Increased Cardiovascular Disease Risk Indices in HIV-Infected Women

Sara E. Dolan, MSN, NP, Colleen Hadigan, MD, MPH,* Kathleen M. Killilea, BS,* Meghan P. Sullivan, MS,* Linda Hemphill, MD,‡ Robert S. Lees, MD,‡ David Schoenfeld, PhD,† and Steven Grinspoon, MD**

Summary: Little is known regarding cardiovascular disease risk indices in HIV-infected women. This study investigated cardiovascular disease risk indices in 100 consecutively recruited HIV-infected women and 75 healthy female control subjects. Subjects were recruited from hospital- and community-based health care providers. C-reactive protein (CRP), interleukin-6 (IL-6), adiponectin, lipid, and glucose levels were the main outcome measures. CT scan, dual-energy x-ray absorptiometry (DXA), and anthropometry were used to assess body composition. Although similar in age, weight, and racial composition, HIV-infected women demonstrated higher CRP (4.6 ± 0.7 vs. 2.3 ± 0.4 mg/L, $P = 0.007$), IL-6 (2.7 ± 0.2 vs. 1.8 ± 0.1 pg/mL, $P = 0.02$), triglyceride (1.84 ± 0.21 vs. 0.85 ± 0.05 mM, $P = 0.0002$), 2-hour glucose after oral glucose challenge (6.88 ± 0.22 vs. 5.72 ± 0.17 mM, $P = 0.0003$), and fasting insulin (81 ± 8 vs. 45 ± 2 pM, $P = 0.0002$) and lower high-density lipoprotein cholesterol (1.17 ± 0.03 vs. 1.45 ± 0.05 mM, $P < 0.0001$) and adiponectin (5.4 ± 0.3 vs. 7.6 ± 0.5 mg/L, $P = 0.0001$) levels compared with the control population. HIV-infected women had more abdominal visceral fat and less extremity fat by CT and DXA scan and demonstrated a higher waist-to-hip ratio (WHR) than the control population. Within the HIV group, CRP and other indices were significantly related to body composition in stepwise regression models. Among all subjects, WHR, but not HIV status, was significantly related to CRP and other cardiovascular disease risk indices. HIV-infected women demonstrate significantly increased risk factors for cardiovascular disease in association with abnormal fat distribution.

Received for publication September 24, 2004; accepted January 12, 2005.

From the *Program in Nutritional Metabolism and †Biostatics Unit, General Clinical Research Center (GCRC), Massachusetts General Hospital and Harvard Medical School, Boston, MA; and ‡Boston Heart Foundation and the Harvard–MIT Division of Health Sciences and Technology, Cambridge, MA.

Supported by NIH DK 59535, 3-M01-RR 01066-25S1, the Harvard AIDS Center for AIDS Research (CFAR, NIH P30 AI42851), and the Mary Fisher Clinical AIDS Research and Education (CARE) Fund. Dr. Grinspoon has received unrestricted research grant funding from Serono, GlaxoSmith Kline, Abbott, and Boeringer Ingelheim and has served as a consultant for Bristol-Myers Squibb and Thera-Technologies, Inc. He has received grant support from Gilead Pharmaceuticals and Serono, Inc. No funding source played a role in the writing, review, or approval of the manuscript.

Reprints: Steven Grinspoon, Neuroendocrine Unit and Program in Nutritional Metabolism, Massachusetts General Hospital, 55 Fruit Street, LON207, Boston, MA 02114 (e-mail: sgrinspoon@partners.org).

Key Words: HIV, women, cardiovascular disease, C-reactive protein, waist-to-hip ratio

(*J Acquir Immune Defic Syndr* 2005;39:44–54)

Metabolic and body composition abnormalities, including insulin resistance, dyslipidemia, subcutaneous fat loss, and increased visceral adiposity, have been shown to occur frequently among HIV-infected patients in the era of potent antiretroviral therapy. Among patients treated with potent antiretroviral therapy, recent data demonstrate increased cardiovascular events,[1] although the extent of increased cardiovascular disease (CVD) risk and relationship to antiretroviral therapy remains controversial.[2] Prior studies have shown increased risk and metabolic abnormalities, particularly among patients with fat redistribution, suggesting that changes in fat distribution may be an important marker for CVD risk in this population.[3,4] In these studies, only limited information is available comparing HIV-infected men and women, and few data are available on the relationship of coronary heart disease to antiretroviral therapy in women. In the data collection on adverse events of anti-HIV drugs (DAD) study of adverse events of antiretroviral medications, 76% of patients were male. The adjusted relative rate of myocardial infarction was 1.26/year of combination antiretroviral exposure among all subjects, and male gender was an independent risk factor.[1]

In contrast, few prior studies have been performed to evaluate metabolic and body composition risk factors exclusively in the growing population of HIV-infected women.[5–7] In a study based on insurance claims to Medi-Cal from 1994–2000, Currier et al[8] demonstrated an increased relative risk of coronary heart disease of 1.67 (95% CI: 1.41, 1.97) vs. non-HIV-infected women in younger (aged 35–44 years) but not older female HIV-infected subjects. With the advent of highly active antiretroviral therapy, deaths due to HIV infection decreased significantly between 1994 and 2000. Cardiac disease is the 2nd leading cause of death for young African American HIV-infected patients and the 3rd leading cause among young Hispanic and white HIV-infected women. In contrast, HIV is the 10th leading cause of death for white HIV-infected women and 3rd and 4th leading causes of death, respectively, for young African American and Hispanic HIV-infected women.[9]

Recent evidence suggests that C-reactive protein (CRP),[10] interleukin-6 (IL-6),[11] and adiponectin[12] may independently contribute to CVD risk in non-HIV-infected patients. To our

knowledge, prior studies have not simultaneously assessed hormonal factors, nor measured newer inflammatory markers of increased CVD risk in HIV-infected women. In this study, we evaluated metabolic and body composition indices in HIV-infected women in comparison to a control group of simultaneously recruited healthy women similar in age, weight, and race. In addition to increased traditional risk factors (high triglycerides, low high-density lipoprotein [HDL] cholesterol, and impaired glucose tolerance), our data show increased CRP and IL-6 and reduced adiponectin. We demonstrate the degree to which fat redistribution and CVD markers are associated in HIV-infected women and demonstrate the need to modify CVD risk factors, even among relatively young HIV-infected women.

METHODS

Subjects

A planned sample of 100 HIV-infected women and 75 HIV-negative female controls was recruited through community advertisement and primary care provider referral. The HIV-infected subjects were recruited from March 2000 to July 2002 and the control subjects from October 2000 to October 2003. Consecutive HIV-infected and control subjects between 18 and 60 years of age with a body mass index (BMI) >20 kg/m^2 were enrolled without regard to fat distribution based on the following eligibility criteria. Excluded from participation were subjects who had used megestrol acetate, ketoconazole, antidiabetic agents, steroids, growth hormone, oral contraception pills, medroxyprogesterone acetate, testosterone, or any anabolic agent within 3 months of the study; who engaged in substance abuse; were pregnant or had breast-fed in the past year; who had a history of oophorectomy; or who had had an acute infection within 3 months of the study.

HIV-infected subjects recruited for this study were similar in age, race and geographical representation to HIV-infected subjects in the Commonwealth of Massachusetts and nationally.[13] Of the 100 HIV-infected women in the study, 40% were recruited from hospital-based health care providers, 20% from community-based providers, 12% from community agencies, 12% from community advertising, 11% from patient referral, and 5% from advertising at AIDS housing complexes. Subjects were recruited from a diverse geographic background within the state—Boston (40%), Metro-west (31%), and Western (12%) regions of the state—similar to the geographic representation of HIV-infected patients in Massachusetts based on analysis of zip codes.[14]

For the HIV-infected subjects, duration of HIV and antiretroviral medication use was obtained via patient interview. Patients were asked about all current antiretroviral medications and dates of all prior antiretroviral medication use, including the start and stop dates for each medication. Current smoking history as well as total duration and amount of smoking (pack-years) were determined by questionnaire. Subjects who had changed or initiated antiretroviral medication within 2 months of the study were excluded.

Control subjects met the same entrance criteria and enzyme-linked immunosorbent assay (ELISA) testing verified HIV-negative status. Control subjects were healthy, without known acute or chronic diseases, and were not receiving medication for diabetes, hypertension, or dyslipidemia.

Earlier studies suggest increased menstrual abnormalities in HIV-infected women.[15] HIV-infected and control subjects were characterized as eumenorrheic (normal menstrual function) or oligomenorrheic (<3 menstrual periods in the 3 months prior to study) to determine clinically significant menstrual dysfunction. Estradiol and gonadotropin levels were measured to assess menopausal status in the early follicular phase (within 10 days of initiation of menses) to avoid midcycle changes in hormone levels.

All subjects gave written informed consent. The study was approved by the Human Research Committee at the Massachusetts General Hospital and the Committee on the Use of Humans as Experimental Subjects at the Massachusetts Institute of Technology. Bone density data, but not metabolic data, were previously reported on a subset of the study population.[16]

Study Procedures

Eligible subjects were seen at the General Clinical Research Center at the Massachusetts Institute of Technology. All testing was performed following a 12-hour overnight fast. Insulin and glucose were measured at 0 and 120 minutes after a standard 75-g oral glucose challenge.

Biochemical Indices

CRP was measured by ELISA (DSL, Webster, TX) with intra-assay and interassay coefficients of variation (CVs) ranging from 1.7%–3.9% and 2.8%–5.1%, respectively. The sensitivity of the assay is 0.002 mg/L. IL-6 was measured by a high-sensitivity quantitative sandwich enzyme immunoassay technique (R&D Systems, Minneapolis, MN) with intra-assay and interassay CVs ranging from 6.9%–7.8% and 6.5%–9.6%, respectively. The sensitivity is 0.04 pg/mL. Tumor necrosis factor-α (TNF-α) was measured by enzyme immunoassay (Alpco Diagnostics, Windham, NH) with intra-assay and interassay CVs of ± 8.3 and ± 10.8% and a sensitivity of 4.8 pg/mL. Adiponectin was measured using a radioimmunoassay (LINCO Research, St. Charles, MO). Intra-assay and interassay CVs range from 1.78%–6.21% and 6.90%–9.25%, respectively. Insulin levels were measured in serum using a radioimmunoassay (Diagnostic Products Corp., Los Angeles, CA). Intra-assay and interassay CVs range from 3.1%–9.3% to 4.9%–10.0%, respectively. Low-density lipoprotein (LDL) cholesterol was measured directly (Genzyme Diagnostics, Cambridge, MA). Total cholesterol, HDL cholesterol, triglyceride, and glucose were measured using standard techniques.

Serum estradiol was measured by radioimmunoassay kit (Diagnostic Systems Laboratories, Webster, TX) with an intra-assay CV of 6.5%–8.9%. Serum luteinizing hormone was measured using a solid-phase immunoradiometric assay (Diagnostic Products Corp.) with an intra-assay CV of 1.0%–1.6%. Serum follicular stimulating hormone was measured using a solid-phase immunoradiometric assay (Diagnostic Products Corp.) with an intra-assay CV of 2.2%–3.8%.

Immune Function

CD4 count was determined by flow cytometry (Becton Dickinson Biosciences, San Jose, CA) and HIV viral load was determined by ultrasensitive assay (Amplicor HIV-1 Monitor Assay, Roche Molecular Systems, Branchburg, NJ) with limits of detection 50–75,000 RNA copies/mL.

Body Composition

Weight and anthropometric measurements were determined in the morning, prior to breakfast. Anthropometric measurements were obtained using an inelastic tape measure by the Bionutrition Staff of the Massachusetts Institute of Technology General Clinical Research Center. The waist-to-hip ratio (WHR) was calculated dividing the waist circumference measured at the iliac crest by the hip circumference measured at the horizontal level of maximum extension of the buttocks.[17] All measurements were obtained in triplicate, with the patient undressed, and then averaged, with variability of the WHR averaging 0.004 for the HIV-infected and 0.006 for the non-HIV-infected between repeated measurements on the same patient. To assess abdominal visceral and subcutaneous adipose tissue area (VAT and SAT, respectively), a cross-sectional abdominal CT scan at the level of the L-4 pedicle was performed. Scan parameters for each image were standardized (144 table height, 80 kV, 70 mA, 2 seconds, 0.25 cm × 4 slice thickness, 48 field of view (FOV). Fat attenuation coefficients were set at −50 to −250 Hounsfield Units (HU).[18] Total fat was measured by dual-energy x-ray absorptiometry (DXA) (Hologic, Waltham, MA) with a precision error of 3.0%.[19] Regional fat in the extremities was determined as previously described.[20]

Nutrition Evaluation

Participants received instructions and completed a 4-day food record prior to their baseline visit. These records were reviewed with each patient by a registered dietician and analyzed using a computerized nutrition software product (NDS Version 4.01 and 4.02-NDS-R, Regents of the University of Minnesota, Minneapolis, MN) to quantify total caloric, protein, carbohydrate, fat, cholesterol, fiber, and folate intake.

Statistical Analysis

Comparison of demographic variables was made by HIV status using Student *t* test for continuous variables and the χ^2 test for noncontinuous variables (Table 1). Differences between the HIV and control groups for biochemical and body composition endpoints were determined in multivariate regression analyses, controlling for smoking (pack-years), current smoking, hormone replacement therapy (HRT) use, and race (Table 2). Use of lipid-lowering medication was additionally controlled for in the comparisons of CRP and lipid parameters between the groups. Use of antihypertensive medication was controlled for in the comparison of systolic and diastolic blood pressure between the groups.

Within the HIV-positive group, separate stepwise regression models were constructed for each of the primary study endpoints (CRP, IL-6, adiponectin, cholesterol, LDL cholesterol, triglyceride, HDL cholesterol, fasting insulin, and 120-minute glucose) as dependent variables (Table 3A). Age,

TABLE 1. Demographic Characteristics

	HIV-Infected (n = 100)	HIV-Negative (n = 75)	*P*-Value
Demographics			
Age (y)	41 ± 1	40 ± 1	0.39
Race (caucasian:non-caucasian)	40:60	34:41	0.48
African American	36	27	
Hispanic	14	9	
Asian	1	3	
Native American	2	0	
Other	7	3	
BMI (kg/m^2)	26.1 ± 0.5	26.9 ± 0.5	0.27
Current smoking (%)	47%	27%	0.006
Smoking pack-years	9.4 ± 1.2	2.5 ± 0.6	<0.0001
% Hormone replacement therapy	7%	1%	0.08
Medication exposure/disease status			
% Current antiretrovirals	81%	NA	NA
% Current PI	44%	NA	NA
% Current lopinavir/ritonavir	8%	NA	NA
% Current indinavir	8%	NA	NA
% Current saquinavir (soft gel)	5%	NA	NA
% Current saquinavir (capsules)	1%	NA	NA
% Current nelfinavir	25%	NA	NA
% Current ritonavir	4%	NA	NA
% Prior PI	69%	NA	NA
Duration PI (mo)	21 ± 2	NA	NA
% Current NRTI	80%	NA	NA
% Current tenofovir	2%	NA	NA
% Current abacavir/ lamivudine/zidovudine	6%	NA	NA
% Current zidovudine/ lamivudine	25%	NA	NA
% Current ddI	14%	NA	NA
% Current abacavir	16%	NA	NA
% Current zidovudine	1%	NA	NA
% Current lamivudine	27%	NA	NA
% Current emtricitabine	1%	NA	NA
% Current stavudine	35%	NA	NA
% Prior NRTI	92%	NA	NA
Duration of NRTI (mo)	43 ± 4	NA	NA
% Current NNRTI	27%	NA	NA
% Current delaviridine	1%	NA	NA
% Current efavirenz	22%	NA	NA
% Current nevirapine	3%	NA	NA
% Prior NNTRI	50%	NA	NA
Duration NNRTI (mo)	8 ± 1	NA	NA
Log HIV viral load (RNA copies/mL)	1.9 (1.7, 3.7)	NA	NA
% Undetectable HIV viral load	46%	NA	NA
CD4 count (cells/mm^3)	407 ± 24	NA	NA

Comparison by Student's *t*-test for continuous variables and the χ^2 test for noncontinuous variables. PI indicates protease inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor. Results are mean ± SEM. For viral load, results are median (interquartile range).

TABLE 2. Biochemical, Body Composition, and Clinical Characteristics

	HIV Infected (n = 100)	HIV Negative (n = 75)	*P* Value*	Normal Range
Biochemical indices				
CRP (mg/L)	4.6 ± 0.7	2.3 ± 0.4	0.007	<3.0†
II-6 (pg/mL)	2.7 ± 0.2	1.8 ± 0.1	0.02	0.4–2.1
Adiponectin (mg/L)	5.4 ± 0.3	7.6 ± 0.5	0.001	NA
TNF α (pg/mL)	20.4 ± 4.5	33.6 ± 11.6	0.4	NA
Cholesterol (mmol/L) [mg/dL]	4.87 ± 0.13 [188 ± 5]	4.58 ± 0.10 [177 ± 4]	0.3	3.11–5.15 [120–199]
Triglycerides (mmol/L) [mg/dL]	1.84 ± 0.21 [163 ± 19]	0.85 ± 0.05 [75 ± 4]	0.0002	0.23–1.81 [20–160]
Direct LDL (mmol/L) [mg/dL]	2.87 ± 0.10 [111 ± 4]	2.72 ± 0.10 [105 ± 4]	0.53	<3.37 [<130]
HDL (mmol/L) [mg/dL]	1.17 ± 0.03 [45 ± 1]	1.45 ± 0.05 [56 ± 2]	<0.0001	0.91–2.2 [35–85]
Fasting glucose (mmol/L) [mg/dL]	4.66 ± 0.06 [84 ± 1.0]	4.55 ± 0.06 [82 ± 1.1]	0.2	3.89–6.61 [70–110]
2-hour glucose (mmol/L) [mg/dL]	6.88 ± 0.22 [124 ± 4]	5.72 ± 0.17 [103 ± 3]	0.0003	3.89–6.66 [70–120]
Fasting insulin (pmol/L) [μIU/mL]	81 ± 8 [11.7 ± 1.1]	45 ± 2 [6.5 ± 0.3]	0.0002	<104.2 [<15]
2-hour insulin (pmol/L) [μIU/mL]	496 ± 47 [71.4 ± 6.7]	267 ± 22 [38.4 ± 3.1]	<0.0001	<583.4 [<84]
% Normal glucose tolerance	78%	92%	0.03	NA
Body composition				
BMI (kg/m^2)	26.1 ± 0.5	26.9 ± 0.5	0.16	NA
Waist (cm)	92.0 ± 1.3	87.8 ± 1.3	0.15	NA
Hip (cm)	100.0 ± 1.2	105.6 ± 1.1	0.002	NA
Waist to hip ratio (WHR)	0.92 ± 0.01	0.83 ± 0.01	<0.0001	NA
Total fat (kg)	22.7 ± 0.9	26.0 ± 1.0	0.007	NA
Upper extremity fat (kg)	2.6 ± 0.1	3.0 ± 0.1	0.01	NA
Lower extremity fat (kg)	7.6 ± 0.4	10.5 ± 0.5	<0.0001	NA
SAT (cm^2)	244 ± 12	280 ± 14	0.05	NA
VAT (cm^2)	103 ± 6	71 ± 5	0.001	NA
VAT:SAT Ratio	0.49 ± 0.04	0.27 ± 0.02	<0.0001	NA
Hormonal function				
Estradiol (pmol/L) [pg/mL]	158 ± 15 [43 ± 4]	180 ± 18 [49 ± 5]	0.38	73.4–532.3 [20–145]
FSH (IU/L) [mIU/mL]	19.6 ± 3.1	15.8 ± 3.9	0.92	30–20
LH (IU/L) [mIU/mL]	7.8 ± 1.0	7.6 ± 1.2	0.42	2–15
% Eumenorrheic	68%	84%	0.03	
Dietary intake				
Total energy (kcal/day)	1960 ± 62	1818 ± 64	0.15	NA
Fat (g/day)	74 ± 3	67 ± 3	0.28	NA
Carbohydrates (g/day)	253 ± 9	228 ± 8	0.04	NA
Protein (g/day)	76 ± 3	74 ± 3	0.65	NA
Cholesterol (mg/day)	292 ± 16	249 ± 16	0.24	NA
Fiber (g/day)	13 ± 1	15 ± 1	0.9	NA
Folate (μg/day)	486 ± 36	484 ± 34	0.6	NA
Clinical parameters				
SBP (mmHg)	111 ± 1	108 ± 2	0.59	90–140
DBP (mmHg)	71 ± 1.0	68 ± 1.2	0.12	65–90

*Adjusted for current smoking, race, smoking pack-years and HRT (hormone replacement therapy) use. CRP, LDL cholesterol, total cholesterol, HDL, and triglycerides comparison also adjusted for use of lipid lowering medication. Systolic and diastolic blood pressure comparison also adjusted for use of antihypertensive medication.

†CRP >3.0 mg/L indicates high risk category.[22]

Results are mean ± SEM (SI units).

To convert to traditional units, divide by the following conversion factors (cholesterol, mmol/L to mg/dL, by 0.0259; triglyceride, mmol/L to mg/dL, by 0.0113; LDL, mmol/L to mg/dL, by 0.0259; HDL, mmol/L to mg/dL, by 0.0259; glucose, mmol/L to mg/dL, by 0.0555; insulin pmol/L to μIU/mL, by 6.945; estradiol, pmol/L to pg/mL, by 3.671; FSH, IU/L to mIU/mL, by 1.00; LH, IU/L to mIU/mL by 1.00). Serological data are missing from 10 HIV-infected and 3 control subjects.

SAT indicates subcutaneous adipose tissue; VAT, visceral adipose tissue.

BMI, use of HRT, current and total smoking pack-years, HIV viral load, CD4 count, total duration protease inhibitor use, total duration nucleoside reverse transcriptase inhibitor (NRTI) use, total duration nonnucleoside reverse transcriptase inhibitor (NNRTI) use, and WHR were tested for entry into each model at $P = 0.1$. Final models were constructed from the significant terms entering each model. Individual antiretroviral medications used by >10% of the subjects were tested in regression modeling.

Additional models were constructed examining waist and hip circumference as separate independent variables, rather than as a ratio. Models were also constructed using DXA

TABLE 3A. HIV-Infected Subjects, n = 100

CRP (mg/L)				Adiponectin (mg/L)				IL-6 (pg/mL)			
Parameter	**Estimate**	r^2	***P* Value**	**Parameter**	**Estimate**	r^2	***P* Value**	**Parameter**	**Estimate**	r^2	***P* Value**
WHR	2.65	0.10	0.008	WHR	−1.35	0.11	0.004	BMI	0.11	0.19	0.0001
HRT use	4.40	0.19	0.006	CD4	−0.003	0.18	0.02	WHR	0.73	0.30	0.001
Age	−0.22	0.26	0.01	Age	0.13	0.26	0.01	HRT use	0.75	0.35	0.04
Current smoking	−1.51	0.32	0.02								
BMI	0.25	0.36	0.06								
CD4	−0.006	0.40	0.05								

Fasting Insulin (μIU/mL)				120-minute Glucose (mg/dL)				Total Cholesterol (mg/dL)			
Parameter	**Estimate**	r^2	***P* Value**	**Parameter**	**Estimate**	r^2	***P* Value**	**Parameter**	**Estimate**	r^2	***P* Value**
WHR	5.12	0.15	0.0006	WHR	11.91	0.07	0.02	HIV viral load	−0.0005	0.07	0.02
HIV viral load	−0.0001	0.19	0.07					Current smoking	−13.34	0.13	0.04
								Age	1.58	0.19	0.03
								BMI	1.50	0.23	0.06

Direct LDL (mg/dL)				Triglycerides (mg/dL)				HDL (mg/dL)			
Parameter	**Estimate**	r^2	***P* Value**	**Parameter**	**Estimate**	r^2	***P* Value**	**Parameter**	**Estimate**	r^2	***P* Value**
Current smoking	−9.49	0.07	0.03	WHR	31.31	0.12	0.003	WHR	−5.23	0.11	0.004
BMI	1.45	0.12	0.05	PI (mo)	0.71	0.16	0.08	Age	0.63	0.22	0.002
Antilipid meds	11.60	0.18	0.03								

Within the HIV-positive group, separate stepwise regression models were constructed for each of the study endpoints (CRP, IL-6, adiponectin, cholesterol, LDL cholesterol, triglyceride, HDL cholesterol, insulin, and glucose). Age, BMI, use of HRT (hormone replacement therapy), smoking (current and pack-years), viral load, CD4, total duration protease inhibitor (PI) use, total duration nucleoside reverse transcriptase (NRTI) use, total duration nonnucleoside reverse transcriptase (NNRTI) use, and waist to hip ratio (WHR) were tested for entry into each model at $P = 0.10$. For CRP and lipid endpoints, use of lipid lowering medications was also tested for entry into the models. Final models were constructed from the significant terms entering each model. r^2 is cumulative and therefore r^2 for whole model is shown for final parameter to enter each model. Serological data were not available from 10 subjects. Parameter estimate for 0.1 unit change in WHR.

Viral load indicates cells/mL; CD4, cells/mm^3; HRT, hormone replacement therapy.

parameters (extremity fat and trunk fat) and CT scan parameters (abdominal visceral and subcutaneous fat area) in addition to WHR. Similar regression models, excluding terms for HIV medications, immune parameters, and HRT, were constructed for the same study endpoints within the control group (Table 3B).

Regression modeling was also performed in a combined analysis among HIV and control subjects to assess the contribution of HIV status, age, BMI, WHR, HRT use, and current and total smoking pack-years for the same study endpoints, testing for interactions between HIV and WHR in each model (Table 4). In additional models, body composition parameters (anthropometric, DXA, and CT parameters) were included to determine the body composition parameters most discriminant for the differences in CVD risk indices between the HIV and control populations.

TABLE 3B. Control Subjects, n = 75

CRP (mg/L)				Adiponectin (mg/L)				IL-6 (pg/mL)			
Parameter	**Estimate**	r^2	***P* Value**	**Parameter**	**Estimate**	r^2	***P* Value**	**Parameter**	**Estimate**	r^2	***P* Value**
BMI	0.36	0.18	0.0003	Age	0.17	0.06	0.04	WHR	0.81	0.21	0.0001
				WHR	−1.77	0.13	0.02				

Fasting Insulin (μIU/mL)				120-minute Glucose (mg/dL)				Total Cholesterol (mg/dL)			
Parameter	**Estimate**	r^2	***P* Value**	**Parameter**	**Estimate**	r^2	***P* Value**	**Parameter**	**Estimate**	r^2	***P* Value**
BMI	0.31	0.24	<0.0001	no variables entered model				Age	1.20	0.06	0.04
Current smoking	−0.82	0.28	0.04					Current smoking	−10.81	0.12	0.04

Direct LDL (mg/dL)				Triglycerides (mg/dL)				HDL (mg/dL)			
Parameter	**Estimate**	r^2	***P* Value**	**Parameter**	**Estimate**	r^2	***P* Value**	**Parameter**	**Estimate**	r^2	***P* Value**
WHR	13.30	0.07	0.03	WHR	20.77	0.14	0.001	WHR	−7.96	0.11	0.005
Current smoking	−9.93	0.13	0.04					Age	0.65	0.21	0.005

Within the control group, separate stepwise regression models were constructed for each of the study endpoints (CRP, IL-6, adiponectin, cholesterol, LDL cholesterol, triglyceride, HDL cholesterol, insulin, and glucose). Age, BMI, smoking (current and pack-years), and waist to hip ratio (WHR) were tested for entry into each model at $P = 0.10$. Final models were constructed from the significant terms entering each model. r^2 is cumulative and therefore r^2 for whole model is shown for final parameter to enter each model. Serological data were not available from 3 subjects. No variables entered the model for 120-minute glucose. Parameter estimate for 0.1 unit change in WHR.

Similar results were obtained using log transformations of nonnormally distributed data. The linearity of associations between continuous predictors was assessed and confirmed. Models were reconstructed after exclusion of outlying data points and did not differ, except that the strength of the association between WHR and triglyceride levels improved within the HIV group. In addition, we performed a number of sensitivity and quality control analyses to verify the conclusion of this study. Regression models including race were not substantially different from models in which race was not included and the results did not change if a 0.2 criterion was used to enter the models. Backward and forward selection models were performed with equivalent results. We also examined regression models in which HIV status and medication use were added sequentially and removed sequentially to assess the impact on the relationship of WHR and medication use to individual CVD risk indices. These models demonstrated that WHR, but neither medication use nor HIV status, was significant in models in which WHR was added first or last after HIV status or medication use.

Serologic data could not be obtained in 10 HIV-infected and in 3 control subjects due to limited IV access. HIV-infected subjects for whom blood could not be obtained were not different from HIV subjects for whom blood could be obtained, in terms of age, race, BMI, WHR, and use of antiretroviral medication (data not shown).

The planned sample size of 100 HIV-infected and 75 non–HIV-infected subjects was sufficient to detect a clinically significant 0.5 SD difference between groups with 85% power at a 2-sided 5% significance level.

Statistical analyses were performed using SAS JMP software (Version 4.04; SAS, Cary, NC). All values are expressed as mean values ±SEM unless otherwise indicated. Table 5 summarizes the analyses.

RESULTS

Demographic Variables

A total of 124 HIV-infected women and 107 HIV-negative patients were screened for the study. Two HIV-positive subjects and 9 control subjects were found ineligible at screening. Twenty-two HIV-positive and 23 control subjects met eligibility but did not continue participation after screening. One hundred HIV-infected subjects and 75 control subjects were enrolled into the study.

The HIV-infected and control subjects recruited for this study were similar in age (41 ± 1 vs. 40 ± 1 years, $P = 0.39$), BMI (26.1 ± 0.5 vs. 26.9 ± 0.5 kg/m^2, $P = 0.27$), and race (60 vs. 55% nonwhite, $P = 0.48$, HIV-infected vs. control) (Table 1). Eighty-one percent of HIV-infected subjects were using antiretroviral medication (44% a protease inhibitor, 80% an NRTI, and 27% an NNRTI).

Total lifetime duration of protease inhibitor, NRTI, and NNRTI medication use was 21 ± 2, 43 ± 4, and 8 ± 1 months, respectively. Current use of individual antiretroviral medications is also summarized in Table 1. The mean CD4 cell count was 407 ± 24 cells/mm^3, and 46% of patients had undetectable HIV viral load. The mean duration of HIV infection for HIV-positive study participants was 8.1 ± 0.4 years. A larger

TABLE 4. HIV-Infected and Control Patients, n = 175

CRP (mg/L)				Adiponectin (mg/L)				IL-6 (pg/mL)			
Parameter	**Estimate**	**r^2**	***P* Value**	**Parameter**	**Estimate**	**r^2**	***P* Value**	**Parameter**	**Estimate**	**r^2**	***P* Value**
HRT use	4.43	0.16	<0.001	WHR	−1.84	0.16	<0.001	WHR	0.74	0.19	<0.0001
WHR	2.07	0.26	<0.001	Age	0.15	0.22	0.004	BMI	0.08	0.25	0.0005
Current smoking	−1.10	0.31	0.001					HRT use	0.79	0.29	0.004
BMI	0.20	0.33	0.05								
Age	−0.12	0.34	0.06								

Fasting Insulin (μIU/mL)				120-minute Glucose (mg/dL)				Total Cholesterol (mg/dL)			
Parameter	**Estimate**	**r^2**	***P* Value**	**Parameter**	**Estimate**	**r^2**	***P* Value**	**Parameter**	**Estimate**	**r^2**	***P* Value**
WHR	4.10	0.18	<0.0001	WHR	10.13	0.13	<0.0001	Current smoking	−15.6	0.05	0.005
				HIV	5.74	0.15	0.06	Age	1.26	0.10	0.003
								WHR	7.12	0.14	0.01
								Smoking (pk y)	0.68	0.16	0.08

Direct LDL (mg/dL)				Triglycerides (mg/dL)				HDL (mg/dL)			
Parameter	**Estimate**	**r^2**	***P* Value**	**Parameter**	**Estimate**	**r^2**	***P* Value**	**Parameter**	**Estimate**	**r^2**	***P* Value**
Current smoking	−12.40	0.05	0.008	HIV	31.41	0.09	0.001	WHR	−6.24	0.19	<0.0001
WHR	7.27	0.10	0.003	WHR	27.39	0.12	0.05	Age	0.64	0.28	<0.0001
Smoking (pk y)	0.59	0.12	0.06					HIV	−2.75	0.30	0.02

In a combined analysis among all subjects, separate stepwise regression models were constructed for each of the study endpoints (CRP, IL-6, adiponectin, cholesterol, LDL cholesterol, triglyceride, HDL cholesterol, insulin, and glucose). Age, BMI, use of HRT, smoking (current and pack years), and waist-to-hip ratio (WHR) were tested for entry into each model at $P = 0.10$. Final models were constructed from the significant terms entering each model. For CRP and lipid endpoints, use of lipid lowering medications was also tested for entry into the models. r^2 is cumulative and therefore r^2 for whole model is shown for final parameter to enter each model. Serological data are not available from 10 HIV-infected patients and 3 control subjects. Parameter estimate for 0.1 unit change in WHR.

Viral load indicates cells/mL; CD4, cells/mm^3; HRT, hormone replacement therapy.

TABLE 5. Summary of Analyses

Analysis	Method	Table
Demographic comparison	*t*-test or χ^2	1
Endpoint comparison	Regression analysis	2
Body composition effects on CVD endpoints within the HIV group*	Stepwise regression analysis	3A
Body composition effects on CVD endpoints within the control group*	Stepwise regression analysis	3B
Body composition and HIV effects on CVD endpoints within combined grop*	Stepwise regression analysis	4

*Additional sensitivity analyses performed using forward and backward selection, liberalized criteria for entry, and nested models with sequential addition of variables (see text for full details).

percentage of the HIV group were current cigarette smokers (47% vs. 27%, $P = 0.006$) with higher number of pack-years smoked (9.4 ± 1.2 vs. 2.5 ± 0.6 pack-years, $P < 0.0001$). The use of HRT did not differ significantly in the HIV vs. control group (7% vs. 1%, $P = 0.08$). Nine percent and 12% of the HIV group were using antihypertension and lipid-lowering medication, respectively, compared with none of the control subjects.

Biochemical Indices

CRP, IL-6, triglyceride, 2-hour glucose, fasting insulin, and 2-hour insulin concentrations were significantly higher in the HIV group compared with the control subjects (Table 2 and Fig. 1). A larger percentage of HIV patients demonstrated a high-risk CRP >3.0 mg/L[21] (39.3% vs. 23.6%, $P = 0.03$). Conversely, HDL cholesterol and adiponectin were significantly lower in the HIV group compared with the control subjects (Fig. 1). Similar results for cardiovascular indices were obtained in analyses excluding patients receiving lipid-lowering or antihypertensive medication. Significant differences were not seen in TNF-α between the groups. Fasting glucose was not different between the groups. Based on World Health Organization criteria, fewer HIV-positive subjects demonstrated normal glucose tolerance (2-hour blood glucose <7.77 mM; 140 mg/dL) compared with the control group (78% vs. 92%, $P = 0.03$). Similarly, 22% of the HIV group vs. none of the control group demonstrated a fasting insulin >104.2 pM (15 μIU/mL) [90th percentile for healthy young female subjects] ($P < 0.0001$). Thirty-seven vs. 9% of HIV-infected vs. control subjects demonstrated either impaired glucose tolerance or fasting hyperinsulinemia ($P < 0.0001$).

Body Composition

Total body fat, extremity fat, subcutaneous abdominal fat area, and hip circumference were significantly lower in the HIV-positive patients than the control subjects (Table 2). Abdominal visceral fat area was higher among the HIV-infected subjects compared with control subjects. Both WHR and ratio of visceral to subcutaneous fat area were significantly higher in the HIV group than in control subjects.

Hormonal Function

A smaller percentage of the HIV group was eumenorrheic (68% vs. 84%, $P = 0.03$), but hormone levels including estradiol and gonadotropin levels were not different between the groups (Table 2).

Dietary Intake

Total calorie, fat, protein, cholesterol, fiber, and folate intake were not different between the groups (Table 2). HIV-infected patients consumed more carbohydrates than the control subjects. Cholesterol intake was on average <300 mg/d in both groups. Fat intake represented 34% of caloric intake in the HIV group and 33% in the control group.

Blood Pressure

Systolic blood pressure was not different between the groups. Diastolic blood pressure was slightly increased in the HIV group, but this difference was not statistically significant (Table 2).

Regression Modeling of Cardiovascular Disease Risk Parameters in the HIV-Infected Group

Stepwise regression models were constructed assessing the relationship of HRT use; age, BMI, current and total smoking pack-years, WHR, HIV viral load, CD4 cell count, total duration of protease inhibitor, NRTI, and NNRTI use, for each of the primary study endpoints (CRP, IL-6, adiponectin, insulin, glucose, and lipids) (Table 3). For each variable, order of entry into the model, parameter estimate (beta coefficient, β), and cumulative r^2 are shown. Only those variables that entered the model at $P < 0.1$ are included in each final model. WHR was the most significant variable in the models for CRP ($P = 0.008$), adiponectin ($P = 0.004$), fasting insulin ($P = 0.0006$), 120-minute glucose ($P = 0.02$), and HDL cholesterol ($P = 0.004$). For adiponectin, a higher CD4 count was associated with a lower adiponectin level. Conversely, lower HIV viral load was associated with an increased cholesterol level. Antiretroviral drug exposure was not significant in any of the models. Similarly, use of individual antiretroviral agents was not associated with CVD risk indices and did not enter the models.

Additional models were constructed examining waist and hip as separate independent variables, rather than as a ratio, but these models were not generally stronger than those using the WHR ratio (eg, for CRP, the r^2 for the model using waist and hip separately was 0.39 vs. 0.40 for the model using the WHR). Models were also constructed using DXA parameters (extremity fat and trunk fat) and CT scan parameters (abdominal visceral and subcutaneous fat area) in addition to WHR. CT visceral fat area rather than WHR entered into models using multiple body composition parameters and was the most significant predictor for CRP ($P < 0.0001$, overall r^2 for the model = 0.38) and IL-6 ($P < 0.0001$, overall r^2 for the model = 0.47). In contrast, visceral fat area did not enter the models and WHR remained the most significant predictor for adiponectin, HDL cholesterol, and fasting insulin. Use of more specific body composition parameters did not strengthen the overall r^2 of the models for many of the variables; eg, for CRP the r^2 for the model using WHR was 0.40, for waist and hip independently,

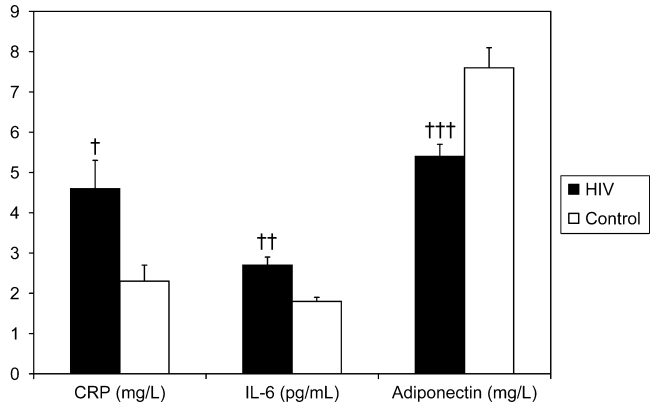

FIGURE 1. CRP, IL-6, and adiponectin concentrations in HIV (n = 90) and control subjects (n = 72). †P = 0.007, ††P = 0.02, †††P = 0.001 for HIV vs. control subjects in regression analysis adjusting for HRT, age, race, BMI, current smoking and smoking pack-years, and use of lipid-lowering medication for CRP analysis.

0.39, for DXA parameters 0.29, for CT parameters 0.38, and for all body composition parameters simultaneously, 0.38.

Stepwise Regression Modeling for Cardiovascular Disease Risk Parameters in the Control Group

Within the control group, WHR was most significant in the models for adiponectin (P = 0.02), IL-6 (P = 0.0001), LDL cholesterol (P = 0.03), triglycerides (P = 0.001), and HDL cholesterol (P = 0.005) (Table 3). In contrast to the HIV subjects, BMI (P = 0.0003) but not WHR was significant in the model for CRP.

Assessment of the Overall Effect of WHR in the HIV and Control Subjects

Stepwise regression modeling was performed to simultaneously assess the contribution of HIV status and WHR to CVD risk parameters among all study subjects (HIV and control together in the same model, n = 175), controlling for use of HRT, age, BMI, and current and total smoking pack-years (Table 4). HIV status did not enter the models for CRP, adiponectin, IL-6, or insulin, whereas WHR was a significant factor in regression models for these variables among all subjects. For 120-minute glucose, triglycerides, and HDL cholesterol, HIV status and WHR were both significant in the models, demonstrating independent effects of HIV status and increased WHR on these variables. For example, triglyceride levels increased 0.35 mM (31 mg/dL) in association with positive HIV status and 0.31 mM (27 mg/dL) for each 0.1 increase in WHR, in regression modeling (Table 4).

The interaction of HIV and WHR was tested. There was a positive interaction between HIV status and WHR for fasting insulin only. The slope of the relationship between WHR and insulin was steeper for the HIV group (insulin = $-34.2 + 49.8 \times$ WHR, P = 0.002) compared with the control group (insulin = $-1.2 + 9.3 \times$ WHR, P = 0.06).

The strength of the modeling for CVD risk indices was not substantially improved in models using all body composition parameters compared with models using WHR alone among all the study subjects (n = 175). Using all body composition parameters simultaneously in modeling among all subjects (anthropometric, DEXA, and CT parameters), visceral abdominal fat area by CT entered the models and was the most significant predictor of CRP ($P < 0.0001$, overall r^2 for the model = 0.36), IL-6 ($P < 0.0001$, overall r^2 = 0.34), and cholesterol (P = 0.0003, overall r^2 = 0.18). However, WHR remained the most significant factor in modeling for adiponectin, fasting insulin, 120-minute glucose, and HDL. Furthermore, the use of more specific body composition parameters did not substantially improve the overall strength of the models (overall r^2 0.34 vs. 0.36 for modeling with WHR vs. all body composition parameters for CRP; overall r^2 0.29 vs. 0.34 for modeling with WHR vs. all body composition parameters for IL-6; overall r^2 0.16 vs. 0.18 for modeling with WHR vs. all body composition parameters for cholesterol). No significant interaction was seen between visceral fat area and HIV status for any variable.

DISCUSSION

In this study, we demonstrate increased CVD risk indices in HIV-infected women, investigating both traditional and newer inflammatory risk markers. Increased CRP, IL-6, triglycerides, insulin, and 2-hour glucose and reduced adiponectin and HDL cholesterol were observed. Simple markers of abnormal fat distribution, such as WHR, were significantly associated with inflammatory markers, such as CRP, IL-6, and adiponectin.

Few prior studies have investigated body composition and metabolic abnormalities exclusively in HIV-infected women. In contrast to most previous studies, we did not select subjects on the basis of fat redistribution or use of antiretroviral medication, but instead assessed consecutive HIV-infected women and controlled for these variables in regression analyses within both the HIV group and a well-matched control group of HIV-negative women. Our subjects, though a convenience sample, are representative of HIV-infected subjects throughout the state of Massachusetts and nationally, based on demographic information, and were treated with antiretroviral medications at a rate similar to that seen in the HIV Epidemiology Research Study (HERS) and Women's Interagency HIV Study (WIHS) studies.[22] Subjects were prospectively recruited HIV-infected women from a variety of sources to avoid bias as much as possible. Control subjects were healthy and representative of the general female population in the United States; eg, the WHR of 0.83 in the control population was similar to that demonstrated in healthy women of similar age and BMI in the Framingham study (0.82 ± 0.01).[3] Prior studies identified increased triglycerides, insulin, and low HDL cholesterol in HIV-infected women and increased risk for CVD using a prediction model[23] but have not evaluated CRP, IL-6, adiponectin, or TNF-α in comparison to a well-characterized control group, controlling for detailed measures of body composition.

Differences in body composition were noted in the HIV-infected women compared with the healthy control group. In a large study, Galli et al[24] documented adipose tissue alterations in 30% of consecutively recruited HIV-positive men and

42% of HIV-positive women but did not compare specific body composition parameters with those of a control group. We identified marked differences in WHR between the groups using simple anthropometry. More specific measures of abdominal visceral fat and extremity fat showed a similar pattern of central fat distribution and decreased extremity fat compared with the control group. Menstrual irregularities were somewhat more common in the HIV group, but estradiol and gonadotropin levels were similar between the 2 groups, indicating that differences in body composition and other endpoints are not due to differences in menopausal status.

A primary finding in this study was the observation of significantly increased CRP in HIV-positive women compared with control subjects. CRP is known to be a strong independent risk marker for CVD among non-HIV-infected women.[10,25] There was limited evidence for increased HRT use, although at a relatively low level (7%), in the HIV group compared with 1% in the control subjects. HRT is associated with increased CRP in non–HIV-infected women.[26] Use of HRT independently contributed to increased CRP among HIV-infected women in this study, but differences in CRP between the HIV and control group remained significant controlling for HRT as well as smoking.

In the recently published DAD study, a large international prospective cohort study of adverse events in HIV-infected patients, myocardial infarction rates increased with increased duration of combination antiretroviral therapy.[1] CRP and other inflammatory markers were not measured in the DAD study, which included mostly men (76%). CRP concentrations were shown to be associated with overall mortality in younger HIV-infected women in a recent study by Feldman et al.[7] However, CRP levels were not compared with a control population and cardiovascular mortality was not assessed. The relationship between CRP and CVD in HIV-infected women remains unknown but should be investigated given the marked increases in this population.

A number of possibilities may explain the observation of increased CRP in the HIV-infected group, including viral factors related to inflammation,[27] other cytokines such as IL-6,[28] and differences in body composition between the groups.[29] WHR, but not HIV status, was highly significant in stepwise regression modeling for CRP among all subjects, suggesting that differences in body composition, rather than HIV status, account in part for increased CRP in the HIV group. These results were confirmed in regression models in which WHR was sequentially added after HIV status and medication use. Medication use was not significant in either stepwise or sequential regression models. Although causality and sequence cannot be definitively determined in a cross-sectional study, our data strongly suggest that the changes in body composition associated with highly active antiretroviral therapy, not the medication use itself or HIV status, contribute to abnormal CVD risk indices among HIV-infected women. WHR performed comparably to more specific body composition measurements in modeling for CVD risk indices. Neither waist nor hip circumference measurement performed as well individually as the WHR. Within the HIV group, a 0.1 increase in WHR was associated with a 2.7-mg/L increase in CRP ($P = 0.008$), controlling for immune function, antiretroviral use, smoking, and HRT. In contrast, CRP was more strongly related to BMI than WHR in the control group. Our data demonstrate that CVD risk indices are strongly related to body composition parameters in HIV-infected women, as measured by more sophisticated CT and DXA techniques, and comparably well to simple, easily performed measures such as WHR. WHR thus functions well as a marker of overall fat distribution, eg, relative increase in visceral adiposity and loss of subcutaneous extremity fat, each of which may contribute to overall cardiovascular risk and metabolic abnormalities.

We demonstrate significantly increased IL-6 concentrations in association with increased WHR in the HIV-infected subjects. These data are consistent with data in non–HIV-infected women, demonstrating increased IL-6 and CRP in women with abdominal adiposity.[29] CRP is not known to be made in adipocytes per se, but primarily in the liver.[27] A stimulatory effect of adipocyte-secreted IL-6 on CRP,[28,30,31] rather than a direct effect of fat mass per se, is one potential mechanism to explain the markedly increased CRP in this population. Increased adipogenic expression of IL-6 was recently identified in subcutaneous fat of HIV-infected patients with lipodystrophy, suggesting a possible source of increased IL-6 in this population.[32] IL-6 was shown to be independently associated with increased coronary heart disease in non–HIV-infected women,[11] and further studies are needed to determine the relationship between increased IL-6 and CVD in HIV-infected women. WHR, but not HIV status, was significantly associated with IL-6 in regression modeling among the combined group.

Serum TNF-α concentrations were not different between HIV-infected and control subjects. Johnson et al[33] recently showed increased in vitro TNF-α release from stimulated subcutaneous adipocytes in HIV-infected men and women with lipodystrophy but did not report serum concentrations in comparison to age-matched controls. TNF-α is associated with increased cardiac events in older non–HIV-infected subjects,[34] but unlike IL-6 and CRP, the association between TNF and cardiac events in women has not been established.

We demonstrate lower plasma adiponectin levels in HIV-infected women compared with control subjects. To our knowledge, this is the first measurement of adiponectin in a study exclusively comprised of women and gender-matched control subjects. Among HIV-infected men, we and others[35–37] have shown decreased adiponectin. Both increased truncal fat and decreased extremity fat were associated with decreased adiponectin in prior studies,[35] and thus it is not surprising that increased WHR is strongly associated with decreased adiponectin, independent of HIV status. Adiponectin is thought to be an important modulator of intramuscular fatty acid oxidation and insulin resistance.[38,39] Reduced adiponectin concentrations are independently associated with coronary artery disease and endothelial dysfunction in non–HIV-infected men.[12,40, 41] Studies are needed to investigate the effects of adiponectin on CVD in HIV-infected women. PPAR-γ (peroxisomal proliferator-activated receptor-γ) agonists increase adiponectin in the HIV population.[42]

In addition to increased CRP, IL-6, and reduced adiponectin, the HIV-infected subjects demonstrated traditional CVD risk factors, including increased triglyceride and reduced HDL cholesterol. Increased fasting insulin and impaired glucose tolerance

were demonstrated, which may further place HIV-infected women at risk for CVD. In the DAD study, diabetes was a significant independent variable associated with myocardial infarction rates, and similar studies should be performed in large cohorts of HIV-infected women.[1] Our study shows that WHR is strongly associated with insulin, 2-hour glucose, and HDL cholesterol, controlling for smoking and HRT use, as well as virologic and medication parameters. Interestingly, we showed an interaction between HIV and WHR in the model for insulin, indicating a steeper slope in the relationship between insulin and WHR in the HIV group compared with the control group and suggesting an as-yet unknown biologic interaction between HIV itself and body composition contributing to increased insulin levels, independent of medication use.

Smoking rates were increased in the HIV-infected group and smoking is therefore a major modifiable risk factor that should be addressed by physicians caring for HIV-infected women. However, we controlled for smoking in all analyses, and differences in CVD indices remained, suggesting that other factors, including abnormal body composition, increase risk indices in the HIV population. Dietary factors were relatively similar between groups. Fat and cholesterol intake were similar between the groups and not generally above the recommended guidelines. Blood pressure did not differ significantly between the groups although there was a modest trend toward increased diastolic blood pressure in the HIV group, controlling for antihypertensive medication use.

This study has a number of strengths, including investigation of a well-matched control population for age, race, and BMI. The cross-sectional nature of the study limits the conclusions as to causality, but the modeling approaches used strongly suggest that WHR and changes in body composition, more than other factors, including medications or HIV status, are associated with abnormal CVD risk indices. While the associations with WHR and other body composition endpoints were highly significant, caution should be used in interpreting borderline statistical associations for other variables in the regression modeling. Patients receiving diabetes medication and oral contraceptives were excluded as they might significantly affect the endpoints assessed, and therefore our results cannot be generalized to this population. Also we excluded patients with active drug abuse to adequately consent subjects and ensure greater reliability of scheduling and avoid metabolic complications associated with drug or alcohol abuse. Our results cannot be generalized to the group of HIV-infected women actively using drugs.

In conclusion, this study demonstrates marked abnormalities in inflammatory and traditional CVD risk indices in HIV-infected women. Simple measures of body composition such as WHR are strongly associated with abnormal biochemical indices in this population. Moreover, in sequential and stepwise regression modeling, WHR, but not HIV status, was significantly associated with increased CRP, IL-6, insulin, glucose, cholesterol, and direct LDL and decreased adiponectin. Further studies are critically needed to determine whether increased cardiovascular risk indices translate into increased cardiovascular events among HIV-infected women. In addition, treatment strategies to modify CVD risk and improve abnormal fat distribution are needed.

ACKNOWLEDGMENTS

The authors thank the participants in this study, the nursing and bionutrition staffs of the MGH and MIT General Clinical Research Centers for their dedicated patient care, and Jeff Breu for his performance of the radioimmunoassays.

REFERENCES

1. Friis-Moller N, Sabin CA, Weber R, et al. Combination antiretroviral therapy and the risk of myocardial infarction. *N Engl J Med*. 2003;349:1993–2003.
2. Bozzette SA, Ake CF, Tam HK, et al. Cardiovascular and cerebrovascular events in patients treated for human immunodeficiency virus infection. *N Engl J Med*. 2003;348:702–710.
3. Hadigan C, Meigs JB, Corcoran C, et al. Metabolic abnormalities and cardiovascular disease risk factors in adults with human immunodeficiency virus infection and lipodystrophy. *Clin Infect Dis*. 2001;32:130–139.
4. Hadigan C, Meigs JB, Wilson PW, et al. Prediction of coronary heart disease risk in HIV-infected patients with fat redistribution. *Clin Infect Dis*. 2003;36:909–916.
5. Gervasoni C, Ridolfo AL, Trifiro G, et al. Redistribution of body fat in HIV-infected women undergoing combined antiretroviral therapy. *AIDS*. 1999;13:465–471.
6. Dong KL, Bausserman LL, Flynn MM, et al. Changes in body habitus and serum lipid abnormalities in HIV-positive women on highly active antiretroviral therapy (HAART). *J Acquir Immune Defic Syndr*. 1999;21: 107–113.
7. Feldman JG, Goldwasser P, Holman S, et al. C-reactive protein is an independent predictor of mortality in women with HIV-1 infection. *J Acquir Immune Defic Syndr*. 2003;32:210–214.
8. Currier JS, Taylor A, Boyd F, et al. Coronary heart disease in HIV-infected individuals. *J Acquir Immune Defic Syndr*. 2003;33:506–512.
9. Centers for Disease Control and Prevention. *Deaths Among Persons with AIDS, HIV/AIDS Surveillance Report,* 2002;8(No. 1).
10. Ridker PM, Hennekens CH, Buring JE, et al. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med*. 2000;342:836–843.
11. Pradhan A, Manson J, Rossouw J, et al. Inflammatory biomarkers, hormone replacement therapy, and incident coronary heart disease: prospective analysis from the Women's Health Initiative Observational Study. *JAMA*. 2002;288:980–987.
12. Pischon T, Girman CJ, Hotamisligil GS, et al. Plasma adiponectin levels and risk of myocardial infarction in men. *JAMA*. 2004;291:1730–1737.
13. Massachusetts Department of Public Health HIV/AIDS Surveillance Program. *HIV/AIDS in Massachusetts: An Epidemiologic Profile*. Boston, MA: HIV/AIDS Bureau; 2002.
14. Massachusetts Metropolitan Area Planning Council (MMAP). *Annual Report.* Boston, MA: MMAP; 1999.
15. Clark RA, Mulligan K, Stamenovic E, et al. Frequency of anovulation and early menopause among women enrolled in selected adult AIDS Clinical Trials Group studies. *J Infect Dis*. 2001;184:1325–1327.
16. Dolan SE, Huang JS, Killilea KM, et al. Reduced bone density in HIV-infected women. *AIDS*. 2004;18:475–483.
17. Kuczmarski RJ, Carroll MD, Flegal KM, et al. Varying body mass index cutoff points to describe overweight prevalence among U.S. adults: NHANES III (1988 to 1994). *Obes Res*. 1997;5:542–548.
18. Borkan GA, Gerzof SG, Robbins AH, et al. Assessment of abdominal fat content by computed tomography. *Am J Clin Nutr*. 1982;36:172–177.
19. Mazess RB, Barden HS, Bisek JP, et al. Dual-energy x-ray absorptiometry for total-body and regional bone-mineral and soft-tissue composition. *Am J Clin Nutr*. 1990;51:1106–1112.
20. Hadigan C, Miller K, Corcoran C, et al. Fasting hyperinsulinemia and changes in regional body composition in human immunodeficiency virus-infected women. *J Clin Endocrinol Metab*. 1999;84:1932–1937.
21. Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation*. 2003;107:499–511.
22. Hader SL, Smith DK, Moore JS, et al. HIV infection in women in the United States: status at the millennium. *JAMA*. 2001;285:1186–1192.

23. Saves M, Chene G, Ducimetiere P, et al. Risk factors for coronary heart disease in patients treated for human immunodeficiency virus infection compared with the general population. *Clin Infect Dis*. 2003;37:292–298.
24. Galli M, Veglia F, Angarano G, et al. Gender differences in antiretroviral drug-related adipose tissue alterations: women are at higher risk than men and develop particular lipodystrophy patterns. *J Acquir Immune Defic Syndr*. 2003;34:58–61.
25. Ridker PM, Rifai N, Rose L, et al. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N Engl J Med*. 2002;347:1557–1565.
26. Bermudez EA, Rifai N, Buring J, et al. Interrelationships among circulating interleukin-6, C-reactive protein, and traditional cardiovascular risk factors in women. *Arterioscler Thromb Vasc Biol*. 2002;22:1668–1673.
27. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. *J Clin Invest*. 2003;111:1805–1812.
28. Moshage HJ, Roelofs HM, van Pelt JF, et al. The effect of interleukin-1, interleukin-6 and its interrelationship on the synthesis of serum amyloid A and C-reactive protein in primary cultures of adult human hepatocytes. *Biochem Biophys Res Commun*. 1988;155:112–117.
29. Rexrode KM, Pradhan A, Manson JE, et al. Relationship of total and abdominal adiposity with CRP and IL-6 in women. *Ann Epidemiol*. 2003; 13:674–682.
30. Kern PA, Ranganathan S, Li C, et al. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. *Am J Physiol Endocrinol Metab*. 2001;280:E745–E751.
31. Mohamed-Ali V, Goodrick S, Rawesh A, et al. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. *J Clin Endocrinol Metab*. 1997;82:4196–4200.
32. Kannisto K, Sutinen J, Korsheninnikova E, et al. Expression of adipogenic transcription factors, peroxisome proliferator-activated receptor gamma co-activator 1, IL-6 and CD45 in subcutaneous adipose tissue in lipodystrophy associated with highly active antiretroviral therapy. *AIDS*. 2003; 17:1753–1762.
33. Johnson JA, Albu JB, Engelson ES, et al. Increased systemic and adipose tissue cytokines in patients with HIV-associated lipodystrophy. *Am J Physiol Endocrinol Metab*. 2004;286:E261–E271.
34. Cesari M, Penninx BW, Newman AB, et al. Inflammatory markers and onset of cardiovascular events: results from the Health ABC study. *Circulation*. 2003;108:2317–2322.
35. Tong Q, Sankale JL, Hadigan CM, et al. Regulation of adiponectin in human immunodeficiency virus-infected patients: relationship to body composition and metabolic indices. *J Clin Endocrinol Metab*. 2003;88: 1559–1564.
36. Vigouroux C, Maachi M, Nguyen TH, et al. Serum adipocytokines are related to lipodystrophy and metabolic disorders in HIV-infected men under antiretroviral therapy. *AIDS*. 2003;17:1503–1511.
37. Addy CL, Gavrila A, Tsiodras S, et al. Hypoadiponectinemia is associated with insulin resistance, hypertriglyceridemia, and fat redistribution in human immunodeficiency virus-infected patients treated with highly active antiretroviral therapy. *J Clin Endocrinol Metab*. 2003;88:627–636.
38. Yamauchi T, Kamon J, Waki H, et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med*. 2001;7:941–946.
39. Fruebis J, Tsao TS, Javorschi S, et al. Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. *Proc Natl Acad Sci USA*. 2001; 98:2005–2010.
40. Kumada M, Kihara S, Sumitsuji S, et al. Association of hypoadiponectinemia with coronary artery disease in men. *Arterioscler Thromb Vasc Biol*. 2003;23:85–89.
41. Shimabukuro M, Higa N, Asahi T, et al. Hypoadiponectinemia is closely linked to endothelial dysfunction in man. *J Clin Endocrinol Metab*. 2003; 88:3236–3240.
42. Hadigan C, Yawetz S, Thomas A, et al. Metabolic effects of rosiglitazone in HIV lipodystrophy: a randomized controlled trial. *Ann Intern Med*. 2004;140:786–794.

