

Lipodystrophy in patients with HIV-1 infection: effect of stopping protease inhibitors on TNF- α and TNF-receptor levels, and on metabolic parameters

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Objective: To evaluate the effects of stopping treatment with protease inhibitors (PIs) on tumour necrosis factor (TNF)- α and TNF-receptor levels, and on the metabolic and morphological abnormalities seen in patients with lipodystrophy.

Design: Longitudinal study.

Methods: Ten HIV-positive patients with lipodystrophy (LD) were studied whilst on PIs (LD1) and 3 months after stopping PIs (LD2) together with 10 HIV-positive subjects on PIs without LD (controls). TNF- α and TNF-receptor levels, insulin resistance parameters, lipid and hormonal profiles, body composition and fat distribution were measured in all subjects.

Results: TNF- α , TNF-receptor I (-RI) and TNF-RII levels were significantly lower in controls ($P=0.02$) than in subjects with LD, and there was a significant decrease in TNF-RI and TNF-RII levels ($P=0.01$ and 0.03 , respectively)

on stopping PIs. Insulin levels and the homeostasis model assessment for insulin resistance (HOMA-IR) index were significantly higher in LD1 subjects ($P=0.02$) than in controls but did not alter when PIs were stopped. Bioelectrical impedance analysis showed a significant decrease on stopping PIs but CT scans showed no significant difference in fat distribution. Apart from high-density lipoprotein, there was no change in lipid parameters on stopping PIs. There was no difference in the level of testosterone, sex hormone binding globulin and cortisol between the three groups.

Conclusion: Our results show that TNF- α activity in patients with LD is modulated by PIs. This was not accompanied by significant changes in body habitus or insulin resistance, although this may have been a consequence of the short follow-up in this study.

Introduction

Highly active antiretroviral therapy (HAART), although highly effective in reducing HIV-related morbidity and mortality [1], has also been associated with toxicity [2]. Particular attention has focused on the lipodystrophy (LD) syndrome, which is characterized by both progressive fat wasting and intra-abdominal or localized fat accumulation, in addition to metabolic abnormalities such as dyslipidaemia and insulin resistance [3].

Both protease inhibitors (PIs) and nucleoside analogue reverse transcriptase inhibitors (NRTIs) have been implicated in the pathogenesis of LD [4–6]. PIs in particular have been associated with fat accumulation and insulin resistance, while NRTIs can independently lead to fat wasting [7]. Nevertheless, longitudinal studies have shown that PIs exert a dominant influence and act synergistically with NRTIs to increase the risk of fat wasting [8]. The predominant influence of PIs on

the pathogenesis of LD has thus led to several switch studies. Unfortunately, most of these studies have been disappointing in that they have failed to show a beneficial effect on either insulin resistance or on fat wasting, despite changes in the blood lipid profile [7,9,10].

Defects in the function of, and metabolism within, adipocytes are thought to be important in the pathogenesis of LD [11]. These cells are not only responsive to insulin, but also to various other molecules including cytokines [12]. For example, TNF- α , a multi-functional cytokine, is known to modulate adipocyte differentiation and apoptosis [13], and is involved in mediating insulin resistance in adipocytes [14], with resulting disturbances of triglyceride and glucose metabolism. Such effects have led to a consideration of the role of TNF- α in the pathogenesis of LD. TNF- α is a pro-inflammatory cytokine that becomes elevated in many infectious diseases including HIV [15]. A flow

cytometric study has recently shown that TNF- α homeostasis in T cells is dysregulated by HAART, with the dysregulation being more dramatic in subjects with LD [16], and is associated with atherogenic markers in peripheral blood. This suggests that HAART-mediated dysregulation of TNF- α synthesis may be linked to LD development in HIV-positive patients. However, whether this dysregulation is related to PIs, NRTIs or both is unknown.

In this study, we have undertaken a detailed examination of patients switching from PI-based regimens to non-PI regimens, concentrating on morphological and endocrine abnormalities, as well as on the effects on circulating TNF- α and TNF-receptor levels.

Materials and methods

Subjects and controls

All HIV-positive subjects were recruited from the HIV clinics at North Manchester General Hospital. Local research ethics committee approval was granted and written informed consent was obtained from each subject upon recruitment. Ten HIV-positive male Caucasian patients on one or more PIs who had clinical evidence of LD and who were due to switch to a PI-sparing regimen as part of their clinical management, were recruited and studied whilst on PIs

(hereafter termed LD1), and again 3 months after switching to a PI-sparing regimen (LD2) (Table 1).

LD syndrome was diagnosed on the basis of the clinician's confirmation of patient self-report or on the clinician's observation alone following the start of PI therapy, by methods used previously [17]. These subjects were found to have either fat wasting of the face, arms, legs or buttocks (with prominence of leg and arm veins), and/or fat accumulation in the abdomen or over the dorsocervical spine. Additionally, patients had to have either fasting hyperlipidaemia (cholesterol >5.5 mmol/l or triglyceride >2.0 mmol/l), and/or abnormal (although not diabetic) fasting glucose (6.1–7.0 mmol/l). None of the subjects had suffered any active AIDS illness in the 3 months preceding recruitment, nor were they taking any testosterone or anabolic steroids, lipid-lowering or oral hypoglycaemic agents. Confirmed cases of diabetes were excluded from recruitment.

For the control group, we recruited 10 HIV-positive patients (hereafter referred to as controls), matched for age, sex, ethnic background and body mass index (BMI), who had undergone PI therapy for at least 1 year without any evidence of LD, as defined by the above criteria. The controls were studied on one occasion. All controls in the study had also received various NRTIs, in combination with one or more PIs, as part of

Table 1. Characteristics of HIV-positive subjects with and without lipodystrophy

Variable	With LD, on PIs (LD1) <i>n</i> =10	With LD, off PIs (LD2) <i>n</i> =10	Without LD (controls) <i>n</i> =10
Demographics			
Age, years	43.8 \pm 8.8	43.8 \pm 8.8	35.3 \pm 4.7
BMI, kg/m ²	25.3 \pm 3.0	25.3 \pm 3.0	23.2 \pm 2.3
Duration HIV infection, years	7.9 \pm 3.8	7.9 \pm 3.8	7.2 \pm 4.7
CDC C (%)	7 (70%)	7 (70%)	4 (40%)
CD4 count, cells $\times 10^6$ /l	361 \pm 202	376 \pm 217	444 \pm 212
HIV-1 RNA, log copies/ml	2.6 \pm 1.7	2.6 \pm 1.5	2.2 \pm 0.8
Total therapy duration, months			
All PIs	27.7 \pm 7.5	27.7 \pm 7.5	34.9 \pm 11.2
All NRTIs	55.7 \pm 25.1	58.7 \pm 25.1	55.6 \pm 30.4
Zidovudine	30.8 \pm 20.5	31.1 \pm 20.1	30.6 \pm 20.9
Lamivudine	26.1 \pm 13.5	27.9 \pm 12.9	27 \pm 14.5
Stavudine	24.0 \pm 7.1	26.7 \pm 7.4	26.1 \pm 16.6
Didanosine	9.8 \pm 11.0	10.1 \pm 10.8	10.2 \pm 14.8
Zalcitabine	9.3 \pm 12.5	9.3 \pm 12.5	4.8 \pm 13.2
Abacavir	0	1.6 \pm 1.5	1.8 \pm 5.7
All NNRTIs	1.2 \pm 3.8	3.9 \pm 4.0	5.3 \pm 11.2
Nevirapine	2.5 \pm 5.3	2.8 \pm 5.2	2.6 \pm 8.2
Efavirenz	0	2.4 \pm 1.3	0

Data are presented as mean \pm SD, except when stated as percentages (%). LD1 = on PIs; LD2 = off PIs for 3 months. PI, protease inhibitor; NRTI, nucleoside analogue reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; BMI, body mass index.

their HAART regimen. The clinical characteristics of the patients with and without LD are shown in Table 1.

Viral and immunological measurements

HIV viral load and CD4 cell counts were measured on the first study day for the LD1, LD2 and control groups. Plasma was assayed for HIV RNA by a quantitative reverse transcriptase PCR assay (HIV Amplicor Roche, UK; limit of detection 50 (1.7 log₁₀) copies/ml) and CD4 T cells were quantified by flow cytometry, according to a standard procedure.

Fat redistribution and anthropometric measurements

Weight and stadiometer height were measured and the BMI (kg/m²) calculated. Anthropometric measurements were made by the same investigator on study day 1 for all three groups (LD1, LD2 and controls) using standardized methods [18,19]. Abdominal (at umbilicus), triceps and subscapular skinfolds were also measured (to the nearest 0.2 mm) using skinfold calipers (Holtain Ltd, Dyfed, UK). All measurements were made in triplicate on both sides of the body by the same investigator, and the mean value was used in the analysis. Bioelectrical impedance analysis (BIA) (Holtain Body Composition Analyser, Dyfed, UK; calibrated to 400 ohms before each use) was used to estimate fat-free mass and fat mass, by deduction from body weight using the equation described by Kotler *et al.* [20]. Total body water and percentage body fat were also calculated.

Single slice CT scans were performed at the umbilicus (L4 vertebral level) in both the LD1 and LD2 groups to determine the ratio of visceral abdominal fat to total abdominal fat (VAT:TAT), along with subcutaneous fat (SCF), by methods previously described. An abnormal VAT:TAT ratio was defined as >0.4 [21,22].

Assessment of glucose tolerance and insulin resistance

Glucose tolerance was assessed using the oral glucose tolerance test (OGTT), where subjects ingested a 75 g dextrose solution after a 12-h overnight fast, and blood samples were collected at 0, 30, 60, 90 and 120 min thereafter for determination of glucose, and at 0 and 120 min to determine insulin, proinsulin and C-peptide levels, as previously described [23]. The area under the curve (AUC) for glucose was determined using Arcus Quickstat Biomedical Software (Research Solutions, Cambridge, UK).

Insulin sensitivity was assessed using a modified insulin tolerance test (ITT) [24]. After a 12-h overnight fast, a 0.1 U kg⁻¹ body weight bolus of human actrapid insulin (Novo Laboratories, Basingstoke, UK) was given as an intravenous bolus through a butterfly

needle inserted into a left forearm vein. Arterialized venous blood was then taken every minute for 15 min (via a retrograde cannula inserted in the back of each subject's right hand and warmed in an 'arm-warming' device to enhance flow) for glucose determination. Insulin sensitivity was indicated by the first order rate constant for disappearance rate of glucose, K_{ITT} , estimated from the slope of the regression line of the logarithm of blood glucose against time during the first 3–15 min of the ITT [24].

We also estimated insulin resistance by the homeostasis model assessment for insulin resistance (HOMA-IR), using the equation:

$$\text{HOMA-IR} = [\text{fasting insulin (m IU/l)} \times \text{fasting glucose (mmol/l)}] / 22.5$$

An abnormal fasting HOMA-IR at time 0 was defined as >2.56 [25]. Serum insulin, pro-insulin and C-peptide levels were also measured at time 0 and 15 min.

Biochemical measurements

Fasting lipids [total cholesterol, triglycerides and high-density lipoprotein (HDL)], liver function tests (alkaline phosphatase, gamma glutamyl transferase, alanine transaminase and bilirubin), glucose, serum and urine creatinine were all measured by standard methods using Roche reagents on a Hitachi 747 or 911 analyser (Roche, Mannheim, Germany).

Insulin was measured using the Mercodia enzyme linked immunosorbent assay (ELISA) for intact insulin (Uppsala, Sweden). The detection limit was 5 pmol/l and inter-assay coefficients of variation (CVs) were <8% across the working range of the assay. Cross-reactivity of the insulin ELISA with pro-insulin was <0.1%. Pro-insulin was measured using the Dako Intact proinsulin ELISA (Dako Diagnostics Ltd, Ely, Cambridgeshire, UK). The detection limit was 1.0 pmol/l, the CVs <6% across the working range of the assay and cross-reactivity with insulin was 0%. C-peptide was measured using a radioimmunoassay (RIA) supplied by DPC (Euro/DPC Ltd, Gwynedd, UK). The CVs were <10% across the range 200–6000 pmol/l and the detection limit was 140 pmol/l. Serum cortisol was measured using an automated enzyme immunoassay (EIA) using the Bayer Immuno 1 analyser (Bayer plc, Newbury, Berkshire, UK). The working range of the assay was 25–1600 nmol/l and the CVs were <10% across the working range; the limit of detection was 10 nmol/l. Urinary cortisol was measured by RIA after extraction from urine using dichloromethane. Total testosterone was measured using a Coat-A-Count RIA from DPC. The working range of the assay was 0.7–55.5 nmol/l and the CVs across this range were

<12%. Sex hormone binding globulin (SHBG) was measured using an in-house radioassay that employs charcoal treatment of the sample and then incubation with ^{14}C -labelled testosterone. After removal of unbound hormone, the amount of radioactivity was directly proportional to the SHBG capacity. The working range of the assay was 5–120 nmol/l and the CVs across this range were <10%.

Human TNF- α was measured using an EIA (R&D Systems, Oxford, UK). The working range of the assay was 0.5–32.0 pg/ml with CVs <10% across this range. Human sTNF-RI was measured using an EIA from R&D Systems with a working range of 7.8–500.0 pg/ml; the CVs were <8.0% across this range (3.7% at 252 pg/ml). Human sTNF-RII was measured using an EIA from R&D Systems with a working range of 7.8–500.0 pg/ml; the CVs were <10% across this range (3.5% at 197 pg/ml).

Genotyping for the TNF- α promoter region polymorphisms

The polymorphisms in the TNF- α promoter region at positions -308 and -238 are both G→A transitions, and were detected by PCR-restriction fragment length polymorphism (RFLP) analysis, as described previously [26].

Statistical analyses

All statistical analyses were performed by Arcus Quickstat Biomedical Software (Research Solutions, Cambridge, Cambridgeshire, UK). HIV-1 viral loads

and levels of SCF measured by CT scanning were log₁₀ transformed prior to analysis. Demographic and clinical characteristics in the HIV-positive patients with and without LD syndrome were determined by paired and unpaired Student's *t*-test and the Mann–Whitney test, as appropriate (after assessing whether the data were normally distributed). A *P* value of ≤ 0.05 was accepted as being statistically significant.

Results

Patient characteristics and antiretroviral treatment history

The clinical characteristics of the patients and the antiretrovirals taken before and after stopping PIs are shown in Table 1. In addition, Table 2 shows the drugs taken by each patient before and after the switch from PIs, together with the drugs taken by each corresponding control. The LD and control groups were well matched clinically, apart from the fact that controls were slightly younger ($P=0.03$). Drug exposure between the LD and control groups was similar. However, as would be expected following a switch in therapy, differing changes were observed in LD1 compared with the LD2 group (Table 1). Importantly, viral load and CD4 cell counts did not differ between the groups.

Body composition and fat distribution

BMI did not change after stopping PIs (25 kg/m²), and was not different from that seen in controls

Table 2. Antiretroviral drugs taken by HIV-positive subjects with lipodystrophy before and after the switch from PIs, and the matched controls

Subject	With LD, on PIs (LD1) <i>n</i> =10	With LD, off PIs (LD2) <i>n</i> =10	Control	Without LD (controls) <i>n</i> =10
A	Stavudine, lamivudine, ritonavir	Efavirenz, abacavir, stavudine	1	Stavudine, lamivudine, indinavir
B	Stavudine, lamivudine, indinavir	Efavirenz, abacavir, stavudine	2	Stavudine, lamivudine, nelfinavir
C	Stavudine, lamivudine, indinavir	Efavirenz, abacavir, lamivudine	3	Zidovudine, lamivudine, indinavir
D	Stavudine, didanosine, nevirapine, indinavir	Efavirenz, abacavir, lamivudine, stavudine	4	Stavudine, didanosine, nelfinavir, saquinavir
E	Stavudine, lamivudine, ritonavir, saquinavir, nevirapine	Efavirenz, abacavir, stavudine, didanosine	5	Stavudine, lamivudine, nelfinavir
F	Stavudine, lamivudine, nelfinavir, saquinavir	Efavirenz, abacavir, stavudine	6	Stavudine, lamivudine, ritonavir
G	Stavudine, lamivudine, indinavir	Efavirenz, lamivudine, stavudine	7	Stavudine, abacavir, nelfinavir, ritonavir
H	Stavudine, lamivudine, nelfinavir	Stavudine, lamivudine, nevirapine	8	Stavudine, didanosine, nevirapine, hydroxyurea, nelfinavir, ritonavir
I	Stavudine, lamivudine, indinavir	Efavirenz, abacavir, lamivudine	9	Zidovudine, lamivudine, nelfinavir
J	Stavudine, lamivudine, ritonavir	Efavirenz, lamivudine, stavudine	10	Stavudine, lamivudine, indinavir

LD1 = on PIs; LD2 = off PIs for 3 months.

(23.2 kg/m²). Of the parameters measured, only bioelectrical impedance altered in the LD group on stopping PIs from 406 \pm 63 (mean \pm SD) to 384 \pm 49 ohms ($P=0.03$). Similarly, no changes were observed in any of the anthropometric measurements in the LD group on stopping PIs, apart from a small reduction in hip circumference from 97.5 \pm 6.9 to 95.5 \pm 6.7 cm ($P<0.01$). Single slice CT scans performed before (LD1) and after (LD2) stopping PIs at the level of L4 showed that there was no significant difference in the VAT:TAT ratio (0.6 \pm 0.2 vs 0.6 \pm 0.2) or in SCF (9.2 \pm 0.7 vs 9.2 \pm 0.5). Both LD1 and LD2 groups had an abnormal VAT:TAT ratio, defined as greater than 0.4 [21].

Metabolic parameters

Comparison of the lipid profiles in the control group and patients with LD prior to stopping PIs (LD1) showed that HDL levels were significantly higher in the control group, although none of the other parameters showed a difference. There was a small increase in HDL levels in the patients with LD (LD2) after the switch in antiretrovirals, although this just failed to reach statistical significance (Table 3).

One subject in the LD group became frankly diabetic after stopping PIs and was therefore excluded from analysis of the OGTT and modified ITT. No differences in glucose AUC were detected amongst the three groups (Table 3). Fasting insulin levels were significantly lower in the control group compared with the LD1 group ($P=0.02$); however, there were no significant changes in insulin, proinsulin and C-peptide levels on stopping PIs (Table 3).

Insulin resistance, assessed using HOMA-IR, showed that both the LD1 and LD2 groups had abnormally elevated fasting HOMA-IR values of 4.9 and 4.6, respectively. The fasting HOMA-IR was below the defined normal (2.56) in the control group, and was significantly lower when compared with the LD1 group. The HOMA-IR did not change in the LD group 3 months after switching from PIs. Insulin resistance was also assessed using the modified ITT [24], using the glucose disappearance rate, K_{ITT} , as a measure of insulin sensitivity. There was a significant correlation between the K_{ITT} and HOMA-IR ($r=-0.5$, $P=0.01$). However, there was no significant difference in the K_{ITT} between the control group ($P=0.03 \pm 0.01$) and patients with LD (LD1; $P=0.02 \pm 0.02$), which did not change on stopping PIs (LD2; $P=0.03 \pm 0.02$).

Steroid hormones

Testosterone and SHBG did not vary between the control and LD groups, and did not change on stopping PIs (Table 4). Similarly, plasma cortisol and 24-h urinary cortisol were not different between the different groups.

TNF- α and TNF-receptor levels

TNF- α , TNF-RI and TNF-RII levels were all significantly lower in the control group when compared with the LD group (Table 5). There was also a significant decrease in both the TNF-RI and TNF-RII levels in the LD group on stopping PIs, but no changes in the TNF- α levels. There was no correlation between TNF- α , TNF-RI and TNF-RII levels and either viral load or

Table 3. Metabolic characteristics of HIV-positive patients with lipodystrophy on and off PIs, compared with patients without lipodystrophy

Variable	With LD, on PIs (LD1)	With LD, off PIs (LD2)	<i>P</i> value (LD1 vs LD2)	Without LD (controls)	<i>P</i> value (LD1 vs controls)
Fasting lipids, mmol/l					
Total cholesterol	5.5 \pm 0.7	6.0 \pm 1.7	0.5	5.5 \pm 1.3	1.0
Triglycerides	3.3 \pm 2.7	3.1 \pm 2.8	0.71	2.9 \pm 1.9	0.97
HDL, mmol/l	0.7 \pm 0.4	1.0 \pm 0.6	0.07	1.2 \pm 0.1	0.003
LDL ratio	3.7 \pm 0.6	3.8 \pm 1.2	0.88	3.1 \pm 1.2	0.47
Glucose, mmol/l					
AUC ₁₂₀	909 \pm 180.4	915.6 \pm 150.7	0.99	847.4 \pm 123.4	0.90
Insulin, mU/l					
T ₀	18.5 \pm 15.1	19.9 \pm 14.0	0.56	6.6 \pm 5.1	0.02
HOMA-IR T ₀	4.9 \pm 4.8	4.6 \pm 3.4	0.62	1.5 \pm 1.3	0.04
Proinsulin, mU/l					
T ₀	4.2 \pm 3.5	5.0 \pm 4.9	0.41	2.4 \pm 1.1	0.21
C-peptide, mU/l					
T ₀	448.6 \pm 285.5	738.1 \pm 746.3	0.31	613.1 \pm 316.1	0.17

Data are presented as mean \pm SD. LD1 = on PIs; LD2 = off PIs for 3 months. LDL, low-density lipoprotein; HDL, high-density lipoprotein.

Table 4. Hormonal parameters in HIV-positive subjects with and without lipodystrophy

Parameter	With LD, on PIs (LD1)	With LD, off PIs (LD2)	P value (LD1 vs LD2)	Without LD (controls)	P value (LD1 vs controls)
Testosterone, nmol/l	16.8 ±7.2	15.0 ±6.8	0.43	22.2 ±5.4	0.11
SHBG, nmol/l	36.8 ±12.5	31 ±11.9	0.2	38.2 ±19.4	0.45
Cortisol, nmol/l	253.1 ±114.5	331.2 ±97.2	0.11	370.6 ±158.5	0.09
24 h urinary free cortisol, nmol/l/min	106.0 ±47.7	144.1 ±53.7	0.1	118.9 ±61.2	0.7
Creatinine clearance, ml/min	98.1 ±43.1	94.9 ±34.7	0.81	83.1 ±26.4	0.72

Data are presented as mean ±sd. LD1 = on PIs; LD2 = off PIs for 3 months. SHBG, sex hormone binding globulin.

Table 5. TNF- α , TNF-RI and TNF-RII levels in HIV-positive subjects with and without lipodystrophy

Variable	With LD, on PIs (LD1)	With LD, off PIs (LD2)	P value (LD1 vs LD2)	Without LD (controls)	P value (LD1 vs controls)
TNF- α , pg/ml	8.4 ±3.1	7.3 ±3.4	0.45	5.6 ±1.0	0.02
TNF-RI, pg/ml	2026 ±723	1680 ±585	0.01	1406 ±377	0.02
TNF-RII, pg/ml	5982 ±1897	5170 ±1876	0.03	3868 ±1042	0.02

Data are presented as mean ±sd. LD1 = on PIs; LD2 = off PIs for 3 months. TNF, tumour necrosis factor; TNF-RI and TNF-RII, TNF-receptor I and II.

CD4 cell counts. There was a good correlation between TNF-RI and TNF-RII levels ($r=0.9$, $P<0.0001$). There was also a correlation between the HOMA-IR and TNF-RI ($r=0.5$, $P=0.006$) and TNF-RII ($r=0.4$, $P=0.03$) levels, but not with TNF- α levels ($r=-0.03$, $P=0.9$).

Genotyping at the -238 position of the TNF- α gene showed that four out of the 10 patients with LD were heterozygotes, while all of the 10 patients in the control group were homozygote wild-types. In the 20 HIV-positive subjects studied, TNF- α levels in the heterozygotes were significantly higher than in the wild-types (9.4 ± 1.2 vs 6.3 ± 2.6 pg/ml; $P=0.03$). Genotype did not affect the change in TNF- α levels on stopping PIs (data not shown). Genotyping at the -308 position of the TNF- α gene showed that three and four patients had at least one variant allele in the LD and control groups, respectively. There was no difference in the TNF- α levels between those patients with the variant and common alleles at the -308 position (7.0 ± 3.1 vs 7.4 ± 2.8 pg/ml).

Discussion

Several hypotheses relating to retinoic acid-dependent cell signalling, mitochondrial genomic or biochemical toxicity, cytokine effects and immune restoration have been proposed to explain the clinical and epidemiological observations of HIV-associated LD syndrome. Currently available clinical data suggest that the manifestations of the LD syndrome may be due to partially overlapping toxicities of the NRTI and PI classes by

different and as yet not fully elucidated mechanisms [7,27]. In the longitudinal study presented here, we have undertaken a detailed examination of HIV-positive subjects with LD who switched from PI-based to non-PI regimens in order to evaluate the effects of PIs on the metabolic and morphological abnormalities, as well TNF- α and TNF-receptor levels.

The LD and control groups were well matched in both the types of drug and length of time exposed to the various antiretrovirals. All subjects (both the LD group and controls) had received zidovudine, stavudine or lamivudine, in combination with one or more PIs, as part of their HAART regimen. Three separate methods of quantifying body composition and fat distribution were used in the study, namely BIA, anthropometric measurements and CT scanning at the L4 vertebral level. The LD1 group showed no significant change in any of their anthropometric variables following the switch from the PI-containing regimen (LD2). There was also no significant difference in the VAT:TAT ratio or the SCF distribution between the LD1 and LD2 groups. These findings are consistent with other switch studies in which objective improvement in fat wasting has not been demonstrated in any PI switch study where concurrent NRTI treatment has been continued or intensified [7,9,10].

TNF- α has complex effects, many of which could help account for many of the recognized features of the LD syndrome. For example, research in non-HIV patients has shown that TNF- α influences insulin resistance [28], although this does not seem to be the case

in healthy volunteers [29]. TNF- α is also involved in triglyceride and glucose metabolism [12], adipocyte differentiation and metabolism [13], and enhances plasminogen activator inhibitor levels [30]. Previous studies have shown that TNF- α is over-expressed in HIV subjects with the LD syndrome [16], and it is correlated with both insulin resistance [31] and hyperlipidaemia [32]. Furthermore, a dramatic increase in the percentage and absolute number of T cells synthesizing TNF- α following PI administration has been shown [16]; this correlated positively with total cholesterol, total triglycerides, apoB concentrations and the apoB/apoA1 ratio, and inversely with the HDL concentration. Adipocytes from HIV-positive individuals show changes in TNF- α levels and sterol regulatory element-binding protein 1 (SREBP1) [31], which together may be involved in the pathogenesis of some of the features of LD.

In view of this, we studied the effect of stopping PIs on TNF- α levels using a highly sensitive assay. We show that plasma TNF- α levels were significantly higher in the group with LD when compared with the control group without LD, but showed little change on stopping the PIs in the LD group. Interestingly, TNF-receptor levels were also high in LD patients, but unlike TNF- α levels, fell significantly on stopping PIs. TNF-receptor levels exist in a complex feedback regulatory relationship with TNF- α [33] representing a buffer system prolonging the biological effect of TNF- α by forming a 'slow release' reservoir and impeding the spontaneous denaturation of the cytokine. Moreover, TNF-receptor concentrations increase with increasing local TNF- α action in skeletal muscle and adipose tissue [34]. Taken together, the findings suggest that plasma TNF- α levels may not be a good marker of TNF- α activity in tissues, and TNF-receptors should be considered to be a more sensitive indicator [35]. In accordance with this, TNF-receptor levels have been shown to be elevated in other conditions associated with insulin resistance such as myotonic dystrophy [35], obesity [12] and in young obese subjects with impaired glucose tolerance [36]. Additionally, in accordance with previous literature [37], there was a correlation between TNF-receptor levels and HOMA-IR in our subjects. TNF- α levels as well as insulin resistance are known to alter with BMI [28,32]. Our patients were matched for BMI, and thus the differences seen in our study in TNF- α and insulin resistance on switching from PI-based to non-PI regimens are unlikely to be due to anthropometric differences between the LD and control groups. Furthermore, it is important to note that all our patients had been free of acute illness for more than 3 months preceding the study and were virologically well controlled. Taking everything into consideration, our results would suggest that the elevation in TNF- α and

TNF-receptor levels is at least partly a direct effect of the PIs, a finding that is supported by *in vitro* studies from our laboratory [38]. However, as revealed by our *in vitro* results [38], it is unlikely that the effect of different PIs will be the same, and the mechanism by which PIs modulate TNF- α secretion needs further study.

TNF- α levels are known to vary greatly in human populations [39], and are partly under genetic control [40]. It has recently been shown in two studies that the TNF- α promoter region -238 gene polymorphism is associated with LD [41,42]. Functional analysis of this polymorphism *in vitro* has produced contradictory findings [43-45]. Our data, using the whole cohort of patients recruited in this study, show that the TNF-238 GA genotype was associated with higher TNF- α levels than the GG genotype, suggesting that the -238 promoter region gene polymorphism may predispose to LD by increasing TNF- α secretion in HIV-positive patients.

An important feature of the LD syndrome is insulin resistance. In this study, we used two independent methods for examining insulin resistance: (i) the glucose disappearance rate (K_{ITT}), derived from the blood glucose response to a bolus of exogenous insulin in 15 min [24], which represents insulin-mediated glucose uptake by the tissues [46] and (ii) the HOMA-IR index, based upon fasting insulin and glucose concentrations [25]. Although these methods examine insulin resistance under differing physiological conditions, there was a correlation between HOMA-IR and K_{ITT} . However, there was no significant improvement in insulin resistance on stopping the PIs in the LD group. This contrasts with data presented by Goebal & Walli [47] who showed an improvement in insulin resistance after switching from a PI to abacavir. However, it is important to note that contradictory data have also been produced on the effect on insulin resistance in different switch studies [7], which may be a reflection of patient heterogeneity and the fact that the different antiretrovirals used in the switch studies have differing effects on insulin resistance. This latter point needs further study, and may be important in any future recommendations for therapy switching. It is thus interesting to note that insulin sensitivity does not improve with efavirenz after 1 year of follow-up [10]. In our study, nine out of the 10 subjects in the LD1 group were switched to efavirenz (seven of these combined with abacavir), whilst only one subject was switched to nevirapine.

The molecular aetiology of insulin resistance in HIV disease is likely to be complex and multifactorial. Apart from the effects of TNF- α , it has been shown that PIs directly inhibit the specific insulin sensitive glucose transporter, Glut-4 [48]. Nevertheless, there is

likely to be an interaction between all the components contributing to insulin resistance. For example, TNF- α is known to be involved in the regulation of free fatty acids, Glut-4 transporter numbers, and/or insulin receptor activity [49], all of which will contribute to the final phenotype.

In summary, the results presented in this intensive study show a significant reversal of some, but not all, of the metabolic and morphological abnormalities associated with LD after stopping PIs. The most noticeable effects were on the TNF- α system. Although these data need to be confirmed in larger numbers of patients, our finding of the effect of PIs on TNF- α *in vivo* are consistent with the emerging evidence of the role of this cytokine in the LD syndrome, and are supported by *in vitro* evidence suggesting that PIs may have a direct effect on TNF- α production [11,50,51].

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