Carotid Intima Media Thickness, Inflammatory Markers, and Endothelial Activation Markers in HIV Patients with Lipoatrophy Increased at 48 Weeks Regardless of Use of Rosiglitazone or Placebo

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Abstract

Rosiglitazone may be useful for the treatment of antiretroviral therapy-associated lipoatrophy, but an association with cardiovascular disease (CVD) has been questioned in diabetics. We evaluated rosiglitazone's effect on surrogate markers of CVD in HIV-infected individuals with lipoatrophy. HIV⁺ patients with lipoatrophy on thymidine-sparing regimens were randomized to rosiglitazone vs. placebo for 48 weeks. We serially assessed carotid IMT, fasting metabolic profiles, tumor necrosis factor (TNF)- α , soluble receptors (sTNFR I and II), interleukin (IL)-6, high-sensitivity C-reactive protein (hsCRP), myeloperoxidase (MPO), and endothelial activation markers [von Willebrand factor (vWF), soluble intercellular cell adhesion molecules-1 (sICAM-1), and vascular cell adhesion molecules-1 (sVCAM-1)]. Seventy-one subjects enrolled: 17% were female and 51% were white. Baseline characteristics were similar between groups except for higher total cholesterol in the placebo group ($p=0.04$). At 48 weeks, common carotid artery (CCA) IMT changed significantly ($p\leq 0.05$) within but not between the groups ($p=0.36$): the median (IQR) increase was 0.10 (0.05, 0.25) mm and 0.15 (0, 0.25) mm in the rosiglitazone and placebo groups, respectively. hsCRP, sTNFR I and II, sVCAM-1, and vWF changed significantly ($p\leq 0.02$) within but not between groups. Total cholesterol increased significantly in the rosiglitazone group ($p=0.008$). In our study of virologically controlled subjects with lipoatrophy, rosiglitazone did not independently increase carotid IMT, endothelial activation, and inflammatory cytokines.

Introduction

CARDIOVASCULAR DISEASE (CVD) remains the number one overall cause of mortality in the United States.¹ HIV and antiretroviral therapy (ART) have recently been associated with further increasing risks of CVD and myocardial infarction (MI).^{2,3} Among HIV-infected individuals, CV events have become one of the most common causes of non-AIDS deaths in the United States.⁴ The degree to which traditional CVD risk factors and HIV-related factors such as ART contribute to this increased risk is not clear. Thus, it remains important to characterize the CV and metabolic issues of individuals living with HIV.

ART and HIV itself have been associated with abnormal endothelial function and accelerated atherosclerosis.⁴⁻⁶ For

the assessment of CVD in the HIV-negative population, carotid ultrasound measurement of intima media thickness (IMT) has been established as a reliable noninvasive surrogate.^{7,8} Also, in the general population, a number of inflammatory, thrombogenic, and oxidative measures have been shown to correlate with the presence of CVD.⁹⁻¹¹ Tumor necrosis factor (TNF)- α , mediated by soluble TNF receptors (sTNFR I and II), has been associated with acute coronary syndromes.¹² The cardiovascular biomarker myeloperoxidase (MPO) has been shown to be a predictor of MI in patients with chest pain.¹¹ von Willebrand factor (vWF), soluble intercellular cell adhesion molecules-1 (sICAM-1), and soluble vascular cell adhesion molecule-1 (sVCAM-1) have been shown to increase with activation of endothelial cells.¹⁰ In the HIV-infected population, increased interleukin (IL)-6 has been

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associated with increased mortality at baseline and over time.⁴ Also, high sensitivity C-reactive protein (hsCRP), IL-6, and sVCAM-1 have been shown to decrease after initiation of ART.¹³ Endothelial activation markers have been reported to be increased in HIV-infected ART-naïve individuals compared with HIV-negative individuals.⁶

Another metabolic complication of ART, lipoatrophy, which is distressing to patients and is associated with thymidine nucleoside analogue reverse transcriptase inhibitor (NRTI) downregulation of peroxisome proliferator-activated receptor- γ (PPAR- γ). Individuals with lipoatrophy who have already stopped thymidine NRTIs have few treatment options. The thiazolidinedione rosiglitazone is a potent agonist of PPAR- γ and thus may be a promising additional therapeutic option for these individuals.^{14–20} However, a recent publication has questioned an association between MI and rosiglitazone in diabetics raising concern regarding its use.²¹ Subsequent studies led to conflicting results.^{22,23} Furthermore, studies in the HIV-negative population have suggested that PPAR- γ agonists may reduce inflammatory biomarkers and improve endothelial function.^{24,25}

The objective of our primary study was to evaluate rosiglitazone's effect on DEXA-measured limb fat in HIV-infected individuals with established lipoatrophy. Our results suggested that in the absence of thymidine NRTIs, rosiglitazone significantly improved peripheral lipoatrophy even in subjects without insulin resistance (presented elsewhere).^{15,26} In this cardiovascular substudy in order to further characterize these apparently accelerated atherosclerotic processes and the effects of the PPAR- γ agonist rosiglitazone in HIV-infected individuals, we have evaluated carotid IMT, inflammatory markers, and endothelial activation markers.

Materials and Methods

This randomized double-blind placebo-controlled trial evaluated baseline and serial measurements of carotid IMT, inflammatory markers, and endothelial activation markers in HIV-infected patients with lipoatrophy receiving rosiglitazone or placebo for 48 weeks. The participants were enrolled at the John T. Carey Special Immunology Unit of University Hospitals Case Medical Center and at the Cleveland Clinic in Cleveland, OH.

HIV-infected patients with clinical lipoatrophy were enrolled. Inclusion criteria included currently receiving a stable thymidine-sparing antiretroviral regimen for at least 24 weeks prior to study entry with HIV-1 RNA levels less than 5000 copies/ml at study entry. Additional inclusion criteria included a past history of receiving thymidine analog for at least a cumulative 12 months. Women of childbearing potential had a negative pregnancy test at study entry and were required to use adequate contraception during the study.

Exclusion criteria included diabetes mellitus or receiving metformin. Additional exclusion criteria included heart failure of New York Heart Association class 3 or 4, cirrhosis, pregnancy or breastfeeding, and known sensitivity to the study drug. None was receiving concurrent hormonal supplementation with recombinant growth hormone, anabolic steroids, estrogen, or testosterone (except at replacement doses). Individuals were also excluded if they had any significant laboratory abnormalities including serum transaminases and alkaline phosphatase greater than two times the

upper normal limit (ULN), serum lipase greater than 2.5 times the ULN, creatinine greater than 3 times the ULN, PT/PTT greater than 1.2 times the ULN, absolute neutrophil count less than 750/mm³, hemoglobin less than 9.0 g/dl, platelet count <75,000/mm³, and glucose less than 70 mg/dl.

The subjects were randomized at a central location to rosiglitazone or matching placebo for 48 weeks. Both the study drug and matching placebo were provided by GlaxoSmithKline. After a lead-in period in which the subjects received rosiglitazone 4 mg daily for 4 weeks, they received rosiglitazone 4 mg twice daily for the remainder of the study. All subjects tolerated the lead-in period well and none dropped out during the lead-in period. Participants continued their present antiretroviral regimens and were advised to maintain their current diet and exercise habits.

Study evaluations consisted of clinical examinations including anthropometric evaluations including waist-hip ratio, weight, and height. All past and current medical diagnoses and detailed ART history were obtained by extensive chart review. Fasting metabolic tests, biomarkers, and centralized carotid ultrasound were serially assessed at weeks 0, 24, and 48.

The metabolic assessments were done in a fasting state of at least 8 h at weeks 0, 24, and 48, and included glucose, insulin, and lipoprotein levels. The presence of metabolic syndrome was assessed by evaluating for ≥ 3 of the following parameters: waist circumference ≥ 88 cm (in women) and ≥ 102 cm (in men), triglyceride levels ≥ 150 mg/dl (or hypolipemics known to affect triglyceride levels), HDL cholesterol levels <50 mg/dl (in women) or <40 mg/dl (in men), glucose level ≥ 100 mg/dl, and blood pressure $\geq 130/85$ mm Hg (or anti-hypertensives with a history of hypertension). Insulin resistance was estimated using the homeostasis model assessment of insulin resistance (HOMA-IR): = fasting glucose (mg/dl) $\times$ fasting insulin (μ U/ml)/22.5.²⁷

The following biomarkers were measured: inflammatory cytokines (TNF- α , sTNFRI and II, IL-6, and hsCRP), endothelial activation markers (vWF, sICAM-1, and sVCAM-1), and the cardiovascular marker MPO. The markers were measured in duplicate and averaged using commercially available enzyme-labeled immunosorbent sandwich assays (Searchlight; Thermo Fisher Scientific, Woburn, MA). The median intraassay coefficients of variation for TNF- α , sTNFRI, sTNFRII, IL-6, hsCRP, vWF, sICAM-1, sVCAM-1, and MPO were 15.4%, 8.8%, 8.6%, 11.7%, 6.9%, 13.3%, 8.0%, 8.7%, and 7.5%, respectively. The median interassay coefficients of variation for each assay were 9.8%, 10.8%, 6.0%, 10.4%, 4.5%, 9.0%, 4.2%, 13.4%, and 12.6%, respectively. In addition, CD4⁺ cell count and HIV-1 RNA were concomitantly measured as markers of HIV disease status.

All carotid IMT measurements were performed on the same machine by the same dedicated sonographer and were read by an experienced radiologist (V.D.). Both the sonographer and the radiologist were blinded to treatment assignment. Images of the bilateral distal common carotid arteries (CCA) and internal carotid arteries (ICA) were obtained in longitudinal views separately. IMT was scored as per the protocol of Riley *et al.*²⁸ by one of the investigators (V.D.). Images of the near (proximal) and far wall free of plaques (distal) were acquired with a 7–14 MHz AT 1204 linear array transducer (Toshiba American Medical Systems, Tustin, CA) operating at 14 MHz with differential harmonics.

Three measurements of the IMT were obtained at the near and far wall of each CCA and ICA. The mean of three measurements at each site (right and left side) was used as a final measurement of IMT for that site (for both CCA and ICA the far and near wall had three IMT measurements each, resulting a total of 12 measurements). Plaque was defined, measured, and graded as a plaque index according to published protocols from the Cardiovascular Health Study.²⁹

The Institutional Review Board (IRB) Committees of both institutions approved the study. All patients gave written informed consent.

Data analysis

Baseline characteristics are described by group (Table 1); nominal variables are described with frequencies and per-

cents; continuous variables are described using medians and interquartile ranges (IQR). Two-sample tests were used to determine if any of the variables were different at baseline. Chi squared analysis or Fisher's exact test was used as appropriate for nominal variables and Student's *t* test was used for normally distributed continuous variables and Wilcoxon rank sum test for nonnormally distributed continuous variables.

Outcome measures at baseline (IMT measurements and CV end-points) are described and compared in the same manner (Table 1). Changes from baseline were calculated by subtracting baseline values from 48-week values (Table 2). They are described and outlined above. Distributionally appropriate paired tests (paired *t* tests for normally distributed variables and Wilcoxon signed rank test for nonnormally distributed variables) were used to determine the significance

TABLE 1. BASELINE CHARACTERISTICS

Median (IQR)	Rosiglitazone (n = 34)	Placebo (n = 37)
Age in years	47 (44, 52)	52 (44, 54)
Men, N (%)	29 (85)	30 (81)
White, N (%)	19 (56)	17 (45)
CD4 ⁺ cell count, cells/mm ³	595.5 (437, 706)	690 (422, 860)
Nadir CD4 ⁺ cell count, cells/mm ³	205 (98, 271)	123 (64, 247)
HIV-1 RNA <50 copies/ml, N (%)	28 (82)	29 (78)
Duration HIV diagnosis (months)	146 (122, 194)	170 (122, 231)
Duration on ART (months)	114 (96, 127)	105 (74, 123)
NNRTI at study entry, N (%)	15 (44)	14 (37)
PI at study entry, N (%)	19 (56)	23 (62)
TDF at study entry, N (%)	25 (74)	27 (73)
ABC at study entry, N (%)	16 (47)	15 (45)
Systolic blood pressure, mm Hg	120 (110, 130)	120 (112, 130)
Diastolic blood pressure, mm Hg	80 (72, 88)	80 (74, 85)
BMI, kg/m ²	25.3 (22.5, 30.8)	25.8 (22.8, 27.9)
Waist-hip ratio	0.98 (0.95, 1.05)	0.98 (0.94, 1.03)
Carotid IMT CCA	1.25 (1.05, 1.56)	1.33 (1.18, 1.45)
Carotid IMT ICA	1.35 (1.10, 1.70)	1.53 (1.14, 1.84)
TNF- α , pg/ml	4.0 (1.0, 4.0)	4.0 (1.0, 13.4)
sTNFRI, pg/ml	773.5 (597.0, 957.0)	701.5 (573.1, 891.0)
sTNFRII, pg/ml	564.0 (232.0, 1247.0)	605.0 (315.0, 1208.2)
IL-6, pg/ml	6.0 (2.0, 13.3)	6.0 (3.0, 10.5)
hsCRP, mg/liter	7.6 (4.1, 18.1)	3.2 (1.2, 14.7)
MPO, pg/ml	8052.5 (5630.0, 15,132.2)	5980.0 (4385.0, 17,107.7)
vWF, U/ml	12.9 (8.0, 37.3)	17.0 (11.0, 33.0)
sICAM-1, ng/ml	371.2 (300.3, 612.5)	450.1 (294.0, 628.9)
sVCAM-1, ng/ml	614.6 (400.9, 2625.6)	871.3 (422.3, 3360.6)
Total cholesterol, mg/dl	174.0 (140, 215)	187.0 (172.0, 235.0) ^a
Triglycerides, mg/dl	160.0 (93.0, 331.0)	200.5 (111.0, 301.0)
HDL, mg/dl	35.0 (31.0, 44.0)	38.0 (35.0, 42.0)
Non-HDL, mg/dl	142 (109, 181)	149 (133, 181)
Glucose, mg/dl	91 (86, 97)	88 (81, 92)
Insulin >15 IU/ml at entry, N (%)	14 (41)	12 (32)
Insulin, IU/ml	14 (9, 19)	12 (9, 21)
HOMA-IR	3.2 (1.9, 4.3)	2.4 (1.7, 4.8)
Metabolic syndrome at entry, N (%)	14 (41)	11 (30)
Waist circumference $\geq$ 88 cm, N (%)	12 (35)	8 (22)
Triglycerides >150 mg/dl or hypolipemics, N (%)	22 (64)	24 (65)
HDL <50 mg/dl in women or <40 mg/dl in men, N (%)	21 (62)	24 (65)
Glucose $\geq$ 100 mg/dl, N (%)	8 (24)	4 (11)
Blood pressure $\geq$ 130/85 mm Hg or antihypertensives w/hx HTN, N (%)	15 (44)	11 (30)
Limb fat, g	4695.9 (3643.6, 7758.3)	5966.7 (3514.3, 8434.7)

^aSignificant differences between groups at baseline (*p* = 0.04).

TABLE 2. CHANGE IN CAROTID IMT AND FASTING METABOLIC PARAMETERS BY TREATMENT GROUP FROM BASELINE TO 48 WEEKS

Median (IQR)	Rosiglitazone (n = 30)	p (within group)	Placebo (n = 32)	p (within group)	p (between groups)
Carotid IMT CCA	+0.10 (−0.05, 0.25)	0.05	+0.15 (0, 0.25)	0.001	0.36
Carotid IMT ICA	+0.25 (0, 0.75)	0.03	+0.23 (−0.08, 0.54)	0.01	0.73
TNF- α , pg/ml	+1.1 (−3.3, 33.0)	0.27	+1.5 (−10.9, 16.7)	0.4	0.78
sTNFRI, pg/ml	+733.8 (318.5, 9891.3)	<0.001	+973.3 (373.3, 8662.9)	<0.001	0.98
sTNFRII, pg/ml	+1722.4 (872.9, 5951.9)	<0.001	+1586.9 (994.7, 5552.8)	<0.001	0.97
IL-6, pg/ml	+0.2 (−9.0, 34.2)	0.30	+1.5 (−5.2, 16.9)	0.29	0.92
hsCRP, mg/liter	−4.6 (−11.1, −0.9)	<0.001	−1.5 (−8.8, 0.18)	0.02	0.11
MPO, pg/ml	+1992.3 (−4865.7, 11,266.9)	0.46	+4482.0 (−8740.0, 9934.7)	0.44	0.88
vWF, U/ml	−5.9 (−21.4, −0.8)	<0.001	−8.0 (−16.9, −1.3)	<0.001	0.99
sICAM-1, ng/ml	−29.0 (−160.5, 131.8)	0.79	−17.4 (−203.7, 171.2)	0.67	0.77
sVCAM-1, ng/ml	+2150.1 (754.0, 3544.7)	<0.001	+2311.6 (941.2, 3675.1)	<0.001	0.67
Total cholesterol, mg/dl	+22 (−8.0, 37.0) (n = 27)	0.02	−8 (−23.0, 13.0) (n = 29)	0.22	0.008
TG, mg/dl	+19 (−32.0, 66.0) (n = 27)	0.46	−1 (−61.0, 24.0) (n = 29)	0.38	0.19
HDL, mg/dl	0 (−2.0, 8.0) (n = 27)	0.28	0 (−1.0, 3.0) (n = 29)	0.51	0.68
Non-HDL, mg/dl	+8 (−10.0, 32.0) (n = 27)	0.10	−2 (−17.0, 15.0) (n = 29)	0.38	0.05
Glucose, mg/dl	−2 (−9, 6)	0.30	−3 (−7, 9)	0.63	0.6
Insulin,	−4.0 (−7.0, 2.0)	0.01	−0.5 (−4.0, 5.5)	0.58	0.02
HOMA-IR	−0.8 (−1.9, 0.4)	0.01	−0.3 (−0.8, 1.4)	0.76	0.03
Metabolic syndrome at 48 weeks, N (%)	13 (43) (n = 30)		7 (23) n = 31		0.1
Waist circumference $\geq$ 88 cm at 48 weeks, N (%)	11 (37) (n = 30)		9 (31) (n = 31)		0.6
Triglycerides $>$ 150 mg/dl or hypolipemic	19 (63) (n = 30)		20 (63%) (n = 31)		1
HDL $<$ 50 mg/dl in women or $<$ 40 mg/dl in men	21 (70) (n = 30)		17 (53%) (n = 32)		0.2
Glucose $\geq$ 100 mg/dl	2 (7) (n = 30)		4 (13) (n = 32)		0.7
Blood pressure $\geq$ 130/85 mm Hg or antihypertensives w/hx HTN	13 (43)		12 (38)		0.7
SBP, mm Hg	−8 (−11, 4)	0.18	−1 (−14, 10)	0.30	0.82
DBP, mm Hg	−1 (−8, 8)	0.50	−1.5 (−10, 5)	0.26	0.76
Limb fat, g	+448 (138, 1670)	<0.001	+153 (−100, 682)	<0.03	0.02
BMI, kg/m ²	+0.9 (0.08, 1.6)	<0.001	+0.5 (−0.08, 1.3)	0.03	0.24
Waist-hip ratio	−0.003 (−0.020, 0.029)	0.97	0.008 (−0.024, 0.025)	0.83	0.96

for changes within groups. Two-sample tests were used to determine the significance for changes between groups. A p value of 0.05 was considered statistically significant. All analyses were carried out using SAS, v 9.2 (The SAS Institute, Cary, NC).

Results

Between July 2006 and December 2007, 71 subjects enrolled in this prospective study. The baseline characteristics by group are listed in Table 1. Total cholesterol was significantly ($p = 0.04$) higher at baseline in the placebo group. Overall, nine subjects were lost to follow-up (four on rosiglitazone). Of these, only one (in the rosiglitazone group) discontinued the study for a possible adverse event. This subject had exacerbation of prediagnosed documented coronary artery disease as manifested by increasing chest pain; he was receiving atazanavir ritonavir (ATV/r) and coformulated emtricitabine/tenofovir. Four patients (one on rosiglitazone) had grade 2 elevations of transaminases, which were felt to be secondary to underlying chronic hepatitis C and not study related. None of the subjects added or stopped protease inhibitors (PIs) or nonnucleoside reverse transcriptase inhibitors (NNRTIs). In the rosiglitazone group, one subject changed ART from nevirapine to efavirenz. In the placebo group, one subject switched from lopinavir/ritonavir (LPV/r) to ATV/r and another switched from ATV/r to LPV/r.

Carotid IMT

The common carotid artery (CCA) IMT values did not significantly differ between the rosiglitazone and placebo groups as detailed in Table 2 and Fig. 1. However, the changes in CCA IMT increased significantly ($p \leq 0.005$) from baseline to week 48 in both the rosiglitazone and placebo groups. In the rosiglitazone group the baseline median (IQR) CCA IMT was 1.25 (1.05, 1.56) mm and the median (IQR) increase was 0.10 (−0.05, 0.25) mm. In the placebo group the baseline CCA IMT was 1.33 (1.18, 1.45) mm and the median (IQR) increase was 0.15 (0, 0.25) mm. Similarly, the ICA IMT also increased significantly ($p < 0.05$) within both the rosiglitazone and placebo groups from baseline to week 48 (Fig. 2). The median

FIG. 1. Carotid IMT CCA by treatment group. (A) Rosiglitazone group. (B) Placebo group.

FIG. 2. Carotid IMT ICA by treatment group. (A) Rosiglitazone group. (B) Placebo group.

(IQR) ICA values were 1.35 (1.10, 1.70) mm at baseline and the median (IQR) increase was 0.25 (0, 0.75) mm in the rosiglitazone arm. In the placebo arm, the ICA value was 1.53 (1.14, 1.84) mm and the median (IQR) increase was 0.23 (−0.08, 0.54) mm. These changes were also not significantly different between the two groups.

Inflammatory cytokines and endothelial activation markers

From baseline to week 48, the hsCRP, sTNFR1 and II, sVCAM-1, and vWF levels all changed significantly ($p \leq 0.02$) within each group but were not significantly different between the two study arms as detailed in Table 2. For the markers sVCAM-1 and TNFR1 and II, there were significant (all $p \leq 0.006$) changes from baseline between 0 and 24 weeks in both the rosiglitazone and placebo arms. For the markers hsCRP and vWF, the changes occurred later, as there were no significant differences between 0 and 24 weeks. The MPO, sICAM-1, TNF- α , and IL-6 levels did not change within or between groups at 48 weeks.

We did not find statistically significant correlations between the changes in carotid ICA or CCA and changes in cardiovascular biomarkers except between the change in CCA IMT and TNF- α ($R = -0.49$, $p = 0.025$) in the rosiglitazone group. Additionally, we did not find statistically significant correlations between the changes in carotid ICA or CCA and those in BMI, insulin, or lipids.

Metabolic parameters

DEXA-measured limb fat increased significantly ($p = 0.02$) more in the rosiglitazone group compared with the placebo group median (IQR): +448 (138, 1670) and 153 (−100, 682). The median (IQR) change of HOMA-IR and insulin levels between the rosiglitazone arm and the placebo arm [−0.8 (−1.9, 0.4) μ U/ml vs. −0.3 (−0.8, 1.4) μ U/ml and −4.0 (−7.0, 2.0) μ U/ml vs. −0.5 (−4.0, 5.5) μ U/ml; $p = 0.03$ and 0.02] was significant at 48 weeks. The waist-hip ratio did not change between or within groups.

Six subjects (three on rosiglitazone) started a lipid-lowering agent during the study and were excluded from the lipid analysis. The only lipid parameter with a significant difference between the groups over 48 weeks was total cholesterol: median (IQR) change was 22 (−8.0, 37) mg/dl in the rosiglitazone group vs. −8 (−23, 13) mg/dl in the placebo group ($p = 0.008$). By 24 weeks of treatment, there was a significant ($p < 0.001$) difference between the groups as well as a significant ($p = 0.004$) within-group change only in the rosiglitazone group.

At baseline, 14/34 (41%) of subjects in the rosiglitazone group and 11/37 (30%) in the placebo group met the criteria for metabolic syndrome ($p = 0.1$). The frequency of metabolic syndrome did not change significantly from baseline to 48 weeks within either group (rosiglitazone group $p = 0.65$; placebo group $p = 0.26$). Of the individual components of the metabolic syndrome, the lipid criteria were the most frequently met (Tables 1 and 2). As expected, the frequency of subjects who met the glucose definition of metabolic syndrome in the rosiglitazone arm significantly ($p = 0.01$) decreased over the course of the study: 8/34 (24%) and 2/30 (7%) at 48 weeks. Otherwise, the frequency of the four other individual components of the metabolic syndrome remained unchanged over the study period. At week 48, there were no significant changes within or between groups in HIV parameters of CD4⁺ cell count or HIV-1 RNA levels. At both baseline and week 48, median HIV-1 RNA levels were <50 copies/ml. At baseline, three subjects had baseline HIV-1 RNA levels >400 copies/ml; only one subject had baseline HIV-1 RNA levels >400 copies/ml at 48 weeks.

Discussion

Several recent studies have highlighted the significance of CVD in the HIV-infected individuals.⁴ These studies suggest that the HIV-infected population is at risk of accelerated CVD. CVD in HIV remains a poorly understood complication, with a potential contribution from HIV infection, enhanced inflammation, and ART.

In addition to its role as an antidiabetic agent, rosiglitazone may potentially be used in nondiabetic HIV-infected individuals with HIV lipodystrophy.²⁶ A recent meta-analysis questioned an association between rosiglitazone and MI in diabetic patients²¹ raising concerns regarding the use of rosiglitazone. Subsequent studies failed to show this association.^{22,23} Even if there is an association between rosiglitazone and CVD in diabetics, this cannot be automatically translated to different populations, such as people living with HIV.

Although carotid IMT, inflammatory, and endothelial activation markers did not significantly differ between the rosiglitazone and placebo groups, we noted both ICA and CCA IMT values as well as the biomarkers hsCRP, TNFRI, TNRFRII, vWF, and sVCAM-1 changed significantly from baseline to week 48 within both arms. As some studies have suggested that ICA may be a better surrogate,^{30,31} we evaluated separately both carotid IMT CCA and ICA. A prior study has reported that carotid IMT progresses more rapidly in HIV-infected compared with HIV-negative individuals.³² This observation may have been related in part to lack of matching of HIV-infected cases to HIV-negative controls as, for example, smoking was significantly ($p < 0.01$) more prevalent in the HIV-infected individuals in that study. Our

study similarly found that carotid IMT significantly increased longitudinally over 48 weeks regardless of rosiglitazone treatment assignment.

Several inflammation markers (hsCRP, sTNFRI, sTNFRII) and the endothelial activation markers (sVCAM-1 and vWF) did not differ significantly between the arms but changed significantly in both arms from baseline to 48 weeks. This finding is consistent with results of prior studies.^{4–6} Two of these prior studies suggest that the increased immune activation and enhanced inflammatory state are related to uncontrolled viremia. The first trial reported a rapid improvement of endothelial function after initiation of ART,⁵ with HIV-1 RNA levels being the only factor associated with improvement of brachial artery flow-mediated dilation. The other trial reported an increased risk of incident CVD in subjects with intermittent CD4⁺ cell count-driven ART interruption when compared with those who were on continuous ART.⁴ Also, hsCRP, IL-6, and sVCAM-1 decreased after initiation of ART.¹³

An interesting and novel finding in our trial, however, is that sTNF receptors and endothelial markers increased significantly from baseline to week 48 in the setting of continuous and stable ART and suppressed viremia. The worsening over time of these markers happened in the setting of unchanged smoking status and BMI. Also, these markers worsened over time in the face of improvements in insulin resistance and lipodystrophy, improvements that should have led to decreased CVD risk. Our finding of worsening IMT and marker levels over 48 weeks may be related to worsening lipid parameters over this same time period. However, this is unlikely as we found no correlation between IMT and total or non-HDL cholesterol changes. Additionally, the lipid values decreased or stayed the same in the placebo group, which would not explain the observed worsening IMT and biomarkers in the placebo group.

We acknowledge the limitations to this study, mainly the relatively small sample size and the fact that the surrogate markers of CVD presented here were secondary endpoints of a trial that primarily aimed to study and that showed that rosiglitazone increased limb fat in HIV-infected individuals. Thus, although our data do not support that rosiglitazone independently worsens carotid IMT or endothelial markers, we cannot completely preclude a smaller effect on CV events. Although the sample size was relatively small, the markers were assessed in a randomized trial over 48 weeks with consistent outcomes. The present cardiovascular substudy provides the first report of the CVD effect of rosiglitazone in HIV and the first observation of significant increases in sTNF receptors and endothelial markers in the setting of continuous and stable ART and suppressed viremia.

In summary, in our study of HIV-infected patients with good virological control and established lipodystrophy, rosiglitazone did not independently increase carotid IMT, endothelial activation, and inflammatory cytokines. However, in both study arms, carotid IMT and several of the inflammation and endothelial biomarkers worsened after 48 weeks, consistent with worsening CVD risk. It is important to note that this worsening CVD risk happened in the setting of stable ART, continuous virological control, stable CD4⁺ cell count, and improvement of lipodystrophy and insulin resistance (results presented elsewhere).²⁶ Additional studies are needed to evaluate cardiovascular disease risks in HIV-infected subjects

and the biochemical characterization of the underlying mechanisms.

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