DOI: 10.1111/j.1468-1293.2007.00527.x

ORIGINAL RESEARCH

Early changes in adipokine levels and baseline limb fat may predict HIV lipoatrophy over 2 years following initiation of antiretroviral therapy*

A Calmy,[1] D Carey,[2] PWG Mallon,[2] H Wand,[2] M Law,[2] DA Cooper,[1,2] A Carr[1] on behalf of the INITIO Trial International Co-ordinating Committee and HAMA study coordination team†

[1]St Vincent's Hospital, Sydney, Australia and [2]National Centre in HIV Epidemiology and Clinical Research, University of New South Wales, Sydney, Australia

Background

No biological marker has been identified that predicts the development of lipodystrophy (LD). We investigated whether metabolic and body composition parameters could predict the development of LD over 2 years in adults initiating antiretroviral therapy (ART).

Methods

We used stored plasma collected at baseline and weeks 12, 24 and 48 from adults initiating combination ART. Adipocytokine, inflammatory cytokine, lipid and glycaemic parameters were measured and related to subsequent lipoatrophy (loss of limb fat mass of at least 2 kg from weeks 24 to 96 by dual-energy X-ray absorptiometry) and an increase in visceral adipose tissue (VAT; an increase of at least 18 cm^2 from baseline to week 48 by abdominal computed tomography). Risk factors associated with limb fat loss and VAT gain were analysed by logistic regression.

Results

Fifty-four HIV-infected, treatment-naïve adults were included in the study: 53 (98%) of them were men, and they had a median age of 39 years [interquartile range (IQR) 34–48 years] and a median body mass index of 22.6 kg/m^2 (IQR 20–24.8 kg/m^2). In multivariate analysis, a higher baseline limb fat percentage, and a 1 mmol/L increase in plasma leptin levels during the first 6 months of ART, independently predicted a peripheral fat loss of $\geq$2 kg [odds ratio (OR) 2.58, 95% confidence interval (CI) 1.04–6.41; OR 3.15, 95% CI 1.34–7.35, respectively). VAT changes showed a borderline association with high baseline tumour necrosis factor-α levels and hip circumference (OR 1.04, 95% CI 1.00–1.07; OR 1.44, 95% CI 1.07–1.95, respectively).

Conclusions

In ART-naïve men, higher baseline limb fat and an early increase in leptin concentrations may predict the subsequent development of lipoatrophy. We did not find the same risk factors in the two different groups of patients with peripheral fat loss and central fat gain, suggesting a partially independent pathogenesis.

Keywords: HIV, leptin, limb fat, lipoatrophy, lipodystrophy, predictors

Received: 21 June 2007, accepted 18 October 2007

Introduction

The metabolic abnormalities (dyslipidaemia, insulin resistance and hyperlactataemia) and morphological changes [peripheral lipoatrophy and relative central (visceral) fat accumulation] of HIV lipodystrophy (LD) are of concern [1]. Physical changes are very common [2], and may stigmatize patients and reduce adherence to antiretroviral therapy (ART) [3], while dyslipidaemia and insulin

*Presented at the 8th International Workshop on Adverse Drug Reactions and Lipodystrophy in HIV, 24–26 September 2006, San Francisco, CA, USA.

†The INITIO Trial International Co-ordinating Committee and HAMA study coordination team are listed in the Appendix.

Correspondence: Dr Alexandra Calmy, HIV, Immunology and Infectious Diseases Unit, St Vincent's Hospital, Sydney, 2010 NSW, Australia. Tel: + 61 (2) 8382 3872; fax: + 61 (2) 8382 4749; e-mail: acalmy@gmail.com

resistance appear to increase the risk of cardiovascular disease [4,5].

The only intervention that has been shown to be effective for lipoatrophy is switching from a thymidine-based nucleoside reverse transcriptase inhibitor (tNRTI) to a non-tNRTI. This switch, however, leads to only modest improvements in limb fat mass assessed by dual-energy X-ray absorptiometry (DEXA) over 2 years [6]. Switching to an NRTI-sparing regimen has also produced modest increases in peripheral and visceral fat over 2 years, but metabolic profiles were adversely affected [7]. Similarly, although a small study on rosiglitazone, a thiazolidinedione, showed a modest increase in limb fat mass [8], larger and longer trials failed to show any significant benefit of rosiglitazone [9]. Promising results have been obtained for pioglitazone and uridine in randomized studies, but these results have not been confirmed [10,11]. Protease inhibitor (PI) cessation has not been shown to be effective [12]. Prevention of lipoatrophy, therefore, appears to be the best approach, but this strategy may be limited over time by the development of resistance to available drug classes. Therefore, there is a need to identify a simple, accurate marker that would identify at-risk patients prior to any clinically evident subcutaneous fat loss and that would enable clinicians to modify ART promptly when possible.

Although LD has been associated with the use of drugs such as stavudine (d4T) and zidovudine (ZDV) [13,14], no biological marker has been consistently found to predict its development [15]. We wished to investigate whether any markers potentially involved in LD pathogenesis could usefully predict the development of lipoatrophy and increased visceral fat following the initiation of combination ART. We evaluated plasma levels of adipokines (adiponectin and leptin), cytokines [tumour necrosis factor (TNF)-α], C-reactive protein (CRP), anion gap, fasting glucose, insulin, triglycerides and cholesterol as predictors of lipoatrophy and visceral fat accumulation.

Materials and methods

Participants

To be eligible for this analysis, subjects had to be ART-naïve and have body composition assessed by DEXA and computed tomography (CT) prior to starting ART. Fifty-four HIV-infected adults who commenced initial ART as part of two clinical studies, INITIO LD, a substudy of INITIO [16] ($n = 39$), and HIV Infection and Metabolic Abnormalities (HAMA) [17] ($n = 15$), were included in this study. INITIO participants were randomized in a 1:1:1 ratio to receive the NRTIs didanosine (ddI) and d4T, together with efavirenz (EFV) [a nonnucleoside reverse transcriptase inhibitor (NNRTI)], nelfinavir (NFV) (a PI), or EFV plus NFV [16]; 39 participants co-enrolled in the INITIO LD substudy at five clinical sites in Australia and New Zealand and were followed for 144 weeks. Participants in HAMA [17], a nonrandomized, 96-week observational study of body composition and metabolic abnormalities associated with ART, were allocated to receive either a PI or an NNRTI plus two NRTIs chosen by the treating physician. Between September 2003 and September 2004, all antiretroviral (ARV)-naïve, HIV-infected patients attending the HIV clinic at St Vincent's Hospital, Sydney, Australia who required ART were invited to enrol in the HAMA study. Fifteen patients were recruited through the Outpatients' Clinic at St Vincent's Hospital.

Assessments

All participants were clinically and biologically assessed at baseline and 12-weekly thereafter until week 96 in HAMA and until week 144 in INITIO LD [18]. Unless otherwise stated, assessments in the INITIO LD and HAMA studies were identical. Anthropometric parameters (weight, umbilical waist circumference and maximum hip circumference) were measured at each visit. Height was recorded at baseline. LD case definition scores were calculated using the validated equation [18].

Blood was collected for fasting total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, glucose and insulin. HAMA participants also had blood collected for leptin and CRP at weeks 0, 24 and 48. At each visit, an additional 10-mL fasting blood sample was collected and following centrifugation was stored at $-70\ ^{\circ}C$. Data from blood collected at the following INITIO LD and HAMA study time-points were utilized: baseline [week 0 or screening (week −2) if week 0 was not available] and weeks 12, 24 and 48. INITIO LD stored samples from these time-points were used for measurement of leptin, adiponectin, TNF-α and CRP. HAMA stored samples were used for measurement of adiponectin and TNF-α. Body composition in INITIO LD participants was quantified at baseline and weeks 24, 48, 96 and 144 by DEXA and CT. In HAMA, body composition was quantified at baseline and at weeks 24, 48 and 96 by DEXA and at baseline and weeks 24 and 48 by CT. Visceral adipose tissue (VAT) was measured using a single L4 slice in INITIO LD patients and at three levels, L2–L4, in HAMA participants. Only measurements at L4 were used in this analysis. Common protocols were used for DEXA and CT data acquisition [12] and all scans were analysed at a single site by blinded technicians (one each for DEXA and for CT scans).

Laboratory assays

Plasma leptin and adiponectin levels were measured using radioimmunoassays (Linco Research, St Charles, MO, USA) and TNF-α levels were measured using an immunoenzymometric assay (BioSource Europe SA, Nivelles, Belgium). CRP was measured using a high-sensitivity assay (Roche/Hitachi Modular P Analyser; Roche Diagnostics, Mannheim, Germany). All samples were quantified in one laboratory. Limits of detection for leptin, adiponectin, CRP and TNF-α were 0.5, 0.5 ng/mL, 0.03 mg/L and 3 pg/mL, respectively, and the inter-assay coefficients of variation were 8.0–9.9% for TNF-α, 3.5–4.2% for leptin, 10% for CRP and 7.7–13.0% for adiponectin.

Statistical analysis

Baseline characteristics were summarized without formal between-study comparison. Unless stated otherwise, median [and interquartile range (IQR)] values are presented. Changes in potential risk factors predicting long-term lipodystrophy development were calculated as the simple difference between measurements of continuous risk factors at baseline and at various time-points during the study. The primary endpoint for the assessment of long-term lipoatrophy was the change in limb fat mass between weeks 24 and 96; lipoatrophy was defined as a reduction in limb fat of at least 2 kg from week 24. Change in VAT was the change between baseline and week 48; a VAT increase was defined as an increase of at least 18 cm^2. We first conducted an analysis to explore the relationship between the variables of interest at baseline and week 24 and the primary endpoint. The nonparametric Spearman test was used to assess correlations.

Risk factors for fat loss (defined as a limb fat decrease greater than or equal to the median 2 kg from week 24 to 96) and visceral fat (defined as a gain of at least the median 18 cm^2) were assessed using logistic regression analysis. For the limb fat percentage, the odds ratio (OR) was expressed per 5% increment of limb fat percentage at baseline. For subcutaneous abdominal fat (SAT), the OR was expressed per unit representing a 2-cm^2 SAT increment. Multivariate models considered all variables statistically significant ($P<0.05$) in initial analyses and used forward stepwise methods. Receiver–operator characteristic (ROC) curves were constructed to assess the performance of early changes in serum markers and body composition in predicting long-term LD development. Statistical analysis was performed using STATA Release 8.2 (Stata Statistical Software, Release 8.0; Stata Corporation, College Station, TX, USA).

Results

The characteristics of the 54 participants are shown in Table 1. Almost all (98%) participants were male, 13 (24%) had AIDS and 35 (65%) commenced a PI-containing regimen [30 (86%) nelfinavir and five (14%) lopinavir/ritonavir]. Eight participants (15%) commenced either d4T or ZDV with lamivudine (3TC) as their NRTI backbone and 39 (72%) started d4T plus ddI, reflecting the INITIO study design and clinical practice at the time at which both HAMA and INITIO commenced. Other NRTI combinations included abacavir and 3TC ($n=3$) and tenofovir plus 3TC ($n=4$). All median baseline metabolic and body composition parameters were within normal limits.

Fasting total cholesterol levels rose substantially by week 12 (1.1 mmol/L) and remained elevated during the study. By week 12, HDL cholesterol levels had risen by 0.3 mmol/L and this increase was sustained until week 48. In contrast, triglyceride and insulin concentrations remained stable. TNF-α levels decreased substantially (-14 pg/mL by week 12 of therapy) and this reduction was maintained until week 48.

Limb fat mass increased from 5.3 kg at baseline to 6.2 kg at week 24, a median increase of 0.9 kg (17%), and limb fat percentage increased from 17.5% at baseline to 18.5% at week 24 (Fig. 1a). From week 24 onwards, there was a progressive loss of limb fat, with a median loss of 1.9 kg (IQR 0.4 to -3.5 kg) at week 96. In contrast to limb fat, VAT increased to week 48 and the increased VAT level was maintained until week 96 (median increase 18.0 cm^2; IQR 4–44) (Fig. 1b), although there was a modest decline between weeks 48 and 96. Of the 21 patients with a loss of at least 2 kg of limb fat at week 96, five (23%) had LD according to the LD case definition. Of the 24 patients with an increase of at least 18 cm^2 in VAT from baseline to week 48, 11 (45%) had LD according to the LD case definition.

Correlations with body fat changes

We explored the relationship between metabolic and anthropometric variables and limb fat loss occurring after week 24. Baseline body mass index (BMI) ($r=-0.36$; $P=0.003$), VAT ($r=-0.40$; $P=0.01$), SAT ($r=-0.65$; $P=<0.0001$), limb fat mass ($r=-0.49$; $P=0.002$) and limb fat percentage ($r=-0.51$; $P=0.0009$) were significantly and negatively correlated with changes in limb fat mass between weeks 24 and 96. Negative correlations between the change in limb fat mass from week 24 to week 96 and leptin levels at baseline and at week 24 were also found ($r=-0.34$, $P=0.066$; $r=-0.42$, $P=0.009$, respectively). Of note, there was a significant correlation between the changes in limb fat and in plasma leptin from baseline

Table 1 Patient characteristics

Parameter	Week 0		Week 24			Week 48	
	n		*n*		*P*-value*	*n*	
Patients							
Sex (male %)	54	98		–			–
Age (years)		39 (34–48)		–			–
AIDS (%)		28		–			–
Waist circumference (cm)		82.5 (75.3–88.0)		85.0 (80.0–90.5)			87.0 (80.0–90.0)
Hip circumference (cm)		91.0 (87.0–95.0)		92.0 (87.5–97.0)			92.25 (86.6–96.3)
Body mass index (kg/m^2)		22.6 (20.0–24.8)		23.7 (21.5–24.9)			23.2 (21.2–25.3)
Antiretroviral therapy							
Protease inhibitor (%)		65		65			63
ZDV or d4T (%)		85		83			80
NNRTI regimen (%)		65		63			63
CD4 lymphocyte count (cells/μL)		208 (102–320)		312 (187–468)			378 (247–506)
Metabolic							
Glucose (mmol/L)	50	3.7 (3.4–4.9)	51	4.9 (4.6–5.3)	0.11	49	5.1 (4.7–5.3)
C-reactive protein (mg/L)	52	1.9 (1.1–3.5)	51	1.8 (0.8–3.7)	0.57	49	2.2 (0.7–4.9)
Total cholesterol (mmol/L)	49	4.2 (3.4–4.9)	55	5.1 (4.2–5.9)	<0.0001	52	5.1 (4.5–6.1)
HDL cholesterol (mmol/L)	48	0.9 (0.7–1.1)	53	1.2 (0.9–1.5)	<0.0001	53	1.1 (1.1–1.4)
Lactate (mmol/L)	47	1.0 (0.9–1.3)	40	1.6 (1.0–2.0)	0.008	43	1.5 (1.2–2.4)
Anion gap (meq/L)	47	12.0 (9.0–14.7)	52	13.9 (10.1–15.9)	0.004	53	13.2 (10.0–16.0)
Insulin (mU/L)	44	5.7 (3.7–9.0)	53	6.0 (3.7–8.8)	0.22	51	5.6 (3.8–7.8)
Triglycerides (mmol/L)	47	1.5 (1–2.1)	53	1.5 (1.0–2.4)	0.12	51	1.5 (0.9–2.4)
Parameter	Week 0		Week 24		Week 48		
	n		*n*		*P*-value	*n*	
Adipocytokines							
Leptin (ng/mL)	52	2.3 (1.3–4.7)	50	2.9 (1.7–5.1)	0.07	48	2.6 (1.7–4.2)
Adiponectin (ng/mL)	52	10.3 (7.1–15.1)	51	10.4 (7.3–15.0)	0.94	49	9.0 (5.4–10.4)
TNF-α (pg/mL)	52	36.5 (23.8–48.2)	51	18.8 (13.3–23.6)	<0.0001	49	17.1 (12.7–21.2)
Body composition							
Limb fat mass (kg)	48	5.3 (3.3–7.1)	49	6.2 (4.0–8.0)	0.0023	49	5.8 (4.4–8.9)
Limb fat percentage	48	17.5 (11.9–22.0)	49	18.5 (13.0–24.1)	0.003	49	18.1 (14.9–23.0)
Visceral adipose tissue (cm^2)	53	49 (24–82)	51	72 (39–105)	0.0005	50	85 (56–123)
Subcutaneous adipose tissue (cm^2)	53	78 (50–138)	51	81 (56–147)	0.003	50	100 (59–141)

Values are expressed as median [interquartile range (IQR)] or number (%) unless otherwise stated.
Anion gap = Na–(Cl + HCO_3).
*P-value refers to the statistical difference between week 24 and week 0 (baseline) in metabolic parameters.
d4T, stavudine; HDL, high-density lipoprotein; NNRTI, nonnucleoside reverse transcriptase inhibitor; TNF-α, tumour necrosis factor-α; ZDV, zidovudine.

to week 24 ($r = 0.462$; $P = 0.002$). Changes from baseline to weeks 12, 24 and 48 in lipids, insulin, lactate, TNF-α and HIV RNA were not significantly related to limb fat change between weeks 24 and 96. Baseline TNF-α levels and the changes between baseline and weeks 24 and 48 were strong predictors of VAT change over the first 48 weeks of ART ($r = -0.53$, $P = 0.0002$; $r = -0.38$, $P = 0.008$; $r = -0.36$, $P = 0.02$, respectively).

Risk factors for lipoatrophy

The median change in limb fat mass between weeks 24 and 96 (-1.9 kg) was used to define the cut-off for the logistic regression analysis. Factors associated with limb fat loss ≥ 2 kg between weeks 24 and 96 are presented in Table 2. In the univariate analysis, risk factors associated with a ≥ 2-kg limb fat loss were a higher baseline limb fat mass [OR 1.54, 95% confidence interval (CI) 1.10–2.15; $P = 0.010$], higher baseline SAT (OR 1.09, 95% CI 1.02–1.16; $P = 0.007$), increases from baseline to week 24 in limb fat mass and leptin concentration (OR 3.10, 95% CI 1.85–6.63, $P = 0.003$; OR 2.03, 95% CI 1.10–3.72, $P = 0.022$, respectively) and increases from baseline to week 48 in adiponectin concentration (OR 1.80, 95% CI 0.90–3.60; $P = 0.016$). For every 5% increment in baseline limb fat percentage, the risk of a ≥ 2-kg limb fat loss doubled (OR 2.01, 95% CI 1.15–3.49). Similarly, every 5% increase in limb fat percentage from baseline to week 24 was associated with a fivefold increased risk of a ≥ 2-kg limb fat loss after week 24 (OR 5.51, 95% CI 1.43–21.1). The only

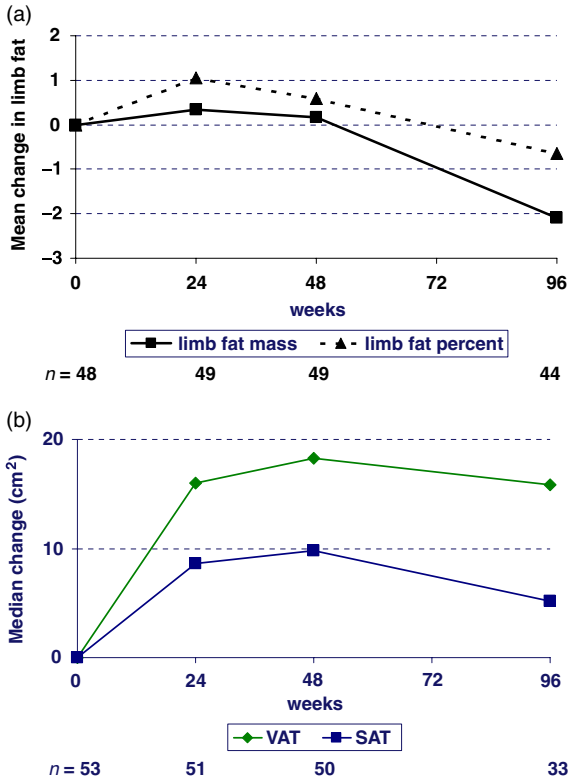

Fig. 1 Changes in body composition with treatment. Median changes in (a) limb fat mass (kg) and limb fat percentage and (b) visceral adipose tissue (VAT; cm^2) and subcutaneous adipose tissue (SAT) with treatment are shown.

factor associated with a reduced likelihood of a $\geq$2-kg limb fat loss was an increase in adiponectin levels between baseline and week 48 (OR 0.83, 95% CI 0.71–0.96; $P = 0.016$). Interestingly, neither CD4 cell count (per 100-cell increase) nor HIV RNA (log-transformed) was significantly associated with limb fat loss (OR 1.12, 95% CI 0.75–1.69, $P = 0.56$; OR 2.23, 95% CI 0.78–6.30, $P = 0.13$, respectively).

In the multivariate analysis, a high baseline limb fat percentage and a 1 ng/mL increase in plasma leptin levels during the first 6 months of ART independently predicted a peripheral fat loss of $\geq$2 kg (OR 2.58, 95% CI 1.04–6.41, $P = 0.041$; OR 3.15, 95% CI 1.34–7.35, $P = 0.008$, respectively). Together, these two variables explained 46% of the changes in limb fat occurring between weeks 24 and 96.

We explored with a ROC curve whether limb fat at baseline and early increases in leptin concentrations jointly predicted the risk of a $\geq$2-kg limb fat loss between weeks 24 and 96. These analyses showed that any increase from baseline in leptin levels and a high baseline limb fat percentage were good predictors of a $\geq$2-kg limb fat loss (ROC area = 0.90) (Fig. 2).

Risk factors for increase in VAT

The median change in VAT from baseline to week 48 was investigated. Factors significantly associated with an increase in VAT of at least the median 18 (IQR 4–44) cm^2

Table 2 Factors associated with a reduction in limb fat of at least 2 kg between weeks 24 and 96

		Univariate analysis		Multivariate analysis	
Parameter	**Time**	**OR (95% CI)**	***P*-value**	**OR (95% CI)**	***P*-value**
Anthropometry					
Weight (kg)	Change at week 24	1.14 (0.98–1.33)	0.073	–	–
Body composition					
Limb fat mass (kg)	Baseline	1.54 (1.10–2.15)	0.010		
	Change at week 24	3.10 (1.45–6.63)	0.003	–	–
Limb fat %*	Baseline	2.01 (1.15–3.49)	0.013	2.58 (1.04–6.41)	0.041
	Change at week 24	5.51 (1.43–21.1)	0.013		
Subcutaneous adipose tissue (cm^2)†	Baseline	1.09 (1.02–1.16)	0.007	–	–
	Change at week 24	1.23 (1.04–1.45)	0.012	–	–
Metabolic parameters					
Anion gap (mmol/L)	Baseline	1.45 (1.07–1.94)	0.014	–	–
Leptin (ng/mL)	Change at week 24	2.03 (1.10–3.72)	0.022	3.15 (1.34–7.35)	0.008
Adiponectin (ng/mL)	Change at week 48	0.83 (0.71–0.96)	0.016	–	–
Cholesterol (mmol/L)	Change at week 48	1.80 (0.90–3.60)	0.093	–	–
HIV surrogate markers					
HIV RNA (log)	Baseline	2.23 (0.78–6.30)	0.13	–	–

*Odds ratios (ORs) are expressed per 5% higher limb fat percentage at baseline; there is a 2.58 risk of limb fat loss $\geq$2 kg for each 5% higher baseline limb fat percentage.
†Odds ratios are expressed per 2 cm^2 greater amount of baseline subcutaneous adipose tissue.
All other odds ratios are expressed per unit increase (1 mmol, 1 kg etc.). Only variables with a *P*-value $\leq$0.1 are included in this table. Nonsignificant parameters at all time-points in univariate analysis were: waist, hip, age, VAT, glucose, triglycerides, high-density lipoprotein cholesterol, cholesterol, insulin and CD4 cell count.
CI, confidence interval; VAT, visceral adipose tissue.

between weeks 0 and 48 are shown in Table 3. No baseline anthropometric measures predicted long-term VAT change after 1 year of therapy. In the univariate analysis, a VAT increase at week 24 and an increased hip circumference at week 24 were associated with a greater increase in visceral fat at 48 weeks (OR 1.04, 95% CI 1.01–1.06, $P = 0.007$; OR 1.44, 95% CI 1.10–1.49, $P = 0.008$, respectively). Higher baseline TNF-α levels and change in TNF-α levels between baseline and week 24 were significantly associated with a VAT increase (OR 1.06, 95% CI 1.01–1.12, $P = 0.01$; OR 0.95, 95% CI 0.91–0.99, $P = 0.034$, respectively). Early changes in leptin, adiponectin and lipid levels were not associated with VAT changes in the univariate analysis. In the multivariate analysis, baseline TNF-α levels and change in hip circumference at week 24 showed a borderline association with a VAT increase between weeks 0 and 48 (OR 1.04, 95% CI 1.00–1.07, $P = 0.066$; OR 1.44, 95% CI 1.07–1.95, $P = 0.018$, respectively).

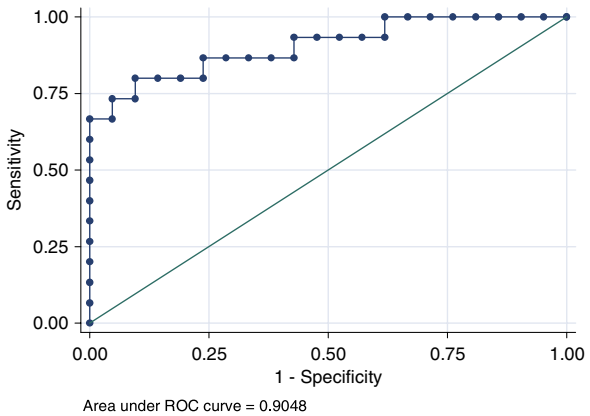

Fig. 2 Receiver–operator curve (ROC) modelling fat mass at baseline and leptin increase from baseline to week 24.

Discussion

We explored factors associated with limb fat loss over 96 weeks in 54 patients commencing their first regimen of ART. We showed that baseline factors such as BMI, plasma leptin levels, limb fat mass, SAT and VAT were significantly associated with peripheral fat loss from 6 months after treatment initiation. In multivariate analysis, we showed that any 5% higher baseline limb fat percentage was associated with an increased risk of developing clinically significant peripheral fat loss (OR 2.58, $P = 0.041$). Similarly, a 1 ng/mL increase in plasma leptin levels during the first 6 months of therapy translated into a threefold greater risk of peripheral fat atrophy (OR 3.15, $P = 0.008$).

Although changes in leptin levels have been shown to correlate with changes in BMI in lipoatrophic adults [19], our results suggest that leptin and limb fat percentage independently predict the selective loss of limb fat that occurs from week 24 onwards. Our findings also suggest that adipocytokines and body composition data may be more useful in predicting peripheral fat atrophy than lipids and glycaemic parameters, the parameters most strongly associated with lipoatrophy in cross-sectional studies [2].

Table 3 Factors associated with an increase in visceral adipose tissue (VAT) of at least 18 cm^2 between weeks 0 and 48

		Univariate analysis		Multivariate analysis	
Parameter	**Time**	**OR (95% CI)**	***P*-value**	**OR (95% CI)**	***P*-value**
Anthropometry					
BMI (kg/m^2)	Baseline	1.19 (0.96–1.47)	0.10	–	–
	Change at week 24	1.64 (1.03–2.62)	0.035		
	Change at week 48	1.51 (1.04–2.18)	0.027	–	–
Hip circumference (cm)	Change at week 24	1.44 (1.10–1.89)	0.008	1.44 (1.07–1.95)	0.018
Waist circumference (cm)	Baseline	1.07 (0.99–1.15)	0.052	–	–
Body composition					
VAT (cm^2)	Change at week 24	1.04 (1.01–1.06)	0.007	–	–
Metabolic parameters					
TNF-α (ng/mL)	Baseline	1.06 (1.01–1.12)	0.010	1.04 (0.00–1.07)	0.066
	Change at week 24	0.95 (0.91–0.99)	0.034	–	–
Adiponectin (ng/mL)	Change at week 24	0.81 (0.64–1.02)	0.076	–	–
HIV surrogate markers					
HIV RNA (log)	Baseline	2.67 (0.99–7.17)	0.051	–	–
	Change at week 24	0.5 (0.24–1.18)	0.122	–	–

Only variables with a *P*-value ≤ 0.1 are included in this table. Nonsignificant parameters at all time-points in univariate analysis were: waist, hip, limb fat mass and percentage, body mass index (BMI), subcutaneous adipose tissue, glucose, high-density lipoprotein cholesterol, triglycerides, insulin, anion gap and CD4 cell count.
CI, confidence interval; OR, odds ratio; TNF, tumour necrosis factor; VAT, visceral adipose tissue.

There is a well-described association between leptin levels and limb fat mass [20]. However, leptin secretion is not only dependent on fat mass but is also regulated by several cytokines and hormones [21,22]. Leptin is a mediator of long-term regulation of energy balance and induces weight loss both by suppression of food intake and by stimulation of metabolic rate [22]. Thus, increased leptin could potentially cause long-term fat loss. Leptin replacement in HIV-infected adults with lipoatrophy, hypoleptinaemia (<3 ng/mL) and insulin resistance was associated with a marked reduction in visceral fat with no improvement of peripheral lipoatrophy, suggesting the critical role of leptin in abnormal fat distribution [23]. Our study was not designed to gain an insight into the pathogenesis of lipoatrophy but to determine whether there were clinically applicable early predictors of limb fat loss. In this setting, an early change in leptin, although biologically only reflecting the changes in limb fat over the same period, may identify those at subsequent risk of fat loss, without being necessarily linked to its pathogenesis. As such, leptin appears to be both a clinically and a biologically relevant marker.

We chose to investigate only baseline parameters and changes that occurred up to week 24 of therapy, as markers can only be predictive before an outcome and the most useful will be those present at baseline or those that change the soonest. We included in our analysis standard anthropometric measures, a wide range of metabolic parameters, many of which are routinely measured, and body composition parameters. Additional markers such as leptin, adiponectin and TNF-α were included, as altered expression of adipocytokines is observed in adipocyte cell models [24] and in subcutaneous, lipoatrophic fat biopsies [25]. NRTIs and PIs may induce secretion of cytokines, including TNF-α, thereby enhancing insulin resistance and lipolysis, and increasing adipocyte apoptosis [26,27]. We defined long-term lipoatrophy as limb fat loss of at least 2 kg occurring between week 24 and week 96 following ART initiation. This pattern of peripheral fat loss has been well described in prospective studies of HIV-infected individuals commencing initial ARV regimens [14]. The strong, inverse correlations of baseline BMI and body composition parameters such as limb fat, limb fat percentage and VAT with subsequent limb fat loss suggest that individuals with greater pretreatment limb fat are more likely to experience peripheral fat loss, perhaps because such patients have more fat to lose. This finding conflicts somewhat with earlier cross-sectional studies that showed that lipoatrophy was more common in patients who were emaciated [2].

The association between plasma leptin levels and the development of lipoatrophy was investigated in lipoatrophic HIV-infected males in a nested case–control study (10 cases and 87 controls) from the Swiss HIV Cohort Study. At 2 years, there was no difference in leptin levels between patients who developed clinically assessed lipoatrophy and controls, with age the only predictor of lipoatrophy [19]. This study did not assess lipoatrophy with DEXA, and leptin concentrations were only measured at baseline and after 2 years of ART.

Recently, an exploratory pharmacogenetic substudy of ACTG 5005 [28] investigated whether genetic testing might assist in predicting long-term abnormal fat distribution [29]. The authors examined 135 genes and 285 single nucleotide polymorphisms in 189 HIV-infected adults (88% male; 56% Caucasian) and found that a single variant of the resistin gene was associated with a cluster of patients who experienced adverse metabolic changes and greater limb fat loss. Resistin polymorphisms and plasma levels appear to be interesting candidates for exploring further the long-term morphological and metabolic abnormalities of ART.

Our data suggest that standard anthropometric measures, together with early changes in leptin levels, are independent markers of subsequent limb fat loss following initiation of ART: a reduction in baseline leptin levels adjusted for BMI predicted a median 2-kg fat loss at 96 weeks with sensitivity and specificity approaching 75%. Moreover, baseline limb fat mass assessed by DEXA was significantly associated with peripheral fat loss from 6 months after treatment initiation, suggesting that a DEXA scan at baseline and then at 6 months may detect peripheral fat loss before it becomes clinically apparent. Our results do not support the use of routine CT scans, as none of its parameters was included in the final model. Moreover, exposure to radiation and cost may greatly limit the routine use of CT. The questions of whether these associations have the same predictive power in more diverse populations commencing other ART regimens, and also of whether early pharmacological intervention can prevent development of clinically relevant lipoatrophy remain unanswered. Given the exploratory nature of our analysis, these findings need further validation in routine clinical care models.

In the current study, predictors of limb fat loss differed from those associated with visceral fat increase over 48 weeks. TNF-α was associated with VAT increases at 48 weeks, but not with peripheral fat loss. At baseline, TNF-α concentrations were elevated, reflecting high pre-ART levels of HIV-1 replication, but decreased dramatically following treatment initiation. The reduction in TNF-α levels was associated with visceral fat gain over 96 weeks, supporting the hypothesis that peripheral fat loss and central visceral fat gain may constitute different processes, at least in part.

Our study has a number of limitations: most subjects were male, and the sample size was relatively small and only representative of those infected with HIV-1 in Western countries. d4T with ddI, the most common NRTI backbone, is no longer recommended as initial therapy as this combination has been associated with an increased risk of lipoatrophy and peripheral neuropathy [14,25]. Therefore, the extension of these findings to other ART regimens should be carried out with caution. Moreover, in wealthy countries at least, d4T is mainly used in patients with a long history of ART, and whether our findings apply in these situations is unexplored. It is likely that some INITIO LD and HAMA participants changed NRTI therapy during the 96 weeks, but this study was not designed to investigate the impact of individual components of initial ART on LD. Lastly, we combined two trial populations for this analysis, but this is unlikely to have affected our findings as common protocols were used for reading and interpreting CT and DEXA scans and because baseline values were similar across the two study populations.

With no therapies for lipoatrophy imminent, prevention of abnormal fat distribution is vital. Larger prospective studies to determine the role of genetic polymorphisms and to validate the potential for simple clinical and metabolic measures to predict the development and severity of LD are required.

Acknowledgements

The study was supported by the HIV, Immunology and Infectious Diseases Unit, St Vincent's Hospital, Sydney, Australia.

References

1 Carr A, Samaras K, Burton S *et al.* A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors. *AIDS* 1998; **12**: F51–F58.

2 Miller J, Carr A, Emery S *et al.* HIV lipodystrophy syndrome: prevalence, severity and correlates of risk in Australia. *HIV Med* 2003; **4**: 293–301.

3 Ammassari A, Murri R, Pezzotti P *et al.* Self-reported symptoms and medication side effects influence adherence to highly active antiretroviral therapy in persons with HIV infection. *J Acquir Immune Defic Syndr* 2001; **28**: 445–449.

4 Henry K, Melroe H, Huebsch J *et al.* Severe premature coronary artery disease with protease inhibitors. *Lancet* 1998; **351**: 1328.

5 Maggi P, Serio G, Epifani G *et al.* Premature lesions of the carotid vessels in HIV-1-infected patients treated with protease inhibitors. *AIDS* 2000; **14**: F123–F128.

6 Martin A, Smith DE, Carr A *et al.* Reversibility of lipoatrophy in HIV-infected patients 2 years after switching from a thymidine analogue to abacavir: the MITOX extension study. *AIDS* 2004; **18**: 1029–1036.

7 Boyd MA, Carr A, Ruxrungtham K *et al.* Changes in body composition and mitochondrial nucleic acid content in patients switched from failed nucleoside analogue therapy to ritonavir-boosted indinavir and efavirenz. *J Infect Dis* 2006; **194**: 642–645.

8 Hadigan C, Yawetz S, Thomas A, Havers F, Sax PE, Grinspoon S. Metabolic effects of rosiglitazone in HIV lipodystrophy: a randomized, controlled trial. *Ann Intern Med* 2004; **140**: 786–794.

9 Carr A, Workman C, Carey D *et al.* No effect of rosiglitazone for treatment of HIV-1 lipoatrophy: randomised, double-blind, placebo-controlled trial. *Lancet* 2004; **363**: 429–438.

10 Slama L, Lanoy E, Valentin MA *et al.* Effect of pioglitazone on HIV-1 related lipoatrophy: a randomized double-blind placebo-controlled trial (ANRS 113) with 130 patients. *13th CROI, 2006*. Late breaker [Abstract 151LB].

11 Sutinen J, Walker UA, Sevastianova K, Hakkinen AM, Ristola M, Yki-Jarvinen H. Uridine supplementation increases subcutaneous fat in patients with HAART-associated lipodystrophy (HAL) – a randomized, placebo-controlled trial. *Abstracts of the 7th International Workshop on Adverse Events and Lipodystrophy in HIV.* Dublin, Ireland, November 13–16, 2005 [abstract 7].

12 Carr A, Hudson J, Chuah J *et al.* HIV protease inhibitor substitution in patients with lipodystrophy: a randomized, controlled, open-label, multicentre study. *AIDS* 2001; **15**: 1811–1822.

13 Lichtenstein KA, Ward DJ, Moorman AC *et al.* Clinical assessment of HIV-associated lipodystrophy in an ambulatory population. *AIDS* 2001; **15**: 1389–1398.

14 Joly V, Flandre P, Meiffredy V *et al.* Increased risk of lipoatrophy under stavudine in HIV-1-infected patients: results of a substudy from a comparative trial. *AIDS* 2002; **16**: 2447–2454.

15 Mallon PW, Miller J, Cooper DA, Carr A. Prospective evaluation of the effects of antiretroviral therapy on body composition in HIV-1-infected men starting therapy. *AIDS* 2003; **17**: 971–979.

16 INITIO Trial International Co-ordinating Committee. Virological and immunological outcomes at 3 years after starting antiretroviral therapy with regimens containing non-nucleoside reverse transcriptase inhibitor, protease inhibitor, or both in INITIO: open-label randomised trial. *Lancet* 2006; **368**: 287–298.

17 HAMA (HIV infection And Metabolic Abnormalities). Accessed 30 August 2006 via http://med.unsw.edu.au/nchecr/.

18 Carr A, Law M, for the HIV Lipodystrophy Case Definition Study Group. An objective lipodystrophy severity grading scale derived from the lipodystrophy case definition score. *J Acquir Immun Defic Syndr* 2003; **33**: 571–576.
19 Wunder D, Bersinger NA, Fux C *et al*. Plasma leptin levels in men are not related to the development of lipoatrophy during antiretroviral therapy. *AIDS* 2005; **19**: 1837–1842.
20 Klock MD, Jacobsdottir S, Drent ML. The role of leptin and ghrelin in the regulation of food intake and body weight in humans: review. *Obesity Rev* 2007; **8**: 21–34.
21 Anderson PD, Metha NN, Wolfe ML *et al*. Innate immunity modulates adipokines in humans. *J Clin Endocrinol Metab* 2007; **92**: 2272–2279.
22 Havel PJ. Role of adipose tissue in body-weight regulation: mechanisms regulating leptin production and energy balance. *Proc Nut Soc* 2000; **59**: 359–371.
23 Khatami H, Mulligan K, Schwartz JM *et al*. Effect of leptin treatment on glucose and lipid metabolism and fat distribution in HIV + patients with lipoatrophy and hypoleptinemia. *Antiviral Ther* 2006; **11**: L15 [Abstract 22].
24 Jones SP, Janneh O, Back DJ, Pirmohamed M. Altered adipokine response in murine 3T3-F442A adipocytes treated with protease inhibitors and nucleoside reverse transcriptase inhibitors. *Antivir Ther* 2005; **10**: 207–213.
25 Kotler DP, Ionescu G, Johnson JA *et al*. Studies of adipose tissue metabolism in human immunodeficiency virus-associated lipodystrophy. *Clin Infect Dis* 2003; **37** (Suppl. 2): S47–S51.
26 Gougeon M-L, Penicaud L, Fromenty B, Leclercq P, Viard J-P, Capeau J. Adipocytes targets and actors in the pathogenesis of HIV-associated lipodystrophy and metabolic alterations. *Antivir Ther* 2004; **9**: 161–177.
27 Sutinen J, Korsheninnikova E, Funahashi T, Matsuzawa Y, Nyman T, Yki-Jarvinen H. Circulating concentration of adiponectin and its expression in subcutaneous adipose tissue in patients with highly active antiretroviral therapy-associated lipodystrophy. *J Clin Endocrinol Metab* 2003; **88**: 1907–1910.
28 Dube MP, Parker RA, Tebas P *et al*. Glucose metabolism, lipid, and body fat changes in antiretroviral-naive subjects randomized to nelfinavir or efavirenz plus dual nucleosides. *AIDS* 2005; **19**: 1807–1818.
29 Parker R, Flint O, Parker R *et al*. A polymorphism in the resistin gene is associated with early adverse metabolic outcome and predicts future fat loss on HAART: pharmacogenetic association study of ACTG5005s. *Antiviral Ther* 2006; **11**: L10.

Appendix: INITIO trial international co-ordinating committee

D. A. Cooper (joint chair NCHECR), P. Yeni (joint chair Groupe Hospitalier Bichat). J.-P. Aboulker (INSERM SC10), F. Antunes (National Principal Investigator, Portugal), A. Babiker (MRC CTU), M. Becker (Roche), N. Boukli (INSERM SC10), F. Brun-Vezinet (joint chair Virology Group), D. Carey (NCHECR), D. Churchill (joint National Principal Investigator, UK), B. Conway (National Principal Investigator, Canada), C. Chazallon (INSERM SC10), J. Darbyshire (MRC CTU), S. De Wit (National Principal Investigator, Belgium), B. Dusak (Dupont), S. Emery (NCHECR), M. Flepp (National Principal Investigator, Switzerland), M. Floridia (ISS), J. Gatell (National Principal Investigator, Spain), P.-M. Girard (National Principal Investigator, France), R. L. Goodall (MRC CTU), R. Hemmer (National Principal Investigator, Luxembourg), M. Hooker (MRC CTU), M. Law (NCHECR), C. Loveday (joint chair Virology Group) J. Lundgren (National Principal Investigator, Denmark), D. Manion (Dupont), V. Meiffredy (INSERM SC10), A. Mijch (National Principal Investigator, Australia and NZ), F. Mulcahy (National Principal Investigator, Ireland), A. Orani (National Principal Investigator, Italy), C. Pharo (GSK), M. Ristola (National Principal Investigator, Finland), B. Salzberger (joint National Principal Investigator, Germany), E. Sandstrom (National Principal Investigator, Sweden), M. Schechter (National Principal Investigator, Brazil), S. Schnittman (Bristol Myers Squibb), M. Seligmann (chair Immunology Group), S. Staszewski (joint National Principal Investigator, Germany), M. Stek (Merck), W. Verbiest (VIRCO) J. Weber (joint National Principal Investigator, UK).

Co-ordinating trial centres

Australia/New Zealand/Brazil: National Centre in HIV Epidemiology and Clinical Research, University of NSW, Sydney (D. Carey, S. Emery, W. Lee, S. Phipps, T. Sharkey). Canada: Department of Pharmacology and Therapeutics, University of British Columbia, Vancouver (B. Conway, R. Dimayuga, M. Jones, S. Jutha, D. Kraus, B. Zastre). Denmark/Sweden/Finland: Copenhagen HIV Programme, Hvidovre (U. Dragsted, A. Grønholdt, K. Jensen, J. Ludgren, D. Mollerup, L. Skinnes). France/Belgium/Luxembourg/Spain/Portugal: INSERM SC10, Paris (J.-P. Aboulker, N. Boukli, C. Chazallon, B. Guillon, S. Kahi, L. Léger, V. Meiffrédy, A.-S. Rodier, Y. Saïdi), UASP – Hospital Clinic, Barcelona (A. Cruceta). Germany: Co-ordinating Centre for Clinical Studies (KKS), Philipps University, Marburg (B. Lenz, J. Rochon, C. Schade-Brittinger, M. Wittenberg, H. Wolf). Italy: Laboratory of Virology and Department of Clinical Research and Evaluation, Istituto Superiore di Sanità, Rome (R. Bucciardini, M. Floridia, V. Fragola, C. M. Galluzzo, E. Germinario, M. Guidi, F. Innocenti, M. Massella, A. Mattei, M. Mirra, M. I. Paoloni, C. Polizzi, M. Pirillo, S. Vella). Switzerland: Clinic for Infectious Diseases and Hospital Epidemiology, University Hospital, Zurich (M. Flepp, E. Gremlich, A. Mosimann). UK and Ireland: MRC

Clinical Trials Unit, London (A. Babiker, J. Darbyshire, R. Goodall, M. Hooker, F. Hudson, D. Johnson, P. Kelleher, A. Poland, K. Taylor, J. Wait, R. Withnall).

Overall co-ordination of the trial was carried out by the MRC CTU (London) with databases held and maintained at the National Centre in HIV Epidemiology and Clinical Research, University of NSW, Sydney, Australia; INSERM SC10, Paris, France; Co-ordinating Centre for Clinical Studies (KKS), Philipps University, Marburg, Germany; Laboratory of Virology and Department of Clinical Research and Evaluation, Instituto Superiore di Sanità, Rome, Italy; and MRC Clinical Trials Unit, London, UK.

HAMA Co-ordinating committee

Paddy Mallon, Andrew Carr, Alexandra Calmy, Richard Norris, Martina Rafferty, Mark Lacey, Donald Chisholm, Katherine Samaras and Michael Feneley.