

Low density lipoprotein apolipoprotein B metabolism in treatment-naïve HIV patients and patients on antiretroviral therapy

A Margot Umpleby^{1*}, Satyajit Das², Michael Stolinski¹, Fariba Shojaee-Moradie¹, Nicola C Jackson¹, William Jefferson¹, Nicky Crabtree¹, Peter Nightingale³ and Mohsen Shahmanesh²

¹Department of Diabetes and Endocrinology, St Thomas' Hospital, GKT School of Medicine, Kings College, London, UK

²Departments of HIV, Nuclear Medicine and Wellcome Trust Clinical Research Facility, University Hospitals Birmingham, UK

³Wellcome Trust Clinical Research Facility, Queen Elizabeth Hospital, Birmingham, UK

*Corresponding author: Tel: +44 (0)1483 688579; Fax: +44 (0)1483 688501; E-mail: m.umpleby@surrey.ac.uk

Background: Dyslipidaemia and lipodystrophy have been described in treated HIV patients and in a small percentage of untreated HIV patients. Lipodystrophy in these patients has been shown to be associated with a lower expression of low density lipoprotein (LDL) receptors.

Methods: We have investigated the effect of antiretroviral treatment with either a protease inhibitor (PI) or a non-nucleoside reverse transcriptase inhibitor (NNRTI) on body fat distribution and LDL apolipoprotein B (apoB) kinetics in 12 HIV-negative controls and 52 HIV-infected patients, including antiretroviral treatment-naïve (TN) patients ($n=13$) and patients taking two nucleoside analogues plus either a PI ($n=15$) or an NNRTI ($n=24$).

Results: LDL cholesterol was not different between groups. Compared with the controls, LDL apoB absolute synthetic rate (ASR) and fractional catabolic rate (FCR) were lower and residence time (RT) was higher in the PI and NNRTI

groups ($P<0.05$). In the TN patients, LDL ASR was lower ($P<0.05$) and there was a trend for a lower FCR and higher RT compared with the controls ($P=0.07$). LDL apoB pool size was greater in the PI group compared with the controls ($P<0.05$). In the PI group, patients on ritonavir (RTV)-containing regimens had a lower LDL apoB ASR ($P=0.009$) and a trend to a lower LDL apoB FCR and increased RT compared with non-RTV-containing PI regimens ($P=0.05$). There was a positive correlation between LDL apoB FCR and limb fat/lean body mass ($P=0.004$) in all subjects.

Conclusions: Decreased LDL FCR, despite unchanged LDL cholesterol, was demonstrated in both treated and untreated HIV patients. It was more marked with RTV-containing regimens and was associated with reduced limb fat. The increased LDL RT may lead to an increased risk of atherogenesis thus contributing to the risk for cardiovascular disease in these patients.

Introduction

Antiretroviral treatment of HIV infection is associated with disturbances in body fat distribution [1–3], dyslipidaemia [1,4] and insulin resistance [2,4,5]. Lipodystrophy [6] and dyslipidaemia [7] have also been demonstrated in a small percentage of antiretroviral treatment-naïve (TN) patients. The exact mechanisms of these changes have yet to be determined but may be related to the effect of HIV infection [6], the adverse effects of some antiretroviral drugs and the long-term consequence of antiretroviral therapy on regional fat distribution. We have previously shown that patients treated with protease inhibitors (PIs) or non-nucleoside reverse transcriptase inhibitors (NNRTIs) with mild dyslipidaemia have a decreased clearance of very low density lipoprotein (VLDL) and intermediate density lipoprotein (IDL) apolipoprotein B (apoB) which was correlated with peripheral fat loss [8].

Low density lipoprotein (LDL) cholesterol has been shown to be reduced in TN HIV-infected patients compared with control subjects [9] while other studies have reported LDL cholesterol levels to be normal [10,11] and in the D:A:D study (Data collection on Adverse events of anti-HIV Drugs) approximately 12% of TN patients were shown to have LDL cholesterol >4.1 mmol/l [7]. There are also conflicting reports on the effect of highly active antiretroviral therapy (HAART) on LDL cholesterol. Treatment with PIs in one study has been reported to result in a small increase in LDL cholesterol [12] while other studies report no effect of PIs on LDL cholesterol [13] and in the D:A:D study approximately 32% of patients on PIs had LDL cholesterol >4.1 mmol/l. The NNRTI component of HAART may also increase LDL cholesterol. NNRTI-treated patients have been shown to have higher LDL

cholesterol levels than TN patients [12]. In a study which switched PI-treated patients to nevirapine (NVP) there was a significant decrease in LDL cholesterol [14].

The mechanisms for the changes in LDL cholesterol in HIV infection and with different treatment regimes are unclear. However, a recent study of treated and untreated HIV patients with and without lipodystrophy showed that HIV lipodystrophy was associated with a lower expression of LDL receptors [15]. We hypothesized that HIV infection and antiretroviral treatment may reduce LDL clearance and that this may be related to changes in body fat distribution. To investigate this, we have undertaken a large cross-sectional study of LDL apoB kinetics and body fat distribution in 15 patients treated with a PI, 24 patients treated with NNRTIs, 13 TN patients and 12 HIV-negative controls.

Methods

Patients

A cross-sectional study was performed on 39 HIV-positive patients who were taking two nucleoside reverse transcriptase inhibitors (NRTIs) plus either a PI ($n=15$), or the NNRTIs NVP ($n=11$) or efavirenz (EFV) ($n=13$) for between 1–6 years. Patients on PI were taking nelfinavir ($n=6$), lopinavir/ritonavir (RTV) ($n=3$), RTV alone ($n=2$), indinavir/RTV ($n=2$), saquinavir/RTV ($n=1$) or indinavir alone ($n=1$). There was no difference in the nucleoside backbone between treatment groups. Most patients were on a zidovudine/lamivudine combination (nine PI, 17 NNRTI). Other combinations were stavudine/lamivudine (two PI, four NNRTI), stavudine/didanosine (three PI, one NNRTI), zidovudine/abacavir (one NNRTI), abacavir/lamivudine (one NNRTI) and zidovudine only (one PI). Thirteen HIV-positive TN patients were also studied as well as 12 presumed HIV-negative controls. A negative HIV test within the last 3 months was required if risk history revealed a risk of HIV (two subjects). Data on VLDL and IDL apoB metabolism in these patients have been published previously [8]. Three patients were taking statins (two PI and one NNRTI) which were stopped 6 weeks prior to tests.

Exclusion criteria were: fasting glucose >6.0 mmol/l, glucocorticoids, any drugs which affect lipid metabolism, hypothyroidism, creatinine >150 mmol/l, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) greater than five times upper limit of normal, anaemia and $>10\%$ loss in body weight in the preceding 6 months. Ethical approval was obtained from South Birmingham Local Research Ethics Committee and all subjects gave written informed consent.

Study protocol

Following an overnight fast, patients were admitted to the Wellcome Trust Clinical Research Facility and an

intravenous line placed in the antecubital vein of each arm – one for blood sampling and one for administration of $1\text{-}^{13}\text{C}$ leucine (^{13}C enrichment 99%; Cambridge Isotope Laboratory, Andover, MA, USA) as a primed (1 mg/kg) constant infusion (1mg/kg/h^{-1}) for 9 h. A baseline blood sample was taken prior to the infusion then samples were taken at 30 mins and then every hour for 9 h. Body fat distribution was measured by whole body dual energy X-ray absorptiometry (DEXA) scan within 1 month of the study. A total of 55 subjects were scanned using a Hologic QDR 4500A (version 11.2.3; Hologic Inc, Waltham, MA, USA). Eight subjects were scanned using a Lunar DPX-L (version 1.3g; GE Medical Systems, Milwaukee, WI, USA). One patient refused a scan. Results are presented as limb fat (arm plus leg) or trunk fat (g) divided by lean body mass (LBM; g).

Experimental methods

After removal of VLDL and IDL by sequential ultracentrifugation [8], LDL was isolated by adjusting plasma density to 1.063 g/ml and spinning for 20 h on a Beckman Coulter Optima LE80-K ultracentrifuge (High Wycombe, UK). ApoB-100 was precipitated from the LDL fraction with tetramethylurea, delipidated and hydrolysed with 6M hydrochloric acid. The isotopic enrichment of leucine in LDL apoB was measured as the *N*-acetyl, *n*-propyl-ester derivative using gas chromatography combustion-isotope ratio mass spectrometry (GC-IRMS) (GC: Hewlett-Packard 5890; Hewlett Packard, Bracknell, UK. Combustion unit – Orchid; Europa Scientific, Crewe, UK. IRMS-SIRA 10; VG Isotech, Middlewich, UK) as previously described [16]. The GC was equipped with an AT-1 capillary column (60 m, 0.25 mm internal diameter, 1.0- μm film thickness; Alltech, Stamford, UK). Isotope abundance was expressed relative to pulse peaks of reference CO_2 gas. Data were analysed using the manufacturers' software (Orchid Post Processor, Version 2.3c; Europa Scientific). The isotopic enrichment of $\alpha\text{-KIC}$ was determined by selected ion monitoring of the quinoxalinol-*tert*-butyldimethylsilyl derivative at m/z 259 and 260 by GC/MS (Hewlett Packard 5971A MSD) with electron impact ionization.

ApoB-100 LDL concentration was determined by an immunoturbidimetric method (Immunoturb Kit; Immuno Ltd, Dunton Green, UK; interassay CV 4%). Enzymatic methods were used to measure plasma total, LDL cholesterol and triglyceride (ABX Diagnostics, Shefford, UK) and HDL cholesterol (Alpha Laboratories, Eastleigh, UK). Insulin was measured by ELISA (Mercodia, Uppsala, Sweden) and glucose was measured using a glucose analyser (Roche Diagnostics, Lewes, UK). LDL oxidation was measured by ELISA (Mercodia).

Data analysis

Insulin resistance was calculated using the homeostasis assessment model (HOMA_{IR}) [17]. The fractional secretion rate (FSR) of LDL was calculated using a simple regression model as previously described [18] using LDL enrichment between 4 and 9 h when the enrichment curves were linear. The precursor compartment for the incorporation of ¹³C leucine into the LDL particles was the steady state enrichment of α -KIC [19]. FSR was thus calculated as:

$$\text{FSR (pools/day)} = \frac{\text{Rate of increase of leucine enrichment in LDL apoB (APE/h)}}{\text{Steady state enrichment of } \alpha\text{-KIC}} \times 24$$

Patients were in a steady state in the study as determined by the constant LDL apoB concentration (data not shown). In this case, the FSR equals the fractional catabolic rate (FCR). The LDL apoB absolute synthetic rate (ASR) (mg/kg/day) was calculated from the product of the FSR (pools/day) and the apoB pool size (mg) divided by body weight. The LDL pool size was calculated from the product of the mean LDL apoB concentration (mean concentration of apoB in four pooled samples) and the plasma volume. Plasma volume was calculated using Pearson *et al.*'s formula [20]. Residence time (RT) was calculated as 1/FCR (pools/day). The ratios of cholesterol and triglyceride to apoB were calculated with units of g/l.

Initial comparison between the four groups was by one-way analysis of variance or Kruskal–Wallis followed by Bonferroni's or Dunn's multiple comparison test (SPSS 10.0.7 for Windows; SPSS Inc, Chicago, IL, USA). HDL cholesterol was gender adjusted. Fisher's exact test was used for categorical data between groups and associations were analysed by Spearman's rank correlation test. A stepwise linear regression model examined the effect of variables on

LDL metabolism. Variables entered were age, sex, ethnicity (Caucasian vs non-Caucasian), smoking, family history of diabetes, family history of cardiovascular disease, alcohol intake, limb fat/LBM, trunk fat/LBM, non-esterified fatty acids (NEFA) and HOMA.

Results

The demographics of the patients are shown in Table 1. Age and BMI were not significantly different between groups. Patients were significantly longer on PI than NNRTI ($P=0.001$). Serum AST, gamma glutamyl transpeptidase, alkaline phosphatase and haemoglobin were not different between groups. No patient had hepatitis C or was a carrier for hepatitis B. There was no difference in current CD4 counts between HIV groups. Current and pre-treatment viral loads and pre-treatment CD4 counts were not different in the treatment groups. Two patients in the PI group had a detectable viral load (800 and 1100 copies/ml). Viral loads were below the detection limit of the assay (<50 copies/ml) in all other treated patients.

Plasma triglycerides (TGs) were significantly higher ($P<0.05$) in the PI group (2.28 ± 0.34 mmol/l) than in the control group (1.1 ± 0.12 mmol/l) but were not different in the TN (1.49 ± 0.29 mmol/l) and NNRTI groups (1.71 ± 0.23 mmol/l). HDL cholesterol was lower ($P<0.05$) in the TN (1.12 ± 0.08 mmol/l), PI (1.22 ± 0.14 mmol/l) and NNRTI groups (1.24 ± 0.05 mmol/l) compared with the controls (1.9 ± 0.11 mmol/l). NEFA concentration was not different between groups (data shown previously [8]).

Only four patients had clinical evidence of lipodystrophy (one in the PI group, three in the NNRTI group). Limb fat/LBM was significantly reduced in the PI and NNRTI groups ($P<0.05$) but not in the TN group compared with the control group (Table 2).

Table 1. Baseline characteristics (mean \pm SD) in HIV-negative patients (controls), TN patients and those on HAART antiretroviral-containing regimens containing PIs or NNRTIs

	Control (n=12)	TN (n=13)	PI (n=15)	NNRTI (n=24)
Age, years	32.8 \pm 12.2	39.7 \pm 10.8	43.6 \pm 9.3	37.5 \pm 10.0
BMI	23.0 \pm 3.6	24.0 \pm 2.9	24.4 \pm 3.6	23.2 \pm 3.1
Male	5	9	13	20
Caucasian	10	6	13	18
Homosexual	2	6	11	20
Treatment, months	—	—	48.7 \pm 14.7	31.0 \pm 14.7*
Log pre-treatment viral load, copies/ml	—	4.3 \pm 0.8	5.1 \pm 0.5	4.8 \pm 0.8
Pre-treatment CD4, cells/dl	—	—	125 \pm 122	181 \pm 127
Current CD4, cells/dl	—	377 \pm 238	435 \pm 187	479 \pm 266

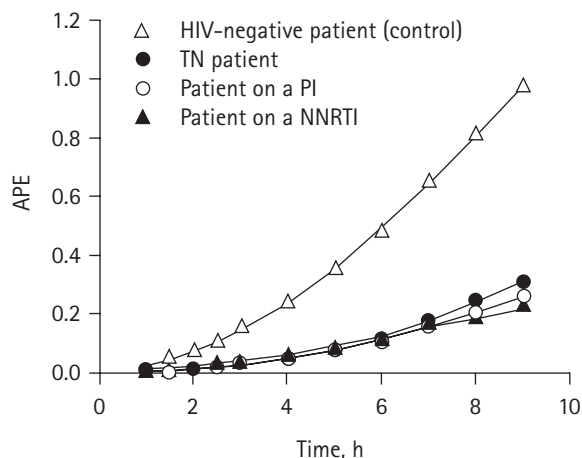
*NNRTI versus PI ($P<0.001$). BMI, body mass index; HAART, highly active antiretroviral therapy; NNRTI, non-nucleoside transcriptase inhibitor; PI, protease inhibitor; TN, treatment-naive.

When the analysis was confined to the patients who had body fat measurements made on the same DEXA (55 subjects), the findings were similar with a significantly reduced limb fat/LBM in the PI [0.09 (0.07–0.16) g/LBM] and NNRTI groups [0.10 (0.06–0.16) g/LBM] compared with the control group [0.21 (0.14–0.29) g/LBM; $P < 0.05$]. Trunk fat was not significantly different between groups (Table 2). Although insulin resistance, measured by HOMA, was not significantly different in any of the HIV patient groups from the control subjects, HOMA values within each group were very variable (previously reported in [8]).

LDL cholesterol was not different between groups (Table 3). LDL apoB ASR and FCR were lower and LDL RT higher in the PI and NNRTI groups than in the control group ($P < 0.05$) (Table 3). A typical enrichment curve for one subject in each group is shown in Figure 1. In the TN group, LDL apoB ASR was lower ($P < 0.05$) and there was a trend for LDL apoB FCR to be lower and LDL RT to be higher ($P = 0.07$) than in the control subjects. The PI group exhibited a higher LDL apoB pool size ($P < 0.05$) compared with controls. LDL TG, LDL TG/apoB and LDL cholesterol/apoB were not different between groups. Oxidized LDL was not different between groups.

There were no differences in apoB kinetics between NVP- and EFV-treated patients in the NNRTI group. The PI group patients on RTV-containing regimens had

Figure 1. Typical LDL apoB enrichment curves in an HIV-negative patient, TN patient and patients on HAART regimens containing a PI or an NNRTI



apoB, apolipoprotein B; HAART, highly active antiretroviral therapy; LDL, low density lipoprotein; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; TN, treatment-naive.

a lower LDL apoB ASR ($P = 0.009$) and a trend to a lower LDL apoB FCR and increased RT compared with non-RTV-containing PI regimens ($P = 0.05$) (Table 4). Antiretroviral-treated patients with LDL cholesterol > 3 mmol/l (six PI, three NNRTI) had lower LDL FCR

Table 2. Body fat (median and IQR) measured by DEXA in HIV-negative patients (controls), TN patients and those on antiretroviral regimens containing PIs or NNRTIs

	Control (n=12)	TN (n=13)	PI (n=14)	NNRTI (n=24)	P value between groups
Limb fat/LBM	0.21 (0.14–0.29)	0.15 (0.09–0.21)	0.09* (0.07–0.15)	0.08* (0.06–0.14)	$P = 0.001$
Trunk fat/LBM	0.16 (0.11–0.21)	0.16 (0.09–0.19)	0.14 (0.11–0.19)	0.14 (0.08–0.20)	NS

* $P < 0.05$ versus control. DEXA, dual energy X-ray absorptiometry; IQR, interquartile range; LBM, lean body mass; NNRTI, non-nucleoside transcriptase inhibitor; NS, not significant; PI, protease inhibitor; TN, treatment-naive.

Table 3. LDL kinetics and concentration (median and IQR) in HIV-negative patients (controls), TN patients and those on antiretroviral regimens containing PIs or NNRTIs

	Control (n=12)	TN (n=13)	PI (n=15)	NNRTI (n=24)	P value between groups
LDL ASR, mg/kg/day	9.07 (7.39–9.66)	5.54* (3.77–7.05)	5.12* (4.21–6.87)	6.24* (3.78–7.53)	$P = 0.003$
LDL FCR, pools/day	0.48 (0.34–0.54)	0.27 (0.25–0.36)	0.24* (0.10–0.35)	0.27* (0.17–0.43)	$P = 0.002$
LDL RT, days	2.08 (1.87–2.94)	3.71 (2.77–4.05)	4.11* (2.85–10.55)	3.67* (2.34–6.08)	$P = 0.002$
LDL apoB pool size, g	1.21 (1.03–1.46)	1.36 (1.04–1.56)	1.87* (1.37–2.47)	1.63 (1.20–2.07)	$P = 0.016$
LDL cholesterol, mmol/l	1.79 (1.40–2.44)	1.85 (1.43–2.22)	2.50 (1.87–3.15)	2.20 (1.78–2.74)	NS
LDL cholesterol/apoB ratio	1.56 (1.49–1.63)	1.51 (1.41–1.58)	1.57 (1.39–1.67)	1.54 (1.47–1.61)	NS
LDL triglyceride, mmol/l	0.14 (0.09–0.18)	0.14 (0.12–0.18)	0.20 (0.13–0.23)	0.14 (0.10–0.17)	NS
LDL triglyceride/apoB, ratio	0.28 (0.18–0.33)	0.30 (0.21–0.34)	0.29 (0.23–0.35)	0.23 (0.19–0.27)	NS
Oxidised LDL/apoB, U/g	108.9 (86.9–117.2)	105.9 (95.3–119.8)	97.1 (78.7–111.5)	91.2 (72.3–112.3)	NS

* $P < 0.05$ versus control. apoB, apolipoprotein B; ASR, absolute synthetic rate; FCR, fractional clearance rate; IQR, interquartile range; LDL, low density lipoprotein; NNRTI, non-nucleoside transcriptase inhibitor; NS, not significant; PI, protease inhibitor; RT, residence time; TN, treatment-naive.

($P < 0.02$), increased LDL apoB ($P = 0.001$) and increased LDL TG ($P < 0.001$) compared with those with LDL cholesterol levels < 3 mmol/l. LDL ASR was not different in the two groups.

LDL apoB FCR correlated with limb fat/LBM (Figure 2, $r_s = 0.36$, $P = 0.004$), and negatively correlated with LDL apoB pool size ($r_s = -0.67$, $P < 0.001$), LDL cholesterol ($r_s = -0.59$, $P < 0.001$) and LDL triglyceride ($r_s = -0.44$, $P < 0.001$) in all subjects. HOMA correlated positively with trunk fat/LBM ($r_s = 0.38$, $P = 0.002$) and negatively with LDL apoB ASR ($r_s = -0.33$, $P = 0.008$) and LDL apoB FCR (Figure 2, $r_s = -0.42$, $P = 0.001$).

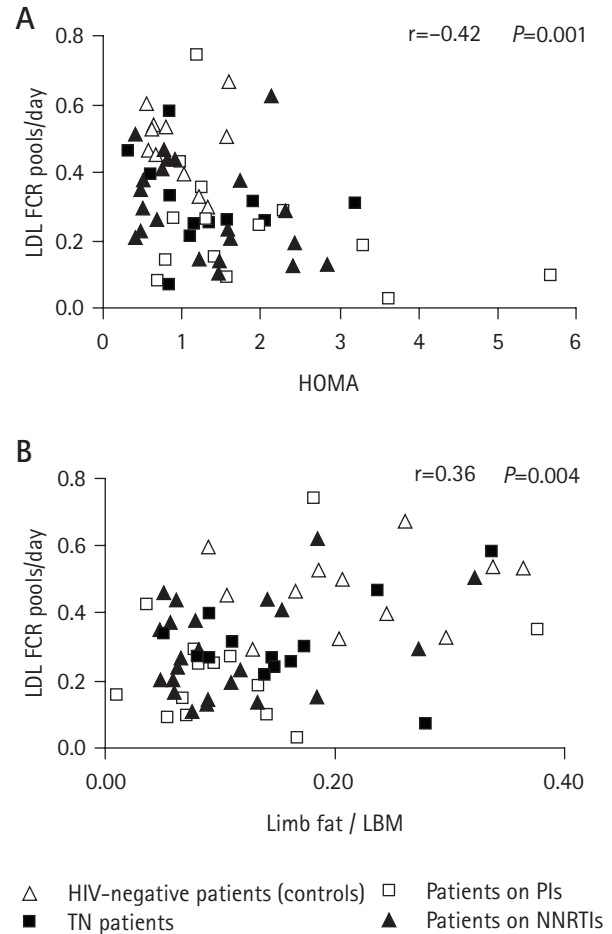
In a linear regression model, HOMA predicted LDL RT and FCR and inversely predicted LDL ASR ($P = 0.011$ and $P = 0.007$, respectively). NEFA concentration predicted LDL apoB pool size ($P < 0.001$), LDL cholesterol ($P = 0.006$) and LDL TG ($P < 0.001$). Limb fat/LBM inversely predicted LDL cholesterol ($P = 0.04$).

Conclusions

This study, which is the largest kinetic study of LDL metabolism in HIV patients, showed a reduction in LDL apoB FCR and LDL ASR in both TN patients and HIV patients taking antiretroviral medication with PI- or NNRTI-containing regimens compared with a control group. In the PI group, LDL apoB FCR and ASR were lower in patients taking RTV compared with patients taking other PIs. LDL apoB FCR in all subjects correlated positively with limb fat and negatively with insulin resistance.

The study demonstrated that LDL kinetics were abnormal in HIV-infected patients before treatment, even though LDL cholesterol levels were normal. In TN patients, LDL cholesterol has been reported to be reduced [9], normal [10,11] and increased in some patients [7]. It has also been shown in untreated

Figure 2. (A) Negative correlation of LDL FCR with HOMA in all subjects ($r = -0.42$, $P = 0.001$). (B) Correlation of LDL FCR with limb fat/LBM in all subjects ($r = 0.36$, $P = 0.004$)



FCR, fractional clearance rate; HOMA, homeostasis assessment model; LBM, lean body mass; LDL, low density lipoprotein; NNRTI, non-nucleoside transcriptase inhibitor; PI, protease inhibitor; TN, treatment-naive.

Table 4. LDL kinetics and concentrations (median and IQR) in patients on antiretroviral regimens containing the PI RTV and those receiving other PIs

	RTV (n=8)	Non-RTV (n=7)	P value
LDL ASR, mg/kg/day	4.56 (2.58–5.10)	6.87 (5.28–8.47)	$P = 0.009$
LDL FCR, pools/day	0.15 (0.09–0.26)	0.27 (0.24–0.42)	$P = 0.05$
LDL RT, days	6.86 (3.99–11.64)	3.76 (2.36–4.11)	$P = 0.05$
LDL apoB pool size, g	2.06 (1.47–3.01)	1.83 (1.30–2.43)	NS
LDL cholesterol, mmol/l	2.95 (1.88–4.03)	2.10 (1.58–3.06)	NS
LDL cholesterol/apoB ratio	1.59 (1.46–1.72)	1.45 (1.33–1.64)	NS
LDL triglyceride, mmol/l	0.22 (0.13–0.45)	0.16 (0.11–0.21)	NS
LDL triglyceride/apoB ratio	0.29 (0.23–0.39)	0.29 (0.19–0.30)	NS
HOMA	1.40 (0.79–3.31)	1.66 (0.97–6.19)	NS
Oxidized LDL/apoB, U/g	90.9 (70.8–109.2)	104.3 (76.2–124.7)	NS

apoB, apolipoprotein B; ASR, absolute synthetic rate; FCR, fractional clearance rate; HOMA, homeostasis assessment model; IQR, interquartile range; LDL, low density lipoprotein; NS, not significant; PI, protease inhibitor; RT, residence time; RTV, ritonavir.

HIV-infected patients that LDL cholesterol is related to immune status, with reduced LDL cholesterol in patients with low CD4 counts [21], which may account for the low LDL cholesterol in some studies. Since LDL cholesterol concentration is determined by the LDL ASR and FCR, a normal concentration will result if there is a decrease in both LDL ASR and FCR, as found in all HIV patient groups in our study. The demonstration that LDL kinetics can be abnormal when LDL cholesterol concentrations are in the normal range shows the importance of measuring the kinetics of lipoprotein metabolism.

The finding of normal LDL cholesterol in the PI and NNRTI patient groups may seem unusual. While most studies have shown that LDL cholesterol is increased following treatment with PIs or NNRTIs [12,22], not all patients develop dyslipidaemia and there are reports that treatment with PIs has no effect on LDL cholesterol [13]. The D:A:D study demonstrated that only 30–40% of patients treated with PIs and NNRTIs developed dyslipidaemia [7]. In the current study, patients on lipid-lowering treatment had their treatments stopped for 6 weeks prior to study. Only three patients had previously been on lipid-lowering treatment and only two patients had severe dyslipidaemia (cholesterol >7 mmol/l and/or triglyceride >5 mmol). A subgroup of treated patients in this study had LDL cholesterol >3 mmol/l. In these patients, LDL FCR was significantly lower than in treated patients with LDL cholesterol <3 mmol/l whereas LDL ASR was not different, demonstrating that the increase in LDL cholesterol in these patients was due to a larger decrease in LDL FCR. The change in lipid levels in response to infection with HIV and following treatment is clearly variable and may be related to the lipodystrophy [1]. Only four patients in the current study had clinical lipodystrophy but measurement of body fat by DEXA demonstrated a significant decrease in limb fat in the treated patients and there was a significant relationship between limb fat and LDL FCR.

The decrease in LDL ASR may be due to a reduction in the direct hepatic secretion of LDL [23] and/or reduced production of LDL formed as a result of remodelling of IDL and VLDL. We have previously reported that VLDL and IDL apoB FCR are reduced in these HIV patients [8] and, while this may be a contributory factor, reduced hepatic secretion may also have a role. LDL turnover studies using radioactive tracers and different mathematical approaches have shown that approximately two-thirds of the LDL pool is degraded by a saturable, receptor-dependent pathway, the remainder is cleared by a receptor-independent pathway in humans [24]. A study of treated and untreated HIV patients with and without lipodystrophy showed that HIV lipodystrophy was associated

with a lower expression of LDL receptors and that this was not related to PI treatment [15]. Although LDL kinetics were not different in the PI and NNRTI groups compared with the TN group in this study, when the patients in the PI group were subdivided into those treated with RTV and those treated with other PIs, it was found that the abnormal LDL kinetics were exacerbated by RTV treatment. LDL FCR in the RTV group was extremely low and similar to levels reported in patients with LDL receptor deficiency [25] and familial defective apoB-100, in which binding of LDL to the LDL receptor is impaired [26]. This suggests that LDL receptor numbers may be considerably reduced in this patient group. LDL cholesterol was not significantly increased by RTV since there was a decrease in both LDL FCR and ASR. One study has shown RTV to be more strongly associated with lipodystrophy than other PIs [27], which may suggest that the decrease in LDL receptor may be related to lipodystrophy [27].

Other mechanisms may also play a role. We previously speculated [8] that the decrease in VLDL and IDL FCR may be due to a decrease in lipoprotein and hepatic lipase [11,28]. While hepatic lipase contributes to the remodelling of apoB-containing lipoproteins, it also participates with surface proteoglycans and LDL receptor-like protein (LRP) as a ligand for the hepatic uptake of apoB-containing remnant lipoproteins and LDL [29]. Decreased hepatic lipase activity may thus contribute to the decrease in LDL FCR. It has also been suggested that PIs may inhibit LRP since the catalytic region of HIV-1 protease, to which PIs bind, has approximately 60% homology to regions within LRP [30]. The greater effect of RTV to reduce LDL FCR could be due to an additional effect of this PI on LRP.

The LDL receptor is regulated by the cholesterol content of cells but is also under hormonal control and is up-regulated by insulin [31,32]. Although insulin resistance measured by HOMA was not significantly different between groups in the present study, HOMA values within each group were very variable. The negative correlation between HOMA and LDL FCR in the current study suggests that insulin resistance, which may down-regulate LDL receptors, may contribute to the observed decrease in LDL FCR.

A consequence of a decrease in LDL FCR is an increase in LDL apoB RT. Recently it has been shown that LDL apoB RT, measured using stable isotope techniques is closely positively related to surrogate markers of LDL apoB oxidation in healthy subjects and patients with familiar defective apoB-100 [33]. In the present study, oxidized LDL was not different between groups and did not correlate with LDL FCR. This may be due to the different methods used for measuring LDL apoB oxidation. The previous study measured the oxidation of LDL apoB proline and arginine residues to

γ -glutamyl semialdehyde, whereas in the current study, LDL oxidation was measured by an ELISA with an antibody to malondialdehyde-LDL.

In conclusion, our data suggest that HIV-infected subjects not taking antiretroviral drugs have a reduced LDL apoB FCR and consequently an increased LDL RT. The same abnormalities were also found in patients treated with either a PI- or NNRTI-containing HAART regimen, with more marked changes in LDL kinetics in the patients treated with regimens containing the PI RTV. The observed association of LDL apoB FCR with limb fat suggests a common mechanism for lipotrophy and abnormal LDL metabolism. The latter may be due to down-regulation of the LDL receptor or LDL-related receptor, a decrease in hepatic lipase or a combination of all these. Insulin resistance may also be a contributing factor. The increased LDL RT may lead to an increased risk of atherogenesis thus contributing to the increased risk for cardiovascular disease reported in patients taking antiretroviral therapy [34].

Acknowledgements

This research was supported by a grant from the British Heart Foundation (PG/2001/153) and the Wellcome Trust (064571). We are grateful to Premila Croos and Ben Wheeler for their technical assistance, Robert Cramb for performing the insulin assays and Gerry Gilleran for helping to recruit patients.

References

- Carr A, Samaras K, Burton S, Law M, Freund J, Chisholm DJ & Cooper D. A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors. *AIDS* 1998; **12**:F51-F58.
- Carr A, Samaras K, Thorisdottir A, Kaufmann GR, Chisholm DJ & Cooper D. Diagnosis and prediction and natural course of HIV protease inhibitor (PI)-associated lipodystrophy, hyperlipidaemia and diabetes mellitus: a cohort study. *Lancet* 1999; **353**:2093-2099.
- Saint-Marc T, Partisani M, Poizot-Martin I, Rouviere O, Bruno F, Avellaneda R, Lang JM, Gastaut JA & Touraine JL. Fat distribution evaluated by computed tomography and metabolic abnormalities in patients undergoing antiretroviral therapy: preliminary results of the LIPOCO study. *AIDS* 2000; **14**:37-49.
- Behrens G, Dejam A & Schmidt H. Impaired glucose tolerance, beta cell function and lipid metabolism in HIV patients under treatment with protease inhibitors. *AIDS* 1999; **13**:F63-F70.
- Vigouroux C, Gharakhanian S, Salhi Y, Nguyen TH, Chevenne D, Capeau J & Rozenbaum W. Diabetes, insulin resistance and dyslipidaemia in lipodystrophic HIV-infected patients on highly active antiretroviral therapy (HAART). *Diabetes & Metabolism* 1999; **25**:225-232.
- Miller J, Carr A, Emery S, Law M, Mallal S, Baker D, Smith D, Kaldor J & Cooper DA. HIV lipodystrophy: prevalence, severity and correlates of risk in Australia. *HIV Medicine* 2003; **4**:293-301.
- Fontas E, van Leth F, Sabin CA, Friis-Moller N, Rickenbach M, d'Arminio Monforte A, Kirk O, Dupon M, Morfeldt L, Mateu S, Petoumenos K, El-Sadr W, de Wit S, Lundgren JD, Pradier C & Reiss P; D:A:D Study Group. Lipid profiles in HIV-infected patients receiving combination antiretroviral therapy: are different antiretroviral drugs associated with different lipid profiles? *Journal of Infectious Diseases* 2004; **189**:1056-1074.
- Shahmanesh M, Das S, Stolinski M, Shojae-Moradie F, Jackson NC, Jefferson W, Cramb R & Umpleby AM. Antiretroviral treatment reduces VLDL and IDL apolipoprotein B fractional catabolic rate in HIV infected patients with mild dyslipidaemia. *Journal of Clinical Endocrinology & Metabolism* 2005; **90**:755-760.
- Zangerle R, Sarletti M, Gallati H, Reibnegger G, Wachter H & Fuchs D. Decreased plasma concentrations of HDL cholesterol in HIV-infected individuals are associated with immune activation. *Journal of Acquired Immune Deficiency Syndromes* 1994; **7**:1149-1156.
- Pernerstorfer-Schoen H, Jilma B, Perschler A, Wichlas S, Schindler K, Schindl A, Rieger A, Wagner OF & Quehenberger P. Sex differences in HAART-associated dyslipidaemia. *AIDS* 2001; **15**:725-734.
- Grunfeld C, Pang M, Doerrler W, Shigenaga J, Jensen P & Feingold K. Lipids, lipoproteins, triglyceride clearance and cytokines in human immunodeficiency virus infection and the acquired immunodeficiency syndrome. *Journal of Clinical Endocrinology & Metabolism* 1992; **74**:1045-1052.
- van der Valk M, Kastelein JJ, Murphy RL, van Leth F, Katlama C, Horban A, Glesby M, Behrens G, Clotet B, Stellato RK, Molhuizen HO & Reiss P; Atlantic Study Team. Nevirapine-containing antiretroviral therapy in HIV-1 infected patients results in an anti-atherogenic lipid profile. *AIDS* 2001; **15**:2407-2414.
- Berthold HK, Parhofer KG, Ritter MM, Addo M, Wasmuth JC, Schliefer K, Spengler U, & Rockstroh JK. Influence of protease inhibitor therapy on lipoprotein metabolism. *Journal of Internal Medicine* 1999; **246**:567-575.
- Negredo E, Ribalta J, Paredes R, Ferrer R, Sirena G, Ruiz L, Salazar J, Reiss P, Masana L & Clotet B. Reversal of atherogenic lipoprotein profile in HIV-1 infected patients with lipodystrophy after replacing protease inhibitors by nevirapine. *AIDS* 2002; **16**:1383-1389.
- Petit JM, Duong M, Duvillard L, Florentin E, Portier H, Lizard G, Brun JM, Gambert P & Verges B. LDL-receptors expression in HIV-infected patients: relations to antiretroviral therapy, hormonal status, and presence of lipodystrophy. *European Journal of Clinical Investigation* 2002; **32**:354-359.
- Christ ER, Cummings MH, Jackson N, Stolinski M, Lumb PJ, Wierzbicki AS, Sönksen PH, Russell-Jones DL & Umpleby AM. Effects of growth hormone replacement therapy on LDL apolipoprotein B100 kinetics in adult patients with growth hormone deficiency: a stable isotope study. *Journal of Clinical Endocrinology & Metabolism* 2004; **89**:1801-1807.
- Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF & Turner RC. Homeostasis model assessment: insulin resistance and cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; **28**:412-419.
- Cohn JS, Wagner DA, Cohn SD, Millar JS & Schaefer EJ. Measurement of very low density and low density lipoprotein apolipoprotein (Apo) B-100 and high density lipoprotein Apo A-I production in human subjects using deuterated leucine. Effect of fasting and feeding. *Journal of Clinical Investigation* 1990; **85**:804-811.
- Matthews DE, Schwartz HP, Yand RD, Motil KJ & Bier D. Relationship of plasma leucine and alpha-ketoisocaproate during L[1-13C] leucine infusion in man: a method for measuring human intracellular leucine tracer enrichment. *Metabolism* 1982; **31**:1105-1112.
- Pearson TC, Guthrie DL, Simpson J, Chinn S, Barosi G, Ferrant A, Lewis SM & Najean Y. Interpretation of measured red cell mass and plasma volume in adults: Expert Panel on Radionuclides of the International Council for Standardization in Haematology. *British Journal of Haematology* 1995; **89**:748-756.

21. Constans J, Pellegrin JL, Peuchant E, Dumon MF, Pellegrin I, Sergeant C, Simonoff M, Brossard G, Barbeau P & Fleury H. Plasma lipids in HIV-infected patients: a prospective study in 95 patients. *European Journal of Clinical Investigation* 1994; 24:416–412.
22. Mulligan K, Grunfeld C, Tai VW, Algren H, Pang M, Chernoff DN, Lo JC & Schambelan M. Hyperlipidemia and insulin resistance are induced by protease inhibitors independent of changes in body composition in patients with HIV infection. *Journal of Acquired Immune Deficiency Syndromes* 2000; 23:35–43.
23. Packard CJ, Demant T, Stewart JP, Bedford D, Caslake MJ, Schwertfeger G, Bedynek A, Shepherd J & Seidel D. Apolipoprotein B metabolism and the distribution of VLDL and LDL subfractions. *Journal of Lipid Research* 2000; 41:305–318.
24. Meddings JB & Dietschy JM. Regulation of plasma levels of low-density lipoprotein cholesterol: interpretation of data on low-density lipoprotein turnover in man. *Circulation* 1986; 74:805–814.
25. Millar JS, Maugeais C, Ikewaki K, Kolansky DM, Barrett PH, Budreck EC, Boston RC, Tada N, Mochizuki S, Defesche JC, Wilson JM & Rader DJ. Complete deficiency of the low-density lipoprotein receptor is associated with increased apolipoprotein B-100 production. *Arteriosclerosis, Thrombosis, & Vascular Biology* 2005; 25:560–565.
26. Schaefer JR, Scharnagl H, Baumstark MW, Schweer H, Zech LA, Seyberth H, Winkler K, Steinmetz A & Marz W. Homozygous familial defective apolipoprotein B-100. Enhanced removal of apolipoprotein E-containing VLDLs and decreased production of LDLs. *Arteriosclerosis, Thrombosis, & Vascular Biology* 1997; 17:348–353.
27. Bonfanti P, Gulisano C, Ricci E, Timillero L, Valsecchi L, Carradori S, Pusterla L, Fortuna P, Miccolis S, Magnani C, Gabbuti A, Parazzini F, Martinelli C, Faggion I, Landonio S, Quirino T & Vigevani G; Coordinamento Italiano Studio Allergia e Infezione da HIV (CISAI) Group. Risk factors for lipodystrophy in the CISAI cohort. *Biomedicine & Pharmacotherapy* 2003; 57:422–427.
28. Purnell JQ, Zambon A, Knopp RH, Pizzuti DJ, Achari R, Leonard JM, Locke C & Brunzell JD. Effect of ritonavir on lipids and post-heparin lipase activities in normal subjects. *AIDS* 2000; 14:51–57.
29. Zambon A, Bertocco S, Vitturi N, Polentarutti V, Vianello D & Crepaldi G. Relevance of hepatic lipase to the metabolism of triacylglycerol-rich lipoproteins. *Biochemical Society Transactions* 2003; 31:1074.
30. Carr A, Samaras K, Chilsholm D & Cooper D. Pathogenesis of HIV-1-protease inhibitor-associated peripheral lipodystrophy, hyperlipidaemias, and insulin resistance. *Lancet* 1998; 351:1881–1883.
31. Duvillard L, Florentin E, Lizard G, Petit JM, Galland F, Monier S, Gambert P & Verges B. Cell surface expression of LDL receptor is decreased in type 2 diabetic patients and is normalized by insulin therapy. *Diabetes Care* 2003; 26:1540–1544.
32. Duvillard L, Pont F, Florentin E, Lizard G, Gambert P & Verges B. Significant improvement of apolipoprotein B-containing lipoprotein metabolism by insulin treatment in patients with non-insulin dependent diabetes mellitus. *Diabetologia* 2000; 43:27–35.
33. Pietzsch J, Lattke P & Julius U. Oxidation of apolipoprotein B-100 in circulating LDL is related to LDL residence time. *In vivo* insights from stable-isotope studies. *Arteriosclerosis, Thrombosis, & Vascular Biology* 2000; 20:E63–7.
34. d'Arminio A, Sabin CA, Phillips AN, Reiss P, Weber R, Kirk O, El-Sadr W, De Wit S, Mateu S, Petoumenos K, Dabis F, Pradier C, Morfeldt L, Lundgren JD & Friis-Moller N. Writing Committee of the D:A:D: Study Group. Cardio- and cerebrovascular events in HIV-infected persons. *AIDS* 2004; 18:1811–1817.

Received 4 February 2005, accepted 13 June 2005