Lipoatrophy of the foot pad in HIV-treated patients is associated with increased PAI-1

Short running title: Lipoatrophy of the foot pad in HIV-treated patients

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Abstract

Purpose: To describe lipoatrophy of the plantar pedis fat pads in HIV patients with or without long-term antiretroviral therapy (ART); to compare the characteristics of ART patients with and without plantar pedis lipoatrophy; and to examine the effects of HIV and metabolic/cardiovascular risk parameters and treatment history on plantar pedis lipoatrophy.

Design: One hundred and thirty four patients who started PI-ART in 1996 and 49 treatment naïve patients, recruited 2004, were examined and graded for lipoatrophy of five body compartments including the plantar fat pads eight years after start of ART. Baseline HIV- and ART-related factors were documented together with follow up metabolic/cardiovascular risk parameters.

Results: Plantar pedis lipoatrophy occurred more often among ART patients (60%) than among treatment naïve patients (12%; $p < 0.001$). ART patients with plantar lipoatrophy were older, had higher PAI-1 values, a higher prevalence of lipoatrophy in other body compartments, and longer stavudine and didanosine treatment history as compared to patients without plantar lipoatrophy. In multiple logistic regression, the best predictive model for plantar lipoatrophy was increased PAI-1 when HIV and metabolic/cardiovascular risk parameters were studied and treatment with didanosine when treatment history was studied. Increased PAI-1 was not associated to lioatrophy in any other location.

Conclusions: Plantar lipoatrophy is common among patients on long-term ART and, although often overlooked, may cause significant discomfort. The association to PAI-1, a well known marker of increased cardiovascular risk, is intriguing and further focuses on the need of an active approach to evaluating and lowering cardiovascular risk factors in long-term HIV treatment.

Keywords: Adverse effects, antiviral therapy, body changes, metabolic disorders, plasminogen activator inhibitor 1

Introduction

Since the introduction of protease inhibitors (PI) in antiretroviral therapy (ART) in 1996, the mortality and incidence of AIDS-related conditions has declined dramatically (Mocroft et al., 2003). However, ART is itself associated with metabolic abnormalities including hyperlipidemia, insulin resistance, and subcutaneous and visceral liopdystrophy. The degree of lipodystrophy (loss of subcutaneous fat and the accumulation of visceral fat) is in daily clinical life generally assessed on the basis of subjective observation by the patient and or clinician. ART-associated lipodystrophy has been reported in 11 to 83% of patients receiving ART (Carter et al., 2001; Grinspoon & Carr, 2005; Hansen et al., 2006; Mauss et al., 2002) and several studies suggests that lipodystrophy is associated with negatively affected health-related quality of life (Guaraldi et al., 2008; Nicholas, Kirksey, Corless, & Kemppainen, 2005) and or adherence to therapy (Guaraldi et al., 2008). There is increasing evidence that the subcutaneous lipoatrophy may develop from an independent mechanism to the often co-existing absolute or relative abdominal lipohypertrophy, often combined with increased fat in the liver (Grinspoon & Carr, 2005; Saint-Marc et al., 1999). Peripheral lipoatrophy has been correlated to older age, advanced disease stage, longer duration of ART, and the nucleoside analogue reverse transcriptase inhibitors (NRTI) stavudine, didanosine, and zidovudine (Carr et al., 1998; Lichtenstein, 2005; Mallon, Miller, Cooper, & Carr, 2003; Mauss et al., 2002; Miller et al., 2003; G. J. Moyle, 2005; Saint-Marc et al., 1999) while central lipohypertrophy has been correlated with older age and treatment with stavudine and in particular PIs (Heath et al., 2002; Lichtenstein, 2005; Miller et al., 2003; G. Moyle, 2007). The pathogenic mechanisms suggested for lipoatrophy include deranged fat cell differentiation or apoptosis and mitochondrial toxicity (Lichtenstein, 2005). Stavudine, didanosine, and, to some extent, zidovudine interfere with mitochondrial gamma polymerase (Villarroya, Domingo, & Giralt, 2005). Fat biopsies from patients on NRTI-containing ART have been shown to have lower

mitochondrial DNA content and RNA expression (Mallon et al., 2008). PIs with the exception of atazanavir inhibit lipogenesis and adipocyte development, increase lipolysis and induce insulin resistance directly through the inhibition of glucose transport into the cell and indirectly through abdominal obesity (Grinspoon & Carr, 2005; G. Moyle, 2007; Murata, Hruz, & Mueckler, 2002; Noor, Flint, Maa, & Parker, 2006). Also NRTIs have been found to decrease insulin sensitivity, possibly due to increased lipolysis (Blumer et al., 2008). Plasminogen activator inhibitor type 1 (PAI-1) is an important inhibitor of fibrinolysis, contributes to the pathogenesis of atherosclerosis and is a well known risk factor for coronary artery disease (Kohler & Grant, 2000). Increased PAI-1 has been reported in lipodystrophy (He et al., 2005), seems to be linked to the insulin resistance (De Larranaga, Galich, Puga, Alonso, & Benetucci, 2004), and is possibly produced, together with other cardiovascular risk factors, in the fatty liver (Yki-Jarvinen, 2005). In our clinical practice, we have made a new observation in patients on ART reporting non-neuropathic foot pain while walking and standing. Some of these patients display a pronounced reduction of the plantar pedis fat pads. However, apart from case reports (Stupar & Tibbles, 2008), no systematic studies have been published investigating the prevalence of plantar lipoatrophy and its contributing factors.

The purposes of this study were: 1) to describe and compare the prevalence of lipoatrophy of the plantar pedis fat pads in long term ART patients and ART naïve patients, 2) to describe and compare the characteristics of HIV-infected patients with and without lipoatrophy of the plantar pedis fat pads, and 3) to examine the influence of baseline clinical characteristics, current metabolic and cardiovascular risk parameters, and treatment history on lipoatrophy of the plantar pedis fat pads in ART patients.

Materials and Methods

Patients

Treatment with PIs in the form of indinavir TID or ritonavir BID was introduced at Venhälsan HIV clinic, Stockholm South General Hospital, Stockholm in 1996. All 202 patients who started PI-ART in 1996 were included in a clinical cohort to be monitored prospectively. During year 2004 the remaining 160 patients from this cohort (30 patients had died and 12 had moved to another city or country; ART patients) were, together with an additional 89 consecutive treatment naïve HIV-positive patients (ART naïve control group) visiting the clinic for their routine checkup, invited to participate in the present study. All potential participants received oral and written information about the present study by their responsible staff nurse. Those who agreed to participate were asked to sign a consent form. The patients were invited to give blood samples for the analyses specified below and to have a physical examination of potential lipoatrophy by a plastic surgeon not involved in the care of the patients. The study procedures and research instruments were reviewed and approved by an institutional ethics committee (South Review Board of Research Ethics, Huddinge University Hospital).

Measurements

Lipoatrophy

The plastic surgeon performed a systematic physical examination with "fat mapping" including an "in house" standardized grading of the degree of subcutaneous fat wasting in the following regions: face, gluteal regions, thigh, palm, and sole of the foot. The degree of regional lipoatrophy was rated on a 0–5 scale. In addition, the body mass index (BMI) and waist–hip ratio (WHR) were calculated.

Baseline clinical characteristics

For the ART subsample of patients, data at baseline (*i e* at PI-ART start, year 1996) on age, time since HIV diagnosis, AIDS diagnosis, NRTI experience at the start of PI, HIV-RNA (Roche ultra-sensitive test, version 1.5; frozen samples), and CD4 cell count (lyse-no-wash preparation and "single-platform" procedure with reagents from Becton Dickinson, Hägersten, Sweden) were obtained by reviewing the patients' medical records. Furthermore, the history of ART with the exact treatment time in days on each individual drug was calculated.

Current CD4, metabolic and other cardiovascular risk parameters

All blood was sampled during morning hours with the patients fasting since midnight. CD4 cell measurements were performed by the Swedish Institute for Infectious Disease Control (lyse-no-wash preparation and "single-platform" procedure with reagents from Becton Dickinson, Hägersten, Sweden). All other laboratory tests were performed by the Laboratory of Clinical Chemistry at Huddinge University Hospital (accreditation by the Swedish Board for Accreditation and Conformity Assessment) with standard biochemical methods where not otherwise indicated.

The fasting plasma levels of glucose and insulin were determined. The homeostatic model assessment for insulin resistance (HOMA-IR) was calculated using the formula [HOMA-IR = (insulin (pmol/l) / 6.945 × glucose (mmol/l)) / 22.5]. A HOMA-IR index above 3 indicates insulin resistance. Triglycerides (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), apolipoprotein A1 (Apo A1), apolipoprotein B (Apo B), and lipoprotein (a) (Lp(a)) were determined. Low density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula. High sensitivity C-reactive protein (hsCRP) was determined. The plasma level of PAI-1 was determined on citrated plasma with the Chromolize PAI-1 kit (Biopool AB, Umeå, Sweden).

Statistical methods

Statistical analyses were performed with SPSS for windows version 13.0. The criterion for accepting statistical significance was $p < 0.05$. The non-parametric Mann Whitney *U*-test was used for pair wise group comparisons of continuous and scaled variables. Fisher's exact test was used for group comparisons on frequency data. Logistic regression analyses were performed to study the influence of different potentially related variables on the odds ratio (OR) for development of lipoatrophy of the plantar fat pads among the patients up to eight years after start of PI-ART (follow up). First, the influence of each independent variable on the dependent variable was analysed univariately. Subsequently, those independent variables statistically significant in the univariate analysis were entered simultaneously in a multiple logistic regression. The independent variables investigated were a set of potential risk variables at baseline and the outcome of the different relevant metabolic and cardiovascular risk parameters at follow up. In a second set of analyses, the influence on treatment with didanosine and stavudine on the OR for development of lipoatrophy of the plantar fat pats was performed. The independent variables were dichotomised as follows: age was coded into the lowest quartile (*i e* the youngest patients) versus the other three quartiles; HIV-RNA at baseline was coded by less than 100,000 copies/ml versus equal or above 100,000 copies/ml; CD4 count and time with known HIV infection at baseline were coded as below or equal versus above the group median values; the metabolic and cardiovascular risk parameters at follow up were coded regarding to the laboratory's reference values (*i e* normal versus pathological); the follow up Lp(a) was coded as above or below 300 mg/l; the follow up HOMA-IR index was coded as below or above 3. Treatment with didanosine and stavudine were coded as ever treated or never treated with the respective drug. At last, to study the association between abnormal PAI-1 and lipoatrophy of all investigated body regions, a

multiple logistic regression analysis was performed with PAI-1 as the dependent variable and lipoatrophy in face, gluteal regions, thigh, palm, and sole of the foot as the independent variables.

Results

Study participation

The majority of both ART patients and the ART naïve control patients agreed to take part in the study (134/160 (84%) ART patients: 125 men who hav sex with men (MSM), 7 women, and 2 heterosexual men; 49/89 (55%) ART naïve patients: 48 MSM and 1 heterosexual man).

Representability of the participants

Comparisons between participants and non-participants within the ART patients (n=134 and 26, respectively) and within the ART naïve control group (n=49 and 40, respectively), regarding age, AIDS, CD4, HIV-RNA, and months since HIV diagnosis did not show any statistically significant difference (data not shown).

Baseline characteristics of the ART patients

At PI-ART start (year 1996; baseline; n=134) the median age was 39.5 (inter quartile range (IQR) 34–45, mean 40, standard deviation (SD) 8) years, the median CD4 count 210 (IQR 80–320) $\times 10^6$ cells/l, the median RNA level 61,100 (IQR 9,725–192,825) copies/ml, and the median time with known HIV infection 87.5 (IQR 42–125) months. Forty-seven percent had a CD4 count below 200 $\times 10^6$ cells/l, 30% had been diagnosed with AIDS, and 55% had either a CD4 count below 200 $\times 10^6$ cells/l or AIDS. A majority of the ART patients, 93/134 (69%), were NRTI experienced, usually to zidovudine and or didanosine, when starting PI-ART (118/134 (88%) started with indinavir and 16/134 (12%) with ritonavir). The patients have been treated according to best clinical practice by their individual healthcare team. Most of the patients have changed treatment several times due to viral failure, side effects, or a wish to simplify the regimen. At follow-up (year 2004) the median time since the start of PI-ART was 7.8 years (range 7.1–8.3 years).

Comparisons of demographic, clinically and metabolic characteristics

Comparisons regarding the ART patients' and the ART naïve control patients' demographic, clinically and metabolic characteristics at follow-up are shown in Table 1. Not surprisingly, the ART patients were older and had longer known time with HIV infection as compared to the ART naïve patients. The ART patients had lower weight and BMI and higher WHR than the ART naïve patients. When comparing the metabolic and cardiovascular risk laboratory parameters, the ART patients had higher values of the "bad" lipid levels TG, TC, LDL-C and Apo B, but also of the "good" lipids HDL-C and Apo A1. Furthermore, HOMA-IR was higher among the ART patients, suggesting a higher degree of insulin resistance among the patients on treatment.

Prevalence of lipoatrophy of the plantar pedis fat pads

Lipoatrophy of the plantar pedis fat pads was found in 81/134 (60%) of the ART patients as compared to 6/49 (12%) of the ART naïve control patients (p <0.001). Three of the six ART naïve patients with plantar pedis lipoatrophy had HOMA above 3 indicating insulin resistance.

Prevalence of lipoatrophy in other locations than the plantar pedis

Lipoatrophy of the hand was found in 44/134 (33%) of the ART patients and 4/49 (8%) of the ART naïve control patients (p <0.001), gluteal lipoatrophy was found in 68/134 (51%) of the treated and none of the naïve patients (p <0.001), thigh lipoatrophy was found in 69/134 (51%) of the treated and 3/49 (6%) of the naïve patients (p <0.001), and face lipoatrophy was found in 48/134 (36%) of the treated and 1/49 (2%) of the naïve patients (p <0.001).

Comparison between the ART patients with and without plantar pedis lipoatrophy

Comparisons between the ART patients with and without plantar pedis lipoatrophy are shown in Table 2. The patients with plantar lipoatrophy were older and had higher PAI-1 values indicating a more atherogenic profile as compared to the patients without plantar lipoatrophy. When comparing treatment time on different individual drugs, the patients with plantar lipoatrophy had been longer on didanosine and stavudine as compared to the patients without plantar lipoatrophy. Not surprisingly, the patients with plantar lipoatrophy also had a higher prevalence of lipoatrophy of the other investigated body compartments as compared to the patients without plantar lipoatrophy.

Effects of baseline clinical characteristics and current parameters on plantar pedis lipoatrophy among ART patients

The results of the univariate logistic regression analyses to investigate the effects of baseline clinical characteristics and current metabolic and cardiovascular risk parameters on plantar pedis lipoatrophy are shown in Table 3. Only baseline CD4 count below median and currently abnormal PAI-1 (*i e*, above 15kIE/l) were statistically significant associated with lipoatrophy of the plantar pedis fat pads. The OR for having plantar pedis lipoatrophy was 2.13 for those having a baseline CD4 count below median as compared to those having a baseline CD4 count above median. Further, the OR for having plantar pedis lipoatrophy was 2.69 for those having abnormal PAI-1 as compared to those having PAI-1 within the normal range *(i e,* below 15kIE/l). There was no statistically significant association to NRTI experience at PI-ART start, disease stage, age, HIV-RNA, months with HIV infection, lipid values, HOMA-IR or hsCRP.

In the multivariate logistic regression analysis, when baseline CD4 count and current PAI-1 were entered simultaneously into the equation, only PAI-1 showed a statistically significant association to plantar pedis lipoatrophy. When the effects of baseline CD4 count was adjusted for, the OR for having plantar pedis lipoatrophy was 2.52 for those having abnormal PAI-1 as compared to those having PAI-1 within the normal range.

Effects of treatment history with different antiretroviral drugs on plantar pedis lipoatrophy among ART patients

When the effects of treatment with didanosine and stavudine were investigated in univariate logistic regression analyses, treatment history with didanosine showed a statistically significant association with plantar pedis lipoatrophy. The OR for having plantar pedis lipoatrophy was 3.12 for those having a treatment history with didanosine as compared to those without a treatment history with didanosine (Table 3).

The effects of lipoatrophy at various locations on increased PAI-1 among ART patients

As abnormal PAI-1 was the strongest predictor of plantar pedis lipoatrophy and there was a relation between plantar pedis lipoatrophy and lipoatrophy in the other investigated body regions, a multivariate logistic regression analysis was performed to investigate the effect of lipoatrophy of all investigated body regions on the OR for having an abnormal PAI-1. The result showed that only plantar pedis lipoatrophy had a statistically significant effect on the OR for having abnormal PAI-1. The OR for having abnormal PAI-1 was 2.92 for those having plantar pedis lipoatrophy as compared to those not having plantar pedis lipoatrophy when the effects of lipoatrophy at the other investigated locations were adjusted for (Table 4).

Discussion

This cross-sectional study revealed that the vast majority of the HIV-infected patients on long term ART had a reduction of the plantar pedis fat pads. HIV-associated lipoatrophy of this particular region has, to our knowledge, not been systematically described before. Furthermore, the study also revealed a high prevalence of lipoatrophy in other body regions.

The patients with plantar lipoatrophy were characterised by higher age, higher PAI-1, higher prevalence of lipoatrophy in other regions, and longer didanosine and stavudine exposure. In the multivariate logistic regression analysis including HIV and metabolic parameters, the best predictive model for plantar pedis lipoatrophy was increased PAI-1. Although thinning of the plantar fat pads has been described in non-HIV-infected diabetic patients with polyneuropathy (Bus, Maas, Cavanagh, Michels, & Levi, 2004), we did not find any association to insulin resistance among the ART patients. However, plantar pedis lipoatrophy was also found in 12% of the ART naïve patients, half of whom had signs of insulin resistance.

Autopsy studies have shown the fat pads of the feet to have a significant nerve and blood supply separate from the surrounding musculature and a meshwork of septa arranged in closed-cell configuration, creating a delicate weight bearing structure (Jahss et al., 1992). It is likely that the fat hypotrophy found in this study impairs this cushioning function. The consequences for the patient might be pain and stiffness while standing and walking.

HIV infection, both before and after the introduction of PI-ART, is associated with a pro-atherogenic lipid profile characterized by an increase in triglyceride levels, a decrease in HDL-C, and the presence of small LDL-C particles (Riddler et al., 2003; Salyer, Lyon, Settle, Elswick, & Rackley, 2006). Patients on ART for six years had decreased HDL-C and BMI and increased TG and insulin values as compared to HIV-negative healthy controls (Hansen et al., 2006). In the present study, we found that the ART patients had higher triglycerides,

LDL-C, and HOMA-IR indices and lower BMI as compared to the ART naïve HIV-positive controls. However, we found the HDL-C and ApoA1 levels to be higher among the ART patients, possibly reflecting treatment induced reversion of HIV-induced lipid abnormalities.

PAI-1, but not triglycerides, hsCRP, or HOMA was associated with plantar pedis lipoatrophy. No association of increased PAI-1 was found to lipoatrophy in any other location. Abdominal and liver fat is an important source of PAI-1 (Alessi et al., 1997; Yki-Jarvinen, 2005). PAI-1 is increased in patients with abdominal obesity and insulin resistance (Alessi et al., 1997; Kohler & Grant, 2000) and among ART patients with lipodystrophy (Hadigan et al., 2001; He et al., 2005). We have previously found increased PAI-1 values to be associated to PI-ART (Koppel, Bratt, Schulman, Bylund, & Sandstrom, 2002). Visceral adiposity and insulin resistance is reported to be the main predictor of plasma PAI-1 levels in patients with lipodystrophy (De Larranaga et al., 2004; He et al., 2005). PAI-1, overproduced by the fatty liver is linked to dysregulation of the TNF-α system (He et al., 2005). Increased TNF-α expression in lipoatrophic tissue has been suggested as a possible cause of impaired adipocyte development and apoptosis (Bastard et al., 2002). Thus, PAI-1 might be of importance for the development of plantar pedis lipoatrophy through facilitation of thrombotic micro events and increasing the clots resistance to fibrinolysis in the small interseptal vessels.

Certain study limitations should be emphasised when interpreting the results. Firstly, the great majority of the investigated cohort was male patients who started PI-ART in an advanced stage of HIV infection and had prior experience of NRTI mono- or dual therapy. The inferences drawn from this study might not be applicable to women nor to ART naïve patients starting antiretroviral therapy today in a less advanced stage of the disease and with more metabolically tolerable combinations. With regard to gender, however, a recent study has shown that lipodystrophy is at least as common if not even more so (including fat loss

from lower limbs) in women (Galli et al., 2003), indicating that the present study findings may well be of importance for both sexes. Secondly, as expected, the treatment naïve control group was younger and had had shorter known time with HIV infection as compared to the ART patients. On the other hand, it is our opinion that a comparison with a HIV-positive control group has an advantage in sorting out more specific ART related effects in contrast to using a matched HIV-negative control group. Thirdly, there were some problems motivating the naïve controls to participate in the study. However, there were no apparent demographic or clinical differences between the participating and non-participating control patients.

Conclusion

In conclusion, evaluation of the plantar pedis fat pads in patients on ART should be performed regularly by the responsible physician or nurse specialist, especially when there are signs of lipoatrophy in other areas and regardless of any spontaneously reported leg or foot discomfort. Because of the proposed relation to increased cardiovascular risk, the presence of plantar lipoatrophy and or foot pain might also indicate the need of cardiovascular risk reduction interventions. Furthermore, the presence of plantar pedis lipoatrophy should actualize the need to reconsider the patient's ART regimen with the goal to avoid zidovudine, didanosine and stavudine whenever possible. Further research about the causes of plantar pedis lipoatrophy is needed to prevent and relieve the patients suffering. The strong association with PAI-1 suggests possible treatment options with anticoagulants and or drugs that decrease liver and central fat accumulation.

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Table 1. Comparison between the participating ART and ART-naïve control patients; all values originate from the time point where the systematic physical examination with "fat mapping" was performed

Variable	**ART patients (n=134)**	**ART-naïve patients (n=49)**	
	median (IQR)/ frequency (%)	**Median (IQR)/ Frequency (%)**	***p***
Age (years)	48 (42–53)	40 (36–45)	<**0.001**[a]
Time with HIV (months)	172 (126–209)	50 (25–123)	<**0.001**[a]
CD4 ($\times 10^6$cells/l)	524 (330–656)	445 (353–635)	0.725[a]
Weight (kg)	72 (65–78)	75 (71–86)	**0.002**[a]
BMI	22.5 (20.6–24.0)	23.8 (22.1–26.4)	**0.003**[a]
WHR	0.95 (0.91–0.98)	0.90 (0.87–0.97)	**0.001**[b]
TG (mmol/l)	1.9 (1.1–2.9)	1.2 (0.8–1.9)	<**0.001**[a]
TC (mmol/l)	5.4 (4.6–6.2)	4.2 (3.7–5.0)	<**0.001**[a]
HDL-C (mmol/l)	1.3 (1.1–1.6)	1.1 (0.9–1.4)	**0.007**[a]
LDL-C (mmol/l)	3.1 (2.4–3.8)	2.5 (2.2–3.0)	**0.003**[a]
Lp(a) (mg/l)	163 (91–507)	140 (61–362)	0.316[a]
PAI-1 (kIE/l)	13.5 (8.0–29.3)	9.0 (4.3–16.0)	**0.008**[a]
Apo A1 (mmol/l)	1.3 (1.2–1.5)	1.1 (1.0–1.2)	<**0.001**[a]
Apo B (mmol/l)	1.0 (0.8–1.2)	0.8 (0.7–0.9)	<**0.001**[a]
hsCRP (mg/l)	1.7 (0.8–4.0)	1.3 (0.7–2.7)	0.056[a]
HOMA-IR	2.0 (1.3-3.1)	1.4 (0.8-2.8)	**0.009**[a]

[a]Mann Whitney *U*-test; [Apo A1]apo A1 lipoprotein; [Apo B]apo B lipoprotein; [ART]antiretroviral therapy; [BMI]body mass index; [HOMA-IR]homeostatic model assessment for insulin resistance;

hsCRPhigh sensitivity C-reactive protein; HDL-Chigh density lipoprotein cholesterol; IQRinter quartile range; LDL-Clow density lipoprotein cholesterol; Lplipoprotein; PAIplasminogen activator inhibitor; TCtotal cholesterol; TGtriglycerides; WHRwaist hip ratio

Table 2. Comparison between the ART patients with and without plantar pedis lipoatrophy

Variable	**ART patients with plantar pedis lipoatrophy (n=81)**	**ART patients without plantar pedis lipoatrophy (n=53)**	
	median (IQR)/ frequency (%)	**Median (IQR)/ Frequency (%)**	***p***
Age at PI-ART start (years)	40 (35–46)	38 (32–44)	**0.025**[a]
Time with HIV at PI-ART start (months)	88 (48–121)	87 (34–128)	0.913[a]
HIV-RNA at PI-ART start (log_{10} copies/ml)	4.8 (4.1–5.4)	4.6 (3.9–5.1)	0.174[a]
CD4 at PI-ART start ($\times 10^6$cells/l)	160 (61–290)	230 (115–340)	0.077[a]
CD4 <200 $\times 10^6$cells/l or AIDS at PI-ART start (freq)	49/81 (61%)	24/53 (45%)	0.11[b]
Actual CD4 ($\times 10^6$cells/l)	485 (323–627)	557 (351–689)	0.211[a]
TG (mmol/l)	1.9 (1.2–3.3)	1.9 (1.0–2.7)	0.177[a]
TC (mmol/l)	5.3 (4.6–6.2)	5.4 (4.5–6.1)	0.935[a]
HDL-C (mmol/l)	1.2 (1.1–1.5)	1.3 (1.1–1.6)	0.312[a]
LDL-C (mmol/l)	3.1 (2.4–3.8)	3.2 (2.4–3.8)	0.654[a]
HOMA-IR	2.2 (1.3–3.4)	1.7 (1.1–2.7)	0.180[a]
Lp(a) (mg/l)	178 (105–535)	139 (50–427)	0.240[a]
PAI-1 (kIE/l)	17.0 (9.0–31.5)	11.0 (6.0–25.5)	**0.039**[a]
Apo A1 (mmol/l)	1.31 (1.15–1.51)	1.36 (1.21–1.57)	0.423[a]
Apo B (mmol/l)	1.00 (0.82–1.20)	1.07 (0.83–1.18)	0.943[a]
hsCRP (mg/l)	1.8 (1.1–6.0)	1.3 (0.7–3.4)	0.052[a]

Weight (kg)	71.4 (63.9–77.3)	72.9 (65.2–79.0)	0.568[a]
BMI	22.1 (20.5–23.7)	22.8 (21.3–24.4)	0.280[a]
WHR	0.95 (0.92–0.99)	0.94 (0.90–0.97)	0.078[a]
Exposure to zidovudine (months)	71 (47–92)	78 (44–95)	0.825[a]
Exposure to lamivudine (months)	81 (63–90)	79 (60–90)	0.667[a]
Exposure to didanosine (months)	28 (3–66)	5 (0–32)	**0.002**[a]
Exposure to stavudine (months)	30 (0–53)	12 (0–43)	**0.042**[a]
Lipoatrophy of the face (freq)	37/81 (46%)	11/53 (21%)	**0.003**[b]
Lipoatrophy of the palms (freq)	36/81 (44%)	8/53 (15%)	**<0.001**[b]
Lipoatrophy of the thigh (freq)	49/81 (60%)	20/53 (38%)	**0.013**[b]
Lipoatrophy of the gluteal region (freq)	49/81 (60%)	19/53 (36%)	**0.008**[b]

[a]Mann Whitney *U*-test; [b]Fisher's exact test; [Apo A1]apo A1 lipoprotein; [Apo B]apo B lipoprotein; [ART]antiretroviral therapy; [BMI]body mass index; [freq]frequency; [hsCRP]high sensitivity C-reactive protein; [HDL-C]high density lipoprotein cholesterol; [HOMA-IR]homeostatic model assessment for insulin resistance; [IQR]inter quartile range; [LDL-C]low density lipoprotein cholesterol; [Lp]lipoprotein; [PAI]plasminogen activator inhibitor; [PI]protease inhibitor; [SHBG]sex hormone binding globulin; [TC]total cholesterol; [TG]triglycerides; [WHR]waist hip ratio

Table 3. Logistic regressions; OR for plantar pedis lipoatrophy related to different baseline clinical characteristics, current metabolic and cardiovascular risk parameters, and treatment history among the ART patients. Plantar pedis lipoatrophy was regarded as the dependent variable. The independent factors that showed statistically significant ORs in the univariate logistic regressions related to baseline and metabolic/cardiovascular risk parameters were entered simultaneously in the multiple logistic regression

	Univariate logistic regression			Multiple logistic regression		
	OR	**CI 95%**	***P***	**OR**	**CI 95%**	***p***
Baseline clinical characteristics						
NRTI experience at PI-ART start:						
Naïve	Reference					
NRTI experienced	1.73	0.82–3.65	0.149			
Clinical stage:						
CD4 >200 and no AIDS	Reference					
AIDS or CD4 <200	1.85	0.92–3.73	0.085			
Age:						
Age ≤34 years	Reference					
Age >34 years	2.12	0.99–4.56	0.054			
HIV-RNA:						
<100,000 copies/ml	Reference					
≥100,000 copies/ml	2.02	0.96–4.25	0.064			
CD4:						
>median	Reference					
≤median	2.13	1.05–4.34	**0.038**	1.95	0.94–4.04	0.074
Months with known HIV infection:						
≤median	Reference					

>median	1.05	0.52–2.07	0.918			
Current metabolic/cardiovascular risk parameter						
TG:						
<1.7 mmol/l	Reference					
>1.7 mmol/l	0.91	0.45–1.83	0.795			
TC:						
<6.5 mmol/l	Reference					
>6.5 mmol/l	0.85	0.33–2.18	0.736			
HDL-C:						
>0.9 mmol/l	Reference					
≤0.9 mmol/l	0.82	0.33–2.04	0.673			
HOMA-IR:						
<3.0	Reference					
>3.0	1.51	0.67–3.44	0.322			
Lp (a):						
<300 mg/l	Reference					
>300 mg/l	1.12	0.54–2.34	0.765			
PAI-1						
<15 kIE/l	Reference					
>15 kIE/l	2.69	1.31–5.52	**0.007**	2.52	1.22–5.23	**0.013**
Apo A1:						
>1.1 mmol/l	Reference					
<1.1 mmol/l	0.86	0.34–2.22	0.759			
Apo B:						

<1.5 mmol/l	Reference			
>1.5 mmol/l	1.11	0.25–4.86	0.889	
hsCRP:				
≤1.8 mg/l	Reference			
>1.8 mg/l	1.34	0.67–2.68	0.414	
Treatment history				
Ever treated with didanosine:				
No	Reference			
Yes	3.12	1.44–6.73	**0.004**	NA
Ever treated with stavudine:				
No	Reference			
Yes	1.39	0.68–2.86	0.373	

[Apo A1]apo A1 lipoprotein; [Apo B]apo B lipoprotein; [ART]antiretroviral therapy; [CI]confidence interval; [HDL-C]high density lipoprotein cholesterol; [HOMA-IR]homeostatic model assessment for insulin resistance; [hsCRP]high sensitivity C-reactive protein; [NA]not applicable; [NRTI]nucleoside analogue reverse transcriptase inhibitor; [Lp]lipoprotein; [OR]odds ratio; [PAI]plasminogen activator inhibitor; [PI]protease inhibitor; [TC]total cholesterol; [TG]triglycerides

Table 4. Logistic regressions; OR for abnormal PAI-1 (>15 kIE/l) related to lipoatrophy at different locations when entered simultaneously into a multiple logistic regression

	Multiple logistic regression		
	OR	**CI 95%**	***p***
Lipoatrophy location			
Plantar pedis fat pads	2.92	1.32–6.44	**0.008**
Face	0.78	0.34–1.82	0.566
Palm	0.59	0.26–1.34	0.210
Thigh	1.58	0.66–3.76	0.306
Gluteal region	1.25	0.54–2.88	0.601

[CI]confidence interval; [OR]odds ratio; [PAI]plasminogen activator inhibitor