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

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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-naive (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-naive (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].
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 infection and antiretroviral treatment of 1.13C leucine (13C 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 CO2 gas. Data were analysed using the manufacturers’ software (Orchid Post Processor, Version 2.3c; Europa Scientific). The isotopic enrichment of α-KIC was determined by selected ion monitoring of the quinoxalinol-tert-butylidempethylsilyl 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 immunoturbimetric method (Immunocheck 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<sub>IR</sub>) [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 l<sup>13</sup>C leucine into the LDL particles was the steady state enrichment of α-KIC [19]. FSR was thus calculated as:

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

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 <i>et al.</i>’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 NNRTI group (1.49 ±0.29 mmol/l) and NNRTI groups (1.71 ±0.23 mmol/l). HDL cholesterol was lower ($P<0.05$) in the PI (1.12 ±0.08 mmol/l), NNRTI (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±SD) in HIV-negative patients (controls), TN patients and those on HAART antiretroviral-containing regimens containing PIs or NNRTIs

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

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

<table>
<thead>
<tr>
<th></th>
<th>Control (n=12)</th>
<th>TN (n=13)</th>
<th>PI (n=14)</th>
<th>NNRTI (n=24)</th>
<th>P value between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limb fat/LBM</td>
<td>0.21 (0.14–0.29)</td>
<td>0.15 (0.09–0.21)</td>
<td>0.09* (0.07–0.15)</td>
<td>0.08* (0.06–0.14)</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Trunk fat/LBM</td>
<td>0.16 (0.11–0.21)</td>
<td>0.16 (0.09–0.19)</td>
<td>0.14 (0.11–0.19)</td>
<td>0.14 (0.08–0.20)</td>
<td>NS</td>
</tr>
</tbody>
</table>

*P<0.05 versus control. DEXA, dual energy X-ray absorptiometry; IQR, interquartile range; LBM, lean body mass; NNRTI, non-nucleoside reverse 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

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

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

**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)

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

<table>
<thead>
<tr>
<th></th>
<th>RTV (n=8)</th>
<th>Non-RTV (n=7)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL ASR, mg/kg/day</td>
<td>4.56 (2.58–5.10)</td>
<td>6.87 (5.28–8.47)</td>
<td>0.009</td>
</tr>
<tr>
<td>LDL FCR, pools/day</td>
<td>0.15 (0.09–0.26)</td>
<td>0.27 (0.24–0.42)</td>
<td>0.005</td>
</tr>
<tr>
<td>LDL RT, days</td>
<td>6.86 (3.99–11.64)</td>
<td>3.76 (2.36–4.11)</td>
<td>0.005</td>
</tr>
<tr>
<td>LDL apoB pool size, g</td>
<td>2.06 (1.47–3.01)</td>
<td>1.83 (1.30–2.43)</td>
<td>NS</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/l</td>
<td>2.95 (1.88–4.03)</td>
<td>2.10 (1.58–3.06)</td>
<td>NS</td>
</tr>
<tr>
<td>LDL triglyceride, mmol/l</td>
<td>1.59 (1.46–1.72)</td>
<td>1.45 (1.33–1.64)</td>
<td>NS</td>
</tr>
<tr>
<td>LDL triglyceride/apoB ratio</td>
<td>0.22 (0.13–0.45)</td>
<td>0.16 (0.11–0.21)</td>
<td>NS</td>
</tr>
<tr>
<td>HOMA</td>
<td>1.40 (0.79–3.31)</td>
<td>1.66 (0.97–6.19)</td>
<td>NS</td>
</tr>
<tr>
<td>Oxidized LDL/apoB, U/g</td>
<td>90.9 (70.8–109.2)</td>
<td>104.3 (76.2–124.7)</td>
<td>NS</td>
</tr>
</tbody>
</table>

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/l). 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 lipoatrophy than other PIs [27], which may suggest that the decrease in LDL receptor may be related to lipoatrophy [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 familial 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

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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 lipostrophy 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].

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