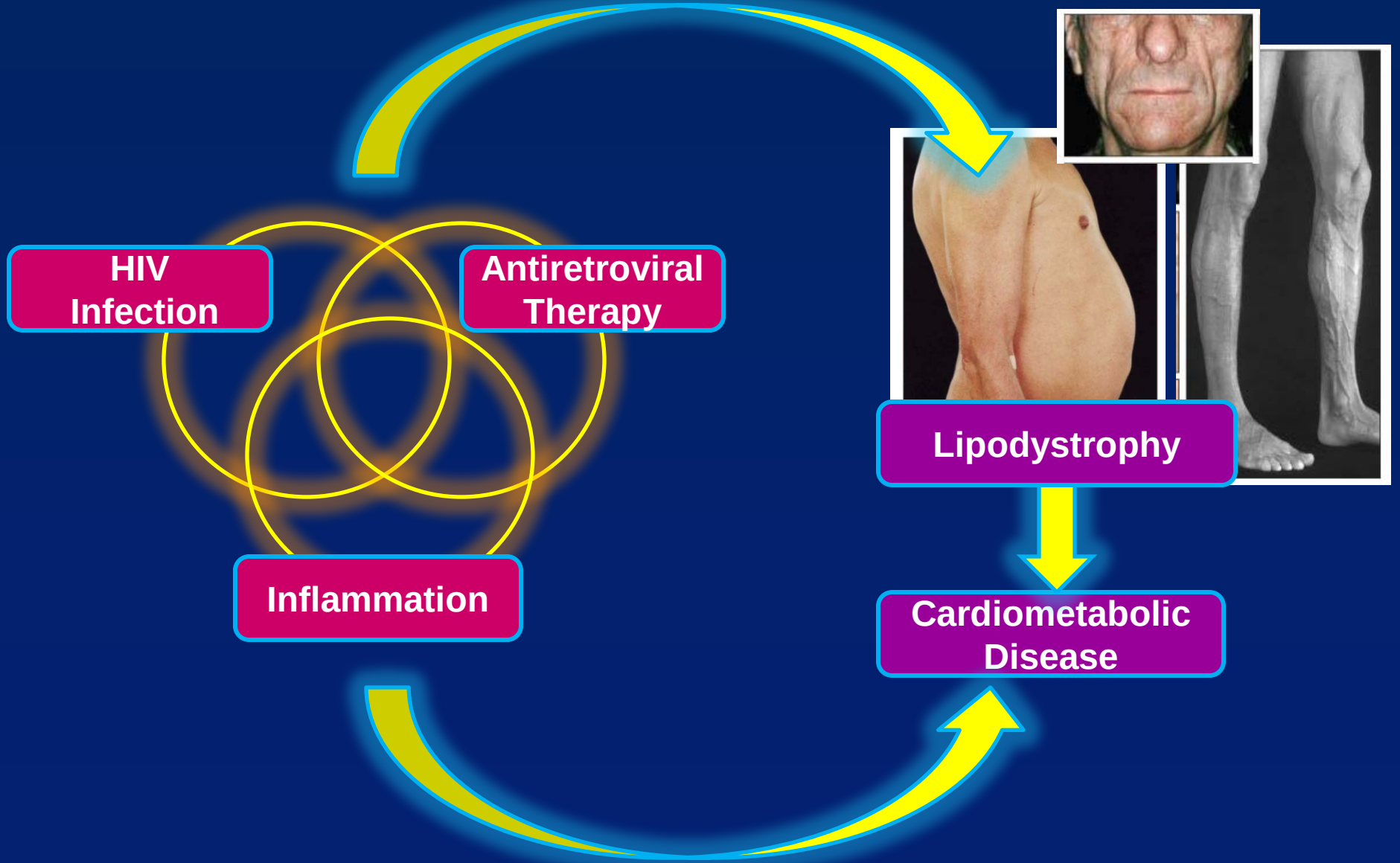
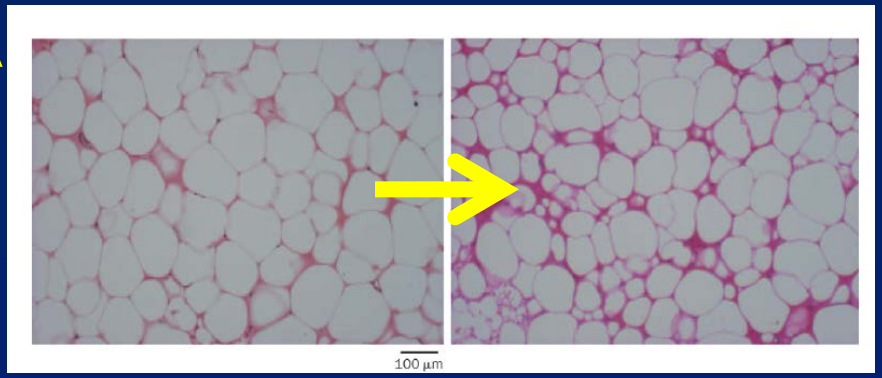
HIV patients are at increased risk for metabolic disease secondary to adipose dysfunction



Mechanisms of adipose redistribution and dysfunction in HIV

Viral Effects
(↓ DICER, ↓ PPARγ)

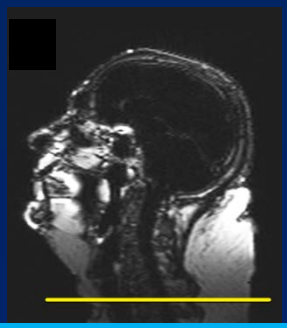
ART Effects
(↓ SREBP ↓Mito Fx)



Functional SC Fat

Dysfunctional SC Fat

?Compensatory

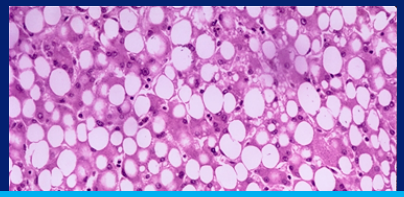


Protective Metabolic Impact:
Brown fat like

Overflow

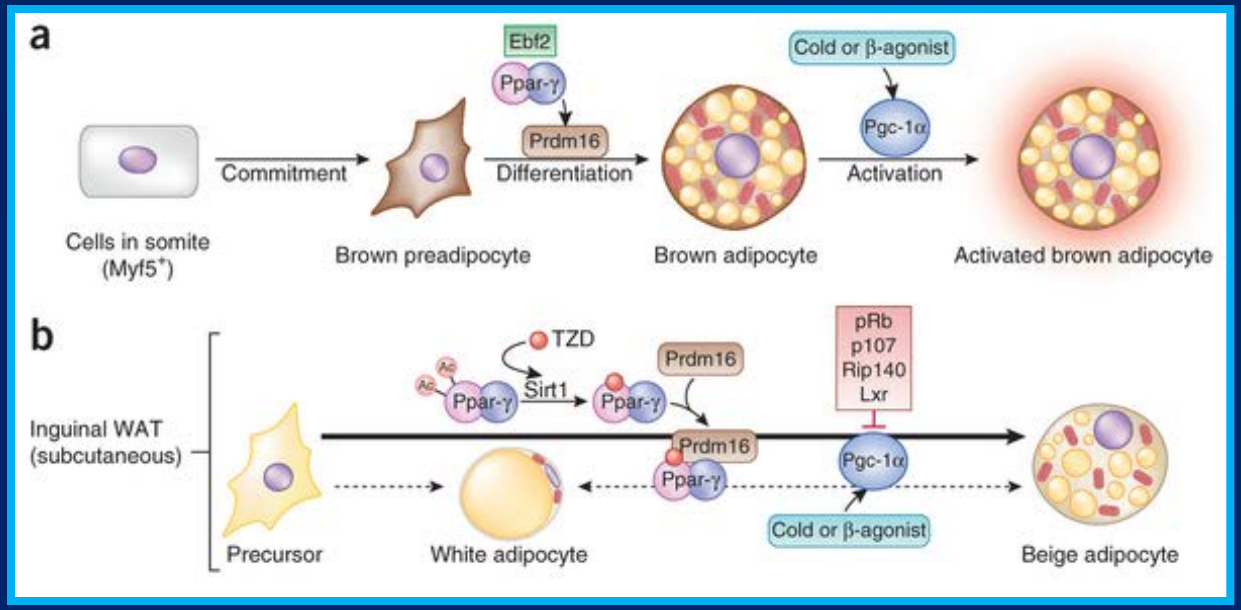
Adverse Metabolic Impact:
↑ VAT

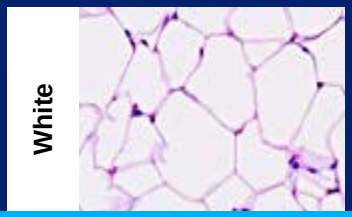
Overflow

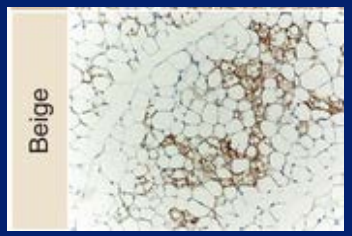


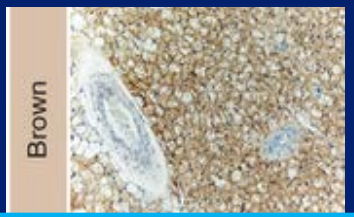
Adverse Metabolic Impact:
↑ NAFLD, IMCL,
cardiac steatosis

Brown or beige fat differentiation may be protective of metabolic disease









Reduced UCP1 Expression
Single Lipid Droplet
Few Mitochondria

Increased UCP1 Expression
Multiple Lipid Droplets
Many Mitochondria

Metabolic Benefit

Adapted from Harms and Seale, *Nature Medicine* 2013.

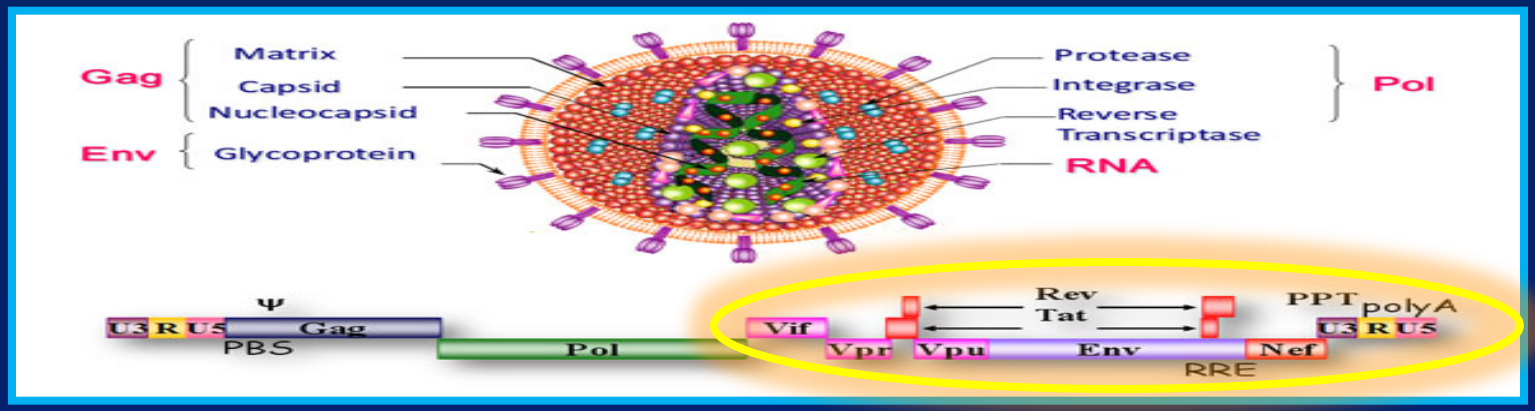
Mechanisms of Adipose Tissue Dysfunction

- Drug Effects

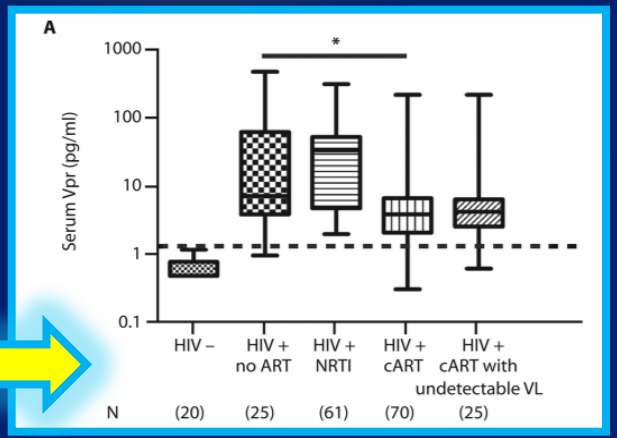
Protease Inhibitors inhibit SREBP and PPARγ in SC fat

Nucleoside Reverse Transcriptase Inhibitors inhibit mitochondrial DNA in SC fat

- Viral Effects

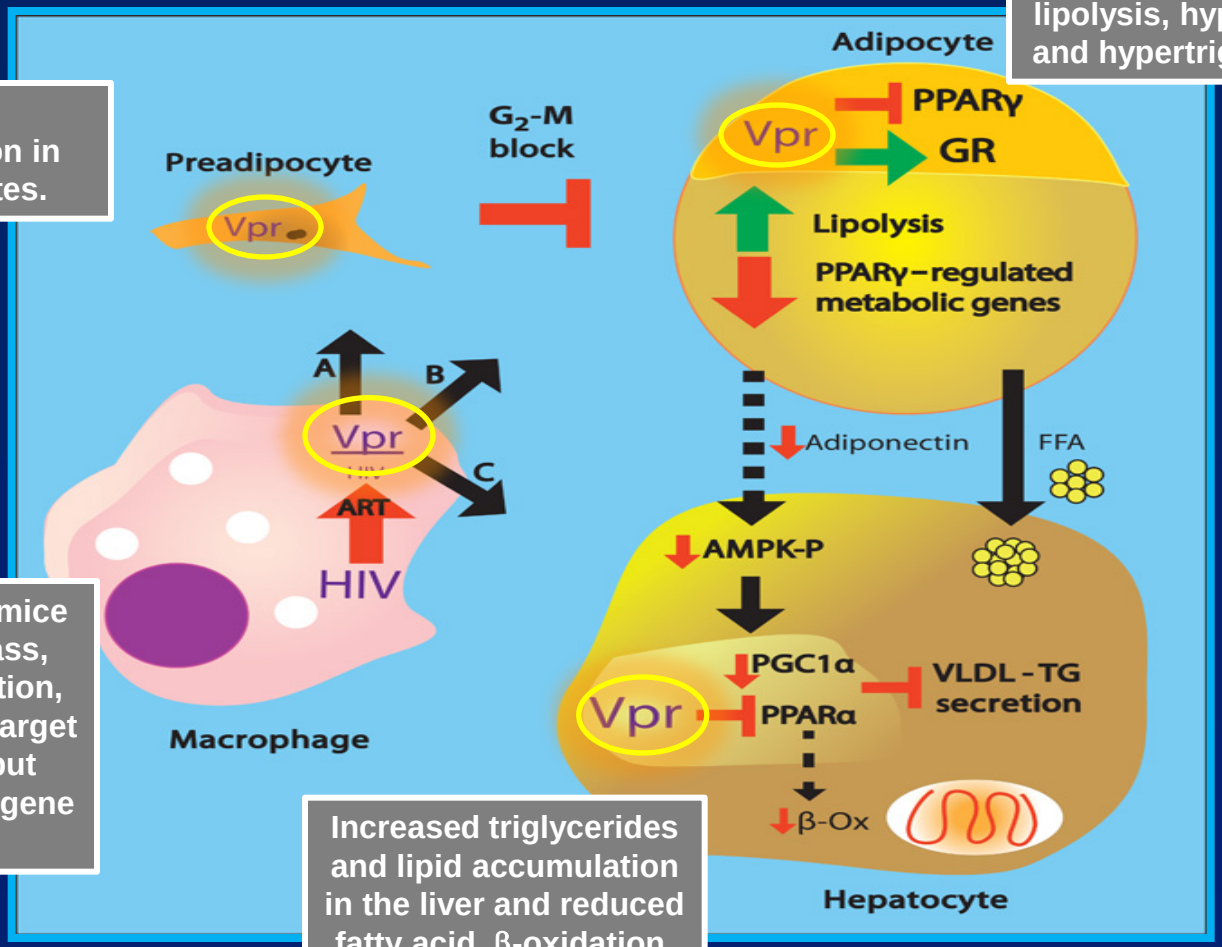


Vpr circulated in the blood of most individuals with HIV, including those on antiretroviral therapy (ART) with undetectable viral load.
(Agarwal, 2013)



HIV VPR mediated effects of adipose dysfunction through the PPARγ and the Glucocorticoid Receptor

In mouse models expressing the Vpr transgene in adipose tissues and liver or infused with synthetic Vpr:



Accelerated whole-body lipolysis, hyperglycemia, and hypertriglyceridemia

Blocked differentiation in preadipocytes.

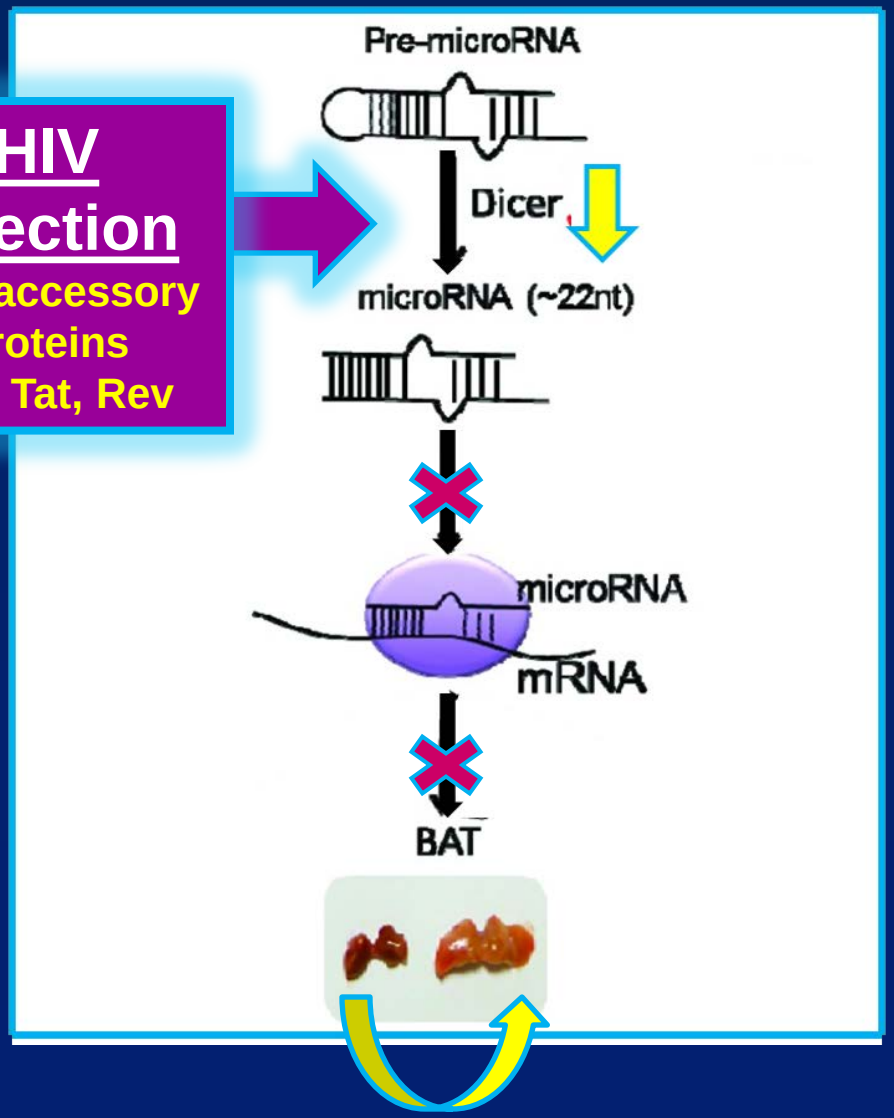
Fat depots in these mice had diminished mass, macrophage infiltration, and blunted PPARγ target gene expression but increased GR target gene expression.

Increased triglycerides and lipid accumulation in the liver and reduced fatty acid β-oxidation.

Agarwal, *Sci Trans Med* 2013
Agarwal, *Scientific Reports 2017*

The endoribonuclease *Dicer* has been linked to modulation of brown and white adipocyte differentiation

HIV Infection
viral accessory proteins Vpr, Tat, Rev



- *Dicer* is a cytoplasmic type III RNase that cleaves pre-micro RNAs into mature microRNAs.
- Downregulation of *Dicer* adversely affects multiple microRNAs that maintain adipocyte identity and play important roles in BAT and WAT differentiation.
- HIV has evolved to suppress *Dicer* expression and activity via viral accessory proteins (Vpr, Tat, and Rev) as a mechanism to enhance infectivity.

Adapted from Xu, Adipocyte 2015.

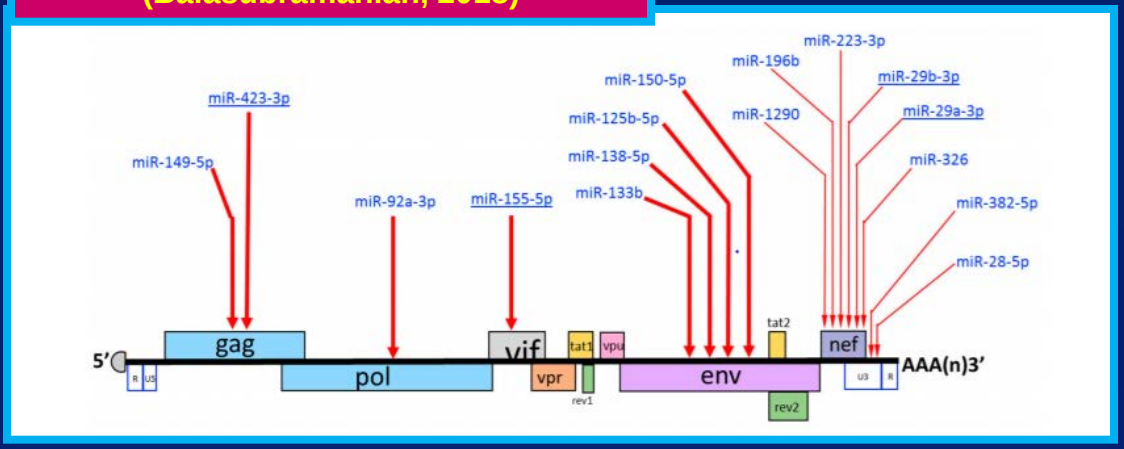
Multiple HIV viral accessory proteins suppress Dicer in order to promote HIV infectivity

Vpr → Triggers depletion of *Dicer* through a ubiquitin ligase complex which enhances macrophage infection (Klockow, 2013).

Tat → Evolved as a suppressor of RNA silencing by reducing *Dicer*'s ability to process dsRNA into siRNA (Bennasser, 2005).

Rev → Suppresses *Dicer* dependent RNAi processing involving the arginine rich motif (Ponia, 2013).

Binding sites for miRNAs which may target suppression of HIV replication (Balasubramanian, 2018)



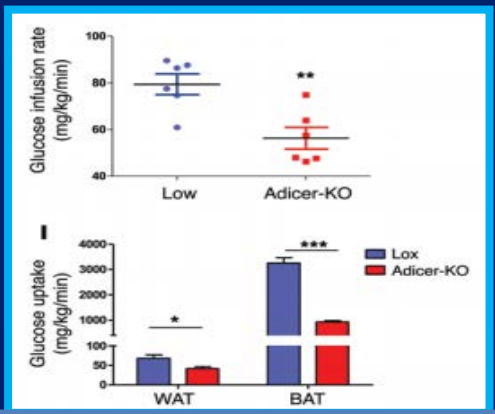
Vpr, Tat , Rev

Reduce Dicer

Reduced Suppression of HIV via Key miRNA's

Adipose specific Dicer-KO mice develop a lipodystrophy phenotype – resembling HIV lipodystrophy

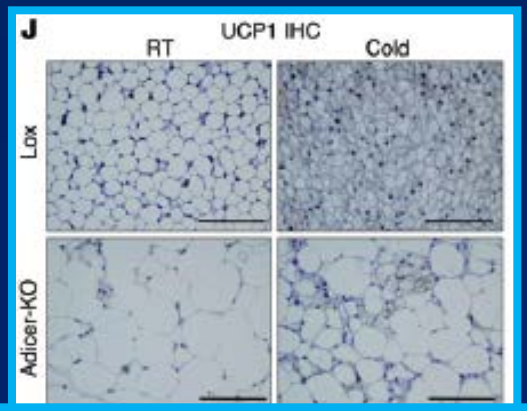
Loss of SC WAT and Increase DC Fat



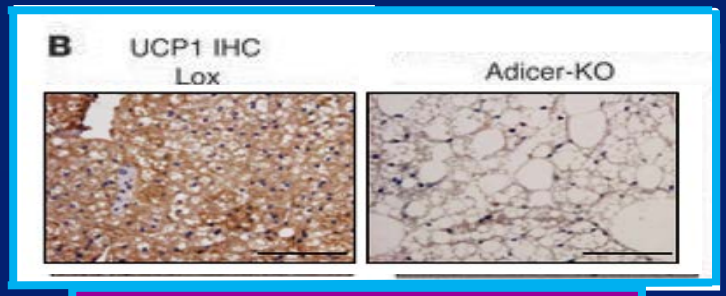
Severe IR,
↓ Gluc uptake in BAT and ↓ HG Suppression

Dicer

↓ miRNA, eg 365



Dysfunctional WAT:
↓ Beige Adipocytes on Cold Exposure; ↑MΦ



↓ Mitochondria and UCP1 in BAT

Adapted from
Mori et al. JCI 2014

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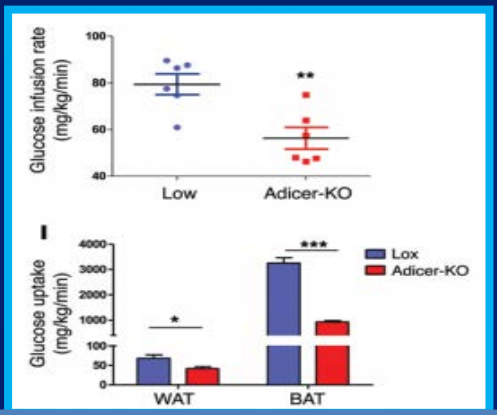
Loss of SC WAT and Increase DC Fat



Severe IR,
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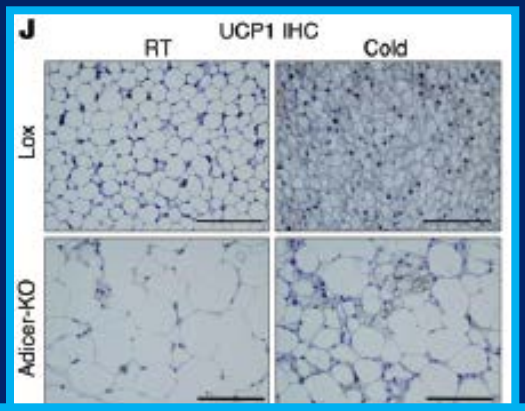
HIV Infection↓
viral accessory proteins
Vpr, Tat, Rev

↓Dicer

↓ miRNA, eg 365



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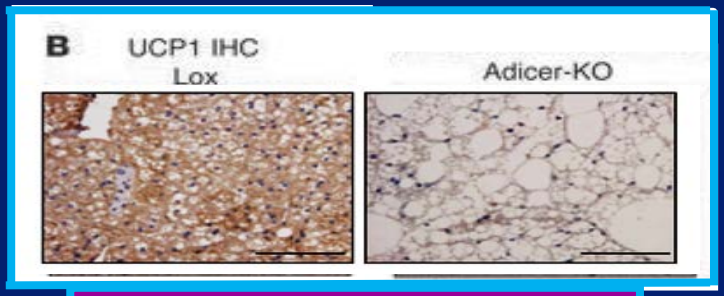

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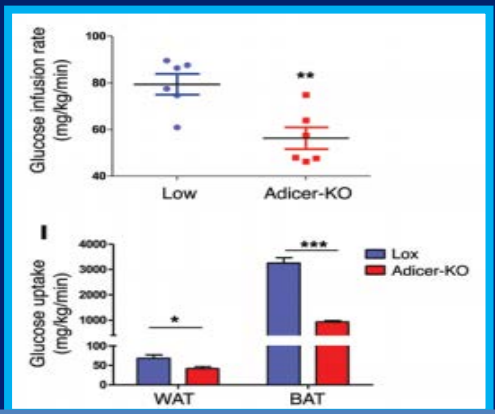
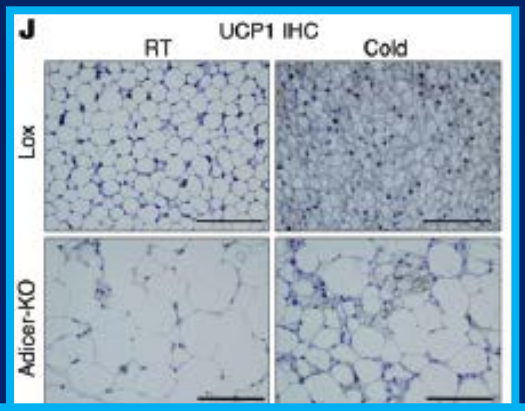


Severe IR,
↓ Gluc uptake in BAT and ↓ HG Suppression

Age ↓
Caloric Restriction↑

↓ *Dicer*

↓ miRNA, eg 365



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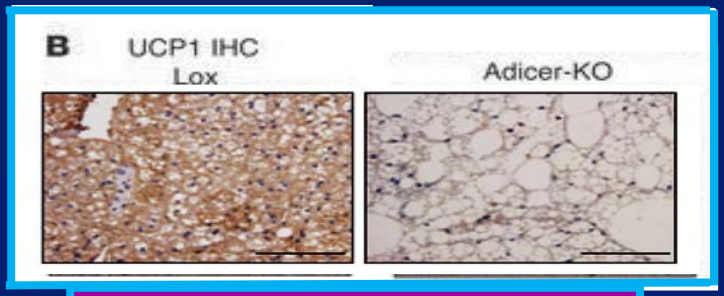


↓ Mitochondria and UCP1 in BAT

Adapted from
Mori et al. JCI 2014

Adipose specific dicer-KO mice develop a pronounced aging phenotype

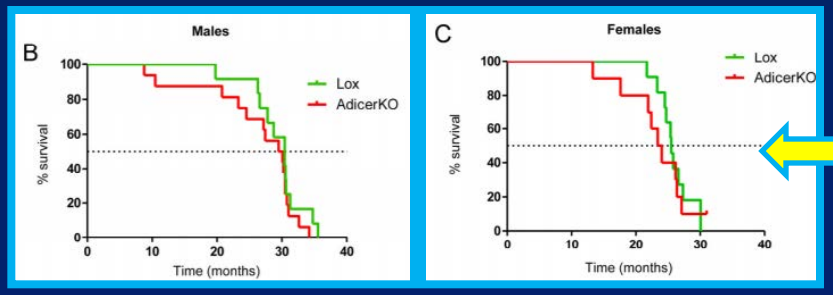
Reis, *Aging* 2016

Adipose specific dicer-KO mice develop a pronounced aging phenotype

Adipocyte Dicer KO mice with more signs of senescence (lipodystrophy, graying, hair loss)

Reis, *Aging* 2016

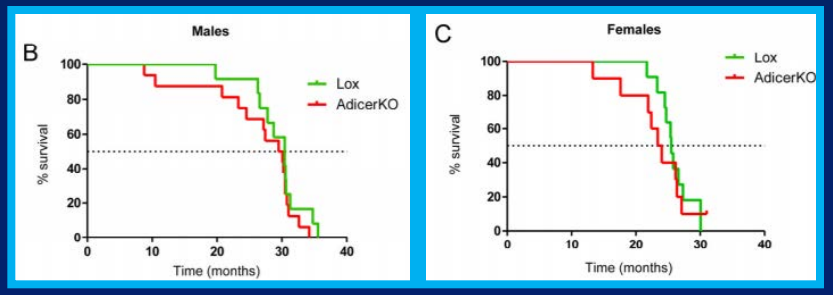
Adipose specific dicer-KO mice develop a pronounced aging phenotype

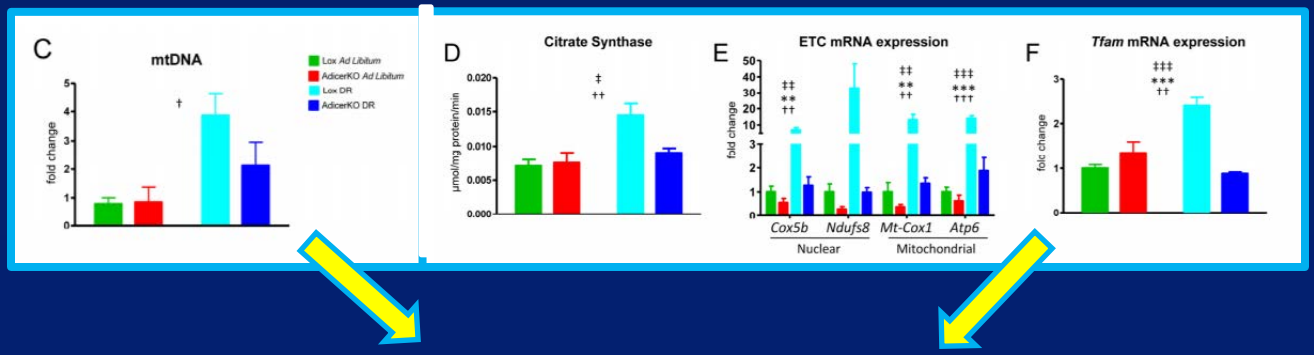


Adipocyte Dicer KO mice with increased premature mortality

Reis, *Aging* 2016

Adipose specific dicer-KO mice develop a pronounced aging phenotype

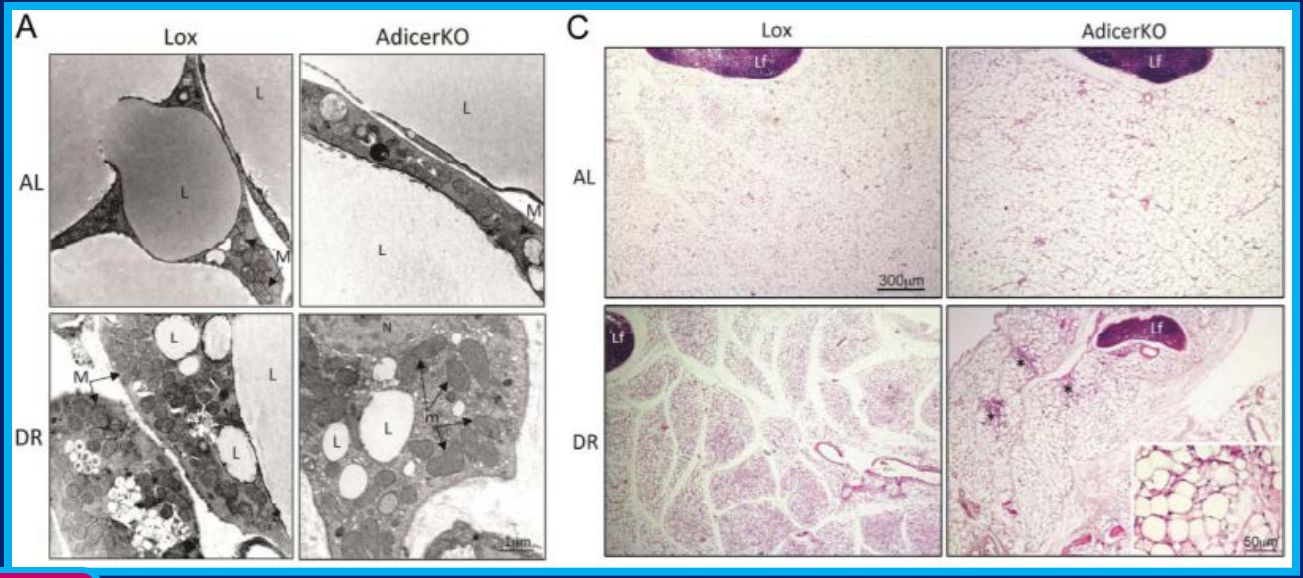




Adipocyte Dicer KO mice with reduced mitochondrial function and biogenesis.

Reis, *Aging* 2016

Adipose specific dicer-KO Mice are resistant to the metabolic benefits of dietary restriction at the level of the adipose



Mitochondria

	Control Mice vs. Adicer KO Mice
Ad Libitum	Less abundant mt in WAT of Adicer KO mice
Dietary Restriction	Healthy looking in controls, Fewer mt with highly irregular shapes and abundant cristae

Adipocytes

	Control Mice vs. Adicer KO Mice
Ad Libitum	Larger adipocytes and more unilocular in Adicer KO mice
Dietary Restriction	More multilocular and filled with cell infiltrate

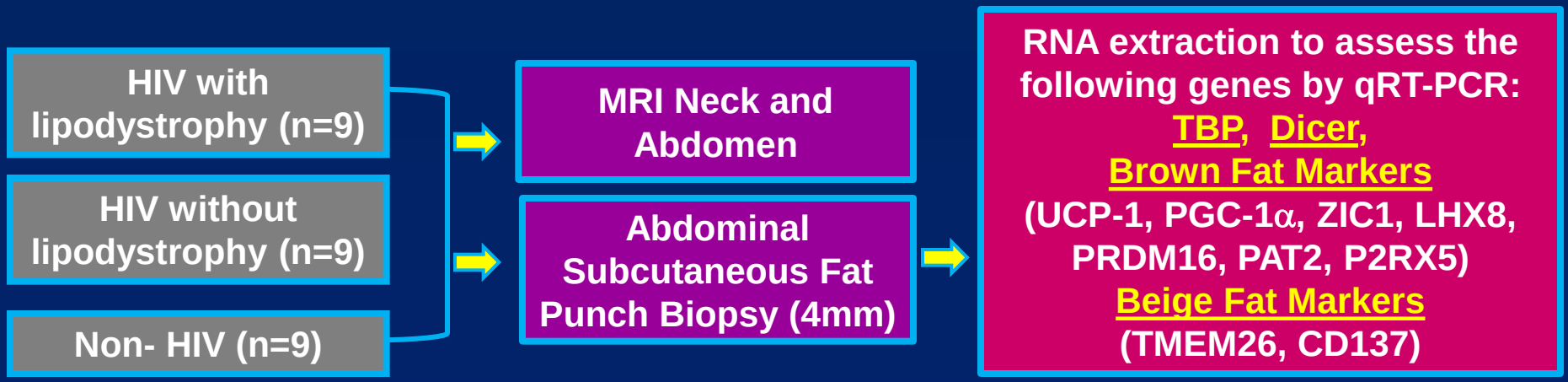
Reis, *Aging* 2016

Objective and Hypothesis

- We investigated *Dicer*, brown fat and beige fat gene expression in nonlipomatous abdominal subcutaneous (SC) adipose tissue of well-characterized HIV individuals with and without lipodystrophic changes in fat redistribution compared to non-HIV individuals.

- We hypothesized dysfunctional subcutaneous adipose tissue characterized by reduced Dicer and related BAT gene expression in HIV subjects with a lipodystrophy phenotype.

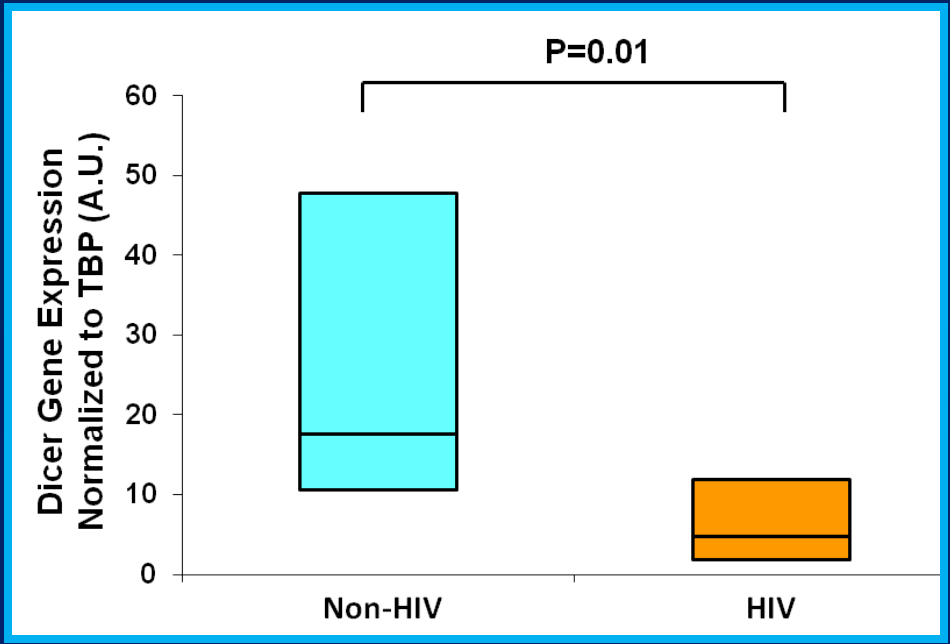
Study Participants and Design

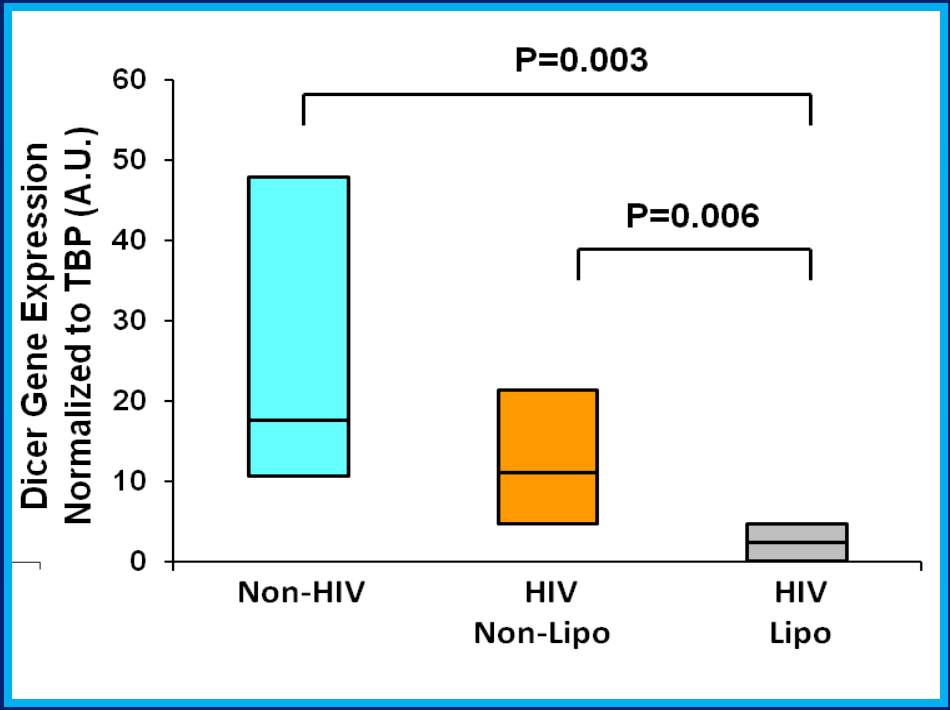


- **Inclusion criteria for all participants:** male gender, age18-60, BMI 18-35.0 kg/m^2.

- **Inclusion criteria for HIV participants**: stable ART regimen ≥ 12 months (lipodystrophy was determined on clinical exam based on presence or absence of dorsocervical adipose tissue [DCAT] accumulation)

- **Major exclusion criteria for all participants**: history of DM, use of glucocorticoids or GH therapies, cr >1.5 mg/dL,





Significant stepwise reduction in *Dicer* expression among Non-HIV, HIV Non-Lipo, and HIV Lipo.

Torriani and Srinivasa, *JCEM* 2016

Individuals with HIV lipodystrophy demonstrate reduced brown and beige fat gene expression in abdominal SC fat

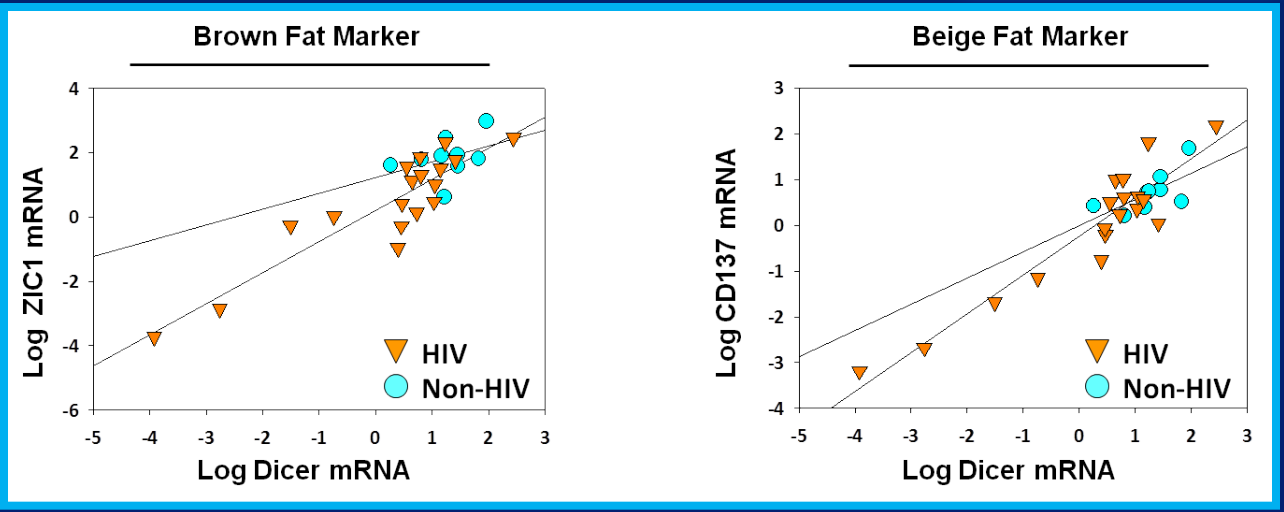
	Non-HIV (n=9)	HIV Non-Lipodystrophic (n=9)	HIV-Lipodystrophic (n=9)	P value HIV Lipo vs. Non-HIV	P value HIV Lipo vs. HIV Non-Lipo
Dicer	17.69 (10.72, 47.91)	11.20 (4.83, 21.45)	2.49 (0.02, 4.88)	**0.003**	**0.006**
Brown adipose tissue markers					
UCP1	0.17 (0.08, 0.28)	0.16 (0.07, 1.45)	0.01 (0.00, 0.20)	--	--
PGC1α	31.12 (7.14, 56.02)	11.71 (0.56, 33.12)	0.03 (0.00, 0.14)	**0.0006**	**0.002**
ZIC1	64.45 (39.43, 185.94)	37.14 (15.14, 138.59)	1.02 (0.06, 2.78)	**0.0006**	**0.004**
LHX8	0.23 (0.08, 0.47)	0.13 (0.06, 0.38)	0.01 (0.00, 0.13)	**0.009**	**0.03**
PRDM16	1.57 (0.23, 2.27)	0.51 (0.30, 1.45)	0.02 (0.00, 0.16)	**0.002**	**0.0008**
PAT2	0.31 (0.18, 0.49)	0.39 (0.28, 0.94)	0.04 (0.00, 0.27)	**0.02**	**0.008**
P2RX5	0.35 (0.17, 1.15)	1.99 (0.20, 4.50)	0.14 (0.00, 0.51)	0.12	**0.02**
Beige adipose tissue markers					
TMEM26	0.78 (0.50, 1.33)	2.54 (0.79, 3.68)	0.24 (0.01, 0.75)	0.07	**0.004**
CD137	5.17 (2.53, 8.61)	4.11 (2.13, 37.84)	0.17 (0.01, 2.00)	**0.006**	**0.008**

Gene expression values are normalized to TBP, results are expressed as ratios in arbitrary units

Torriani and Srinivasa, *JCEM* 2016

Reduced *Dicer* is associated with decreased brown and beige fat gene expression in abdominal SC fat in HIV

	Non-HIV (n=9)		HIV (n=18)	
	ρ	P-Value	ρ	P-Value
Brown adipose tissue markers				
UCP1	0.63	0.07	0.48	**0.04**
PGC1α	0.15	0.70	0.79	**0.0001**
ZIC1	0.42	0.26	0.86	**<0.0001**
LHX8	-0.08	0.83	0.67	**0.003**
PRDM16	0.67	**0.05**	0.84	**<0.0001**
PAT2	0.35	0.36	0.79	**<0.0001**
P2RX5	0.60	0.09	0.74	**0.0004**
Beige adipose tissue markers				
TMEM26	0.72	**0.03**	0.72	**0.0007**
CD137	0.78	**0.01**	0.82	**<0.0001**



Torriani and Srinivasa, *JCEM* 2016

HIV-specific parameters are related to *Dicer* and brown and beige fat gene expression in abdominal SC fat in HIV

	Duration HIV		CD4 Count		CD8 Count	
	r	P-Value	r	P-Value	r	P-Value
Dicer	-0.44	0.07	**0.55**	**0.02**	0.42	0.08
DCAT	0.41	0.09	-0.46	0.06	-0.36	0.14
miRNA-365	-0.42	0.08	-0.0006	0.99	0.16	0.53
Brown adipose tissue markers						
UCP1	-0.34	0.17	0.21	0.41	0.13	0.62
PGC1α	-0.47	**0.05**	0.57	**0.01**	0.58	**0.01**
ZIC1	-0.40	0.10	0.49	**0.04**	0.47	**0.05**
LHX8	-0.25	0.32	0.44	0.07	0.46	0.06
PRDM16	-0.36	0.14	0.61	**0.01**	0.50	**0.04**
PAT2	-0.38	0.12	0.59	**0.01**	0.48	**0.04**
P2RX5	-0.57	**0.01**	0.61	**0.01**	0.45	0.06
Beige adipose tissue markers						
TMEM26	-0.52	**0.03**	0.65	**0.004**	0.41	0.09
CD137	-0.51	**0.03**	0.53	**0.02**	0.42	0.08
Other metabolic markers						
DIO2	-0.55	**0.02**	0.51	**0.03**	0.43	0.08
Leptin	-0.42	**0.08**	0.49	**0.04**	0.50	**0.04**
HSP60	-0.62	**0.006**	0.50	**0.04**	0.48	**0.05**

Unpublished, 2018

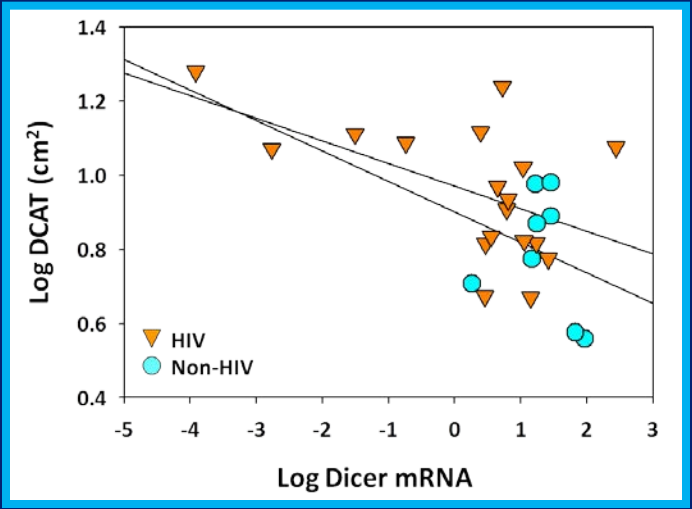
ART-specific parameters are related to reduced *Dicer* and brown and beige fat gene expression in SC fat in HIV

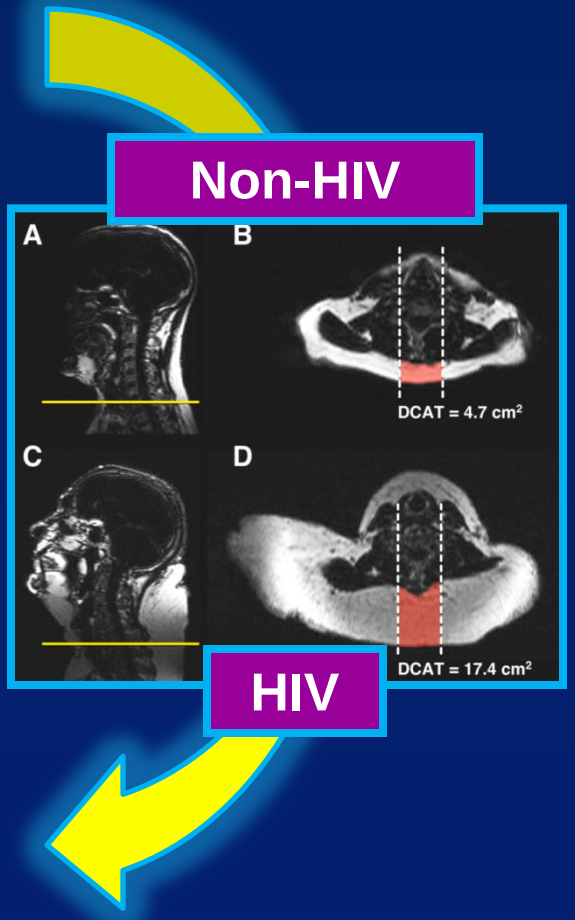
	Duration ART use		Duration NRTI use		Duration NNRTI use		Duration PI use	
	r	P-Value	r	P-Value	r	P-Value	r	P-Value
Dicer	-0.70	**0.001**	-0.37	0.21	-0.12	0.85	-0.71	**0.03**
DCAT	0.59	**0.009**	0.44	0.13	-0.17	0.79	0.80	**0.009**
miRNA-365	-0.44	0.07	-0.49	0.09	0.69	0.19	-0.49	0.18
Brown adipose tissue markers								
UCP1	-0.49	**0.04**	-0.19	0.53	-0.83	0.08	-0.15	0.70
PGC1α	-0.61	**0.007**	-0.31	0.31	-0.20	0.74	-0.73	**0.02**
ZIC1	-0.65	**0.004**	-0.32	0.28	-0.11	0.86	-0.85	**0.004**
LHX8	-0.75	**0.0003**	-0.56	**0.05**	-0.06	0.93	-0.70	**0.04**
PRDM16	-0.67	**0.003**	-0.29	0.34	-0.41	0.49	-0.74	**0.02**
PAT2	-0.75	**0.0004**	-0.44	0.13	-0.06	0.93	-0.74	**0.02**
P2RX5	-0.72	**0.0007**	-0.47	0.11	0.04	0.95	-0.72	**0.03**
Beige adipose tissue markers								
TMEM26	-0.72	**0.0007**	-0.39	0.19	0.11	0.86	-0.67	**0.05**
CD137	-0.62	**0.006**	-0.32	0.28	-0.10	0.87	-0.64	0.06
Other metabolic markers								
DIO2	-0.69	**0.002**	-0.46	0.11	-0.05	0.93	-0.73	**0.03**
Leptin	-0.60	**0.009**	-0.34	0.26	-0.18	0.77	-0.84	**0.004**
HSP60	-0.70	**0.0002**	-0.54	0.06	-0.03	0.97	-0.79	**0.01**

Unpublished, 2018

Increased DCAT accumulation is associated with reduced *Dicer* and brown and beige fat gene expression in abdominal SC fat in HIV

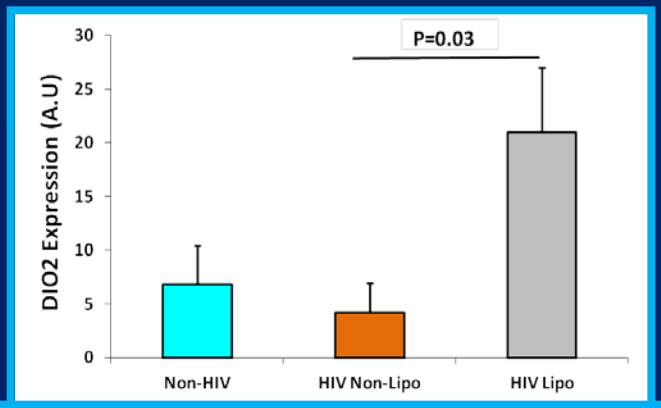
	Non-HIV (n=9)		HIV (n=18)	
	ρ	P-Value	ρ	P-Value
Dicer	-0.24	0.57	-0.50	**0.03**
Brown adipose tissue markers				
UCP1	-0.48	0.23	-0.36	0.14
PGC1α	0.43	0.29	-0.59	**0.01**
ZIC1	-0.55	0.16	-0.49	**0.04**
LHX8	-0.40	0.32	-0.57	**0.01**
PRDM16	-0.19	0.65	-0.71	**0.0009**
PAT2	-0.48	0.23	-0.71	**0.0009**
P2RX5	-0.50	0.20	-0.66	**0.003**
Beige adipose tissue markers				
TMEM26	-0.45	0.26	-0.63	**0.005**
CD137	0.17	0.69	-0.39	0.11



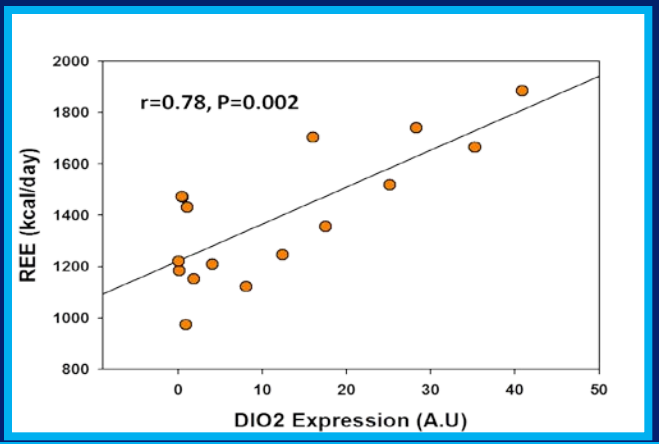


Torriani and Srinivasa, *JCEM* 2016

Lipomatous dorsocervical fat has brown fat features: increased expression of DIO2 and relates to increased EE



DIO2 expression was 3-fold higher in HIV Lipo vs. HIV non-Lipo



In addition to REE, DIO2 expression also relates to UCP-1 (r=0.77, P=0.002), PGC-1α (r=0.81, P=0.01), and CideA expression (r=0.66, P=0.03) in HIV.

Torriani, *JCEM* 2012

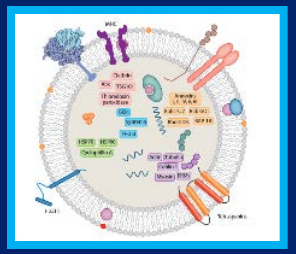
Adipose tissue major source circulating miRNA's: Assessing miRNA signature through serum exosomal profiling

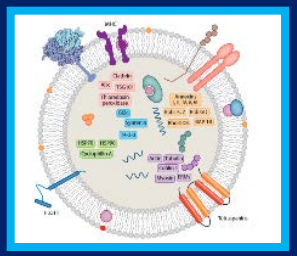
Serum Samples

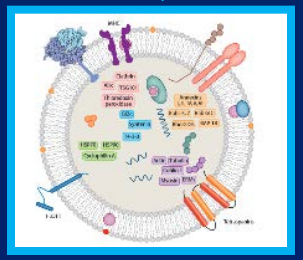
HIV with lipodystrophy (n=9)

HIV without lipodystrophy (n=9)

Non HIV (n=9)





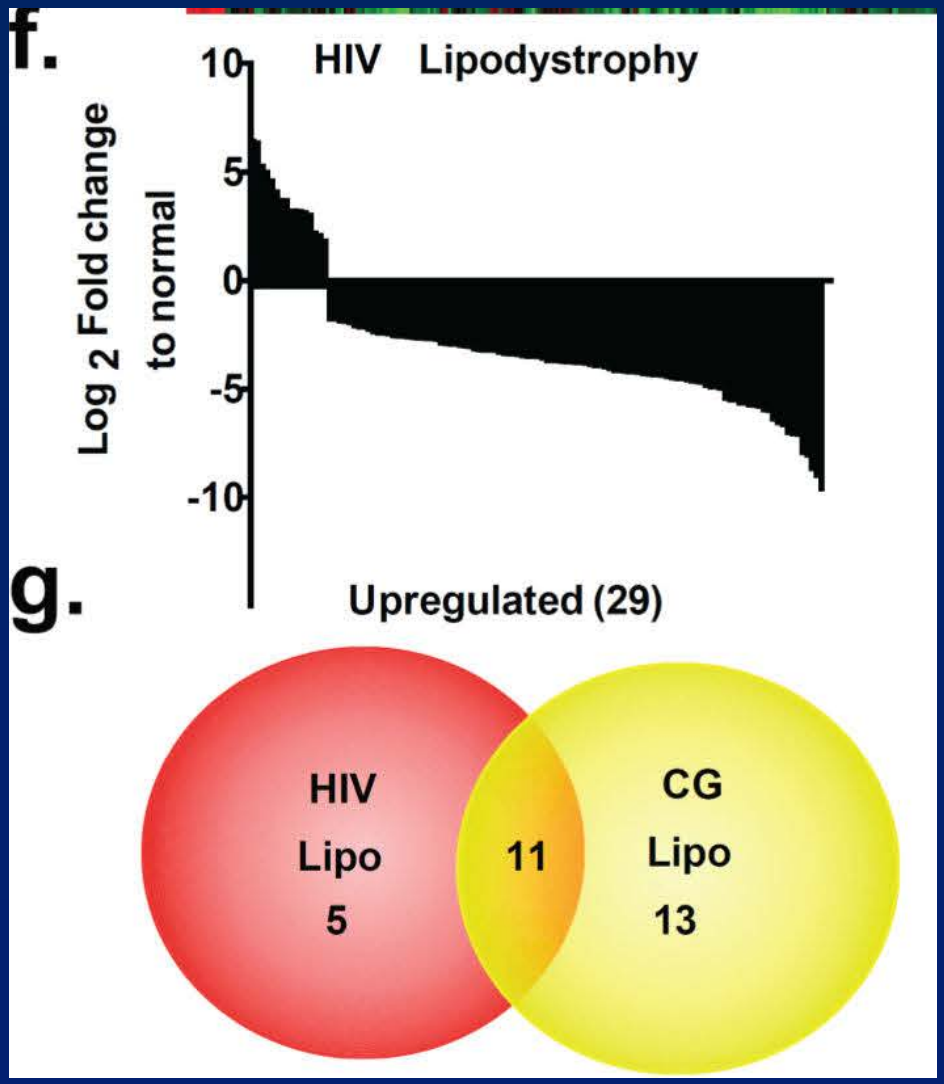


Exosome isolation (ultracentrigufation method)

Exosomal RNA isolation

qPCR-based miRNA profile

Overlapping miRNA Expression Pattern with CGL

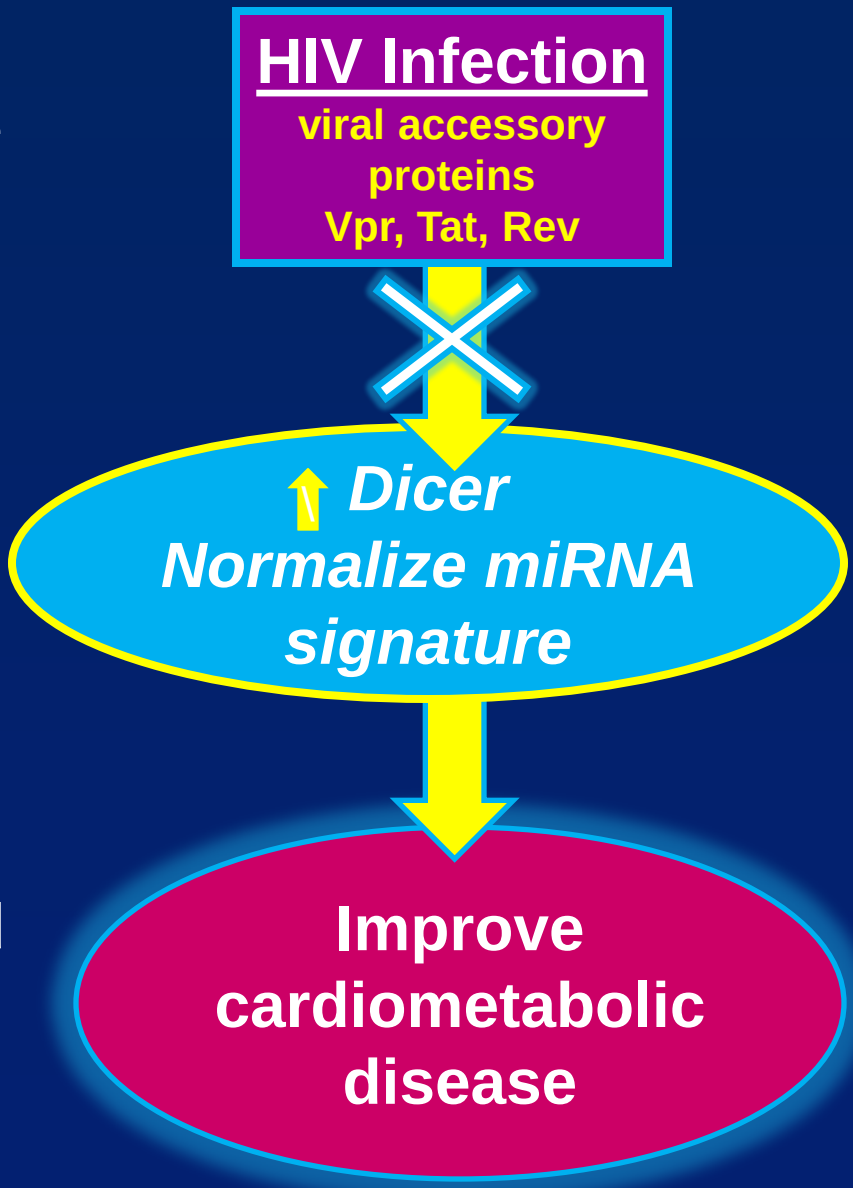


Thomou Nature 2017

Potential treatment strategy for adipose dysfunction and metabolic disease in HIV?

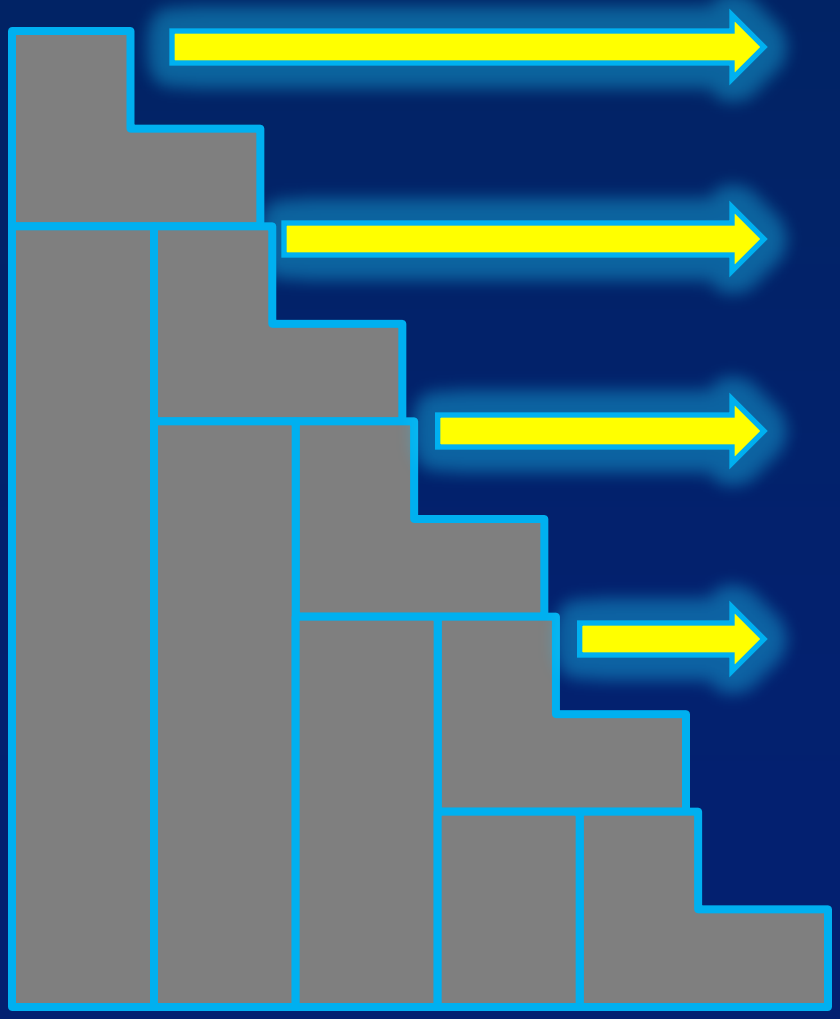
- Our results demonstrate dysfunctional SC adipose tissue marked by reduced *Dicer* which may limit capacity for adipose browning in HIV lipodystrophy and provide a novel mechanism for metabolic dysregulation.

- A potential therapeutic strategy to increase *Dicer* expression or modulate microRNAs may increase “browning” of WAT and improve cardiometabolic health in HIV lipodystrophy.



Summary

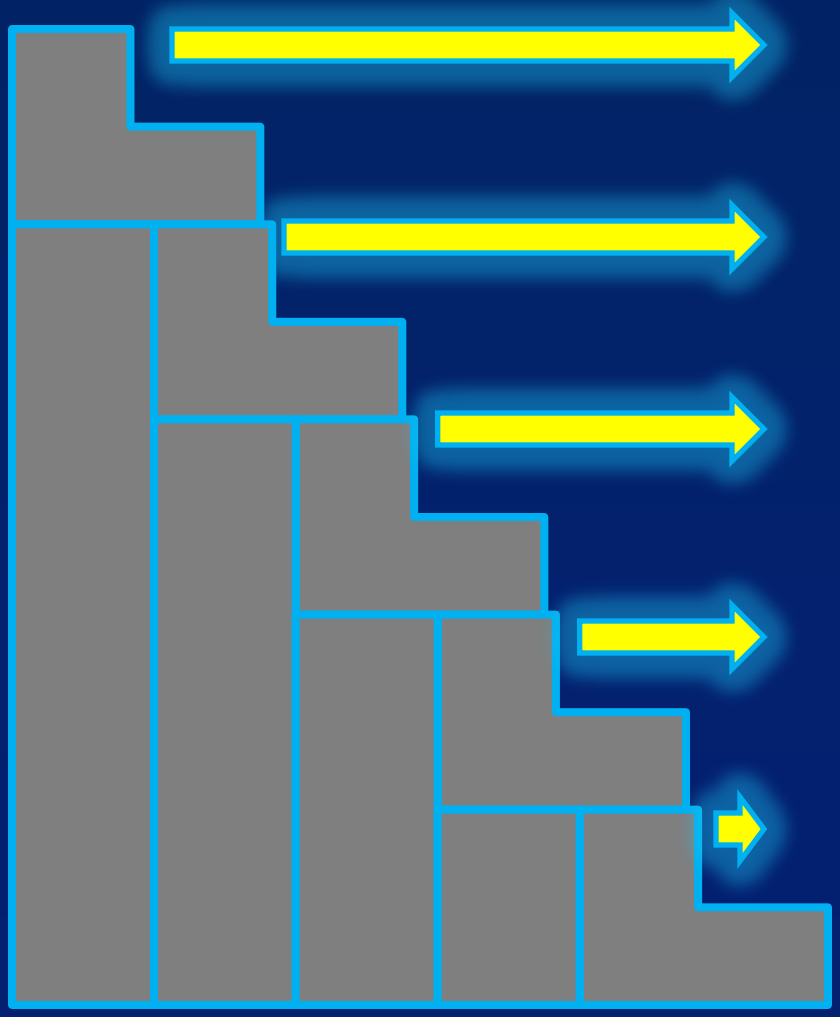
Data to Date Suggest:



- Individuals with HIV have adipose dysfunction, including reduced browning/beiging, which may contribute to metabolic risk.
- HIV viral accessory proteins mediate effects of adipose dysfunction in preclinical models
- In this way HIV may contribute to reduced Dicer, downregulation of brown and beige fat genes, and DCAT accumulation
- Data from the current study in HIV lipodystrophy aligns with evidence from animal studies using adipose specific Dicer KO mice.

Future Directions

What we still don't know:



- Which miRNAs are implicated in adipocyte differentiation and metabolic complications in relation to Dicer expression in HIV? These ongoing studies will help identify a comprehensive panel of panel of miRNAs unique to HIV and that are clinically relevant.

- What are potential treatment strategies that could be harnessed from this novel mechanism, e.g RNA based therapeutics

Funding and Collaborators

- Suman Srinivasa, M.D. (MGH Endocrinology)
- Martin Torriani, M.D. (MGH Radiology)
- Aaron Cypess, M.D. (NIH)
- CR Kahn, M.D. (Joslin Diabetes, Endocrinology)

Thank you!