

Research Article

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# Characteristics Associated to Lipodystrophy Syndrome among HIV-Infected Patients Naïve and on Antiretroviral Treatment

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## Abstract

**Background:** HIV-associated lipodystrophy involves changes in complex metabolic networks that are associated with increased cardiovascular risk. It has been associated with the use of combined antiretroviral treatment (cART), particularly Protease Inhibitors (PI) and thymidine analogs. This study aims to evaluate characteristics and use of ART associated with lipodystrophy, lipohypertrophy, and lipoatrophy among HIV-infected patients.

**Methods:** A cross-sectional study was conducted in HIV-infected patients of both genders, aged 18 years or older, who sought care at an HIV/AIDS referral service for diagnostic confirmation or treatment between June 2006 and December 2008.

**Results:** 1240 out of 1295 patients with HIV infection were included. Among patients on cART, women had a higher risk of lipohypertrophy than men, as well as a time since diagnosis of HIV greater than 6 years (versus <3 years). For lipoatrophy, age, education, lifestyle, and body mass index were associated with increased risk. Metabolic parameters were higher among patients on ART; and cART and PI use were independently associated with lipohypertrophy, lipoatrophy and lipodystrophy. The use of PI can be regarded as responsible for 13% of the association of ART and lipodystrophy, and of 11.5% for the thymidine analogs use, independent of gender, skin color, smoking, CD4, and BMI.

**Conclusions:** Risk factors for lipodystrophy, lipoatrophy and lipohypertrophy are marked among ART users, but also among ART naïve patients.

**Keywords:** HIV; Lipodystrophy; Lipohypertrophy; Lipoatrophy; Risk factors; HAART; Dyslipidemia

## Introduction

The introduction of combined antiretroviral treatment (cART) of HIV was a milestone in the struggle to reduce the rate of morbidity associated with progression of disease toward advanced stages of immunosuppression [1]. HIV-infected patients with lipodystrophy exhibit clinical changes in complex metabolic networks that are associated with increased cardiovascular risk [2-6].

Lipodystrophy is characterized by dyslipidemia, visceral adiposity, and subcutaneous abdominal fat buildup with peripheral wasting. The features of fat redistribution are variable, but they are usually detected by accumulation of fat in the abdomen, chest, breasts, or dorsocervical fat pad ("buffalo hump"), signs of lipohypertrophy, and the wasting of the face, anterior and lateral region of the neck, legs, arms or buttocks, known as lipoatrophy [3,5,7,8]. The HIV-associated lipodystrophy is a multifactorial disorder due to the interaction between virus and host factors related to cART [9]. Lipodystrophy appears to be mediated, even before the start of cART, by the increased inflammatory cytokines resulting from the HIV infection itself and, later, by use of cART [6]. The Protease Inhibitor (PI) reduces the proliferation and differentiation of adipocytes and increases lipolysis by inhibition of CRABP-1 (cytoplasmic retinoic-acid binding protein type1), blocking the activation of transcription factors linked to the PPAR-γ (peroxisome proliferator activated receptor type gamma) [10]. Nucleoside Reverse Transcriptase Inhibitor (especially stavudine-D4T) induce mitochondrial dysfunction [11], leading to lipoatrophy [6,9,10]. The prevalence of lipodystrophy ranges from 18 to 83%, depending on the

criteria used for diagnosis [12] and along with body fat changes, there are metabolic abnormalities including increased levels of triglycerides, LDL cholesterol, total cholesterol, glucose, and insulin, and decreased level of HDL cholesterol [3,5].

There are few data available on ART among HIV-infected people living in low and middle income countries [13,14]. In Senegal, thymidine analogs and PI are among the most commonly used drugs for the treatment of HIV-infected patients, which contributes to the prevalence of lipodystrophy 65% [14]. In another African study, conducted in Rwanda, the prevalence of lipodystrophy was 34%, and the patients were not in treatment with PI [13]. Few studies conducted in Brazil have assessed the prevalence and risk factors of lipodystrophy in HIV-infected patients who are not participating of randomized clinical trials [15]. In a study conducted in São Paulo, lipodystrophy was detected in

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64.3% of patients, 37.4% had lipoatrophy and lipohypertrophy 49.2% [16]. In this study we aimed to verify characteristics associated to lipodystrophy syndrome among HIV-infected patients naïve and on ART.

## Methods

This cross-sectional study included men and women living with HIV/AIDS, aged 18 years or older, who sought HIV diagnostic confirmation or treatment at the HIV/AIDS outpatient care center of Hospital Sanatório Partenon. Pregnant women, patients with intellectual disability, and incarcerated or institutionalized persons were excluded from the study. The study was approved by the institutional review board of the Hospital de Clínicas de Porto Alegre, which is accredited by the Office of Human Research Protections. All participants signed a consent form.

## Study variables

We used a standardized questionnaire to collect data on demographic (age, gender, skin color), socioeconomic (years at school), lifestyle characteristics (smoking and physical activity), HIV-infection related variables: use of antiretroviral treatment (ART), time since HIV diagnosis (categorized as <3, 3-5, and  $\geq 6$ ), and signs and symptoms of lipodystrophy. Age was calculated by subtracting the birth date from the date of interview; with analysis conducted as a categorical variable (18-34, 35-49, and 50-78 years) for the description and as a continuous variable to detect independent associations; skin color was self-reported and categorized as Caucasian or non-Caucasian; and education was measured as the number of years at school, analyzed as a categorical variable (0-4, 5-8, 9-11, or  $\geq 12$  years) in the description and as a continuous variable to assess independent associations. Lifetime tobacco exposure was calculated in former and current smokers by the number of packs smoked per day (1 pack=20 cigarettes) multiplied by the number of years of smoking [17-19]. For purposes of analysis, former or current smokers were stratified into those with  $\geq 20$  and those with <20 pack-years. Physical activity was estimated by the IPAQ (International Physical Activity Questionnaire) instrument [20], and subjects were considered physically active if they engaged weekly in at least 150 minutes of physical activity [21,22]. Body mass index (BMI, kg/m<sup>2</sup>) was assessed as a categorical variable for the description and a continuous variable in order to detect the independent associations. The use of ART was investigated during anamnesis and confirmed with the recorded information in the clinical center for use of any drug during lifetime, protease inhibitors (PI), and cART, which was defined as the use of three or more drugs over the 12 months preceding the study. Patients who have never been exposed to ART were classified as ART naïve. The time of diagnosis of HIV was reported by the patient and confirmed through a review of medical records and the dates of HIV testing.

A 12 hours fasting glucose, lipid profile, CD4-lymphocyte counts and HIV RNA viral load were requested for patients who had not been tested in the last three months. Laboratory tests were performed using standard techniques [23-25]. Dyslipidemia was detected by total cholesterol  $\geq 200$  mg/dL or HDL cholesterol <40 mg/dL [26] and use of lipid-lowering treatment was further investigated.

## Diagnosis of lipodystrophy

Lipodystrophy was identified by objective measurements and by the report of increased or decreased body fat. Objective measures were determined by measurement of waist circumference, hip, arm and neck; and skin folds in infra-orbital, buccal and submandibular regions

with anthropometric caliper. The 10<sup>th</sup> and 90<sup>th</sup> percentiles were used to define the cutoffs for lipoatrophy and lipohypertrophy, respectively [27]. The change in body fat located in face, chin, back of neck, chest or breasts, abdomen, arms, forearms and hands, hips and buttocks, thighs, legs and feet could be determined by the perception of muscular arms or legs, prominent superficial veins, presence of a “buffalo hump”, abdominal enlargement, double chin, hollow cheeks, or changes in the width of the neck or waist. Finally, lipohypertrophy and lipoatrophy were established by the presence of at least two self-perceived signs or objective measurements of hypertrophy or atrophy, respectively [12,28], while those who had one of these conditions were classified as having lipodystrophy.

## Data collection

Data were collected during routine visits, and consisted of blood pressure measurement and anthropometry. Weight (kg) and height (m) were measured, with patients in barefoot and wearing light clothes. Body mass index (BMI) was calculated by dividing the weight (in kg) by the height (in meters) squared. Waist circumference was measured midway between the iliac crest and low costal rib margin [29,30]. Hip circumference was measured at the greater trochanter and the point of greatest gluteal protuberance.

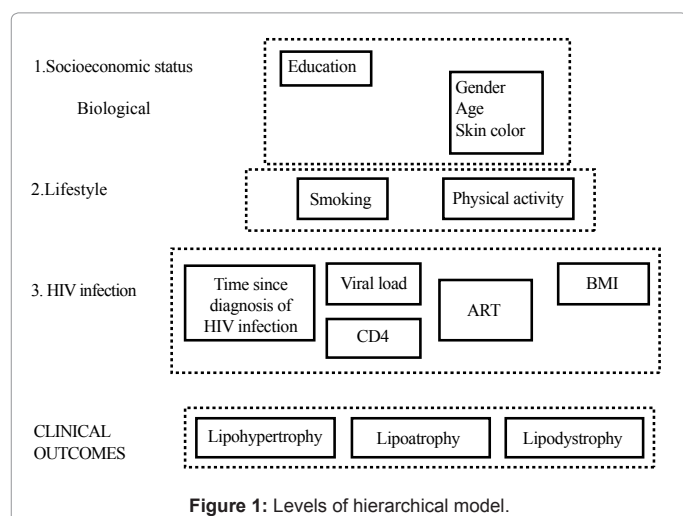
Facial skin fold thickness was measured with a scientific skin fold caliper in the infra-orbital, buccal and submandibular regions. Measurements were done in duplicate and the average was used for determine the abnormal cutoffs.

## Sample size calculation and statistical analysis

Sample size calculation was based on an estimate that 17% of ART naïve patients and 25% of patients on ART would have lipodystrophy. For a statistical power of 80% and 95% confidence interval, with an unexposed to exposed ratio of 0.5, in order to detect a risk ratio (RR) of 1.5, it was needed to enroll at least 984 patients. The sample size was increased in 30% to take into account confounding factors in multivariate analysis.

Pearson's chi-squared test was used for categorical variables and analysis of variance, for continuous variables. All analyses were performed in the Statistical Package for the Social Sciences (SPSS) version 14.0 software environment (SPSS Inc., Chicago, Illinois, USA). Modified Poisson regression was used to test for associations between risk factors and the presence of lipohypertrophy, lipoatrophy, and lipodystrophy, calculating adjusted prevalence ratios and 95% confidence intervals. A trend toward association was defined as  $0.05 < P < 0.1$ .

The analysis was based on a hierarchical framework, which provides a strategy to conduct multivariate analysis in studies where determinants of disease are sought, there are hierarchical relationships among determinants, and these aspects are taken into consideration as well as the literature [29]. The information allowed to testing some characteristics as continuous and categorical variables, and the best fit was used in each statistical test. Characteristics were aggregated into three sets of variables, which contained different hierarchical levels of determination, including in the first level: socioeconomic (education) and biological characteristics (gender, age, and skin color), in the second: lifestyle (smoking and physical activity), and in the third level: HIV-related characteristics (viral load, CD4, time since HIV diagnosis, and BMI). These potential determinants are likely to lead to lipohypertrophy, lipoatrophy, and lipodystrophy (Figure 1). At each level, variables were included in the model based on the strength



of association in the crude analyses ( $P$  value $<0.2$ ), and one regression equation was fitted for each hierarchical level, also including variables from higher levels of determination [30]. We conducted separate analyses according to the ART status (on ART and ART naïve) and for each clinical outcome.

## Results

The cohort included a consecutive sample of 1240, out of 1295, HIV-infected subjects eligible to participate, 15 refused to take part, and 40 were excluded due to age, incarceration, or pregnancy. Participants were, on average,  $38.6 \pm 10.1$  years old. Approximately half were men, most were Caucasians. Most patients had received ART (65.7%) during lifetime, and among those on ART the majority used PI (42.6%), thymidine analogs (31.0%), and d-drugs (31.9%).

Table 1 describes characteristics of patients receiving ART and ART naïve subjects. Patients on cART were older ( $P<0.001$ ), smokers ( $P=0.004$ ), had increased BMI ( $P=0.003$ ), longer time since HIV infection diagnosis ( $P<0.001$ ), had detectable viral load ( $P<0.001$ ), CD4 counts below  $350$  cells/ $\text{mm}^3$  ( $P<0.001$ ), and metabolic parameters were higher than those patients who were cART naïve. There were 51 patients on ART and six, who had not started treatment at the time of the study enrollment, with advanced stages of AIDS.

The prevalence of lipohypertrophy was 50.2% among patients on ART and 37.9% among ART naïve patients, 58.2% and 43.8%, respectively, for lipoatrophy, and 79.1% and 64.7%, respectively, for lipodystrophy. Table 2 presents the distribution of characteristics associated with lipohypertrophy, lipoatrophy, and lipodystrophy separated for patients on cART and ART naïve.

Table 3 shows the characteristics independently associated with risk of lipohypertrophy, lipoatrophy and lipodystrophy in patients on ART. The selection of confounding factors for lipohypertrophy were: gender and age (first level), smoking (second level), and BMI, CD4, and viral load (third level); for lipoatrophy: gender, skin color, and education (first level), none for the second level, and BMI and time since diagnosis of HIV (third level); for lipodystrophy: age, skin color, and education (first level), none for the second level, and BMI and time since diagnosis of HIV (third level). Women had 58% higher risk of lipohypertrophy than men ( $P<0.001$ ), independent of age. After control for age and gender, smoking status remained as a protective factor for lipohypertrophy while an increase in one unit of BMI resulted in

7% elevated risk of lipohypertrophy, after the control for biological, lifestyle, and HIV-related variables. Time since HIV diagnosis was not associated with lipohypertrophy. After the control for gender, skin color, and educations, characteristics as non-white skin color increased 26% the risk of lipoatrophy, in comparison to white ones ( $P<0.001$ ), while education was inversely associated ( $P<0.001$ ). Body mass index was inverse and independently associated to lipoatrophy ( $P<0.001$ ) and longer time since the HIV diagnosis was a risk factor. Lipodystrophy

	Overall (n=1240)	ART (n=815)	No ART (n=425)	P-value
Age (years)	39.1 $\pm$ 10.1	40.5 $\pm$ 9.7	36.5 $\pm$ 10.2	<0.001
Gender				0.4
Male	628 (50.6)	420 (51.5)	208 (48.9)	
Female	612 (49.4)	395 (48.5)	217 (51.1)	
Education (years)	7.5 $\pm$ 4.1	7.4 $\pm$ 4.1	7.7 $\pm$ 4.1	0.2
Skin color				0.9
Caucasian	548 (44.2)	348 (42.7)	182 (42.8)	
Non-Caucasian	692 (55.8)	467 (57.3)	243 (57.2)	
Smoking (pack-years)				0.004
0	422 (34.0)	272 (33.4)	150 (35.3)	
1-19	498 (40.2)	309 (37.9)	189 (44.5)	
$\geq 20$	320 (25.8)	234 (28.7)	86 (20.2)	
Physical activity (min/week)				0.4
$\geq 150$	524 (42.3)	352 (43.2)	172 (40.5)	
<150	716 (57.7)	463 (56.8)	253 (59.5)	
Body mass index (kg/ $\text{m}^2$ )				0.01
<18.5	38 (3.1)	27 (3.3)	11 (2.6)	
18.5-24.9	670 (54.0)	466 (57.2)	204 (48.0)	
25.0-29.9	380 (30.6)	232 (28.5)	148 (34.8)	
$\geq 30.0$	152 (12.3)	90 (11.0)	62 (14.6)	
Time since diagnosis of HIV infection (years)				<0.001
<3	485 (39.1)	211 (25.9)	274 (64.5)	
3-5	350 (28.2)	256 (31.4)	94 (22.1)	
$\geq 6$	405 (32.7)	347 (42.6)	58 (13.4)	
Viral load (copies/mL)				<0.001
<50	713 (58.4)	321 (39.7)	392 (95.1)	
$\geq 50$	508 (41.6)	488 (60.3)	20 (4.9)	
CD4 (cells/ $\text{mm}^3$ )				<0.001
$\geq 350$	751 (61.2)	451 (55.6)	300 (72.1)	
200-349	295 (24.0)	215 (26.5)	80 (19.2)	
<200	181 (14.8)	145 (17.9)	36 (8.7)	
Glucose (mg/dL)	86.8 $\pm$ 27.6	88.2 $\pm$ 29.9	84.3 $\pm$ 22.3	0.02
Triglycerides (mg/dL)	151.9 $\pm$ 107.5	167.9 $\pm$ 121.1	120.6 $\pm$ 63.3	<0.001
Total cholesterol (mg/dL)	183.1 $\pm$ 43.6	187.9 $\pm$ 45.7	173.7 $\pm$ 37.5	<0.001
HDL cholesterol (mg/dL)	51.2 $\pm$ 13.8	51.9 $\pm$ 14.1	49.8 $\pm$ 13.1	0.01
LDL cholesterol (mg/dL)	103.0 $\pm$ 35.8	104.4 $\pm$ 37.4	100.4 $\pm$ 32.4	0.07
Dyslipidemia*				
Yes	608 (49.6)	431 (53.3)	177 (42.5)	<0.001
No	617 (50.4)	378 (46.7)	239 (57.5)	
Use of lipid-lowering treatment				
Yes	29 (2.3)	26 (3.2)	3 (0.7)	0.006
No	1211 (97.7)	789 (96.8)	422 (99.3)	

\* Dyslipidemia: total cholesterol  $\geq 200$  mg/dL or HDL cholesterol  $< 40$  mg/dL

**Table 1:** Characteristics of HIV-infected patients, stratified by ART use [n(%) or mean  $\pm$  SD].

	On ART			ART naïve		
	Lipohypertrophy	Lipoatrophy	Lipodystrophy	Lipohypertrophy	Lipoatrophy	Lipodystrophy
Age (years)						
18-34	127 (51.2)	136 (54.8)	187 (75.4)	84 (37.7)	104 (46.6)	147 (65.9)
35-49	208 (47.5)	265 (60.5)	349 (79.7)	58 (37.9)	64 (41.8)	99 (64.7)
50-78	74 (57.4)	73 (56.6)	109 (84.5)	19 (38.8)	18 (36.7)	29 (59.2)
P value	0.13	0.3	0.11	1.0	0.4	0.7
Gender						
Male	167 (39.8)	248 (59.0)	327 (77.9)	75 (36.1)	90 (43.3)	138 (66.3)
Female	242 (61.3)	226 (57.2)	318 (80.5)	86 (39.6)	96 (44.2)	137 (63.1)
P value	<0.001	0.6	0.4	0.4	0.8	0.5
Education (years)						
0-4	116 (53.0)	136 (62.1)	183 (83.6)	28 (27.5)	56 (54.9)	66 (64.7)
5-8	151 (49.2)	199 (64.8)	252 (82.1)	71 (44.9)	74 (46.8)	114 (72.2)
9-11	96 (49.0)	101 (51.5)	145 (74.0)	42 (36.5)	40 (34.8)	67 (58.3)
≥12	46 (49.5)	38 (40.9)	65 (69.9)	20 (40.0)	16 (32.0)	28 (56.0)
P value	0.8	<0.001	0.007	0.04	0.006	0.06
Skin color						
Caucasian	185 (53.2)	234 (67.2)	297 (85.3)	78 (42.9)	87 (47.8)	127 (69.8)
Non-Caucasian	224 (48.0)	240 (51.4)	348 (74.5)	83 (34.2)	99 (40.7)	148 (60.9)
P value	0.14	<0.001	<0.001	0.07	0.15	0.06
Smoking (pack-years)						
0	160 (58.8)	150 (55.1)	215 (79.0)	61 (40.7)	52 (34.7)	89 (59.3)
1-19	144 (46.6)	182 (58.9)	242 (78.3)	67 (35.4)	83 (43.9)	120 (63.5)
≥20	105 (44.9)	142 (60.7)	188 (80.3)	33 (38.4)	51 (59.3)	66 (76.7)
P value	0.002	0.4	0.8	0.6	<0.001	0.02
Physical activity (min/week)						
≥150	179 (50.9)	276 (59.6)	367 (79.3)	94 (37.2)	121 (47.8)	168 (66.4)
<150	230 (49.7)	198 (56.3)	278 (79.0)	67 (39.0)	65 (37.8)	107 (62.2)
P value	0.7	0.3	0.9	0.7	0.04	0.4
Body mass index (kg/m <sup>2</sup> )						
<18.5	3 (11.1)	27 (100.0)	27 (100.0)	1 (9.1)	11 (100.0)	11 (100.0)
18.5-24.9	170 (36.5)	298 (63.9)	355 (76.2)	49 (24.0)	109 (53.4)	134 (65.7)
25-29.9	156 (67.2)	104 (44.8)	181 (78.0)	64 (43.2)	48 (32.4)	82 (55.4)
≥30.0	80 (88.9)	45 (50.0)	82 (91.1)	47 (75.8)	18 (29.0)	48 (77.4)
P value	<0.001	<0.001	0.001	<0.001	<0.001	0.001
Time since diagnosis of HIV infection (years)						
<3	108 (51.2)	113 (53.6)	160 (75.8)	98 (35.8)	121 (44.2)	170 (62.0)
3-5	128 (50.0)	138 (53.9)	196 (76.6)	41 (43.6)	36 (38.3)	63 (67.0)
≥6	173 (49.9)	223 (64.3)	289 (83.3)	22 (38.6)	29 (50.9)	42 (73.7)
P value	1.0	0.01	0.048	0.4	0.3	0.2
Viral load (copies/mL)						
<50	272 (55.7)	270 (55.3)	390 (79.7)	8 (40.0)	9 (45.0)	11 (55.0)
≥50	134 (41.7)	200 (62.3)	250 (77.9)	148 (37.8)	169 (43.1)	255 (65.1)
P value	<0.001	0.049	0.5	0.8	0.9	0.4
CD4 (cells/mm <sup>3</sup> )						
≥350	242 (53.7)	256 (56.8)	356 (78.9)	130 (43.3)	131 (43.7)	197 (65.7)
200-349	114 (53.0)	126 (58.6)	175 (81.4)	21 (26.3)	30 (37.5)	48 (60.0)
<200	51 (35.2)	90 (62.1)	111 (76.6)	7 (19.4)	19 (52.8)	24 (66.7)
P value	<0.001	0.5	0.5	0.001	0.3	0.6
Dyslipidemia*						
Yes	226 (52.4)	239 (55.5)	339 (78.7)	68 (38.4)	76 (42.9)	117 (66.1)
No	181 (47.9)	230 (60.8)	300 (79.4)	91 (38.1)	105 (43.9)	152 (63.6)
P value	0.2	0.12	0.8	0.9	0.8	0.6
Use of lipid-lowering treatment						
Yes	15 (57.7)	16 (61.5)	22 (84.6)	1 (33.3)	2 (66.7)	2 (66.7)
No	394 (49.9)	458 (58.0)	623 (79.0)	160 (37.9)	184 (43.6)	273 (64.7)
P value	0.4	0.7	0.5	0.9	0.4	0.9

**Table 2:** Distribution of characteristics associated with lipohypertrophy, lipoatrophy, and lipodystrophy in HIV-infected patients according to ART treatment status [n (%)].



	Lipohypertrophy Model 1*	Lipoatrophy Model 1 <sup>f</sup>	Lipodystrophy Model 1 <sup>‡</sup>
Gender			
Male	1.00	1.00	1.00
Female	1.58 (1.37–1.82)	0.92 (0.82–1.03)	1.02 (0.95–1.10)
P value	<0.001	0.16	0.5
Age (years)	1.01 (1.00 – 1.01)	1.00 (1.00–1.01)	1.004 (1.00–1.01)
P value	0.06	0.9	0.04
Skin color			
Caucasian	1.00	1.00	1.00
Non - Caucasian	1.07 (0.94–1.23)	1.26 (1.12–1.41)	1.13 (1.05–1.21)
P value	0.3	< 0.001	< 0.001
Education (years)	1.01 (0.99 – 1.02)	0.97 (0.96 – 0.99)	0.99 (0.98 – 0.99)
P value	0.4	<0.001	0.03
	Model 2**	Model 2 <sup>f</sup>	Model 2 <sup>‡</sup>
Smoking (pack-years)			
0	1.00	1.00	1.00
1-19	0.81 (0.68–0.97)	1.06 (0.92–1.21)	1.00 (0.92–1.08)
≥20	0.81 (0.70–0.95)	1.07 (0.93–1.25)	0.99 (0.91–1.09)
P value	0.01	0.6	1.0
Physical activity (min/week)			
≥150	1.00	1.00	1.00
<150	1.05 (0.91–1.20)	0.96 (0.86–1.08)	1.01 (0.94–1.08)
P value	0.5	0.5	0.9
	Model 3***	Model 3 <sup>ff</sup>	Model 3 <sup>‡‡</sup>
Body mass index (kg/m <sup>2</sup> )	1.07 (1.06–1.09)	0.96 (0.95–0.98)	1.007 (1.00–1.01)
P-value	<0.001	<0.001	0.04
CD4 (cells/mm <sup>3</sup> )			
≥350	1.00	1.00	1.00
200-349	1.11 (0.96–1.28)	0.99 (0.87–1.13)	1.02 (0.95–1.11)
<200	0.83 (0.67–1.05)	0.98 (0.85–1.13)	0.95 (0.86–1.05)
P value	0.049	1.0	0.4
Viral load (copies/mL)			
<50	1.00	1.00	1.00
≥50	0.78 (0.67–0.91)	1.04 (0.93–1.16)	0.96 (0.89–1.04)
P value	0.001	0.5	0.3
Time since diagnosis of HIV infection (years)			
<3	1.00	1.00	1.00
3-5	1.02 (0.87–1.22)	0.97 (0.82–1.14)	1.01 (0.91–1.12)
≥6	1.11 (0.95–1.29)	1.18 (1.02–1.37)	1.12 (1.02–1.22)
P value	0.4	0.005	0.01

\* Adjusted for gender and age

\*\* Adjusted for gender, age, and smoking status

\*\*\* Adjusted for gender, age, smoking status, BMI, CD4, and viral load

<sup>f</sup>Adjusted for gender, skin color and education

<sup>ff</sup>Adjusted for gender, skin color, education, time since HIV diagnosis, and BMI

<sup>‡</sup>Adjusted for age, skin color, and education

<sup>‡‡</sup>Adjusted for age, skin color, education, time since HIV diagnosis, and BMI

**Table 3:** Risk factors for lipodystrophy in HIV – infected patients on antiretroviral treatment (risk ratio and 95% CI).

was markedly affected by biological characteristics, which increased the risk, except by education that was inversely associated. HIV-related characteristics-BMI (P=0.04) and time since the HIV diagnosis (P=0.01) maintained positive associations with lipodystrophy, even after the control for confounding factors.

Table 4 shows the risk factors for lipodystrophy in patients ART naïve, following the same models of control for confounding factors.

The selection of confounding factors for lipohypertrophy were: skin color and education (first level), none for the second level, and BMI and CD4 (third level); for lipoatrophy: age and education (first level), smoking and physical activity (second level), and BMI (third level); for lipodystrophy: skin color and education (first level), smoking (second level), and none for the third level. Non-Caucasian participants were at increased risk of lipohypertrophy (RR 1.31 (95% CI 1.02-1.68); P=0.03),

	Lipohypertrophy Model 1*	Lipoatrophy Model 1 <sup>†</sup>	Lipodystrophy Model 1*
Gender			
Male	1.00	1.00	1.00
Female	1.09 (0.85–1.40)	0.97 (0.79–1.21)	0.92 (0.80–1.06)
P value	0.5	0.8	0.3
Age (years)	1.00 (0.99–1.01)	0.99 (0.98–0.99)	1.00 (0.99–1.00)
P value	0.8	0.035	0.2
Skin color			
Caucasian	1.00	1.00	1.00
Non-Caucasian	1.31 (1.02–1.68)	1.09 (0.88–1.35)	1.12 (0.97–1.29)
P value	0.03	0.4	0.13
Education (years)	1.02 (0.99–1.05)	0.95 (0.93–0.98)	0.99 (0.97–1.01)
P value	0.18	0.001	0.19
	Model 2*	Model 2 <sup>††</sup>	Model 2 <sup>‡</sup>
Smoking (pack-years)			
0	1.00	1.00	1.00
1-19	0.86 (0.67–1.17)	1.16 (0.89–1.52)	1.27 (1.07–1.52)
≥20	0.99 (0.71–1.39)	1.75 (1.31–2.35)	1.06 (0.89–1.26)
P value	0.6	<0.001	0.016
Physical activity (min/week)			
≥150	1.00	1.00	1.00
<150	1.06 (0.83–1.36)	0.80 (0.64–1.00)	0.94 (0.81–1.08)
P value	0.6	0.046	0.4
	Model 3**	Model 3 <sup>†††</sup>	Model 3 <sup>‡</sup>
Body mass index (kg/m <sup>2</sup> )	1.09 (1.07–1.11)	0.94 (0.91 – 0.97)	1.00 (0.99 – 1.02)
P value	<0.001	< 0.001	0.6
CD4 (cells/mm <sup>3</sup> )			
≥350	1.00	1.00	1.00
200-349	0.71 (0.49–1.03)	0.85 (0.63–1.16)	0.90 (0.74–1.09)
<200	0.58 (0.30–1.11)	1.16 (0.85–1.59)	0.98 (0.77–1.26)
P value	0.07	0.3	0.5
Viral load (copies/mL)			
<50	1.00	1.00	1.00
≥50	0.98 (0.57–1.70)	0.88 (0.54–1.42)	1.16 (0.77–1.73)
P value	1.0	0.6	0.5
Time since diagnosis of HIV infection (years)			
<3	1.00	1.00	1.00
3-5	1.12 (0.87–1.45)	0.86 (0.65–1.15)	1.06 (0.90–1.26)
≥6	1.25 (0.88–1.77)	0.96 (0.72–1.29)	1.11 (0.92–1.34)
P value	0.4	0.6	0.5

\*Adjusted for skin color and education

\*\*Adjusted for skin color, education, BMI, and CD4

<sup>†</sup>Adjusted for age and education

<sup>††</sup>Adjusted for age and education, physical activity, and smoking

<sup>†††</sup>Adjusted for age and education, physical activity, smoking, and BMI

<sup>‡</sup>Adjusted for skin color, education, and smoking

**Table 4:** Risk factors for lipodystrophy in HIV– infected patients ART naïve (risk ratio and 95%CI).

	Cholesterol*	HDL cholesterol*	LDL cholesterol*	Triglycerides*	Glucose*
<b>ART</b>					
Lipohypertrophy					
No	179.9 ± 2.2	51.0 ± 0.7	100.2 ± 1.9	150.2 ± 5.9	86.1 ± 1.5
Yes	195.7 ± 2.2	52.7 ± 0.7	108.4 ± 1.9	185.4 ± 5.8	90.2 ± 1.5
P-value	< 0.001	0.09	0.002	< 0.001	0.05
Lipoatrophy					
No	195.6 ± 2.4	52.4 ± 0.8	108.8 ± 2.1	175.6 ± 6.5	89.8 ± 1.6
Yes	182.2 ± 2.0	51.1 ± 0.7	101.1 ± 1.7	162.4 ± 5.5	87.0 ± 1.4
P value	<0.001	0.09	0.005	0.1	0.2
Lipodystrophy					
No	188.5 ± 3.5	52.4 ± 1.1	104.8 ± 2.9	162.5 ± 9.2	86.0 ± 2.3
Yes	187.7 ± 1.8	51.7 ± 0.6	104.2 ± 1.5	169.4 ± 4.7	88.7 ± 1.2
P value	0.8	0.6	0.9	0.5	0.3
<b>ART naïve</b>					
Lipohypertrophy					
No	169.8 ± 2.2	49.0 ± 0.8	98.6 ± 2.0	112.3 ± 3.9	81.6 ± 1.4
Yes	180.5 ± 2.8	51.2 ± 1.0	103.2 ± 2.5	134.0 ± 4.9	88.7 ± 1.7
P-value	0.003	0.08	0.2	0.001	0.001
Lipoatrophy					
No	171.7 ± 2.4	49.1 ± 0.9	98.7 ± 2.1	118.1 ± 4.1	84.8 ± 1.5
Yes	176.3 ± 2.7	50.7 ± 1.0	102.5 ± 2.4	123.8 ± 4.7	83.6 ± 1.7
P-value	0.2	0.2	0.3	0.4	0.6
Lipodystrophy					
No	168.9 ± 3.0	49.3 ± 1.1	97.0 ± 2.6	110.5 ± 5.2	80.9 ± 1.8
Yes	176.3 ± 1.2	50.1 ± 0.8	102.2 ± 2.0	126.1 ± 3.8	86.2 ± 1.3
P-value	0.047	0.6	0.1	0.02	0.02

\*mg/dL.

\*\*Adjusted for age, skin color, smoking status, and time since HIV diagnosis

**Table 5:** Metabolic profile (mean ± SEM) of HIV-infected patients with lipodystrophy\*\*.

	Lipohypertrophy	Lipoatrophy	Lipodystrophy
Antiretroviral treatment (ART)	1.33 (1.15–1.52)*	1.33 (1.18–1.50)*	1.22 (1.13–1.32)*
ART+gender	1.34 (1.16–1.54)*	1.33 (1.18–1.50)*	1.22 (1.13–1.32)*
ART+age (years)	1.31 (1.14–1.51)*	1.34 (1.18 – 1.52)*	1.22 (1.12–1.32)*
ART+skin color	1.34 (1.16 – 1.54)*	1.34 (1.18–1.51)*	1.23 (1.14–1.33)*
ART, complete model <sup>#</sup>	1.49 (1.30 – 1.70)*	1.30 (1.15–1.47)*	1.23 (1.13–1.33)*
Protease inhibitors (PI)	1.16 (1.03–1.31)**	1.22 (1.10–1.36)*	1.14 (1.07–1.22)*
PI+gender	1.13 (1.00–1.27)**	1.23 (1.11–1.36)*	1.14 (1.07–1.22)*
PI+age (years)	1.15 (1.02–1.30) <sup>Δ</sup>	1.22 (1.10–1.36)*	1.14 (1.07–1.21)*
PI+skin color	1.17 (1.04–1.32)**	1.24 (1.12–1.38)*	1.15 (1.08–1.23)*
PI, complete model <sup>#</sup>	1.23 (1.10–1.38)*	1.20 (1.08–1.32)*	1.15 (1.07–1.22)*

\*Adjusted for gender, skin color, smoking status, BMI and CD4.

\*P value<0.001; \*\*P value 0.01 ≤ P ≤ 0.05; <sup>Δ</sup>P value=0.19

**Table 6:** Modified Poisson regression for risk factors associated with lipohypertrophy, lipoatrophy and lipodystrophy (risk ratio and 95%CI).

compared with Caucasians, independent of skin color and education. Furthermore, after adjusting for skin color, education, and CD4, BMI was associated with higher risk [RR 1.09 (95% CI 1.07–1.11); P<0.001]. As for lipoatrophy, age (P=0.035), education (P=0.001), and physical activity (P=0.046) showed an inverse effect on risk, even after the control for confounding factors. Patients who smoked 20 or more pack-years had 75% the risk (RR=1.75 (95%CI 1.31–2.35) in comparison to never smokers. For lipodystrophy, an independent association was detected only with smoking (P=0.016), but there was no dose-response.

Table 5 describes the metabolic parameters observed in patients on cART and not on ART, according to lipohypertrophy, lipoatrophy and lipodystrophy. All metabolic parameters were adjusted for age, skin color, smoking status, and time since HIV diagnosis. Those on

cART who had lipohypertrophy had higher mean levels of nearly all metabolic parameters. Conversely, those with lipoatrophy had lower mean cholesterol (P<0.001) and LDL-cholesterol (P 0.005) levels. Among ART naïve patients, those with lipohypertrophy had higher total cholesterol (P 0.003), triglycerides (P 0.001), and glucose (P 0.001) levels, while for lipodystrophy the metabolic abnormalities were observed (P 0.047, P 0.02, P 0.02, respectively).

Table 6 shows that use of cART and PI was independently associated with lipohypertrophy, lipoatrophy and lipodystrophy. The use of ART increased 23%, 30%, and 49% the risk of lipodystrophy, lipoatrophy, and lipohypertrophy, respectively, while for PI use they were 15%, 20%, and 23%, respectively, after adjustment for biological, lifestyle, and HIV-related characteristics. Additional analysis showed that

thymidine analogs were associated with higher risk of lipohypertrophy (RR=1.47 (95%CI: 1.30-1.36),  $P<0.001$ ), lipoatrophy (RR=1.35 (95%CI: 1.22-1.50),  $P<0.001$ ), and lipodystrophy (RR=1.27 (95%CI:1.20-1.35),  $P<0.001$ ), independent of gender, skin color, smoking, and CD4. In addition, we estimated that use of PI can be regarded as responsible for 13% of the association of ART and lipodystrophy, and of 11.5% for the thymidine analogs use, independent of gender, skin color, smoking, CD4, and BMI.

## Discussion

This study showed that in this population several characteristics associated with lipohypertrophy and lipoatrophy phenotypes were different among those on ART and ART naïve. In addition, it also showed that the lipodystrophy category is not so helpful, since risk factors for lipoatrophy and lipohypertrophy are very different. Although lipodystrophy is more frequent in individuals on ART, this study shows that patients ART naïve were also affected [31] and that clinical outcomes can be attributed to the HIV infection.

Lipodystrophy seems to be mediated, even before the institution of cART, by an increase of inflammatory cytokines resulting from the infection itself [3]. Epidemiological studies suggest that cART and factors not related to therapy alike are potential risk factors for body fat redistribution [6,9]. For instance, among ART naïve patients, the associations between smoking and lipodystrophy, mainly due to the lipoatrophy component, and the inverse relationship between CD4 levels and lipohypertrophy can be related to the progressive wasting that commonly accompanies the decrease of CD4. The results of our study agree with those verified in a large multicenter, cross-sectional [31]. The multi-factorial explanation of lipodystrophy is corroborated in other reports of body fat, outpatient study carried out in the United States, which showed that lipodystrophy was strongly associated with ART and patient-related factors and that lipohypertrophy was linked to correlates of immunologic recovery. The multi-factorial explanation of lipodystrophy is corroborated in other reports of body fat changes in HIV-1 infected patients who were not on PI [32], but were taken nucleoside reverse transcriptase inhibitors (NRTI) and no PI [31,33].

Few studies have been conducted among HIV-infected patients who were not on ART and most of the known associations derived from studies on patients on cART. This report among patients on ART from Southern Brazil confirmed the risk of lipohypertrophy associated with female gender [3,13,34], increasing age [31,35], high BMI [31], hypertriglyceridemia [34], and abnormal lipid profile [35,36]. Regarding lipoatrophy in patients on cART, the results of this study were similar to those described for low BMI [6,36,37], reduced LDL cholesterol, and low total cholesterol. Overall lipodystrophy had confirmed associations with age [35], non-Caucasian skin color, BMI [38], and duration of HIV infection [15]. The comparison of findings among patients on cART and ART naïve allows identifying a common pathway for the association of BMI with lipohypertrophy. Patients with greater fat deposit may slow or mitigate the loss of adipose tissue.

This study also assessed metabolic profile of HIV-infected patients, on ART and ART naïve, mostly associated with metabolic syndrome [39]. A previous multicenter cohort study reported more frequent lipid abnormalities in HIV-infected individuals than in the general population[40]. However, this study showed that lipid levels vary according to the ART status and that the report without taken into account this condition may under detect the prevalence of metabolic abnormalities [41]. A study conducted with patients on cART in Rwanda, showed higher lipid profiles and blood glucose in patients

with lipodystrophy than in those who did not have the condition [13]. This study provided further information since lipid and glucose abnormalities were higher in patients with lipohypertrophy and lower among those with lipoatrophy on cART. However, ART naïve patients who had lipohypertrophy also had higher levels of cholesterol, triglyceride and glucose.

The imbalance of metabolic profile has been attributed to insulin resistance [30], which is increased by ART use, but not exclusively [42]. Sensitivity to insulin is worsen among patients on PI treatment who had lipodystrophy [43] and each additional year of exposure to nucleoside analogue reverse transcriptase inhibitors (NRTI) increased 8% the odds of hyperinsulinemia and 20% for stavudine [42].

There are some potential limitations that should take into account in the interpretation of the results. The cross-sectional design precludes the establishment of causal relationships and may be affected by potential biases. Ascertainment bias, for example, could have biased the diagnosis of clinical outcomes if the observer knew what ART drugs were in use and their adverse effects. However, the diagnosis was based in a combination of objective measurements or the patients' report of changes in body fat. The data collection was carried out with no information about the hypothesis being tested and what criteria would be used for the diagnosis. However, the study enrolled a large sample size, the data collection was meticulously obtained, all measurements were carried out in duplicate and the average was adopted in the analysis. Finally, the control for confounding factors was based on separated modeling for each clinical outcome and the use of modified Poisson regression was able to provide risk estimates appropriated for the cross-sectional design, what may have mitigated the role of biases. Besides, the simultaneous description of both phenotypes according to the ART status provides information to develop specific interventions to minimize the effect of ART and pathways to investigate causality. The results of this study could be generalized to patients with HIV/AIDS who seek care in the public health system of Porto Alegre, Southern Brazil, and its metropolitan area. Therefore, our patients are alike those from other public health centers, which provide care for most of HIV infected patients.

In conclusion, HIV-associated lipodystrophy occurred in patients with longer duration of HIV infection and on treatment with antiretroviral drugs, in whom metabolic abnormalities, especially dyslipidemia, are frequent and present increased cardiovascular risk. Therefore, these patients should undergo frequent laboratory monitoring and, if indicated, specific treatment should be initiated, since ART use is essential to increase life expectancy and to reduce the burden of disease. However, patients ART naïve were also at increased risk of cardiovascular events due to lipid abnormalities, which could be prevented.

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