Proinflammatory Markers, Insulin Sensitivity, and Cardiometabolic Risk Factors in Treated HIV Infection

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Treated HIV infection and HIV-lipoatrophy increases risk of cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM). Circulating inflammatory molecules may, in part, explain this increased risk. This study examined circulating inflammatory molecules in treated HIV infection in relation to insulin sensitivity, lipids total body, and intramyocellular fat, compared to insulin-resistant obesity (an index group at high risk of diabetes). Detailed metabolic phenotypes were measured in 20 treated HIV-infected men (with and without subcutaneous lipoatrophy) vs. 26 insulin-resistant obese men (IR-O, *n* = 26), including inflammatory molecules, insulin sensitivity, total body fat (TBF), visceral fat (visceral adipose tissue (VAT)), and intramyocellular lipid (IMCL). C-reactive protein (CRP) levels in treated HIV were similar to those in IR-O, despite lower TBF and greater insulin sensitivity in treated HIV. In HIV-lipoatrophy, CRP was higher than that found in IR-O. Adiponectin was similar between treated HIV and IR-O, but significantly lower in those with HIV-lipoatrophy. In treated HIV, subjects with higher CRP had significantly higher total cholesterol, VAT, and IMCL. In treated HIV, subjects with lower adiponectin had significantly lower HDL and higher triglycerides, glucose, VAT, and IMCL. In conclusion, a proinflammatory milieu equivalent to that of insulin-resistant obesity characterizes lean men with treated HIV infection, worse in those with subcutaneous lipoatrophy. These factors may contribute to the accelerated diabetogenesis and cardiac risk observed in treated HIV infection.

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INTRODUCTION

Treatment of HIV with highly active antiretroviral therapy (HAART) increases the risk for cardiovascular disease (CVD) (1,2) and type 2 diabetes mellitus (T2DM) (3,4). Recent studies have found the relative risk of myocardial infarction in HIV-infected patients to be almost doubled after adjustment for traditional risk factors (2). The large Data Collection on Adverse Events of Anti-HIV Drugs study recently estimated the risk of myocardial infarction following HAART-initiation at 16% per annum (1). Disorders of glucose metabolism have been reported at 23% after only 2 years of protease inhibitor therapy (3) and recent studies have reported a fourfold increase in T2DM risk in HIV-infected HAART recipients (4). HAART in HIV infection produces widespread metabolic complications, including hyperlipidemia, insulin resistance, lipodystrophy (LD) (peripheral fat wasting (lipoatrophy) with abdominal/visceral fat accumulation (lipohypertrophy)), and disturbances in adipokines (5–18). The role of circulating proinflammatory molecules within this accelerated paradigm of CVD and T2DM development requires further investigation.

There is increasing recognition that adipose tissue and disturbed adipocyte physiology contribute to the inflammatory milieu. C-reactive protein (CRP) levels are strongly associated with adipose tissue mass (19) and predict CVD (20,21) and T2DM (22–24). Low adiponectin is associated with visceral obesity and predicts CVD (25) and T2DM (26). Other proinflammatory adipocyte products such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) are also implicated.

In the most significant and possibly irreversible HAART metabolic complication, lipoatrophy, disturbed adipocyte function may exacerbate preexisting insulin resistance (6), hyperlipidemia and contributes to metabolic syndrome (27–29). In this patient group, we have previously shown that LD (lipoatrophy with abdominal lipohypertrophy) is associated with disturbed glucose metabolism, reduced insulin action, intramyocellular triglyceride accumulation (6), and low adiponectin (27). Disturbed secretion of other adipocyte-derived proinflammatory molecules in treated HIV infection may also contribute to systemic inflammation and its consequences.

This study examined markers of systemic inflammation in HIV-infected men receiving HAART with and without LD

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(lipoatrophy with abdominal lipohypertrophy), compared to fat-free mass–matched men with insulin-resistant obesity, a risk group predisposed to CVD and T2DM, with reference to cardiovascular risk factors and markers of disturbed insulin action (insulin sensitivity and intramyocellular lipids (IMCLs)).

METHODS AND PROCEDURES

All subjects were male. There were 20 HIV-infected HAART recipients ($n = 10$ with clinically evident LD (lipoatrophy with abdominal lipohypertrophy) (HIV+LD) and $n = 10$ without (HIV+)), recruited from a major urban teaching hospital HIV ambulatory care clinic. HIV-infected subjects with LD were matched to those without, for: known duration of HIV infection (HIV+LD: 9.9 ± 1 years vs. HIV+: 7.7 ± 1.5 years, $P = 0.24$), CD4 count ($\times 10^5$/l) (HIV+LD: 493 ± 88 vs. HIV+: 392 ± 102, $P = 0.46$) and HIV viral load (log copies/ml plasma) (HIV+LD: 3.3 ± 0.3 vs. HIV+: 3.2 ± 0.3, $P = 0.94$). There were differences in HAART drug use between the HIV groups: all subjects with HIV+LD had current use of protease inhibitors (vs. none in HIV+); there was roughly equivalent use of nucleoside reverse transcriptase inhibitors: HIV+LD: lamivudine ($n = 8$), stavudine ($n = 5$), zidovudine ($n = 3$), abacavir ($n = 1$), didanosine ($n = 1$); HIV+: lamivudine ($n = 6$), stavudine ($n = 2$), zidovudine ($n = 3$), didanosine ($n = 1$). There were differences in nonnucleoside reverse transcriptase inhibitor use: HIV+LD: efavirenz ($n = 1$); HIV+: nevirapine ($n = 5$). The presence of peripheral lipoatrophy was determined by clinical examination, as described (3).

The control group consisted of $n = 26$ HIV-negative insulin-resistant and obese men (IR-O). The IR-O group consisted of a subgroup of obese, sedentary but otherwise healthy men recruited for an exercise metabolic study; controls were matched to the HIV-infected subjects for age and fat-free mass. Insulin action in the obese control group was determined by glucose infusion rate under hyperinsulinemic conditions, adjusted for fat-free mass, using a median infusion rate of 37.1 µmol/kg fat-free mass·min, as we have previously published (30). A single investigator performed matching of subjects after determination of study hypotheses, statistical plan and prior to any analyses.

No subjects were receiving lipid lowering or diabetic medications. The St Vincent's Hospital Research Ethics Committee approved study protocols and all subjects gave written, informed consent.

Anthropometric measures included: weight (nearest 0.1 kg), height by stadiometer (nearest 0.01 m), waist and hip circumferences (nearest 0.01 m); waist was defined as the narrowest point between the lowest rib and the iliac crest, hip at the level of the greater trochanter.

Total body fat (TBF) and fat-free mass were measured by dual-energy X-ray absorptiometry (Lunar DXPL, Madison, WI). Regional fat was measured in limbs (arms plus legs), trunk and central abdominal fat, which was defined as a 9.8 cm vertical height window from the top of the iliac crest, extending laterally to the inner aspect of the costal margin.

Visceral adipose and subcutaneous adipose tissue (VAT and SAT, respectively) were measured with multiple slices using magnetic resonance imaging. 12 T-1 weighted axial slices (each 0.5 mm) were taken at 0.5 mm intervals between the levels of the L1-2 and L4-5 intervertebral discs. VAT (intraabdominal) and SAT were quantified using planimetric analysis (NIH Image 1.62; National institutes of Health, Bethesda, MD). Volumes of VAT and SAT were derived from interpolation between scanned slices.

IMCL was determined using a 1.5 Tesla medical magnetic resonance scanner (General Electric, Milwaukee, WI) after an 8-h overnight fast, having avoided strenuous physical activity in the preceding 5 days. An extremity coil was used to scan the right lower leg in each subject. A 2.0 × 2.0 × 2.0 cm voxel was positioned within the soleus mUscle, with acquisition of spectra by point-resolved spectroscopy sequence, echo time 135 ms, repetition time 1,500 ms.

Insulin-stimulated glucose disposal was measured using the euglycemic hyperinsulinemic clamp, following a 10-h overnight fast. Bilateral forearm vein cannulation was undertaken: one retrograde with arterialization for blood collection, the other for infusion of insulin and glucose. Insulin was infused at 2 mU·min^{-1}·kg/total body mass for 150 min, to suppress hepatic glucose production and reflect predominantly muscle insulin-stimulated glucose disposal, as previously described (6). Plasma glucose was measured at 10-min intervals with adjustment of the glucose infusion rate to maintain a plasma glucose level of 5.0 mmol/l (YSI 2300 StatPlus; YSI, Yellow Springs, OH). Whole body insulin sensitivity was measured by the glucose infusion rate at steady state, calculated from the last 40 min of the clamp and expressed per fat-free mass from the dual-energy X-ray absorptiometry measurement.

All assays samples were collected after an overnight fast of 12 h. Biochemical analyses: insulin, leptin, adiponectin by radioimmunoassay (Linco Research, St Charles, MO); CRP by a highly sensitive assay, using a Dade Behring Dimension RxL Chemistry Analyzer with reagents and calibrators supplied by Dade Behring Diagnostics (Sydney, New South Wales, Australia) (31); IL-6 and TNF-α by immunoassay by R&D Systems (Minneapolis, MN).

Statistical analyses

Data were analyzed using StatView 4.55 (SAS Institute, Cary, NC). Results were expressed as mean ± s.e. Group comparisons were made using ANOVA, with Fisher's protected least significant difference. Multiple regression analyses included highly related variables. Models were constructed to determine whether the strong relationships between insulin sensitivity and the variables of adipokines, inflammatory factors were exclusive of the close relationships with body and visceral fat.

RESULTS

Insulin-resistant obese HIV-negative controls (IR-O) had a higher BMI than HIV-infected subjects though, importantly fat-free mass was similar between all groups (**Table 1**).

Inflammatory markers and adipokines

CRP, adiponectin, TNF-α, and IL-6 in HIV-infected HAART recipients were similar to that found in insulin-resistant obesity (**Table 1**). Leptin was significantly lower in HIV-infected HAART recipients (**Table 1**).

The presence of LD in HIV-infected HAART recipients (HIV+LD) was associated with significantly higher CRP and lower adiponectin and leptin compared to insulin-resistant obesity (**Table 1**, **Figure 1**). In HIV+LD, adiponectin was also significantly lower than that found in treated HIV without LD (**Table 1**, **Figure 1**). There were no relationships between CRP, adiponectin, TNF-α and IL-6 with CD4 count or viral load (data not shown).

Cardiovascular risk factors, insulin action and IMCL

Overall, HIV-infected HAART recipients had similar cardiovascular risk factors to insulin-resistant obesity, for fasting total cholesterol, triglycerides, apolipoprotein B, and glucose (**Table 1**). HIV-HAART without LD was associated with higher HDL and lower fasting glucose than insulin-resistant obesity (**Table 1**), whereas in HIV+LD, all lipid parameters were similar to insulin-resistant obesity (**Table 1**).

HIV-infected HAART recipients were more insulin sensitive with less IMCL compared to insulin-resistant obesity (**Table 1**). The presence of LD in HIV-infected HAART recipients was associated with similar insulin sensitivity and IMCL to insulin-resistant obesity (**Table 1**).

Body fat partitioning and impact on CRP and adiponectin

Despite matching for fat-free mass, HIV-infected HAART recipients as a group had lower TBF, fat, and SAT but similar VAT and higher VAT:SAT, compared to insulin-resistant obese (Table 1). Similarly, HIV+LD had lower total body and limb fat, lower SAT, similar VAT and a higher VAT:SAT, compared to insulin-resistant obesity, although the differences were more marked (Table 1). In subgroup analyses, HIV-infected subjects without LD had significantly lower VAT compared to controls, though VAT:SAT was similar (Table 1).

Because treated HIV infection had both significantly lower %TBF and higher VAT:SAT than IR-O, analyses were undertaken of CRP and adiponectin adjusted for %TBF and VAT:SAT, to determine whether the differences observed in inflammatory markers were independent of differences in TBF. CRP remained higher in treated HIV infection vs. IR-O after adjustment for %TBF ($P = 0.001$) and VAT:SAT ($P = 0.04$). Adiponectin remained lower in treated HIV infection compared to IR-O after adjustment for %TBF ($P = 0.0001$) and VAT:SAT ($P = 0.007$).

Tertile analyses

Tertile analyses were performed in HIV-infected HAART recipients. Subjects in the highest CRP tertile had significantly higher total cholesterol, %body fat, VAT, IMCL, compared to the lowest CRP tertile (Table 2). Subjects in the lowest adiponectin tertile had significantly higher triglycerides, glucose, insulin, VAT and IMCL, and lower HDL cholesterol (Table 3). Insulin sensitivity (glucose infusion rate expressed per kg lean tissue mass) was not significantly different between low and high adiponectin tertiles (Table 3).

Multiple regression analyses

Models were constructed to evaluate the contribution of adipokines and adipose tissue mass to variance in insulin sensitivity, fasting glucose, cardiometabolic risk factors. Insulin

Table 1 Characteristics of treated HIV-infected subjects, compared to insulin-resistant obese subjects

	Insulin-resistant obesity ($N = 26$)	All treated HIV infection ($N = 20$)	Treated HIV, no LD ($N = 10$)	Treated HIV with LD ($N = 10$)
Age (years)	39.0 ± 1.2	43.2 ± 2.0	42.1 ± 2.7	44.4 ± 3.2
BMI (kg/m^2)	29.6 ± 0.76	24.6 ± 1.0***	25.4 ± 1.6††	23.9 ± 1.3‡‡‡
Inflammatory markers				
C-reactive protein (mg/l))	3.4 ± 0.3	4.6 ± 0.7	3.6 ± 0.5	5.5 ± 1.1‡
Adiponectin (μg/ml)	7.5 ± 0.8	7.2 ± 0.8	9.7 ± 0.9	4.7 ± 0.9‡‡‡,§
TNF-α (pg/ml)	0.08 ± 0.01	0.09 ± 0.03	0.12 ± 0.05	0.06 ± 0.02
IL-6 (pg/ml)	1.0 ± 0.3	1.2 ± 0.6	1.6 ± 1.2	0.87 ± 0.3
Leptin	6.2 ± 0.5	3.5 ± 0.8**	4.3 ± 1.4	2.2 ± 0.7‡‡
Cardiometabolic risk factors				
Total cholesterol (mmol/l)	4.7 ± 0.2	4.5 ± 0.2	4.3 ± 0.3	4.8 ± 0.3
HDL cholesterol (mmol/l)	0.9 ± 0.05	1.0 ± 0.05	1.1 ± 0.07†	0.9 ± 0.05
Triglycerides (mmol/l)	1.9 ± 0.4	2.2 ± 0.4	1.6 ± 0.5	2.9 ± 0.5
Glucose (mmol/l)	5.7 ± 0.1	5.4 ± 0.2	5.2 ± 0.2†	5.5 ± 0.2
Insulin (mU/l)	14.0 ± 1.4	12.8 ± 2.0	10.9 ± 3.5	14.7 ± 1.8
Glucose infusion rate (μmol/min/kg fat-free mass)	39.5 ± 2.9	51.8 ± 5.1*	66.8 ± 6.5†††	36.7 ± 4.2
Intramyocellular lipid (see below)	0.019 ± 0.001	0.016 ± 0.001*	0.012 ± 0.002†††	0.019 ± 0.002
Body composition				
Weight (kg)	90.5 ± 2.3	77.6 ± 2.9***	80.0 ± 4.4†	75.3 ± 4.0‡‡
Total body fat (kg)	29.81 ± 1.62	17.05 ± 2.25***	19.83 ± 3.64††	14.27 ± 2.54‡‡‡
% Total body fat	32.3 ± 1.3	20.8 ± 1.9***	23.4 ± 2.9††	18.1 ± 2.6‡‡‡
Limb fat (kg)	11.96 ± 0.71	6.62 ± 1.03***	9.01 ± 1.63†	4.24 ± 0.75‡‡‡
% Limb fat	30.0 ± 1.4	19.1 ± 2.1***	24.4 ± 2.9	13.7 ± 2.1‡‡‡
VAT	1.51 ± 0.10	1.25 ± 0.18	1.00 ± 0.19†	1.49 ± 0.29
SAT	2.79 ± 0.22	1.51 ± 0.24***	1.77 ± 0.43†	1.26 ± 0.22‡‡‡
VAT:SAT	0.59 ± 0.04	0.93 ± 0.13**	0.61 ± 0.08	1.25 ± 0.21‡‡‡

All data are expressed as mean ± s.e.m. Intramyocellular lipid was quantified as a percentage of the water resonance peak area (see Methods and Procedures).
HDL, high-density lipoprotein; IL-6, interleukin-6; SAT, subcutaneous adipose tissue; TNF-α, tumor necrosis factor-α; VAT, visceral adipose tissue.
Control vs. HIV-infected: *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$. Control vs. HIV, no LD: †$P < 0.05$, ††$P < 0.01$, †††$P < 0.005$. Control vs. HIV+LD: ‡$P < 0.05$, ‡‡$P < 0.01$, ‡‡‡$P < 0.005$. HIV no LD vs. HIV+LD: §$P < 0.005$.

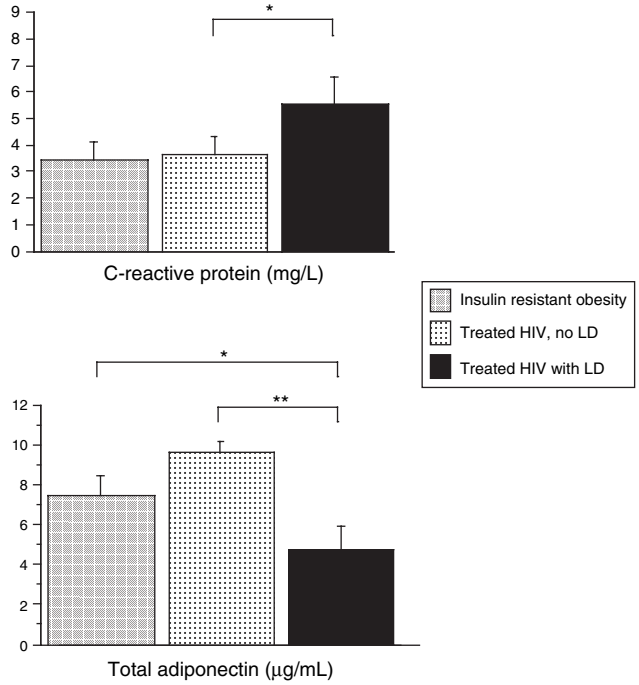

Figure 1 C-reactive protein and adiponectin in treated HIV infection with- and without lipodystrophy, compared to insulin-resistant obesity. $^{*}P < 0.05$, $^{**}P < 0.005$.

Table 2 C-reactive protein is associated with higher total cholesterol, total body fat, and intramyocellular lipid in treated HIV infection

	Top tertile (5.85–13.1 mg/l) *n* = 7	Low tertile (1.92–2.47 mg/l) *n* = 7	*P*
C-reactive protein (mg/l)	7.93 ± 1.00	2.21 ± 0.15	0.0001
Total cholesterol (mmol/l)	5.2 ± 0.38	3.9 ± 0.21	0.01
Triglycerides (mmol/l)	3.1 ± 0.9	1.3 ± 0.3	0.08
HDL cholesterol (mmol/l)	0.96 ± 0.09	0.93 ± 0.12	0.85
Apolipoprotein B (mmol/l)	1.14 ± 0.11	0.89 ± 0.06	0.07
Total body fat (%)	26.9 ± 4.3	15.2 ± 1.1	0.02
Limb fat (%)	24.4 ± 5.1	14.9 ± 1.6	0.10
VAT	1.96 ± 0.29	0.62 ± 0.07	0.0008
VAT:SAT	1.27 ± 0.32	0.66 ± 0.06	0.08
Soleus IMCL[a]	0.019 ± 0.002	0.011 ± 0.001	0.004
Glucose (mmol/l)	5.7 ± 0.27	5.5 ± 0.24	0.74
Insulin (mU/l)	16.2 ± 4.4	11.1 ± 2.6	0.36
Glucose infusion rate[b]	42.3 ± 10.1	55.0 ± 7.1	0.33
Adiponectin (µg/ml)	5.6 ± 1.3	8.5 ± 1.6	0.18
IL-6 (pg/ml)	2.7 ± 1.7	0.40 ± 0.3	0.21
TNF-α (pg/ml)	0.09 ± 0.05	0.03 ± 0.01	0.23

HDL, high-density lipoprotein; IL-6, interleukin-6; IMCL, intramyocellular lipid; SAT, subcutaneous adipose tissue; TNF-α, tumor necrosis factor-α; VAT, visceral adipose tissue.
[a]Intramyocellular lipid was quantified as a percentage of the water resonance peak area (see Methods and Procedures). [b]µmol/min/kg fat-free mass.

action was best predicted by a model including adiponectin and TNF-α, explaining 62% of the variance ($P = 0.0004$); addition of IMCL increased the predictive value of the model to 75% ($P < 0.0001$); addition of IL-6, CRP, %TBF or VAT did not improve the model; removal of TNF-α weakened the model by 9%. Fasting glucose was not predicted by a regression model containing adiponectin, CRP, TNF-α, and IL-6. Total cholesterol was predicted by a model containing triglyceride, visceral fat, CRP, and IL-6, explaining 61% of the variance ($P = 0.005$); addition of adiponectin or TNF-α did not strengthen the model. For HDL cholesterol, a model containing adiponectin, IL-6 and CRP explained 64% of its variance ($P = 0.001$); addition of visceral fat and insulin sensitivity did not improve the model. For triglycerides, 63% of the variance was explained by a model containing visceral fat, IL-6, CRP, and adiponectin ($P = 0.003$). For CRP, 62% of its variance was explained by a model containing visceral fat, total cholesterol, triglycerides, and IL-6 ($P = 0.004$); adding adiponectin, TNF-α, %TBF, or insulin sensitivity did not strengthen the model. For hypoadiponectinemia, 39% of the variance was explained by a model containing insulin sensitivity and CRP ($P = 0.0001$); addition of TNF-α, IL-6 or any measure of body lipid store (%TBF, VAT, or IMCL) did not strengthen the model.

Table 3 Low total adiponectin is associated with higher total cholesterol, total body fat, and intramyocellular lipid in treated HIV infection

	Top tertile (5.85–13.1 mg/l) *n* = 7	Low tertile (1.92–2.47 mg/l) *n* = 7	*P*
Adiponectin (µg/ml)	11.3 ± 0.77	3.5 ± 0.71	0.0001
Total cholesterol (mmol/l)	4.3 ± 0.4	4.9 ± 0.4	0.29
Triglycerides (mmol/l)	1.0 ± 0.2	2.9 ± 0.7	0.03
HDL cholesterol (mmol/l)	1.11 ± 0.1	0.84 ± 0.07	0.03
Apolipoprotein B (mmol/l)	0.87 ± 0.06	1.15 ± 0.10	0.03
Total body fat (%)	18.8 ± 2.6	17.1 ± 1.7	0.58
Limb fat (%)	20.7 ± 3.3	13.1 ± 1.8	0.06
VAT	0.64 ± 0.09	1.52 ± 0.30	0.02
VAT:SAT	0.52 ± 0.04	1.33 ± 0.29	0.02
Soleus IMCL	0.010 ± 0.001	0.020 ± 0.002	0.0003
Glucose (mmol/l)	5.05 ± 0.04	5.96 ± 0.13	<0.0001
Insulin (units)	8.4 ± 1.0	15.7 ± 2.5	0.03
Glucose infusion rate	42.3 ± 10.1	55.0 ± 7.1	0.33
C-reactive protein (mg/l)	3.2 ± 0.5	6.1 ± 1.6	0.11
IL-6 (pg/ml)	0.31 ± 0.17	10.2 ± 0.34	0.09
TNF-α (pg/ml)	0.11 ± 0.06	0.05 ± 0.02	0.39

Intramyocellular lipid was quantified as a percentage of the water resonance peak area (see Methods and Procedures).
HDL, high-density lipoprotein; IL-6, interleukin-6; SAT, subcutaneous adipose tissue; TNF-α, tumor necrosis factor-α; VAT, visceral adipose tissue.

DISCUSSION

This study examined adipokines involved in inflammation in relation to insulin sensitivity, lipid partitioning and cardiovascular risk factors in HIV-infected HAART recipients with and without LD, compared to insulin-resistant obesity, as an index group at high risk of both T2DM and CVD. We found that subjects with treated HIV infection had an inflammatory profile

equivalent to that found in insulin-resistant obesity, despite lower body fat. HIV-infected subjects with LD had evidence of greater systemic inflammation than that seen in insulin-resistant obesity with higher CRP and more pronounced hypoadiponectinemia. Regression models indicated insulin sensitivity was best predicted by adiponectin and TNF-α, but not body fat partitioning. In contrast, adipokine levels and body fat partitioning contributed to the variance in fasting lipids.

Proinflammatory adipokine disturbances in treated HIV infection may affect the generation of atheroma and impaired glucose metabolism, contributing to the increased cardiovascular risk observed in prospective studies. The regulatory roles of these circulating factors in the generation of atheroma and glucose disturbances under usual conditions are the focus of intense investigation. In the circumstances of treated HIV infection, less is understood. Prospective observational studies have documented higher rates of both myocardial infarction and T2DM. The most recent data from the Data Collection on Adverse Events of Anti-HIV Drugs study of over 23,000 HIV-infected patients, report a 16% increased risk of myocardial infarction per year of exposure to one HAART drug class, protease inhibitors (1). This effect was independent of serum lipids (1); suggesting risk may be mediated by other mechanisms. A further study indicated a 75% increase in the risk of myocardial infarction in treated HIV infection, compared to a noninfected cohort, after adjustment for age, gender, race, hypertension, diabetes, and dyslipidemia (2). Higher risk for T2DM in HIV-infected HAART recipients have been reported: we found prevalence rates of 7 and 16% for T2DM and impaired glucose tolerance, respectively, after 2 years of protease inhibitor therapy in a relatively young, nonobese cohort (3). More recently, Brown *et al.* reported a 10% incidence of T2DM in HIV-infected HAART recipients >4 years, compared to 3% in age- and BMI-matched HIV-negative controls (4). HAART-induced disturbances in lipids, insulin resistance and inflammation obviously contribute to the development of atheroma and diabetes in HIV infection treated with HAART.

CRP is recognized to predict coronary disease and is a marker of low-grade systemic inflammation (32,33). Adipose tissue and the liver produce CRP; circulating levels are predicted by adiposity (19). Our study found CRP levels were significantly higher in lipodystrophic subjects, despite much lower levels of TBF, suggestive of dysregulated adipocyte and hepatic hypersecretion of this inflammatory marker. However, the increased visceral obesity found in lipodystrophic subjects may contribute to higher CRP found. Our results concur with other studies finding higher CRP levels in HIV-infected women, which were associated with central adiposity (estimated by waist-to-hip ratio) (34).

This study also found adiponectin in HIV-infected HAART recipients was similar to that found in insulin-resistant obesity; hypoadiponectinemia was more pronounced if LD was present. Adiponectin is an antiatherogenic, antidiabetic adipokine. Hypoadiponectinemia is found in obesity, insulin resistance, metabolic syndrome, and diabetes and identifies individuals at higher risk of myocardial infarction (25). We have previously reported that the inverse relationship between adiponectin and insulin action is independent of circulating, visceral and IMCL (35), suggesting nonlipid pathways may be responsible for the insulin-sensitizing effects of adiponectin. In this study, regression models of insulin sensitivity suggested primacy for adiponectin, as models were not improved by the inclusion of total body or visceral fat or other inflammatory molecules. Hypoadiponectinemia has previously been reported in treated HIV-infected HAART recipients with LD (36–39) and metabolic syndrome (27). Our results concur with prior reports of low adiponectin following adjustment for fat mass (36). The synthesis of published studies to date, suggest HIV-infected HAART recipients have disturbances in adipocyte physiology that result in reduced adiponectin secretion. Our study suggests the disturbance is similar to that found in insulin-resistant obesity, but more profound in the presence of LD.

TNF-α and IL-6 are elevated in obesity, insulin resistance, and diabetes (40,41). In this study, TNF-α did modestly strengthen multivariate models predicting insulin sensitivity. Activation of the TNF system in lipodystrophic HIV-infected HAART recipients has been reported, with increased soluble TNF-α receptor 2 levels (42). Elegant studies of SAT explants in culture have demonstrated increased TNF-α and soluble TNF-α receptor 2 secretion in lipodystrophic HIV-infected HAART recipients (43). TNF-α and IL-6 mRNA expression are increased in peripheral adipocytes from lipodystrophic HIV-infected HAART recipients with expression levels relating to macrophage infiltration (43). IL-6 increases secretion of CRP from both adipocytes and the liver. It is of interest that our multivariate models of adipokines found IL-6 predicted the traditional cardiovascular risk factors of total cholesterol, HDL cholesterol, triglycerides, and CRP.

One of the strengths of this study is matching between the treated HIV groups and control group for fat-free mass, using measures of body composition by dual-energy X-ray absorptiometry. Further strengths include detailed *in vivo* measures including insulin action by hyperinsulinemic euglycemic clamp and IMCL by magnetic resonance spectroscopy. Our control group was selected for insulin resistance and central obesity; insulin action (glucose infusion rate adjusted for fat-free mass—GIR) was similar to that found in other studies of overweight men (35). This GIR is less than that we have found in our prior studies of lean healthy controls who show a wide variation in insulin action, with mean GIR 72 μmol/min/kg fat-free mass (range 52–95 μmol/min/kg fat-free mass) (44). Limitations of this study include the cross-sectional nature of the study and the relatively small numbers of subjects studied, which may have reduced the power of the study to detect differences. Detailed metabolic studies such as we have performed with hyperinsulinemic clamp, spectroscopy, and magnetic resonance are not feasible in larger cohorts however. Simpler surrogate measures (where possible) introduce error and imprecision. A further group of insulin-sensitive fat-free matched men would have provided additional information of inflammatory markers in health for comparison, but was not possible in this study. The cross-sectional nature of the study

does not allow conclusions regarding whether the disturbances noted are because of primary drug effects or secondary to drug-induced complications. Our results may also have been confounded by nonmeasured factors. Prospective studies would assist in this regard. This study was also limited to white men, reflecting the demographics of HIV infection in Australia. Study findings can thus not be extrapolated to women or other ethnic groups. Further, the composition of HAART regimens have changed substantially in recent times and new drug classes (entry inhibitors and integrase inhibitors) currently being evaluated, may alter the face of HAART and its complications. Little or no metabolic data are available for these new classes.

The findings of elevated CRP and hypoadiponectinemia are suggestive of functional impairments in the synthesis or secretion of adipocyte-derived products in HIV-associated LD. In concert with the associations of higher lipids and lipid partitioning (doubled IMCL), these could be expected to promote atherogenesis and contribute to the increased cardiovascular risk found in treated HIV infection. The pathogenetic mechanisms are unclear; "lipotoxic" effects of circulating lipid, visceral obesity, ectopic fat deposition, and IMCL, are only part of the disturbed physiology in association with insulin resistance. Adipokines and inflammatory markers may exert independent effects on atherogenesis, or act through other intermediaries. It has been hypothesized that the reconstitution of the immune response with the instigation of HAART in HIV infection may contribute to the disturbances in inflammatory markers (43). This interesting hypothesis may be extended to encompass the potential adverse effects of adipocyte-derived molecules on atherogenesis. HIV-associated LD may induce a proinflammatory acute-phase responsiveness, marked by higher CRP and lower adiponectin in the setting of higher lipids. In the already metabolically adverse setting of visceral adiposity and insulin resistance, these may accelerate adverse cardiometabolic risk. Prospective studies of HIV-infected patients commencing HAART will assist our understanding of the inflammatory and adipokine disturbances observed in this study and, importantly, give insights into the time course and onset of inflammation, dyslipidemia, insulin resistance, and body fat partitioning. Furthermore, understanding the pathophysiology of treated HIV infection and LD would shed light on the inflammatory changes described in atheroma, T2DM, and obesity.

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DISCLOSURE

The authors declared no conflict of interest.

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