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ScienceDirect
Metabolism Clinical and Experimental 56 (2007) 1518–1526
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

Human subcutaneous adipose tissue *LPIN1* expression in obesity, type 2 diabetes mellitus, and human immunodeficiency virus–associated lipodystrophy syndrome

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Received 31 December 2006; accepted 20 June 2007

Abstract

The aim of this study was to analyze *LPIN1* adipose tissue gene expression levels in 3 clinical insulin-resistant conditions—obesity, type 2 diabetes mellitus, and human immunodeficiency virus (HIV)-associated lipodystrophy—and its relationship with adipogenic and inflammatory markers. Subcutaneous adipose tissue samples were obtained from 2 cohorts: 98 subjects with different degrees of adiposity and with or without the presence of type 2 diabetes mellitus and 37 HIV-infected patients. Real-time polymerase chain reaction was used to measure gene expression of *LPIN1* and adipogenic (*PPARγ*, *SREBP1c*) and inflammatory markers (*IL6*, *TNFα*, *TNFR1*, and *TNFR2*). *LPIN1* messenger RNA expression levels were significantly lower in the obese group ($P = .002$), were similar in type 2 diabetes mellitus patients and control subjects ($P = .211$), and were significantly higher in HIV-infected patients ($P < .001$). *LPIN1* messenger RNA levels positively correlated with insulin sensitivity in all subjects. Moreover, an inverse correlation with proinflammatory cytokines was observed.

1. Introduction

Obesity is a major risk factor for insulin resistance, type 2 diabetes mellitus, heart disease, dyslipidemia, and many other chronic diseases. Paradoxically, lipodystrophy, a condition involving body fat redistribution (central adiposity and peripheral fat wasting), is associated with morbidity normally found in obesity [1]. Both pathologies are characterized by metabolic abnormalities such as hyperlipidemia and insulin resistance.

LPIN1 gene is an important determinant of adipose tissue differentiation and fat cell function, found to participate before *PPARγ* during adipocyte differentiation [2]. It was first described as the mutated gene in the mouse model for fatty liver dystrophy (*fld*) [3]. Mice carrying mutations in the *fld* gene have features of human lipodystrophy [4]. In mouse models, lipin (*LPIN1* protein) absence or inactivity can cause lipodystrophy; but enhanced adipose tissue-specific *LPIN1* levels in transgenic mice promotes obesity and insulin sensitivity [5]. Lipin is phosphorylated in response to insulin by the mammalian target of rapamycin (a Ser/Thr kinase), although the effects of this phosphorylation are unknown [6]. The mammalian target of rapamycin seems to have evolved as a sensor of nutrient availability, and it is involved in insulin-stimulated glucose disposal and directs cellular

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0026-0495/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2007.06.019

growth and proliferation [6]. Whether lipin is a primary regulator of these functions remains to be studied.

Recently, lipin homolog molecular function has been identified as a phosphatidate phosphatase (PAP) in *Saccharomyces cerevisiae*, the enzyme responsible for the synthesis of 1,2-diacylglycerol from phosphatidate acid (PA), involving *LPIN1* directly in the synthesis of triglyceride in the adipocyte [7]. This activity is almost lost in *fld/fld* mice; therefore, *LPIN1* is a major phosphatidate phosphatase (PAP) in vivo in several tissues [8]. Donkor et al [9] showed that *LPIN1* accounts for all of the PAP type 1 activity in white and brown adipose tissue in mice [9]. Moreover, the tissue *LPIN1* expression patterns observed in mice and humans are similar.

Lipin activity is not affected by the phosphorylation state of the protein. Conversely, changing subcellular localization (soluble or membrane bound) is a major mechanism for controlling lipin function. Because insulin increases triacylglycerol (TAG) synthesis but decreases the membrane-bound lipin form (the more active form in TAG synthesis), perhaps changes in lipin localization have roles related to different functions of PA not involving TAG synthesis [8]. Phosphatidate acid is a lipid that could act as a second messenger in pathways that regulate cellular size and proliferation, insulin sensitivity, and glucose homeostasis [10,11].

Regarding human obesity, very few reports on human *LPIN1* gene expression have been performed, suggesting a positive association with insulin sensitivity [12-14] and a negative one with body mass index (BMI) [13,14]. On the other hand, human immunodeficiency virus (HIV)-infected patients who developed lipodystrophy had lower *LPIN1* messenger RNA (mRNA) levels than those without lipodystrophy, in line with findings in the *fld* animal model [15]. Likewise, inflammation has been claimed as being a common disturbance participating in the insulin resistance state observed in obesity, type 2 diabetes mellitus, and HIV-associated lipodystrophy syndrome (HALS), with a key role for a hyperactivity of the tumor necrosis factor (TNF) α system, raised in part by the adipose tissue [16,17].

Here we further investigated the adipose tissue expression of the human *LPIN1* gene in 3 clinical insulin-resistant conditions—obesity, type 2 diabetes mellitus, and HIV-associated lipodystrophy—and its relationship with adipogenic and inflammatory markers.

2. Methods

2.1. Subjects

2.1.1. Obesity and type 2 diabetes mellitus cohort

A group of 98 subjects was recruited at the University Hospital Joan XXIII (Tarragona, Spain). All were white and reported that their body weight had been stable for at least 3 months before the study. They had no systemic disease other than obesity or type 2 diabetes mellitus, and all were free of any infections in the month before the study. Liver and renal diseases were specifically excluded by biochemical workup.

Table 1A
Obesity study: characteristics of the population

	Control subjects (n = 24)	Obese subjects (n = 22)
Age (y)	55.83 ± 15.48	47.23 ± 12.90 *
Sex (female-male)	7:17	17:5 **
BMI (kg/m^2)	25.20 ± 2.90	44.24 ± 11.26 ***
WHR	0.92 ± 0.09	0.95 ± 0.11
Insulin (μIU/mL)	3.77 ± 2.64	7.32 ± 4.92 *
Fasting glucose (mmol/L)	5.56 ± 0.91	5.57 ± 0.77
Cholesterol (mmol/L)	5.09 ± 1.22	5.27 ± 0.74
Glycerol (μmol/L)	383.38 ± 171.74	324.76 ± 163.72

* $P < .05$.
** $P < .01$.
*** $P < .001$.

Obesity was considered positive if BMI was higher than 30 kg/m^2 according to the World Health Organization criteria [18]. With this criterion, there were 24 nonobese and 22 obese nondiabetic subjects for the obesity study (Table 1A). Patients were classified as having type 2 diabetes mellitus according to the American Diabetes Association criteria, with a stable metabolic control in the previous 6 months defined by stable glycated hemoglobin values. According to these criteria, there were 52 type 2 diabetes mellitus subjects. Pharmacological treatment of the patients with type 2 diabetes mellitus was as follows: insulin, 25%; oral hypoglycemic agents, 66.6%; statins, 58.3%; fibrates, 10.6%; and blood pressure–lowering agents, 53.8%. No patient was on thiazolidinedione therapy.

We compared the 22 nondiabetic obese with the 26 type 2 diabetes mellitus obese subjects for the study of type 2 diabetes mellitus, matched for BMI (Table 2A).

The hospital's ethics committee approved the study, and informed consent was obtained from all participants.

2.1.2. HALS cohort

Thirteen HIV-positive patients receiving highly active antiretroviral therapy (HAART) without lipodystrophy (HIV$^+$ HALS$^-$) and a group of 24 HIV-positive patients who developed lipodystrophy under HAART therapy (HIV$^+$ HALS$^+$) were recruited by the Internal Medicine Service of the Hospital Universitari Joan XXIII.

Lipodystrophy was defined by severe fat wasting from the face, buttocks, limbs, and upper trunk with or without central adiposity, buffalo hump, or breast hypertrophy in women according to previously defined criteria [19,20].

As a control group for the HIV-positive cohort, 21 subjects with a mean BMI of 24.66 ± 2.66 kg/m^2 were recruited by the endocrinology service of the University Hospital Joan XXIII (Tarragona, Spain). All subjects were white and reported that their body weight had been stable for at least 3 months before the study. Exclusion criteria included the following: (1) clinically significant hepatic, neurologic, or other major systemic disease, including malignancy; 2) history of drug or alcohol abuse, defined as >80 g of alcohol intake per day in men and >40 g of alcohol intake per day in women, or serum transaminase activity more than twice the normal upper limit;

(3) an elevated serum creatinine concentration; (4) an acute major cardiovascular event in the previous 6 months; and (5) acute illnesses and current evidence of acute or chronic inflammatory or infective diseases.

The hospital's ethics committee approved the study and informed consent was obtained from all participants.

2.2. Anthropometric measurements

Body mass index was calculated as weight (in kilograms) divided by height (in meters) squared. Waist circumference was measured midway between the lowest rib margin and the iliac crest. Hip circumference was determined as the widest circumference measured over the greater trochanter. Waist-to-hip ratio (WHR) was calculated accordingly.

2.3. Insulin sensitivity study

In HIV-positive patients (n = 37), insulin sensitivity (S_I) and glucose effectiveness were measured using the frequently sampled intravenous glucose tolerance test. In brief, the experimental protocol started between 8:00 and 8:30 AM after an overnight fasting. A butterfly needle was inserted into an antecubital vein, and patency was maintained with a slow saline drip. Basal blood samples were drawn at −30, −10, and −5 minutes, after which glucose (300 mg/kg body weight) was injected over 1 minute starting at time 0 and insulin (Actrapid; Novo, Bagsvaerd, Denmark; 0.03 U/kg) was administered at time 20. Additional samples were obtained from a contralateral antecubital vein during 3 hours as previously described [21].

2.4. Analytical methods

Blood samples were drawn from each subject before breakfast, between 8:00 and 9:00 AM, after an overnight rest in bed. Plasma and serum samples were stored at −80°C until analytical measurements were performed, except for glucose and glycated hemoglobin, which were determined immediately after blood was drawn.

Serum glucose was measured with a glucose oxidase method using a glucose analyzer YSI 2300 STAT Plus (YSI, Yellow Springs, OH). Lipid profile (triglycerides, total cholesterol, and high-density lipoprotein cholesterol) was measured by usual enzymatic methods.

Soluble TNF receptor 1 (sTNFR1) and 2 (sTNFR2) were determined by solid phase enzyme immunoassay with amplified reactivity (Bio Source Europe, Nivelles, Belgium). The detection limit was 50 ng/mL for sTNFR1 and 0.1 ng/mL for sTNFR2; and the intra- and interassay coefficients of variation (CVs) were <7% and <9%, respectively. The sTNFR1 assay does not cross-react with sTNFR2. TNFα does not interfere with the assay. Levels of plasma interleukin 6 (IL6) were measured by the highly sensitive quantitative sandwich enzyme immunoassay technique with the Human IL6 Quantikine HS ELISA Kit (R&D Systems, Oxon, UK). The mean of the minimum detectable concentration was 0.039 pg/mL. Intra- and interassay CVs were <9.8% and <11.2%, respectively. Plasma high-sensitivity C-reactive protein was measured by a highly sensitive immunonephelometry kit (Dade Behring, Marburg, Germany). Serum adiponectin concentrations were measured by radioimmunoassay (Linco Research, St Charles, MO). The intra- and interassay CVs were <5%. Serum insulin was measured by radioimmunoassay (Coat-A-Count Insulin; DPC, Los Angeles, CA). Intraassay CV was 6.6%.

2.5. Adipose tissue samples

Adipose tissue samples were obtained from subcutaneous abdominal depots. In the obese and type 2 diabetes mellitus study, samples were obtained during elective abdominal surgical procedures (gastric bypass operation, cholecystectomy, or surgery for abdominal hernia). All patients had fasted overnight; and at the beginning of surgery, 2 to 4 g of subcutaneous fat tissue was removed from each proband and immediately introduced in RNALater (Sigma-Aldrich, St Louis, MO). In the HALS study, 1 to 2 g of subcutaneous fat tissue was removed by scalpel from each proband, immediately frozen in liquid nitrogen, and stored at −80°C until RNA extraction.

2.6. Total RNA isolation and reverse transcription

Total RNA was extracted from 400 to 500 mg of frozen subcutaneous adipose tissue using RNeasy Lipid Tissue Midi Kit (Qiagen Science, Germantown, MD) following manufacturer's instructions. Total RNA was quantified by absorbance measurement, and its purity was assessed by the OD_{260}/OD_{280} ratio.

One microgram of RNA was retrotranscribed to complementary DNA (cDNA) using the Reverse Transcription System (Promega, Madison, WI) in a final volume of 20 μL containing 1× reverse transcriptase buffer (10 mmol/l Tris-HCl [pH 9.0], 50 mmol/L KCl, 0.1% Tritón X-100), 1 mmol/L of each deoxy nucleotide tri-phosphate (dNTP), 1 U/μL Recombinant RNAsin Ribonuclease Inhibitor, 18 U/μg RNA AMV Reverse Transcriptase, and 0.5 μg/μg RNA of random primers. The cDNA reaction was incubated for 15 minutes at 25°C followed by 60 minutes at 42°C and heated for 5 minutes at 95°C.

2.7. Real-time quantitative PCR

2.7.1. Primers

Primer pairs were spanning exon-exon conjunctions and were therefore mRNA/cDNA specific. The following primers were used: 5′-tttccacgtccgctttggg-3′ and 5′-gtggccaggtgcataggg-3′ for *LPIN1*; 5′-ctatggagttcatgcttgtg-3′ and 5′gtactgacatttattt3′ for *PPARγ*; 5′-aaggtgaagtcggcgcgg-3′ and 5′-atcggggctggcaggg-3′ for *SREBP1c*; 5′-gagcactgaaagcatgatcc-3′ and 5′-gctggttatctctcagctcca-3′ for *TNFα*; 5′-cggtggaagtccaagctcta-3′ and 5′-tctaggctctgtggcttgtg-3′ for *TNFR1*; 5′-ggaaactcaagcctgcactc-3′ and 5′-ggatgaagtcgtgttggaga-3′ for *TNFR2*; and 5′-cggtacatcctcgacgg-3′ and 5′-tgatgattttcaccaggc-3′ for *IL6*. The housekeeping genes used to normalize gene

Table 1B
Obesity study: gene expression levels (arbitrary units)

	Control subjects (n = 24)	Obese subjects (n = 22)
LPIN1	3.13 ± 2.31	1.54 ± 1.18 *
PPARγ	4.58 ± 4.21	6.81 ± 8.17
SREBP1c	4.14 ± 4.32	2.44 ± 3.04
IL6	2.36 ± 1.48	24.64 ± 24.21 **
TNFα	0.18 ± 0.15	0.67 ± 0.69 **
TNFR1	3.95 ± 2.80	2.27 ± 1.32 ***
TNFR2	5.24 ± 3.64	6.64 ± 4.35

* $P = .002$.
** $P < .01$.
*** $P < .05$.

expression were β-actin 5′-ggacttcgagcaagagatgg-3′ and 5′-agcactgtgttggcgtacag-3′ and cyclophilin A 5′-caaatgctg-gacccaacac-3′ and 5′-gcctccacaatattcatgccttctt-3′.

LPIN1 primers do not differentiate the α or β isoforms previously described [22]. All primers were synthesized by Sigma (Sigma-Genosys, Haverhill, United Kingdom).

2.7.2. Real-time PCR

Gene expression analysis was performed on a LightCycler Instrument (Roche Diagnostics, Basel, Switzerland) using the SYBR green fluorescence method. Quantification of 2 μL of the cDNA was performed on a volume of 20 μL of a mixture containing 0.3 μmol/L of primers (except for β-actin, *TNFα*, *TNFR1*, and *TNFR2*, which were 0.5 μmol/L, and for *LPIN1*, which was 0.2 μmol/L) and 2 μL of LC-FastStart DNA Master SYBR green I (Roche) on a volume of 20 μL. The final concentration of $MgCl_2$ was adjusted for each gene.

The purity of each amplified product was confirmed by melting curve analysis, and detection of the fluorescent signal was adjusted to avoid primer-dimer detection.

2.7.3. Results analysis

For each sample, the derived gene quantification was calculated from an external standard curve, created with serial dilutions of a cloned PCR fragment from the respective gene, using LightCycler Software version 3.5 (Roche).

Adipose tissue expression levels of each gene were normalized using β-actin (obesity and type 2 diabetes mellitus cohort) and both β-actin and cyclophilin A (HALS cohort). Statistical analysis showed no differences in gene expression for these housekeeping genes among the studied groups.

2.8. Statistical analysis

Sample size was calculated to achieve a difference in logarithm of *LPIN1* mRNA mean levels between the studied groups of 0.3 or greater with a confidence level of 95% and a statistical power of 80%.

Statistical analysis was performed by using the SPSS/PC+ statistical package (version 13.5 for Windows; SPSS, Chicago, IL). Data are expressed as mean value ± SD.

Differences in concentrations and in clinical or laboratory parameters between groups were compared by using either an independent-samples *t* test or analysis of variance when appropriate. Variables that did not have a Gaussian distribution were logarithmically transformed to perform statistical analysis or were analyzed with nonparametric tests. Differences in sex and in retroviral use between studied groups were analyzed by Pearson/Fisher χ^2 test. The relation between variables was tested using Pearson/Spearman correlation analysis and stepwise multiple linear regression analysis. Statistical significance occurred if a computed 2-tailed probability value was less than .05.

3. Results

3.1. Obesity and type 2 diabetes mellitus studies

3.1.1. Subcutaneous adipose tissue gene expression in obese subjects

The main anthropometric and analytical characteristics of the nondiabetic cohort are shown in Table 1A. Obese patients were slightly younger than nonobese subjects, and they were predominantly women.

The expression of *LPIN1*, *PPARγ*, and *SREBP1c* in subcutaneous adipose tissue in nonobese and obese subjects is shown in Table 1B. *LPIN1* mRNA expression levels were significantly lower in the obese group ($P = .002$). *SREBP1c* mRNA levels of obese subjects show a trend toward being lower than that in nonobese subjects, and no differences were found in *PPARγ* mRNA expression between the studied groups.

3.1.2. Subcutaneous adipose tissue gene expression in patients with type 2 diabetes mellitus

The main anthropometric and analytical characteristics of the population selected for the study of subcutaneous gene expression in type 2 diabetes mellitus (obese subjects) are described in Table 2A. The groups were matched for sex, BMI, and WHR.

LPIN1 subcutaneous adipose tissue mRNA expression (Table 2B) showed no differences between type 2 diabetes mellitus patients and control subjects ($P = .211$).

3.1.3. Correlation and regression analysis

We performed a linear correlation analysis between the studied genes and anthropometric and analytical data. The

Table 2A
Type 2 diabetes mellitus study: characteristics of the population

	Subjects without diabetes (n = 22)	Patients with type 2 diabetes mellitus (n = 26)
Age (y)	47.23 ± 12.90	56.42 ± 11.87 *
Sex (female-male)	17:5	18:8
BMI (kg/m^2)	44.24 ± 11.26	41.61 ± 9.40
WHR	0.95 ± 0.11	0.92 ± 0.07
Insulin (μIU/mL)	7.32 ± 4.92	9.27 ± 4.12
Fasting glucose (mmol/L)	5.57 ± 0.77	8.96 ± 2.50 **
Cholesterol (mmol/L)	5.27 ± 0.74	5.10 ± 1.24
Glycerol (μmol/L)	324.76 ± 163.72	351.20 ± 217.34

* $P < .05$.
** $P < .001$.

Table 2B
Type 2 diabetes mellitus study: gene expression levels (arbitrary units)

	Subjects without diabetes (n = 22)	Patients with type 2 diabetes mellitus (n = 26)
LPIN1	1.54 ± 1.18	1.91 ± 1.24
PPARγ	6.81 ± 8.17	10.80 ± 9.08
SREBP1c	2.44 ± 3.04	1.49 ± 1.11
IL6	24.64 ± 24.21	20.33 ± 26.30
TNFα	0.67 ± 0.69	0.54 ± 0.56
TNFR1	2.27 ± 1.32	5.05 ± 3.61 *
TNFR2	6.66 ± 4.35	5.36 ± 3.14

* $P = .001$.

independence of the associations was further evaluated by linear multiple regression analysis, considering all subjects (n = 98) as a whole, controlling for sex and presence of diabetes. In bivariate analysis, *LPIN1* mRNA expression levels correlated positively with age ($r = 0.296$, $P = .003$) and *PPARγ* mRNA expression ($r = 0.223$, $P = .044$) and negatively with BMI ($r = -0.387$, $P < .001$) (Fig. 1A), cholesterol ($r = -0.251$, $P = .026$), plasma insulin ($r = -0.250$, $P = .024$), C-reactive protein ($r = -0.281$, $P = .016$), and *TNFR2* mRNA expression ($r = -0.212$, $P = .038$). In the multiple regression analysis, after adjusting for confounding variables, only plasma insulin levels ($\beta = -.613$, $P = .001$, 95% confidence interval [β] −.051/−.014) were maintained as a determinant of *LPIN1* mRNA levels in women (n = 45) (Fig. 1B).

3.2. HALS study

3.2.1. Clinical and biochemical characteristics of the study groups

Clinical, anthropometric, and analytical characteristics of the subjects studied are shown in Table 3A.

All HIV-positive groups had received HAART for 51.8 ± 18.29 months (range, 14-80 months), and the duration of antiretroviral combination therapy was comparable in HALS-negative and HALS-positive groups (Table 3A). Time of evolution to HALS was 24.41 ± 15.09 months. All patients in both HALS-negative and HALS-positive groups were currently receiving nucleoside reverse transcriptase inhibitors (NRTIs). There were no significant differences between HALS groups in the frequency of receiving non-NRTIs (NNRTIs) and protease inhibitors (PIs).

There were no significant differences between HALS groups in the number of patients who received a specific agent, except for stavudine (Table 3A). Of the NRTI class of antiretroviral agents, lamivudine (33%) was the most frequently used in the HIV^+ $HALS^-$ group, and stavudine (26%) and lamivudine (24%) in the HIV^+ $HALS^+$ group. Of the NNRTIs, efavirenz was the most frequently used in both groups (60% and 68%, respectively). The most common PI in both groups was indinavir (37% and 32%, respectively). There were no significant differences in the length of treatment of each specific agent between groups.

The HIV-positive subjects had higher levels of plasma IL6, and HALS-positive patients showed lower S_I than the HALS-negative group. Adiponectin circulating levels were reduced in HALS-positive patients, although these levels did not reach statistical significance. Nevertheless, adiponectin levels were positively correlated with insulin sensitivity ($r = 0.616$, $P = .001$).

3.2.2. Subcutaneous adipose tissue gene expression

The mRNA expression levels of the different genes studied are shown in Table 3B. *LPIN1* mRNA levels were significantly higher in HIV-positive patients compared with controls, but there were no differences between both HIV-positive groups. We found significant differences in *LPIN1* mRNA levels between different families of HAART

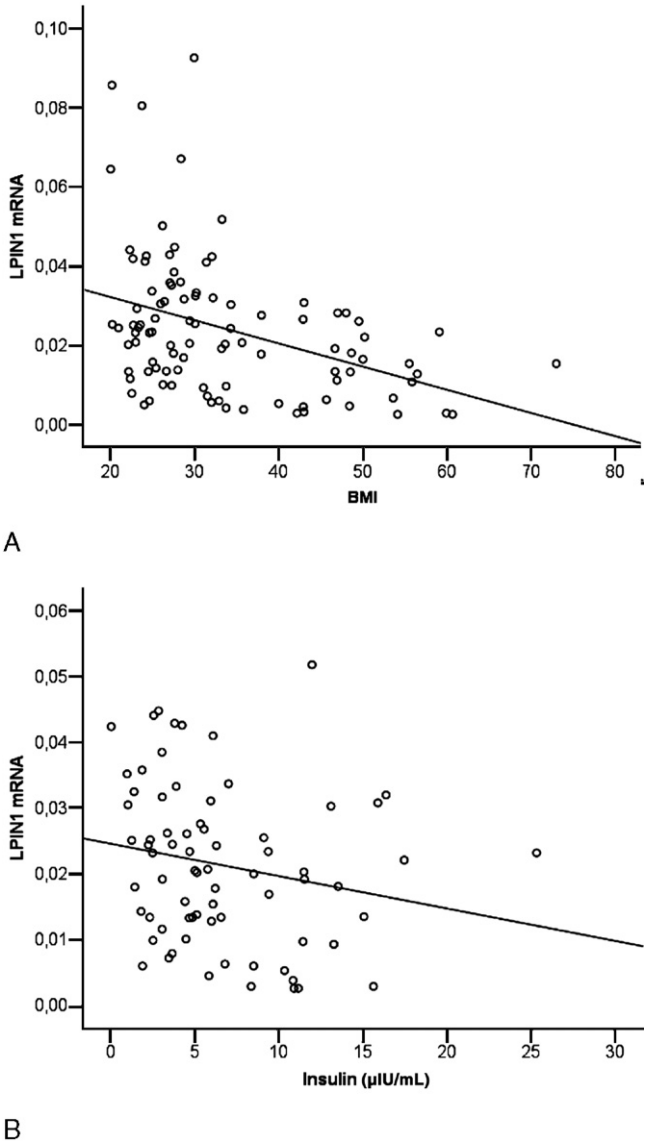

Fig. 1. Linear correlation between *LPIN1* mRNA levels and BMI (A) or serum insulin concentration (B) in the obesity and type 2 diabetes mellitus cohort.

Table 3A
HALS study: characteristics of the population

		HIV$^-$ (n = 21)	HIV$^+$ HALS$^-$ (n = 13)	HIV$^+$ HALS$^+$ (n = 24)
Age (y)		40.86 ± 11.20	39.79 ± 6.96	43.56 ± 8.97
Sex (female/male)		5/16	3/11	5/20
BMI (kg/m^2)		24.66 ± 2.66	23.44 ± 2.59	24.29 ± 2.79
WHR		0.90 ± 0.09	0.89 ± 0.09	0.95 ± 0.09
Fasting glucose (mmol/LM)		5.13 ± 0.56	5.00 ± 0.40	5.21 ± 0.98
Cholesterol (mmol/L)		5.14 ± 1.22	5.12 ± 0.69	5.58 ± 1.42
HDL cholesterol (mmol/L)		1.18 ± 0.38	1.31 ± 0.54	1.10 ± 0.33
LDL cholesterol (mmol/L)		ND	2.96 ± 0.78	3.08 ± 0.97
Triglycerides (mmol/L)		1.88 ± 1.93	2.15 ± 1.27	3.48 ± 2.55
Insulin (μIU/mL) *		3.37 ± 2.00	7.63 ± 3.98	11.79 ± 6.95
S_I **		ND	3.36 ± 0.95	2.17 ± 1.58
CD4 count (cells/mm^3)			628.69 ± 331.10	732.80 ± 292.65
HAART exposure (mo [range])			47.36 ± 29.98 (18-100)	54.32 ± 18.29 (14-80)
No. of patients receiving antiretroviral agents (n [%])	NRTI		13 (100)	24 (100)
	Stavudine **		9 (69)	23 (96)
	NNRTI		6 (46)	7 (29)
	PI		7 (54)	17 (71)
Plasma IL6 (ng/L) *		0.94 ± 1.09	2.68 ± 2.35	2.01 ± 1.20
Adiponectin (μg/mL)		ND	16.12 ± 10.69	13.36 ± 14.30
sTNFR1 (pg/mL) **		1.45 ± 0.84	2.09 ± 0.60	2.11 ± 1.04
sTNFR2 (ng/mL) **		4.52 ± 1.86	4.69 ± 1.57	6.25 ± 2.71

HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; ND , not determined.
* $P < .001$.
** $P < .05$.

therapy in the HALS-positive group (327 ± 205 in HAART with PI [n = 17] vs 508 ± 281 in NRTI + NNRTI [n = 7], $P = .047$). No variation in *LPIN1* mRNA expression was observed between the remaining groups of antiviral in HIV-positive patients.

The *PPARγ* mRNA tended to be lower in HIV-positive compared with HIV-negative subjects and in HALS-positive compared with HALS-negative patients, respectively; but no significant differences were observed (Table 3B). The HIV-positive subjects had significantly higher *IL6* mRNA and a tendency to have higher levels of *TNFα* compared with HIV-negative controls.

3.2.3. Correlation and regression analysis

All HIV subjects (n = 37) were considered as a whole, controlling for sex and presence of lipodystrophy. *LPIN1* mRNA expression levels were positively correlated with S_I ($r = 0.496$, $P = .014$) and *PPARγ* mRNA expression ($r = 0.636$, $P < .001$) and were negatively correlated with BMI ($r = -0.371$, $P = .037$), *IL6* mRNA expression ($r = -0.458$, $P = .006$), and *TNFα* mRNA expression ($r = -0.508$, $P = .002$). In the multiple regression analysis, both *PPARγ* ($\beta = .527$, $P = .006$, CI 0.151-0.756) (Fig. 2A) and *TNFα* mRNA expressions ($\beta = -.467$, $P = .011$, 95% confidence interval [β] −0.710/−0.109) (Fig. 2B) were maintained as determinants of *LPIN1* mRNA levels, independently of sex and presence of lipodystrophy.

Table 3B
HALS study: quantification of gene expression levels (arbitrary units)

	HIV$^-$ (n = 21)	HIV$^+$ HALS$^-$ (n = 13)	HIV$^+$ HALS$^+$ (n = 24)
LPIN1 *	1.36 ± 0.64	4.24 ± 2.68	3.82 ± 2.40
PPARγ	7.78 ± 4.32	6.32 ± 2.26	5.55 ± 2.39
IL6 *	0.067 ± 0.120	0.116 ± 0.067	0.116 ± 0.049
TNFα	0.013 ± 0.010	0.014 ± 0.007	0.016 ± 0.008
TNFR1 **	0.137 ± 0.072	0.273 ± 0.135	0.164 ± 0.121
TNFR2 **	0.116 ± 0.098	0.270 ± 0.158	0.184 ± 0.126

* $P < .001$.
** $P < .01$.

4. Discussion

In the present work, we have analyzed 2 different sets of subcutaneous adipose tissue biopsies: one from patients with increased adiposity and presence of type 2 diabetes mellitus and a second set from HIV-infected lipodystrophic patients. Our findings showed that *LPIN1* mRNA expression decreases with increasing adiposity with independence of the existence of type 2 diabetes mellitus. Likewise, HIV-associated lipodystrophy had no influence in *LPIN1* mRNA subcutaneous adipose tissue expression; but HAART-treated HIV-positive patients had higher *LPIN1* mRNA expression than HIV-negative controls.

We need to stress that the cohort for the obesity study is imbalanced in sex. However, after statistical analysis, no significant differences were found in *LPIN1* mRNA levels between sexes in nonmorbid subjects (data not shown). This prompted us to think that sex would not affect *LPIN1* expression. It will be very interesting to analyze *LPIN1*

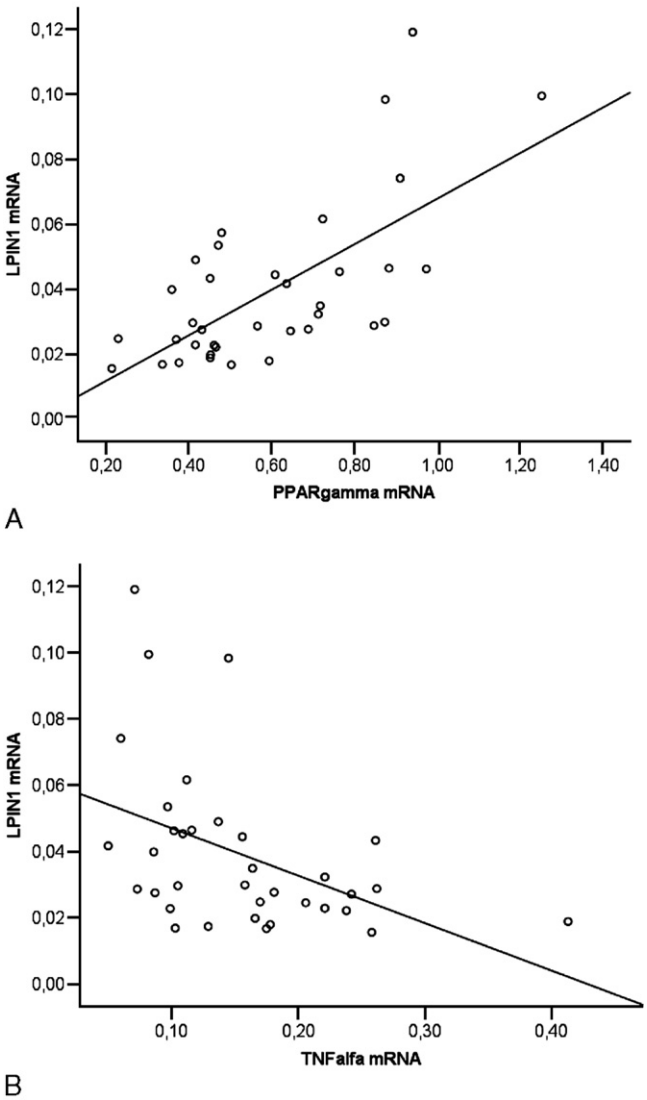

Fig. 2. Linear correlation between *LPIN1* mRNA levels and *PPARγ* mRNA (A) or *TNFα* mRNA (B) concentration in the HIV-positive cohort.

mRNA expression in a morbidly obese cohort with a balanced distribution of sexes.

Although we have not observed any correlation between adipose *LPIN1* mRNA gene expression and plasma levels of adipokines, or cytokine mRNA levels in the obesity cohort, we would require further studies to clarify the causes of the down-regulation of *LPIN1* mRNA levels. *LPIN1* gene response to other factors that affect adipogenesis, such as glucocorticoids or β-adrenergics, needs to be elucidated.

We have observed a relationship between the adipogenic factor *PPARγ* and *LPIN1* gene expression in both sets of biopsies. In mouse models, *LPIN1* has been found to be necessary for adipogenesis and to precede *PPARγ* expression [2]. However, in the transgenic mice model that over-expresses *LPIN1* mRNA specifically in adipose tissue, *PPARγ* mRNA levels are not affected [5]. In mouse liver, *LPIN1* expression is induced by PPARγ coactivator 1α, a transcriptional coactivator for multiple metabolic pathways; and in turn, lipin activates the hepatic PPARα/PPARγ coactivator 1α regulatory pathway by cooperating with it as a transcriptional activator of *PPARα* [23]. All of the above mentioned data suggest that a common activator for *PPARγ* and *LPIN1* could exist in adipose tissue, and the positive association between both genes observed in our population may support this hypothesis.

The relation of *LPIN1* with glucose homeostasis has been analyzed in transgenic mice [5] and in humans [12-15]. Two recent studies showed that *LPIN1* mRNA levels were positively correlated with insulin sensitivity, determined by different approaches [12,13]. Although our patients with type 2 diabetes mellitus showed no differences in *LPIN1* mRNA expression compared with the nondiabetic cohort, we observed a negative association with plasma insulin levels in the whole group. In addition, insulin sensitivity measured by the frequently sampled intravenous glucose tolerance test in HIV-positive patients was positively associated with *LPIN1* adipose tissue expression. The study design does not permit the establishment of causality in this association; however, it has been postulated that *LPIN1* could act as a regulator of the phosphatidate acid cellular levels [7] that participate in several intracellular mechanisms, including glucose homeostasis [10,11,24]. Some PIs have clearly been shown to induce insulin resistance in vivo [25,26]. In our study, HIV-positive patients who received PI tended to have lower insulin sensitivity than patients receiving HAART without PI, although there were no significant differences (data not shown). In addition, HALS-positive patients who received PI-containing HAART expressed significantly lower levels of *LPIN1* mRNA compared with patients having HAART without PI agents. All the above findings may help us to speculate as to a possible role of *LPIN1* as a modulator of insulin resistance induced by PI treatments.

In HAART-treated HIV-positive patients, we observed an increase in *LPIN1* adipose expression regardless of the development of lipodystrophy. It is not possible to attribute this finding to the HIV infection or to the HAART therapy because the cross-sectional design of the study and the absence of a nontreated HIV-positive (naive) control group do not allow us to distinguish between potential effects of HIV or its treatment. Similar results have recently been published concerning HIV^+ $HALS^-$ HAART-treated patients showing an increase in *LPIN1* expression with respect to healthy subjects, with no satisfactory explanation for this finding [15]. In addition, in the said work, the authors also analyzed separately the expression of both *LPIN1* isoforms; and they found similar results: higher expression of both *LPIN1α* and *β* isoforms in the HIV^+ $HALS^-$ HAART-treated patients. Alterations in lipogenesis and in adipocyte differentiation in HIV-positive patients have been hypothesized as an explanation for this *LPIN1* increase. However the absence of differences in *PPARγ* expression between HIV-positive (both $HALS^+$ and $HALS^-$) and HIV-negative subjects would argue against this hypothesis.

When we analyzed the *LPIN1* expression according to proinflammatory cytokines expressed in adipose tissue, we observed an inverse correlation in all studied populations (with *TNFR2* mRNA in obesity and type 2 diabetes mellitus and *TNFα* and *IL6* in HAART-treated HIV patients, respectively).

Elevated mRNA expressions of *TNFα* and *IL6* have been described in adipose tissue of morbidly obese patients and in HIV-positive patients [27-31]. In our population, a clear increase in subcutaneous adipose tissue of cytokine expression was observed in obese and HIV-positive populations. However, after controlling for several confounding factors, only in the HIV cohort was this negative relationship maintained with *TNFα* gene expression. Despite the increased expression level of *LPIN1* observed in HIV-positive patients, the inverse correlation with proinflammatory cytokines was maintained. These findings lead us to think that in HAART-treated HIV patients, there is something different regulating *LPIN1* expression than in other insulin-resistant conditions. It remains to be determined if lipin protein levels differ between control and HIV-infected populations because the present study was conducted only by examining mRNA levels.

The molecular function of this protein remains to be characterized, but it looks promising because all published data to date point to *LPIN1* participation in adipocyte differentiation, lipid metabolism, insulin signaling, and regulation of phosphatidate acid cellular levels, a signal lipid involved in several important cell functions. Further investigations need to be carried out to confirm these possible functions.

In summary, *LPIN1* mRNA expression levels in human subcutaneous adipose tissue are reduced in obesity, do not change in type 2 diabetes mellitus, and are increased in HAART-treated HIV-positive patients. In addition, *LPIN1* mRNA levels are positively correlated with insulin sensitivity in all the subjects analyzed here. Moreover, an inverse correlation with proinflammatory cytokines is observed. We showed that variation in *LPIN1* expression in HIV-positive patients is not influenced by the presence of lipodystrophy despite the fact that we cannot rule out an effect due to the antiretroviral treatment or to a response to HIV infection.

Acknowledgment

This study was supported by the following grants: Marató TV3 (02/1910), FIS 02/1280, FIS 04/0377, FIS 05/1994, FIS 05/1591, FIPSE 36610/06, and REDIMET (RD06/0015/0011).

Appendix A

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