

# Low levels of lipin mRNA expression in adipose tissue from HIV-infected patients with lipodystrophy

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## Introduction

Lipodystrophy (LD) is the major complication of highly antiretroviral therapy in HIV-infected patients, but its pathophysiology is not known. The lipin-deficient *lpl* mouse resembles human patients with generalized lipodystrophy, having genetically reduced adipose tissue throughout the body and insulin resistance. Recently, lipin has been shown to act as an endogenous substrate for the serine/threonine kinase PPAR $\alpha$  and C/EBP $\alpha$ , such that lipin deficiency leads to increased adipocyte differentiation *in vitro* and lipodystrophy *in vivo*. Here we investigate whether lipin expression in adipose tissue is altered in human HIV-associated lipodystrophy, and if not, whether lipodystrophy in HIV-infected patients with lipodystrophy is more severe than lipodystrophy in HIV-infected patients without lipodystrophy. Lipin has been shown to act in adipogenesis upstream of the key transcriptional regulators PPAR $\alpha$  and C/EBP $\alpha$ , such that lipin deficiency leads to impaired adipocyte differentiation *in vitro* and lipodystrophy *in vivo*.

## Methods

HIV-infected male patients without or with lipodystrophy (LD) (n=17 for each group) and 22 healthy controls without the metabolic syndrome participated in a cross-sectional study. All patients were on a nucleoside-analogue therapy. In the LD+ group 12 were receiving protease inhibitors (PI) and 9 non-nucleoside analogues (NNRTI). In the LD- group 9 patients were receiving PI and 3 NNRTI. Body composition were measured by DEXA scanning (Norland XR 36). Lipin mRNA levels were measured in subcutaneous abdominal and femoral-gluteal adipose biopsies by real-time PCR using primers to detect either total lipin expression levels, or expression of two lipin isoforms (Lipin-A and -B). The levels were normalized to the housekeeping gene 18S.

## Results

We found that Lipin mRNA (total mRNA and the isoforms Lipin-A and Lipin-B) was expressed in human adipose tissue. Lipin mRNA expression levels of both Lipin-total, Lipin-A and B were lower in HIV-patients with lipodystrophy compared to HIV-patients without lipodystrophy. Interestingly, Lipin mRNA levels were non significantly elevated in HIV-infected patients without lipodystrophy compared to healthy controls. Low lipin mRNA levels were associated with low limb fat mass and elevated levels of adipose tissue expression of IL-18, IL-18 and elevated plasma-LDL.

## Conclusion

Lipin mRNA levels in adipose tissue can distinguish HIV-patients with and without lipodystrophy. This suggest that individual differences to HAART with those patients having elevated lipin levels having a better prognosis with regard to development of lipodystrophy OR progression with a patient from a state without lipodystrophy to a state with lipodystrophy is associated with a reduction in initially high lipin levels.

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**Background:** Lipodystrophy is a major complication of HAART in HIV-infected patients, but the pathogenesis is still unclear. The lipin-deficient *lpl* mouse resembles human patients with generalized lipodystrophy, having genetically reduced adipose tissue throughout the body and insulin resistance. Recently, lipin has been shown to act as an endogenous substrate for the serine/threonine kinase PPAR $\alpha$  and C/EBP $\alpha$ , such that lipin deficiency leads to increased adipocyte differentiation *in vitro* and lipodystrophy *in vivo*. Here we investigate whether lipin expression in adipose tissue is altered in human HIV-associated lipodystrophy, and if not, whether lipodystrophy in HIV-infected patients with lipodystrophy is more severe than lipodystrophy in HIV-infected patients without lipodystrophy. Lipin has been shown to act in adipogenesis upstream of the key transcriptional regulators PPAR $\alpha$  and C/EBP $\alpha$ , such that lipin deficiency leads to impaired adipocyte differentiation *in vitro* and lipodystrophy *in vivo*.  
**Methods:** HIV-infected male patients without or with lipodystrophy (LD) (n=17 and 22 healthy controls, all men and age matched) participated in a cross-sectional study. HIV-infected patients were on successful and stable HAART. Biopsies were obtained from subcutaneous abdominal and femoral-gluteal adipose tissue after an overnight fast. Lipin mRNA expression were measured by real-time PCR and normalized to 18S. Body composition were measured by whole body DEXA scan. HIV-infected patients had reduced total-, abdominal, and limb fat mass compared with healthy controls, and patients with lipodystrophy had more pronounced fat loss, but not compared with patients without lipodystrophy. Parametric statistical methods were used.  
**Results:** Lipin mRNA was expressed in adipose tissue in all groups. However, significantly lower expression levels were found in HIV-patients with lipodystrophy compared to HIV patients without lipodystrophy in both adipose tissue compartments (abdominal region: Geometric mean 1.1029 (CI 0.78-1.54) vs. 2.061 (30-3.24),  $p < 0.005$ ; femoral-gluteal: 0.6820 (0.41-1.03) vs. 1.3600 (0.82-2.35),  $p = 0.010$ ). Low lipin mRNA levels were associated with low percentage of limb fat mass ( $r = -0.55$ ,  $p < 0.0001$ ) and high LDL-cholesterol ( $r = 0.41$ ,  $p < 0.016$ ).  
**Conclusion:** The findings of low levels of lipin mRNA in adipose tissue from HIV-infected patients with lipodystrophy and a strong correlation between lipin mRNA levels and limb fat mass and LDL-cholesterol suggest that lipin mRNA levels in adipose tissue may be an important parameter to monitor in HIV-infected patients. Further researches target to the treatment of HIV-associated lipodystrophy.

## Patients characteristics

	LD+	LD-	CONTROLS
Age (years)	53 (49-57)	53 (51-55)	51 (78-9)
Duration of HIV infection (years)	11.9 (9.1-14.2)	10.9 (9.2-13.4)	
HIV RNA (copies/ml)	43.8 (21-948.8)	23.3 (18.0-310.1)	
CD4 cell count (cells/ml)	554 (444-685)	616 (496-736)	
Duration of ART (months)	96.4 (83.1-110)	86.9 (48.9-84.8)	
BMI (kg/m <sup>2</sup> )	26.8 (17.2-24.1)*	23.1 (21.9-23.3)	23.9 (23.3-24.6)
WHV	0.55 (0.22-0.99)	0.51 (0.34-0.71)	0.53 (0.35-0.75)
Total fat mass (kg)	9.85 (8.04-11.7)**	14.8 (12.5-17.1)**	19.3 (17.40-21.9)
Total lean mass (kg)	55.4 (50.8-57.9)	55.8 (50.4-60.3)	55.7 (52.7-58.7)
Truncal fat mass (kg)	5.88 (4.48-7.27)**	8.02 (6.49-9.62)*	10.9 (9.28-11.8)
Proximal Truncal Fat (%)	57 (52.7-62.7)	53.0 (49.8-56.2)	53.2 (50.1-54.5)
Limb fat mass (kg)	3.19 (2.53-3.78)**	5.88 (5.3-6.4)**	8.4 (7.25-9.59)
Proximal Limb fat (%)	33.3 (28.7-37.7)**	43.5 (37.3-43.8)	42.3 (41.0-44.4)
Trunk:Limb ratio	1.90 (1.63-2.34)**	1.35 (1.18-1.52)	1.26 (1.17-1.36)
Cholesterol (mmol)	6.04 (5.07-7.01)*	5.03 (4.25-6.0)	5.6 (4.79-5.30)
LDL (mmol)	1.20 (1.01-1.58)	1.40 (1.17-1.83)	1.44 (1.31-1.57)
LDL (mmol)	3.09 (2.04-3.76)	3.21 (2.43-3.96)	3.33 (3.09-3.57)
Triglycerides (mmol)	2.68 (1.76-3.78)**	1.54 (1.27-1.81)	1.33 (0.94-1.54)
Glucose (mmol)	5.12 (4.71-5.52)	5.20 (5.05-5.58)	5.16 (5.00-5.30)
Insulin (mmol)	80.2 (71.2-90.4)	84.9 (71.2-101)	72.8 (61.7-86.8)
TNF- $\alpha$ mRNA abdominal AT	5.403 (70.8-15)**	2.001 (3.54-34)*	1.66 (1.35-2.05)
IL-8 mRNA abdominal AT	2.46 (1.05-5.78)**	0.300 (1.0-0.6)	0.200 (1.0-3.2)
IL-8 mRNA abdominal AT	0.89 (0.33-2.35)**	0.300 (0.7-0.8)	0.180 (0.2-0.9)
IL-18 mRNA abdominal AT	1.75 (1.10-2.77)	0.71 (0.49-1.01)	0.46 (0.3-0.9)

Data expressed as geometric mean (95% CI). Individual significantly different from healthy controls. (\*  $P < 0.01$ , \*\*  $P < 0.0001$ ). \* indicates significantly difference from healthy controls. (\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.0001$ ).

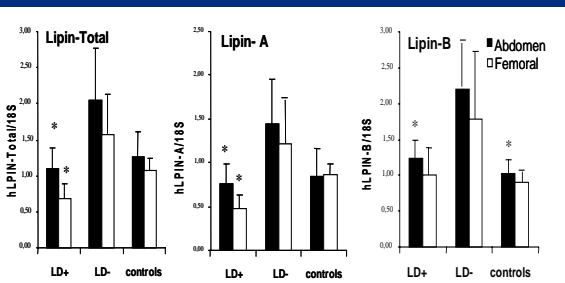
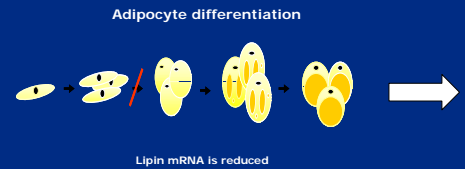


Fig. 1 Lipin mRNA expression (total, Lipin-A, Lipin B) in subcutaneous abdominal and femoral-gluteal adipose tissue in HIV-patients with lipodystrophy (LD+), HIV-patients without LD (LD-) and healthy controls.

\* denotes significant difference from LD-;  $P < 0.05$ .

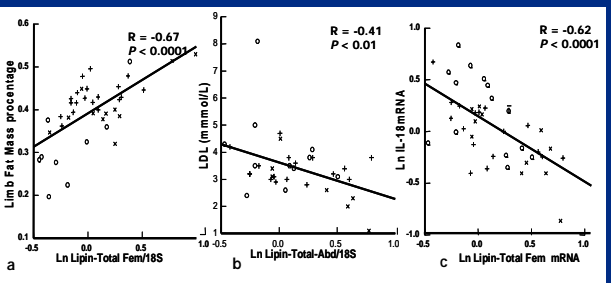


Fig. 2. Correlation between Lipin mRNA in adipose tissue and limb fat mass (a), LDL-cholesterol (b) and interleukin-18 (c).  $\circ$  Indicates patients with lipodystrophy;  $\times$  indicates patients without lipodystrophy; + indicates controls.

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The lipin-deficient *fld* mouse resembles human patients with generalized lipodystrophy, having dramatically reduced adipose tissue stores throughout the body and insulin resistance. Lipin is a nuclear protein with unknown function.

Lipin has been shown to act in adipogenesis upstream of the key transcriptional regulators PPAR $\gamma$  and C/EBP $\alpha$ , such that lipin deficiency leads to impaired adipocyte differentiation *in vitro* and lipodystrophy *in vivo*

	LD	LD	CONTROLS
	(N = 17)	(N = 17)	(N = 22)
Age [years]	53.4(9.1)	50.7(10.8)	51.7(8.9)
Duration of HIV-infection (years)	11.9 (9.71-14.2)	10.9 (8.27-13.6)	
HIV RNA (copies) <sup>†</sup>	43.6 (21.9-86.6)	23.3 (18.0-310.1)	
CD4 cell count (cells/l)	504 (444-569)	678 (490-797)	
Duration of ART (months)	56.4 (49.3-53.0)	60.9 (48.9-64.6)	
WHR	0.95 (0.92-0.99)	0.93 (0.90-0.97)	0.93 (0.91-0.96)
Total fat mass (kg)	8.85 (8.06-11.3) <sup>***</sup>	14.8 (12.5-17.1) <sup>***</sup>	19.9 (17.60-21.9)
Total lean mass (kg)	55.4 (52.8-57.9)	55.6 (50.9-60.3)	56.7 (53.7-59.7)
Truncal fat mass (kg)	5.86 (4.60-7.27) <sup>***</sup>	8.02 (6.43-9.62) <sup>***</sup>	10.57 (9.38-11.8)
Percent Truncal fat (%)	57.7 (52.7-62.7)	53.0 (49.6-56.5)	53.2 (50.1-54.5)
Limb fat mass (kg)	3.15 (2.59-3.72) <sup>***</sup>	5.88 (5.13-6.64) <sup>***</sup>	8.41 (7.55-9.28)
Percent Limb fat (%)	33.0 (28.6-37.4) <sup>***</sup>	40.5 (37.3-43.6)	42.3 (41.0-44.4)
Trunk:Limb ratio	1.93 (1.53-2.34) <sup>***</sup>	1.35 (1.18-1.52)	1.26 (1.17-1.36)
Cholesterol (mmol/l)	6.04 (5.07-7.01) <sup>***</sup>	5.03 (4.25-5.86)	5.05 (4.79-5.30)
HDL (mmol/l)	1.20 (1.0-1.58)	1.40 (1.17-1.63)	1.44 (1.31-1.57)
LDL (mmol/l)	3.99 (3.20-4.78)	3.21 (2.43-3.99)	3.33 (3.09-3.57)
Triglycerides (mmol/l)	2.64 (1.75-3.53) <sup>***</sup>	1.54 (1.27-1.81)	1.13 (0.94-1.32)
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IL-6 mRNA abdominal AT	0.98(0.57-1.71) <sup>***</sup>	0.38(0.27-0.55)	0.34(0.22-0.54)
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