Prior exposure to thymidine analogs and didanosine is associated with long-lasting alterations in adipose tissue distribution and cardiovascular risk factors

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Background: Thymidine analogs and didanosine (ddI) have been associated with redistribution of body fat from subcutaneous adipose tissue (SAT) to visceral adipose tissue (VAT), which, in turn, is a risk factor for cardiovascular disease. We explored differences in adipose tissue distribution between people living with HIV (PLWH) with prior exposure to thymidine analogs and/or ddI, without exposure, and uninfected controls and the association with cardiovascular disease risk factors.

Methods: In all, 761 PLWH from the Copenhagen Comorbidity in HIV Infection study, and 2283 age and sex-matched uninfected controls from the Copenhagen General Population Study were included. PLWH were stratified according to prior exposure to thymidine analogs and/or ddI. VAT and SAT were determined by abdominal computed tomography scan. Hypotheses were tested using regression analyses.

Results: Exposure to thymidine analogs and/or ddI was associated with 21.6 cm^2 larger VAT (13.8–29.3) compared to HIV infection without exposure. HIV-negative status was associated with similar VAT compared to HIV infection without exposure. Cumulative exposure to thymidine analogs and/or ddI [3.7 cm^2 per year (2.3–5.1)], but not time since discontinuation [−1.1 cm^2 per year (−3.4 to 1.1)], was associated with VAT. Prior exposure to thymidine analogs and/or ddI was associated with excess risk of hypertension [adjusted odds ratio (aOR) 1.62 (1.13–2.31)], hypercholesterolemia [aOR 1.49 (1.06–2.11)], and low high-density lipoprotein [aOR 1.40 (0.99–1.99)].

Conclusions: This study suggests a potentially irreversible and harmful association of thymidine analogs and ddI with VAT accumulation, which appears be involved in the increased risk of hypertension, hypercholesterolemia, and low high-density lipoprotein found in PLWH with prior exposure to thymidine analogs and/or ddI, even years after treatment discontinuation.

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Introduction

Fat redistribution from subcutaneous adipose tissue (SAT) to visceral adipose tissue (VAT) is a well known feature in people living with HIV (PLWH) [1,2]. Both VAT accumulation and SAT loss have been hypothesized to represent adverse reactions to antiretroviral therapy (ART) [3,4]. The association between early combination ART (cART) era agents [especially thymidine analogs and didanosine (ddI)] and SAT loss is well established and characterized [5], and further progression can be attenuated with switching to other agents [6]. In contrast, studies reporting a possible effect of ART on VAT accumulation have provided contrasting results [2,5,7].

Despite the introduction of antiretrovirals with less metabolic toxicities, central obesity remains a feature of HIV infection [8]. Whether this phenotype is also still accompanied by the redistribution of abdominal fat from the subcutaneous to the visceral compartment has not yet been determined. The tendency to store adipose tissue as VAT rather than SAT has been linked to increased risk of cardiovascular disease (CVD) [9,10]. Thus, assessing whether PLWH have higher risk of this phenotype and identifying the determinants of it are of primary importance to correctly assess risk of CVD in PLWH.

In the present study, we tested the hypothesis that PLWH are characterized by redistribution of body fat from the subcutaneous to the visceral compartment when compared to uninfected controls, and that thymidine analogs and/or ddI have sustained effect in determining this potentially harmful phenotype. For this purpose, we assessed the association between prior exposure to thymidine analogs and/or ddI with VAT, SAT, and VAT-to-SAT ratio, respectively. Furthermore, we investigated whether prior exposure to thymidine analogs and/or ddI was associated with hypertension, hypercholesterolemia, and low high-density lipoprotein (HDL).

Methods

Study population

People living with HIV were recruited from the Copenhagen Comorbidity in HIV Infection (COCOMO) study – a longitudinal study with the aim of assessing the burden of non-AIDS comorbities in PLWH. In all, 1099 PLWH were enrolled in the COCOMO study. All participants were offered a computed tomography (CT) scan. Procedures for recruitment and data collection have been described in detail elsewhere [11].

Uninfected individuals were recruited from the Copenhagen General Population Study (CGPS). CGPS is an ongoing population study including more than 100 000 individuals residing in the greater Copenhagen area. Only participants aged above 40 years were offered a CT scan. Procedures for recruitment and data collection have been described elsewhere [12–15].

Inclusion criteria for this study were abdominal CT scan available and above 40 years of age. Uninfected controls were age and sex-matched with PLWH in a 3 : 1 ratio using a propensity score matching method. This resulted in 2283 uninfected controls and 761 PLWH included in the present study.

Ethical approval was obtained by the Regional Ethics Committee of Copenhagen (COCOMO: H-15017350; CGPS: H-KF-01-144/01). Written informed consent was obtained from all participants.

Clinical assessments

Identical, structured questionnaires were used in COCOMO study and CGPS to collect information about demographics, physical activity, and smoking.

Data regarding HIV infection were obtained from review of medical charts of COCOMO participants [11].

All physical examinations were performed by trained clinic staff, using identical protocols in both groups [11]. Height and weight measurements and BMI calculations were performed according to WHO guidelines [16].

Blood pressure (BP) was measured on the left arm after 5-min rest with the patient in sitting position, using an automatic Digital Blood Pressure Monitor.

Nonfasting venous blood was collected and analyzed for total cholesterol and high-density lipoprotein (HDL). Blood samples from both COCOMO and CGPS participants were analyzed at Herlev University Hospital, Copenhagen [11].

Clinical outcomes definition

According to Joint National Committee guidelines, hypertension was defined as antihypertensive treatment and/or as having at least 140 mmHg systolic and/or at least 90 mmHg diastolic BP values [17].

According to American College of Cardiology/American Heart Association (ACC/AHA) guidelines, hypercholesterolemia was defined as antidyslipidemic treatment and/or as having total cholesterol above 200 mg/dl [18].

Low HDL was defined as HDL level less than 40 mg/dl in men and less than 50 mg/dl in women [15].

Computed tomography scan measurement of VAT and SAT

Computed tomography imaging was performed using the same 320-multidetector scanner (Aquilion One ViSION Edition, Canon, Japan) in a single rotation (275 ms) in COCOMO and CGPS participants. Field of view (FOV) was 500, tube voltage was 120 kVp, and current was 210 mA (independent of BMI). For measurement of visceral and subcutaneous adipose tissue, an 8-mm section (2×4.0 mm) was reconstructed centered at the level of the fourth lumbar vertebra.

Trained personnel used commercially available CT software (Fat Measurement, Aquilion ONE; Canon, Japan) to measure the cross-sectional area of adipose tissue defined as voxels with attenuation values in the range of -150 to -70 Hounsfield units. From within a manually adjusted region of interest delineated by the muscular compartments, VAT area was automatically calculated. SAT was defined as adipose tissue superficial to the abdominal and paraspinal muscles. Intraintestinal and intramuscular adipose tissues were manually excluded (Supplementary Fig. 1, http://links.lww.com/QAD/B418).

Statistical analysis

Continuous variables were reported as mean and standard deviations (SD), whereas categorical variables were reported as percentage and frequency. Different groups were compared with *t* tests or Mann–Whitney *U* test for continuous data that had normal or non-normal distribution, respectively, and chi-square/Fisher's tests for categorical data.

Univariable and multivariable linear regression models were fitted to test associations between HIV infection and the outcomes of interest. In these analyses, PLWH were stratified according to prior exposure to thymidine analogs (i.e. stavudine and zidovudine) and/or ddI (with and without exposure). Unadjusted and adjusted β coefficient (β/aβ) and 95% confidence intervals (CIs) were computed and reported. Covariates included in the base model were: age, sex, smoking (current, former, never smoker), origin (Scandinavian, other EU, Middle-East and Indian sub-continent, other), physical activity (inactive, moderately inactive, moderately active, very active), and BMI.

In PLWH, univariable and multivariable logistic regression models were fitted to test the association between the presence of hypertension, hypercholesterolemia, and low HDL, and prior exposure to thymidine analogs and/or ddI and VAT area, respectively. In these analyses, the base model was used to adjust the analyses for confounders.

Separate models for PLWH were fitted to assess associations between HIV-related variables and each outcome. The following variables were added to the base model one at a time: $CD4^+$ nadir less than 200/μl, time since cART initiation, and duration of HIV infection.

To more closely evaluate the impact of the exposure to thymidine analogs and ddI on VAT, SAT, and VAT-to-SAT ratio, possible associations between each outcome, and the cumulative period of exposure (as continuous variable and stratified in quartiles) and the cumulative time since discontinuation (as continuous variable and stratified in quartiles) were explored in the same model. Only PLWH with prior exposure to thymidine analogs and/or ddI were included in these analyses.

A P value less than 0.05 was considered statistically significant. Analyses were conducted in R (V.3.3.0).

Results

Demographics

In all, 761 PLWH from the COCOMO cohort and 2283 uninfected individuals from the CGPS cohort were included in the present study. Demographic characteristics of the populations are depicted in Table 1. No differences in age and sex distribution were found. Differences in origin, smoking status, physical activity, and BMI were found between the two groups (Table 1). HIV-specific characteristics of COCOMO participants are shown in Table 1. In all, 451 (60.5%) PLWH had prior exposure to thymidine analogs and/or ddI. Of those, 445 had previous exposure, and 6 individuals were still exposed. The mean cumulative exposure period to thymidine analogs and/or ddI was 6.6 (SD 4.2) years and mean time since discontinuation was 9.4 (SD 2.7) years.

Visceral adipose tissue in PLWH and uninfected controls

No difference in VAT area was found between PLWH and uninfected individuals (104.4 vs. 106.5 cm^2; $P = 0.456$) (Table 1). In multivariable regression analysis, HIV infection was associated with 12.6 cm^2 larger VAT (7.9–17.2) compared to uninfected controls. After stratification according to exposure to thymidine analogs and/or ddI, PLWH with exposure had larger VAT area compared to both PLWH without exposure and uninfected controls (115.5 vs. 88.9 and 106.5 cm^2, respectively) (Supplementary Fig. 2a, http://links.lww.com/QAD/B418). In multivariable analysis, HIV infection with exposure to thymidine analogs and/or ddI was associated with 21.6 cm^2 larger VAT area compared to HIV infection

Table 1. Demographic and clinical characteristics of the study populations.

General characteristics	PLWH ($n=761$)	Controls ($n=2283$)	P value
Age, mean (SD)	54.2 (9.0)	54.4 (9.0)	0.594
Sex, male, *n* (%)	651 (85.5)	1953 (85.5)	1.000
Origin, *n* (%)			<0.001
Scandinavia	570 (76.1)	2122 (94.0)	
Other Europe	77 (10.3)	113 (5.0)	
Middle East and Indian subcontinent	12 (1.6)	18 (0.8)	
Other	90 (12.0)	4 (0.2)	
HIV Transmission mode, *n* (%)			
Heterosexual	166 (22.5)	–	–
IDU	9 (1.2)	–	–
MSM	516 (69.9)	–	–
Other	47 (6.4)	–	–
Current $CD4^+$ group, *n* (%)			
<200	13 (1.8)	–	–
200–349	50 (6.8)	–	–
350–500	116 (15.7)	–	–
>500	560 (75.8)	–	–
$CD4^+$ nadir <200, yes, *n* (%)	337 (45.9)	–	–
cART, yes, *n* (%)	743 (99.8)	–	–
Current viral load <50, *n* (%)	713 (96.2)	–	–
Years since HIV positive test, years, mean (SD)	16.0 (8.9)	–	–
Years since cART initiation, years, mean (SD)	12.1 (6.4)	–	–
Exposure to TA and/or ddI, *n* (%)	451 (60.5)	–	–
Present exposure, *n* (%)	6 (1.4)	–	–
Previous exposure, *n* (%)	445 (98.6)	–	–
HCV co-infection, yes, *n* (%)	36 (4.8)	–	–
Smoking status, *n* (%)			<0.001
Never smoker	256 (34.3)	1042 (46.0)	
Current smoker	196 (26.2)	275 (12.1)	
Ex-smoker	295 (39.5)	950 (41.9)	
Physical activity, *n* (%)			<0.001
Inactive	68 (9.4)	114 (5.0)	
Moderately inactive	259 (35.7)	771 (34.0)	
Moderately active	310 (42.7)	1099 (48.4)	
Very active	85 (12.3)	286 (12.6)	
BMI, mean (SD)	25.2 (3.9)	26.8 (3.9)	<0.001
BMI WHO categories, *n* (%)			<0.001
Underweight, <18.5	18 (0.8)	7 (0.3)	
Normoweight, 18.5–24.9	386 (51.0)	768 (33.7)	
Overweight, 25–29.9	275 (36.3)	1096 (48.0)	
Obese, ≥30	78 (10.3)	411 (18.8)	
Abdominal adipose tissue distribution			
VAT, cm^2, mean (SD)	104.4 (70.6)	106.5 (64.4)	0.456
SAT, cm^2, mean (SD)	140.7 (77.9)	184.8 (83.9)	<0.001
VAT-to-SAT ratio, mean (SD)	1.0 (1.3)	0.6 (0.4)	<0.001

cART, combined antiretroviral therapy; HCV, hepatitis C virus; IDU, intravenous drug use; PLWH, people living with HIV; SAT, subcutaneous adipose tissue; SD, standard deviations; TA, thymidine analogs; VAT, visceral adipose tissue.

without exposure (13.8–29.3) (Table 2). HIV-negative status was associated with similar VAT area compared to HIV infection without exposure to thymidine analogs and/or ddI [aβ 0.2 (−6.4 to 6.8)] (Table 2).

Subcutaneous adipose tissue in PLWH and uninfected controls

People living with HIV had smaller SAT area compared to uninfected individuals (140.7 vs. 184.8 cm^2; $P<0.001$). These results were reproduced when adjusting for confounders, with HIV infection being associated with 22.3 cm^2 smaller SAT area compared to uninfected controls (−27.3 to −17.2). After stratification according to exposure to thymidine analogs and/or ddI, PLWH with exposure had smaller SAT area compared to both PLWH without exposure and uninfected controls (Supplementary Fig. 2b, http://links.lww.com/QAD/B418). In multivariable analysis, HIV infection with exposure to thymidine analogs and/or ddI was associated with 14.8 cm^2 smaller SAT area compared to HIV infection without exposure (−23.3 to −6.3). Conversely, HIV-negative status was associated with 13.0 cm^2 larger SAT area compared to HIV infection without exposure (5.8–20.3) (Table 2).

Visceral-to-subcutaneous adipose tissue ratio in PLWH and uninfected controls

People living with HIV had higher VAT-to-SAT ratio compared to uninfected individuals (1.0 vs. 0.6; $P<0.001$). HIV infection with exposure to thymidine

Table 2. Linear regression model predicting the degree of change (with 95% CI) in cm^2 of VAT and SAT.

	Visceral adipose tissue				Subcutaneous adipose tissue			
	Unadjusted β[a] (95% CI)	*P* value	Adjusted β[a] (95% CI)	*P* value	Unadjusted β[a] (95% CI)	*P* value	Adjusted β[a] (95% CI)	*P* value
Study group								
PLWH without exposure to TA/ddI	Ref		Ref		Ref		Ref	
Uninfected controls	17.6 (9.5; 25.7)	<0.0001	0.2 (−6.4; 6.8)	0.9319	34.2 (24.1; 44.3)	<0.0001	13.0 (5.8; 20.3)	0.0004
PLWH with exposure to TA/ddI	26.6 (16.8; 36.3)	<0.0001	21.6 (13.8; 29.3)	<0.0001	−15.6 (−27.8; −3.4)	0.0122	−14.8 (−23.3; −6.3)	0.0006
Age, per 5 years	10.6 (9.3; 11.9)	<0.0001	7.3 (6.31; 8.4)	<0.0001	−2.6 (−4.3; −0.9)	<0.0001	−2.9 (−4.05; −1.7)	<0.0001
Sex, male	43.9 (37.2; 50.7)	<0.0001	34.9 (29.3; 40.5)	<0.0001	−56.5 (−65.1; −47.9)	<0.0001	−58.5 (−64.4; −52.5)	<0.0001
BMI, per unit	9.1 (8.6; 9.7)	<0.0001	8.7 (8.2; 9.2)	<0.0001	14.8 (14.3; 15.4)	<0.0001	14.5 (13.9; 15.0)	<0.0001

Multivariable models were adjusted for: study group [PLWH without exposure (ref), uninfected controls, PLWH with exposure], age (per 5 years), sex (male vs. female), BMI, physical activity [inactive (ref), moderately inactive, moderately active, very active], smoking [current, former, never smoker (ref)], and origin [Scandinavian (ref), other EU, Middle-East and Indian sub-continent, other]. CI, confidence interval; ddI, didanosine; PLWH, people living with HIV; SAT, subcutaneous adipose tissue; TA, thymidine analogs; VAT, visceral adipose tissue.
[a]β coefficients represent the degree of change in cm^2 of VAT and SAT for every 1-unit of change in the explanatory variables.

analogs and/or ddI was associated with 0.6 higher VAT-to-SAT ratio compared to HIV infection without exposure [aβ 0.6 (0.5−0.7)]. HIV-negative status was associated with similar VAT-to-SAT ratio compared to HIV infection without exposure to thymidine analogs and/or ddI [aβ −0.1 (−0.1 to 0.0)].

Cumulative exposure to and time since discontinuation of thymidine analogs and/or ddI as predictors of VAT and SAT in PLWH

When limiting the analyses to PLWH with exposure to thymidine analogs and/or ddI, each year of cumulative exposure to these agents was associated with 3.7 cm^2 larger VAT (2.3−5.1). These results were reproduced when considering periods of cumulative exposure: less than 3.6 years, reference group; 3.6−6.3 years, aβ 17.5 (0.2−34.8); 6.4−9.2 years, aβ 40.8 (23.2−58.5); more than 9.2 years, aβ 44.4 (26.0−62.7). No association between the time since discontinuation of thymidine analogs and/or ddI and VAT area was found [−1.1 cm^2 per year (−3.4 to 1.1); Table 3].

No association between cumulative time of exposure or time since discontinuation of thymidine analogs, and/or ddI and either SAT (Table 3) or VAT-to-SAT ratio was found.

Visceral adipose tissue and exposure to thymidine analogs and/or ddI as predictors of risk of hypertension, hypercholesterolemia, and low HDL in PLWH

After adjusting for confounders, VAT area in PLWH was positively associated with excess risk of hypertension [adjusted odds ratio (aOR) 1.11 per 20 cm^2 increase of VAT area (1.04; 1.18)], hypercholesterolemia [aOR 1.17 per 20 cm^2 increase of VAT area (1.09; 1.25)], and low HDL [aOR 1.13 per 20 cm^2 increase of VAT area (1.06; 1.20)].

In multivariable analyses, the exposure to thymidine analogs and/or ddI was associated with excess risk of hypertension [aOR 1.62 (1.13−2.31)], hypercholesterolemia [aOR 1.49 (1.06−2.11)], and low HDL, without, however, reaching statistical significance in the latter

Table 3. Association between cumulative exposure to TA and ddI and VAT and SAT.

	VAT Adjusted β[a] (95% CI)	*P* value	SAT Adjusted β[a] (95% CI)	*P* value
Cumulative time of exposure to TA and/or ddI				
<3.6 years	Ref		Ref	
3.6−6.3 years	17.5 (0.2; 34.8)	0.047	−1.3 (−16.2; 13.6)	0.862
6.4−9.2 years	40.8 (23.2; 58.5)	<0.0001	7.6 (−7.6; 22.8)	0.328
>9.2 years	44.4 (26.0; 62.7)	<0.0001	2.2 (−13.6; 18.1)	0.781
Time since discontinuation of TA and/or ddI				
<8.1 years	Ref		Ref	
8.1−9.6 years	−13.5 (−30.8; 3.8)	0.127	8.1 (−6.8; 23.1)	0.288
9.7−10.7 years	1.0 (−16.7; 18.8)	0.906	−4.1 (−19.5; 11.2)	0.595
>10.7	8.62 (−9.4; 26.7)	0.351	14.2 (−1.4; 29.9)	0.075

All the models were adjusted for age, sex, origin, physical activity, smoking, BMI, cumulative time of exposure to TA and/or ddI, and time since discontinuation of TA and/or ddI. CI, confidence interval; ddI, didanosine; SAT, subcutaneous adipose tissue; TA, thymidine analogs; VAT, visceral adipose tissue.
[a]β coefficients represent the degree of change in cm^2 of VAT and SAT, respectively, associated with each level of the explanatory variables.

[aOR 1.40 (0.99–1.99)] (Fig. 1). These associations were lost when further adjusting for VAT area.

Other HIV-specific predictors of visceral and subcutaneous adipose tissue area

Duration of cART was associated with larger VAT area [aβ 9.6 cm^2 per 5 years (6.2; 13.1)]. This result was reproduced when limiting the analysis to PLWH with exposure to thymidine analogs and/or ddI [aβ 17.2 per 5 years (9.5–24.9)], but not in those without (Fig. 2). In multivariable analyses, $CD4^+$ nadir below 200 cells was associated with larger VAT area [aβ 15.6 cm^2 (6.9–24.2)]. This result was reproduced after stratification of PLWH according to exposure to thymidine analogs and/or ddI [with exposure aβ 11.8 cm^2 (0.0–23.6)], but not in those without (Fig. 2). No association between time since HIV infection and VAT area was found. SAT area was not associated with cART duration, $CD4^+$ nadir, and time since HIV infection.

Discussion

The present study resulted in two key findings regarding abdominal adipose tissue distribution in PLWH. First, the redistribution of abdominal adipose tissue as VAT rather than SAT remains a concern in a subpopulation of

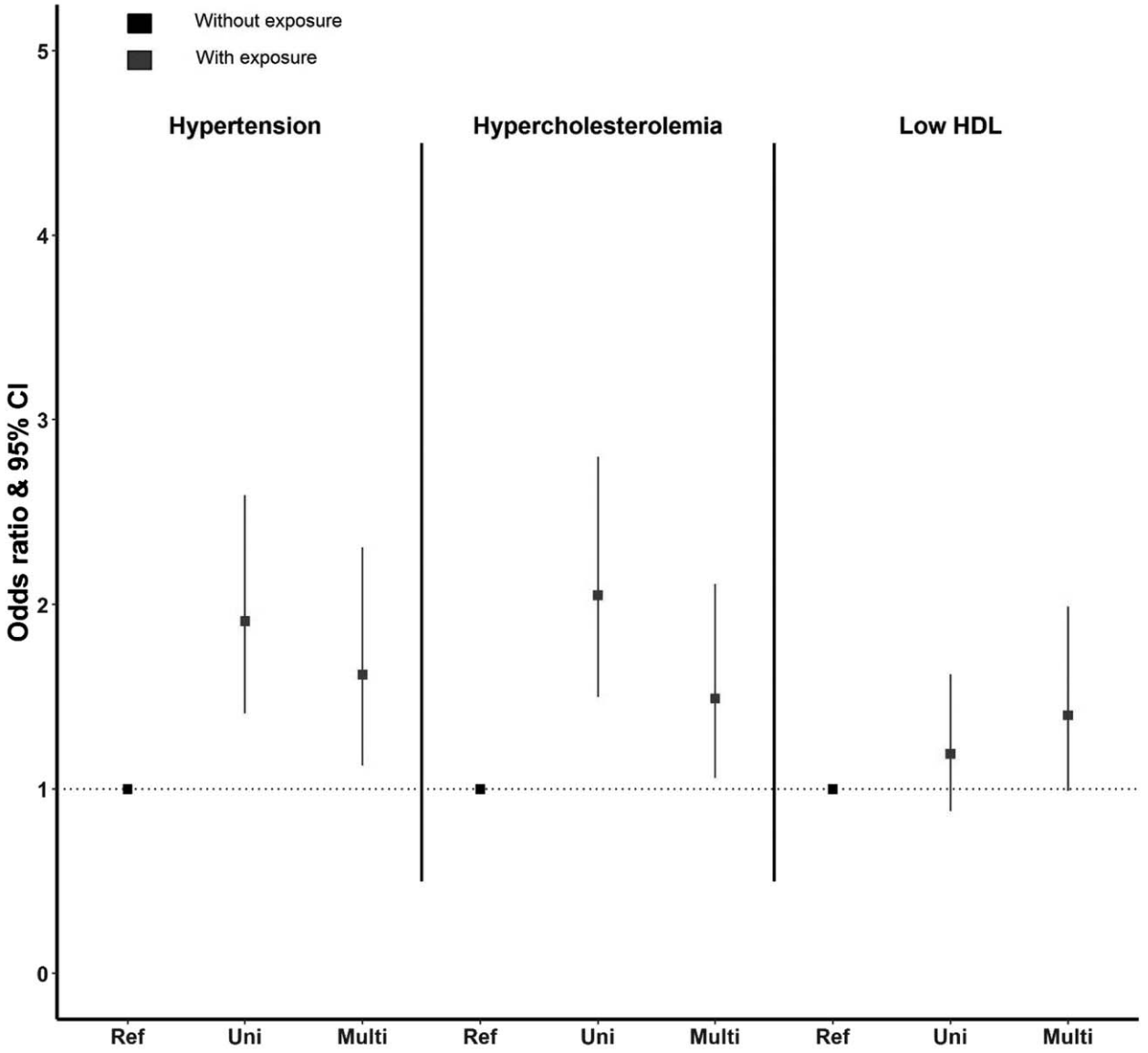

Fig. 1. Association between exposure to thymidine analogs and/or didanosine and hypertension, hypercholesterolemia, and low HDL. Results from univariable and multivariable logistic regression are reported as odds ratios (95% CI). Multivariable models were adjusted for exposure to TA and/or ddI, age, sex, smoking, physical activity, origin, and BMI. CI, confidence interval; ddI, didanosine; PLWH, people living with HIV; TA, thymidine analogs; VAT, visceral adipose tissue.

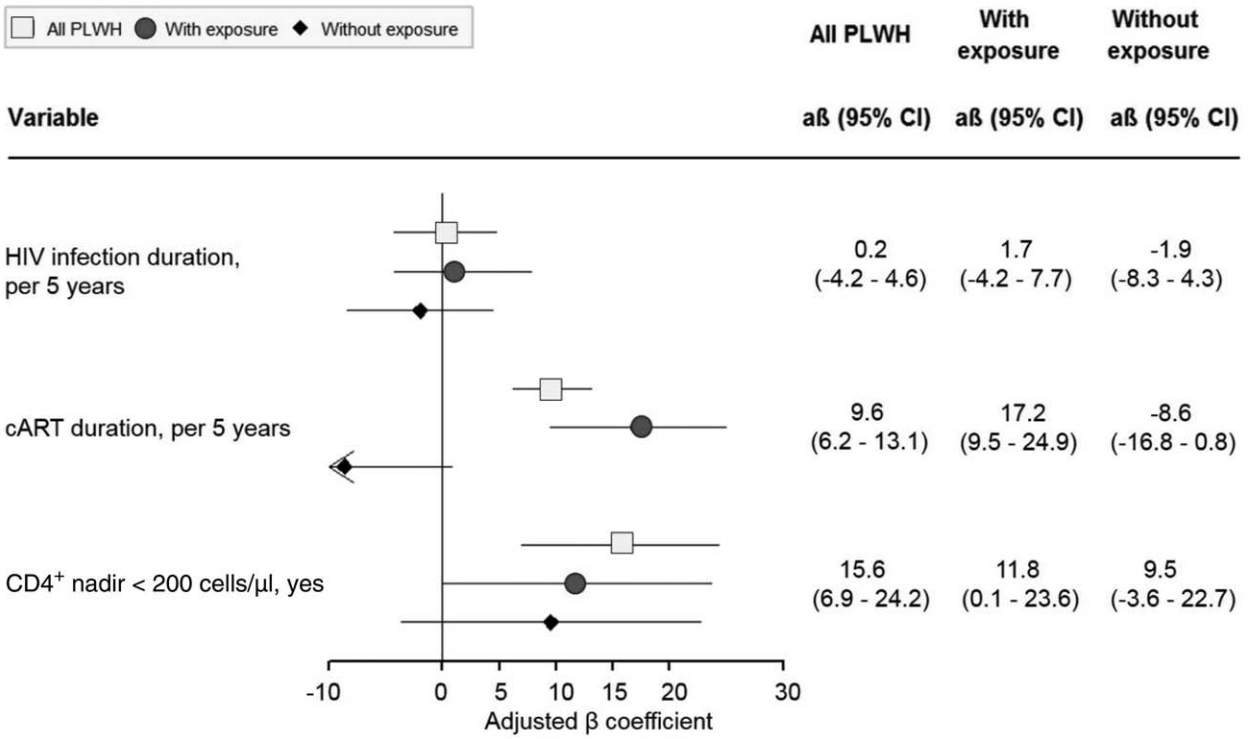

Fig. 2. Association between VAT area and time since HIV infection, cART duration, and CD4$^+$ nadir below 200 cells/ml. These associations were also explored after stratification of PLWH according to the exposure to TA and/or ddI. In addition to the variables shown in the figure, all the models were adjusted for age, sex, smoking, physical activity, origin, and BMI. β coefficients represent the degree of change in cm^2 of VAT for every 1-unit/level of change in the explanatory variables. cART, combination antiretroviral therapy; ddI, didanosine; PLWH, people living with HIV; TA, thymidine analogs; VAT, visceral adipose tissue.

PLWH, possibly due to harmful and irreversible side effects of prior thymidine analogs and/or ddI treatment. Second, our results suggested that PLWH who had been exposed to these agents were characterized by excess risk of hypertension, hypercholesterolemia, and low HDL, even years after treatment discontinuation, which may be mediated by increased VAT accumulation.

The attention towards HIV-associated fat redistribution syndrome has decreased after the introduction of modern antiretroviral regimens with fewer metabolic side effects. However, previous results from our group have shown that abdominal obesity remains a distinct characteristic of PLWH in the contemporary cART era [8]. In the present study, we further characterized this phenotype, by assessing the relative distribution of VAT and SAT at abdominal level. The associations between HIV infection, accumulation of VAT and its determinants have been widely studied. However, whether this phenotype is a direct consequence of HIV infection, a side effect to cART or a back-to-health phenomenon in well treated PLWH is still unclear. In the present study, PLWH with a history of exposure to thymidine analogs and/or ddI had larger VAT area compared to PLWH without exposure, who, on the contrary, had comparable VAT area with uninfected controls. Interestingly, almost all of PLWH with exposure did not report a current use of thymidine analogs and/or ddI, and median since last exposure was more than 9 years, suggesting an irreversible effect of these agents on adipose tissue. This hypothesis was supported by the lack of association between VAT area and the time since discontinuation of thymidine analogs and/or ddI. On the contrary, we described a direct association between the cumulative exposure to these agents and VAT area. Taken together, these results suggest a cumulative and harmful effect of thymidine analogs and/or ddI affecting VAT accumulation, which appears to be irreversible in the time frame considered in the present study. Potential mechanisms leading to irreversibility of this phenotype are many. One may speculate that, once established, the accumulation of VAT, characterized by hypoxia and high content of activated macrophages [19], may lead to a pro-inflammatory environment, known to influence adipocyte proliferation and differentiation [20]. These events may cause a vicious and self-maintaining circle, resulting in the lack of improvement in VAT accumulation after the discontinuation of thymidine analogs and/or ddI. Interestingly, no association between duration of cART and VAT area was found in PLWH without exposure. This finding may suggest that cART regimens not including thymidine analogs or ddI have no deleterious effect on VAT accumulation.

The loss of subcutaneous adipose tissue related to HIV infection has been proposed to be a side effect of thymidine analogs [5]. While our findings support this hypothesis, they also suggest a concomitant role for HIV infection *per se* or modern cART regimens in determining this phenotype. Viral proteins, especially *Vpr* and *Nef*, have previously been described to have a harmful and inhibiting effect on adipogenesis [21,22], which may partly explain the loss of subcutaneous adipose tissue independently of thymidine analogs and/or ddI. Thus, in the present study HIV, infection was associated with smaller SAT area compared to uninfected controls also in PLWH without history of exposure to thymidine analogs or ddI, albeit to a lesser extent compared to those with exposure.

Recently, VAT-to-SAT ratio has been proposed as a better correlate of cardiometabolic risk compared to BMI and absolute abdominal adipose tissue volumes [9]. In the present study, PLWH with exposure to thymidine analogs and/or ddI were characterized by increased VAT-to-SAT compared to both uninfected individuals and PLWH without exposure. This finding may further support the hypothesis that the propensity to store fat viscerally versus subcutaneously in PLWH may be a side effect of the exposure to old generation cART. Fat redistribution syndrome is a well characterized risk factor of CVD [1,23] in PLWH, where a given BMI or waist-to-hip ratio may be associated with different CVD risk depending on the relative distribution of visceral and subcutaneous adipose tissue at abdominal level. Specifically, loss of SAT and VAT accumulation have been associated with increase in renin-angiotensin-aldosterone-system (RAAS) activation, free fatty acid (FFA), and insulin resistance, known risk factors of hypertension and abnormalities in lipid metabolism [24]. Accordingly, we found that increasing VAT area was associated with excess risk of hypertension, hypercholesterolemia, and low HDL, which, in turn, were associated with the use of thymidine analogs and/or ddI even after controlling for traditional risk factors. The latter association disappeared when further adjusting the model for VAT area. This finding may suggest that the harmful association between thymidine analogs and/or ddI, and excess risk of hypertension, hypercholesterolemia, and low HDL may be a result of VAT accumulation induced by these agents.

Thymidine analogs have been proposed to affect adipose tissue distribution due to mitochondrial toxicity [25]. The apparent contrast represented by lipohypertrophy in the visceral and the concomitant lipoatrophy in the subcutaneous compartment as a consequence of the same insult may reflect differences in resistance to mitochondrial toxicity in visceral and subcutaneous adipose tissues [26,27]. In the visceral compartment, the oxidative stress induced by thymidine analogs may result in mild mitochondria dysfunction in the adipocytes, and consequently to a pathological adipose tissue accumulation [28]. Due to lower mitochondrial content and different genes expression [27], SAT has been described to have lower resistance to mitochondrial toxicity compared to the visceral compartment. Thus, an equivalent oxidative insult on adipocytes may be amplified in SAT, causing more severe mitochondrial dysfunction, which may explain the tendency towards atrophy rather than hypertrophy in the subcutaneous compartment [28].

The primary limitation of the present study is the cross-sectional design, where exposure and outcome are assessed at the same time. Specifically, while possible associations between cumulative time of exposure to thymidine analogs and/or ddI, and the time since discontinuation of these agents and our outcomes were explored, causal relationships cannot be drawn because of the lack of longitudinal data. Minor differences in the ethnicity found between the two populations may explain part of the differences in abdominal adipose tissue distribution. However, a possible confounding effect of this variable was reduced by adjusting for, among the others, region of origin in multivariable analyses. The main strength of the present study is the large and well characterized study population who underwent CT scan, comprehensive of a sex and age-matched uninfected control group. Furthermore, all laboratory and CT-scan examinations were performed in identical locations between the two populations, thus eliminating potential bias due to differences in the equipment used.

In conclusion, we present data suggesting prior exposure to thymidine analogs and/or ddI to be associated with long-lasting redistribution of abdominal adipose tissue from SAT to VAT and to negatively impact the risk of hypertension, hypercholesterolemia, and low HDL, even years after treatment discontinuation. If confirmed by prospective studies, our findings may help to identify a subgroup of PLWH who may benefit from more intensive cardiovascular prevention interventions.

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Conflicts of interest

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References

1. Palella FJ, McKibben R, Post WS, Li X, Budoff M, Kingsley L, *et al*. **Anatomic fat depots and coronary plaque among human immunodeficiency virus-infected and uninfected men in the multicenter AIDS Cohort Study.** *Open forum Infect Dis* 2016; **3**:ofw098.
2. Joy T, Keogh HM, Hadigan C, Dolan SE, Fitch K, Liebau J, *et al*. **Relation of body composition to body mass index in HIV-infected patients with metabolic abnormalities.** *J Acquir Immune Defic Syndr* 2008; **47**:174–184.
3. McComsey GA, Kitch D, Sax PE, Tebas P, Tierney C, Jahed NC, *et al*. **Peripheral and central fat changes in subjects randomized to abacavir-lamivudine or tenofovir-emtricitabine with atazanavir-ritonavir or efavirenz: ACTG Study A5224 s.** *Clin Infect Dis* 2011; **53**:185–196.
4. Negredo E, Miró O, Rodríguez-Santiago B, Garrabou G, Estany C, Masabeu A, *et al*. **Improvement of mitochondrial toxicity in patients receiving a nucleoside reverse-transcriptase inhibitor-sparing strategy: results from the Multicenter Study with Nevirapine and Kaletra (MULTINEKA).** *Clin Infect Dis* 2009; **49**:892–900.
5. de Waal R, Cohen K, Maartens G. **Systematic review of antiretroviral-associated lipodystrophy: lipoatrophy, but not central fat gain, is an antiretroviral adverse drug reaction.** *PLoS One* 2013; **8**:e63623.
6. Carr A, Workman C, Smith DE, Hoy J, Hudson J, Doong N, *et al*. **Abacavir substitution for nucleoside analogs in patients with HIV lipoatrophy: a randomized trial.** *JAMA* 2002; **288**:207–215.
7. Guaraldi G, Stentarelli C, Zona S, Santoro A, Beghetto B, Carli F, *et al*. **The natural history of HIV-associated lipodystrophy in the changing scenario of HIV infection.** *HIV Med* 2014; **15**:587–594.
8. Gelpi M, Afzal S, Lundgren J, Ronit A, Roen A, Mocroft A, *et al*. **Higher risk of abdominal obesity, elevated LDL cholesterol and hypertriglyceridemia, but not of hypertension, in people living with HIV: Results from the Copenhagen Comorbidity in HIV Infection (COCOMO) Study.** *Clin Infect Dis* 2018; **67**:579–586.
9. Kaess BM, Pedley A, Massaro JM, Murabito J, Hoffmann U, Fox CS. **The ratio of visceral to subcutaneous fat, a metric of body fat distribution, is a unique correlate of cardiometabolic risk.** *Diabetologia* 2012; **55**:2622–2630.
10. Lee SW, Son JY, Kim JM, Hwang S-S, Han JS, Heo NJ. **Body fat distribution is more predictive of all-cause mortality than overall adiposity.** *Diabetes Obes Metab* 2018; **20**:141–147.
11. Ronit A, Haissman J, Kirkegaard-Klitbo DM, Kristensen TS, Lebech A-M, Benfield T, *et al*. **Copenhagen comorbidity in HIV infection (COCOMO) study: a study protocol for a longitudinal, noninterventional assessment of non-AIDS comorbidity in HIV infection in Denmark.** *BMC Infect Dis* 2016; **16**:713.
12. Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. **Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women.** *JAMA* 2007; **298**:299–308.
13. Vedel-Krogh S, Nielsen SF, Lange P, Vestbo J, Nordestgaard BG. **Blood eosinophils and exacerbations in chronic obstructive pulmonary disease. The Copenhagen General Population Study.** *Am J Respir Crit Care Med* 2016; **193**:965–974.
14. Afzal S, Tybjærg-Hansen A, Jensen GB, Nordestgaard BG. **Change in body mass index associated with lowest mortality in Denmark, 1976–2013.** *JAMA* 2016; **315**:1989–1996.
15. Thomsen M, Nordestgaard BG. **Myocardial infarction and ischemic heart disease in overweight and obesity with and without metabolic syndrome.** *JAMA Intern Med* 2014; **174**:15–22.
16. WHO. **Waist circumference and waist-hip ratio: report of a WHO expert consultation**. World Health Organization. Report of a WHO Expert Consultation 2008; 1: 27–28.
17. James PA, Oparil S, Carter BL, Cushman WC, Dennison-Himmelfarb C, Handler J, *et al*. **2014 evidence-based guideline for the management of high blood pressure in adults: report from the panel members appointed to the Eighth Joint National Committee (JNC 8).** *JAMA* 2014; **311**:507–520.
18. Stone NJ, Robinson JG, Lichtenstein AH, Bairey Merz CN, Blum CB, Eckel RH, *et al*. **2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults.** *Circulation* 2014; **129**:S1–S45.
19. de Souza Dantas Oliveira SH, de Souza Aarão TL, da Silva Barbosa L, Souza Lisbôa PG, Tavares Dutra CD, Margalho Sousa L, *et al*. **Immunohistochemical analysis of the expression of TNF-alpha, TGF-beta, and caspase-3 in subcutaneous tissue of patients with HIV lipodystrophy syndrome.** *Microb Pathog* 2014; **67–68**:41–47.
20. Ye J, Gimble JM. **Regulation of stem cell differentiation in adipose tissue by chronic inflammation.** *Clin Exp Pharmacol Physiol* 2011; **38**:872–878.
21. Otake K, Omoto S, Yamamoto T, Okuyama H, Okada H, Okada N, *et al*. **HIV-1 Nef protein in the nucleus influences adipogenesis as well as viral transcription through the peroxisome proliferator-activated receptors.** *AIDS* 2004; **18**:189–198.
22. Shrivastav S, Kino T, Cunningham T, Ichijo T, Schubert U, Heinklein P, *et al*. **Human immunodeficiency virus (HIV)-1 viral protein R suppresses transcriptional activity of peroxisome proliferator-activated receptor {gamma} and inhibits adipocyte differentiation: implications for HIV-associated lipodystrophy.** *Mol Endocrinol* 2008; **22**:234–247.
23. Grinspoon S, Carr A. **Cardiovascular risk and body-fat abnormalities in HIV-infected adults.** *N Engl J Med* 2005; **352**:48–62.
24. Srinivasa S, Fitch KV, Wong K, Torriani M, Mayhew C, Stanley T, *et al*. **RAAS activation is associated with visceral adiposity and insulin resistance among HIV-infected patients.** *J Clin Endocrinol Metab* 2015; **100**:2873–2882.
25. Pinti M, Salomoni P, Cossarizza A. **Anti-HIV drugs and the mitochondria.** *Biochim Biophys Acta* 2006; **1757**:700–707.
26. Deveaud C, Beauvoit B, Hagry S, Galinier A, Carrière A, Salin B, *et al*. **Site specific alterations of adipose tissue mitochondria in 3′-azido-3′-deoxythymidine (AZT)-treated rats: an early stage in lipodystrophy?** *Biochem Pharmacol* 2005; **70**:90–101.
27. Deveaud C, Beauvoit B, Salin B, Schaeffer J, Rigoulet M. **Regional differences in oxidative capacity of rat white adipose tissue are linked to the mitochondrial content of mature adipocytes.** *Mol Cell Biochem* 2004; **267**:157–166.
28. Caron-Debarle M, Lagathu C, Boccara F, Vigouroux C, Capeau J. **HIV-associated lipodystrophy: from fat injury to premature aging.** *Trends Mol Med* 2010; **16**:218–229.