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Endothelial Function in HIV-Infected Antiretroviral Naïve Subjects Before and After Starting Potent Antiretroviral Therapy: AIDS Clinical Trials Group Study 5152s

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Abstract

Objectives—This study evaluated the effects of three class-sparing antiretroviral therapy (ART) regimens on endothelial function in HIV-infected subjects participating in a randomized trial.

Background—Endothelial dysfunction has been observed in patients receiving ART for human immunodeficiency virus (HIV) infection.

Methods—This was a prospective, multicenter study of treatment-naïve subjects who were randomly assigned to receive a protease inhibitor-sparing regimen of nucleoside reverse transcriptase inhibitors (NRTIs) + efavirenz, a non-nucleoside reverse transcriptase inhibitor-sparing regimen of NRTIs + lopinavir/ritonavir, or a NRTI-sparing regimen of efavirenz + lopinavir/ritonavir. NRTIs were lamivudine + stavudine, zidovudine, or tenofovir. Brachial artery flow-mediated dilation (FMD) was determined by B-mode ultrasound before starting on ART, then after 4 and 24 weeks.

Results—There were 82 subjects (median age 35 years, 91% men, 54% white). Baseline CD4 cell counts and plasma HIV RNA values were 245 cells/mm^3 and 4.8 $\log_{10}$ copies/ml, respectively. At baseline, FMD was 3.68% (interquartile range 1.98 – 5.51%). After 4 and 24 weeks of ART, plasma HIV RNA decreased by 2.1 and 3.0 $\log_{10}$ copies/mL, respectively. FMD increased by 0.74% (−0.62 – +2.74, p=0.003) and 1.48% (−0.20 – +4.30%, p< 0.001), respectively, with similar changes in each arm (p_{KW}>0.600). The decrease in plasma HIV RNA at 24 weeks was associated with greater FMD (r_s=− 0.30, p=0.017).

Conclusions—Among treatment-naïve individuals with HIV, three different ART regimens rapidly improved endothelial function. Benefits were similar for all ART regimens, appeared quickly, and persisted at 24 weeks.

Condensed Abstract—Among 82 treatment-naïve HIV-infected subjects participating in a prospective, multicenter study of three class-sparing antiretroviral therapy regimens, flow-mediated dilation of the brachial artery improved after 4 (+0.74%, p=0.003) and 24 weeks (+1.48%, p< 0.001), with similar changes in each arm (p_{KW}>0.600).

Keywords

Antiretroviral therapy; Cardiovascular disease risk; Endothelial function; Human immunodeficiency virus

Introduction

Since the advent of effective antiretroviral therapy (ART), survival of patients with human immunodeficiency virus (HIV) infection has improved dramatically (1). In observational studies, however, use of ART has been associated with increased cardiovascular disease (CVD) risk (2–4). The observed increase in CVD risk in patients receiving ART appears to be related, in part, to the effects of certain components of ART on CVD risk factors; however, direct effects of ART on the vasculature also may contribute to this problem. Endothelial dysfunction is a key step in atherogenesis that contributes to the initiation, perpetuation, and clinical manifestations of atherosclerosis (5). Abnormal endothelial function has been implicated in early atherogenesis, control of dynamic plaque behavior, and predicts the future occurrence of CVD events (5,6). Although use of HIV protease inhibitors (PIs) has been associated with

endothelial dysfunction (7,8), other components of ART such as nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) also can affect CVD risk factors, and the CVD effects of treating viremia are not well-understood. Furthermore, the effects of newer ART regimens on arterial function, and the mechanisms by which ART, viremia, and risk factors interact to affect CVD risk are unknown. The purpose of this multicenter, prospective study was to evaluate the effects of three class-sparing ART regimens on endothelial function in treatment-naïve HIV-infected subjects participating in a large, prospective, randomized trial.

Methods

Study Design

AIDS Clinical Trials Group (ACTG) Study 5152s is a sub-study of ACTG 5142, a prospective, multicenter randomized clinical trial that investigated time to virologic failure among ART-naïve subjects who were randomly assigned to receive one of three ART-sparing regimens: (i) a protease inhibitor (PI)-sparing regimen of NRTIs plus the NNRTI efavirenz, (ii) a NNRTI-sparing regimen of NRTIs + the PI lopinavir/ritonavir, or (iii) a NRTI-sparing regimen of efavirenz + lopinavir/ritonavir. The NRTIs prescribed in this study were lamivudine plus stavudine (extended release), zidovudine or tenofovir. NRTI use was investigator-selected prior to randomization and was a stratification factor in the parent study. The purpose of major inclusion criteria included HIV-1 infection, and plasma HIV RNA >2.0 $\log_{10}$ copies/mL. Major exclusion criteria included prior use of ART, known cardiovascular disease, diabetes mellitus, and current (within 6 weeks of enrollment) use of lipid-lowering medications, antioxidant vitamin supplements, or hormones at greater than replacement doses. Pharmacological treatment of diabetes mellitus, dyslipidemia, and changes in doses of angiotensin-converting enzyme inhibitors were not permitted during the study. Subjects participating in ACTG 5142 were recruited consecutively from six sites in the United States. Study procedures were performed at baseline and then after 4 and 24 weeks. Laboratory testing was performed at ACTG Central Metabolic Laboratory (Quest Diagnostics, Baltimore, MD). Adiponectin was measured at Northwestern University (Chicago, IL). Treatment arms were blinded and coded until completion and presentation of A5142 in August, 2006 (9).

Evaluation of Endothelial Function

Endothelial function was evaluated by measuring flow-mediated dilation (FMD) of the brachial artery (7,10,11). Brachial artery reactivity studies were performed on the same day as phlebotomy. Subjects were required to be fasting and not use any tobacco-containing products for 8 hours prior to the study. Subjects who were not fasting or who admitted to smoking had their test re-scheduled. Subjects were placed in a supine position in a temperature-controlled room for 10 minutes prior to imaging. A blood pressure cuff was placed on the widest part of proximal right forearm approximately 1 cm distal to the antecubital fossa. The arm was extended 90° from the thorax and placed on an arm board with the elbow positioned downwards and the hand rotated so thumb pointed towards the ceiling. Using a high resolution (≥7 MHz) linear array vascular ultrasound transducer, the brachial artery was located above the elbow and scanned in longitudinal sections with the focus zone set to the depth of the far wall. Time-gain-compensation and overall gain settings were used to optimize images of the lumen/arterial wall interface. Extra-vascular landmarks in each subject were identified and labeled to assure that the imaged segment of the brachial artery was reproduced within and between studies. After recording baseline B-mode images of the brachial artery and spectral Doppler images of flow, the forearm cuff was inflated to 250 mmHg for 4.5 minutes to induce reactive hyperemia. Immediately after deflation, spectral Doppler images were obtained to verify hyperemia. FMD of the brachial artery was measured 1 minute after cuff deflation. After a 15-minute rest period, nitroglycerin-mediated vasodilation (NTGMD) (a marker of endothelium-independent

vasodilation) was measured 3 minutes after administration of sublingual nitroglycerin (400 mcg). FMD was calculated as the ratio of the brachial artery diameter after reactive hyperemia to the baseline diameter and was expressed as a percentage change. NTGMD was calculated in an analogous fashion. Each study was recorded digitally and sent to a core ultrasound laboratory. BA diameters were measured in triplicate with a digital border tracing tool (Access Point 2004, Freeland Systems, Westfield, IN). Measurements were performed by a single reader blinded to subject information and treatment. Using this technique, the median (interquartile range [IQR]) FMD at the core lab is 5.5% (3.7–7.8%), based on studies from 152 non-diabetic individuals without HIV infection (median age 25 years, 25% current smokers, 39% males) performed from May, 2002 through May, 2005. Among 20 non-diabetic HIV-infected individuals on stable PI-containing ART (median age 43 years, 30% current smokers, 90% males), the median FMD at the core lab was 4.9% (3.3–9.1%), based on studies performed from January, 2001 through August 2002.

Several measures were used to minimize inter- and intra-site variability. All sonographers completed a two-day training course at the core ultrasound laboratory using the standards set by the American Society of Echocardiography (12). Following on-site training, each sonographer was required to submit a minimum of three paired mock studies to demonstrate adherence to the study protocol and consistent display of high-quality images and reproducibility of landmarks. Prior to study initiation, ultrasound equipment at each laboratory was evaluated and calibrated using a small parts ultrasound phantom. To evaluate scanning variability, the first 20 subjects that completed week 24 underwent a repeat scan on a separate day within 21 days of the initial 24 week scan. To evaluate reader variability, all scans (week 0, 4, 24, and 26) from the first 15 subjects that completed the study were re-read, blinded to the original reading.

Data Analysis

The primary objective was to compare brachial artery FMD before and after ART within each of the treatment arms. Secondary objectives were to compare changes in brachial artery FMD between arms. Additional objectives were to evaluate the relationships between levels of CD4 cells, plasma HIV-1 RNA, lipids, inflammatory markers, and markers of insulin sensitivity and their changes to FMD and changes in FMD after 4 and 24 weeks. Randomization in the parent study used a permuted-blocks design stratified on screening plasma HIV-1 RNA, choice of NRTI and the presence of active hepatitis infection.

All values are reported as medians (IQRs), unless noted otherwise. Changes in brachial artery FMD measurements and laboratory tests are reported as absolute changes from baseline. The Wilcoxon Signed Rank test was used to assess within-arm changes in FMD and laboratory tests. The Kruskal-Wallis, Wilcoxon Rank Sum, or Fisher's Exact tests were used for between-arm comparisons at specific time points. Spearman correlations were used to evaluate relationships between pairs of continuous variables and were not adjusted for multiple comparisons. For participants who started lipid-lowering agents while on study, results after the start of lipid lowering agents were excluded. Correcting for non-parametric testing and an expected 15% loss-to-follow-up, 25 subjects were needed per arm to achieve 80% power to detect an absolute FMD change of 4% (standard deviation 6%) over 24 weeks with an alpha of 0.05.

Results

Between October, 2002 and December, 2004, 82 treatment-naïve individuals were enrolled from six institutions: 23 subjects were randomized to the PI-sparing arm, 31 to the NNRTI-sparing arm, and 28 to the NRTI-sparing arm in the parent study.

Baseline Characteristics

Baseline characteristics are shown in Table 1. Subjects were well-matched across groups. The median age was 35 (30–40) years, 91% were male, 54% were white, 32% black or Asian, and 15% Hispanic. HIV disease characteristics were similar between groups prior to starting ART. The baseline median (interquartile range) CD4+ cell count was 245 (119 – 356) cells/mm^3 and plasma HIV RNA was 4.8 (4.49 – 5.32) log_{10} copies/mL. Baseline lipid, glucose homeostasis and inflammatory markers were similar in each arm prior to starting ART (Table 2). High-density lipoprotein cholesterol values were similarly low in each arm; however, other median lab values were within their normal ranges. Of the 54 individuals that received NRTIs, 9 (16.7%) received stavudine, 27 (50%) received tenofovir, and 18 (33.3%) received zidovudine with a similar distribution between the arms (p=0.460).

Changes in Laboratory Tests and Other Measurements

After 4 and 24 weeks of ART, HIV RNA decreased by 2.0 (−2.4 – −1.7) and 3.0 (− 3.5 – −2.7) log_{10} copies/mL (p<0.01 for each), respectively, and to a similar extent in each arm. After 24 weeks, 67% of participants had plasma HIV RNA levels below the limit of detection (<50 copies/mL) and 18% had plasma HIV RNA levels between 50 and 100 copies/mL. Of the participants with FMD results at both weeks 0 and 24, only 7% had plasma HIV RNA levels >100 copies/mL at week 24. The increase in CD4 count of 155 (95 – 198) cells/mm^3 after 24 weeks (p<0.01) was similar in each arm (Table 3). After 24 weeks, there was a decrease in heart rate (−2 [−7 – +2] bpm, p=0.023) that was similar in each arm (p_{KW}=0.327), an increase in body-mass index (0.5 [−0.5 – +1.9] kg/m2, p<0.01) that also was similar in each arm (p_{KW}=0.682). Systolic blood pressure did not change over time.

At 4 and 24 weeks, all lipid values increased (p<0.01), but there were differences between arms (Table 3). The increase in total cholesterol in the NRTI-sparing arm was higher than in the other two arms (p≤0.003) at both time points. The increase in high-density lipoprotein cholesterol in the NRTI-sparing arm was higher than in the NNRTI-sparing arm (p=0.015) after 24 weeks. The increase in low-density lipoprotein cholesterol in the NRTI-sparing arm was higher than in the other two arms (p≤0.018) at both time points. The increase in triglycerides was greater in the NRTI-sparing arm than the PI-sparing arm (p≤0.016) at both time points. The total/high-density lipoprotein cholesterol ratio decreased more in the PI-sparing arm than in the NRTI-sparing arm at 4 and 24 weeks (p≤0.005). After 24 weeks, total/high-density lipoprotein cholesterol ratio also decreased more in the PI-sparing arm than in the NNRTI-sparing arm (p=0.043). There was a significant increase in lipoprotein(a) at weeks 4 and 24 (p<0.01) that was similar in each arm.

After 24 weeks, glucose increased in the PI-sparing and NRTI-sparing arms (p=0.040). The differences between the three arms were significant (p_{KW}=0.040) with less increase in glucose in the NNRTI-sparing arm than in each of the other two arms (p≤0.042). Significant between-arms differences in insulin were seen at 4 but not 24 weeks due to higher values in the NRTI-sparing arm p≤0.018). Adiponectin levels decreased in the NRTI-sparing arm after 24 weeks (p=0.040); however, this change was not significantly different than observed in the other arms (p_{KW}=0.442). C-reactive protein increased after 4 weeks (p<0.01) without differences between arms, but differences from baseline after 24 weeks were not significant (p=0.810).

Brachial Artery Reactivity Studies

Technically usable brachial reactivity scans were obtained from 75 subjects at baseline (Table 4). The resting brachial artery diameter was similar in each arm (4.35 [3.85 – 4.63] mm, p_{KW}=0.186). Before starting ART, FMD was similar in each arm (3.68% [1.9 – 5.51%], p_{KW}=0.815), as was NTGMD (14.7% [11.5–17.6%], p_{KW}=0.132). After 4 weeks of ART, the resting brachial artery diameter increased by 0.03 mm (−0.02 – +0.12 mm, p=0.003) from

baseline, with no difference between arms (p_{KW}=0.648). Despite the increase in resting brachial diameter, after 4 weeks of ART, FMD increased significantly (0.74% [−0.62 – +2.74%], p≤ 0.01), with no difference between arms (p_{KW}=0.609). After 24 weeks of ART, the resting brachial artery diameter increased by 0.06 mm (−0.03 – +0.19 mm, p=0.002) from baseline, but there was a difference between arms (p_{KW}=0.002), as significant resting diameter increases were observed in the PI-sparing (p<0.001) and NNRTI-sparing arms (p=0.024), but not the NRTI-sparing arm (p=0.498). Despite the increases in baseline brachial diameter, after 24 weeks of ART, FMD increased significantly (1.48% [−0.20 – +4.30%], p<0.001). The improvement in each arm was of a similar magnitude (p_{KW}=0.778). There was no significant difference between treatment groups in FMD, even after adjustment for changes in brachial artery diameter and the slight decrease in heart rate observed at week 24 (p_{adj}=0.935) (Figure 1). Reactive hyperemic flow was similar in each arm at each week (Table 4). There was a small, statistically significant increase in the time-velocity integral of reactive hyperemic flow after 24 weeks (p=0.01), with a significant increase in the NRTI-sparing arm (p<0.01), however there was no significant difference between treatment groups in FMD after adjustment for reactive hyperemia flow (p_{adj}=0.802).

Of note, the 4 subjects not included in the week 24 FMD analyses of the NRTI-sparing arm had the highest baseline FMD values, an observation that explains why the absolute FMD values in this arm were lower despite a median increase in FMD. Two participants randomized to the NRTI-sparing arm and two participants in the NNRTI-sparing arm were excluded from week 24 because they started lipid-lowering medications between weeks 4 and 24. Additionally, 1 participant each from the NRTI-sparing arm and the PI-sparing arm were excluded from week 24 for non-compliance with ART.

There were no within- or between-arms changes in NTGMD after 4 (p=0.431, p_{KW}=0.560) or 24 weeks (p=0.363, p_{KW}=0.142). Neither CD4 cell counts nor log RNA were significantly correlated with FMD at baseline. There was a weak correlation between triglycerides and FMD at baseline (r_s=0.24, p=0.043) but not at 24 weeks. Significant correlations between FMD and the other variables in Table 1 and Table 2 were not observed at baseline. The change in FMD from baseline to week 24 correlated inversely with the change in log RNA (r_s=−0.30, p=0.017) and positively with adiponectin (r_s=0.26, p=0.041). Significant correlations between changes in FMD and changes in the other variables in Table 1 and Table 2 were not observed after 24 weeks.

In the 20 subjects who underwent repeat scans within 16 days of the week 24 scan, the difference in FMD was only 0.26% (−0.43 – +0.72%, p=0.498). The difference in NTGMD also was small (0.66% [−3.51 – +1.09%], p=0.599). Blinded re-reading of 57 scans revealed strong correlations of 0.97–0.99 (p<0.001) and small median differences of −0.14 – +0.09% at each week. There were no outliers on visual inspection limits of agreement analyses (13).

Discussion

This is the first study of HIV-infected patients where assignment to ART was randomized and a validated marker of arterial function was followed prospectively. Patients with HIV-infection who met criteria for starting ART experienced a rapid improvement in endothelial function. The effect of ART on brachial artery FMD was of similar magnitude for all three ART regimens, appeared after 4 weeks, and persisted at 24 weeks. It persisted after adjustment for the changes in heart rate, brachial artery diameter, and reactive hyperemia observed with ART.

Our findings of improved endothelial function with ART appear to contradict some cross-sectional studies in which use of ART-containing regimens was associated with endothelial dysfunction (7,14,15), a study demonstrating that administration of a PI (indinavir) impaired

endothelial function in healthy men (8) and several studies that identified cellular or molecular mechanisms of endothelial dysfunction associated with use of PIs (16–18). Other investigators, however, have variably found that viral load, CD4 cell count, cardiovascular risk factors, injection drug use, and alcoholism were associated with impaired arterial function, rather than ART (19–22). Although several observational studies have demonstrated that ART and specifically use of PI-containing regimens are associated with increased CVD risk (2–4), short-term decreases in CVD risk and overall mortality have been observed after initiation of ART (23), and the recent SMART study demonstrated that total mortality and possibly CVD risk are increased when ART is discontinued or HIV replication is not suppressed (24). This prospective study helps shed light on this apparent contradictory literature by demonstrating a rapid, consistent, and reproducible improvement in FMD of the brachial artery in three randomly assigned class-sparing ART regimens that was sustained over 24 weeks. In this study, FMD improved despite increases in total cholesterol, low-density lipoprotein cholesterol, triglycerides, and lipoprotein(a); however, high-density lipoprotein cholesterol improved with ART. Changes in an overall measure of lipid change - the total/high-density lipoprotein cholesterol ratio - were small and similar in each arm. None of these parameters, or changes in levels of traditional or inflammatory markers of cardiovascular risk, correlated significantly with changes in FMD. Some studies suggest that HIV infection may directly or indirectly impair arterial function (25–27). HIV has been shown to infect endothelial cells, patients with HIV infection have increased vascular cell adhesion molecule -1 and E-selectin expression from aortic endothelium, and increased circulating markers of endothelial activation (25–27). Although the change in HIV viral load had the strongest association with the change in FMD, the correlation was modest (r_s=−0.30). This may be explained by the robust and homogeneous response of our subjects to each of the ART regimens with 67% of participants having plasma HIV RNA levels below the limit of detection after 24 weeks. This study could not determine if the inverse association between viral load and FMD was due to a direct adverse effect of viremia, a marker of receiving effective treatment, or even a chance finding, since HIV viral load was not associated with the degree of endothelial dysfunction at baseline. The correlation between changes in adiponectin and FMD was of marginal statistical significance, and is difficult to interpret in the absence of changes in glucose, insulin, or body-mass index.

Limitations

Although our study provides further support for the hypothesis that control of HIV replication initially improves endothelial function, our study had only 24 weeks of follow-up. Prolonged and uncorrected lipid abnormalities following ART ultimately may lead to deterioration of endothelial function, and thus to higher cardiovascular risk in the longer term (2–4,7). Also, the effects of ART on arterial function in previously treated patients, or in those whose viremia is less suppressed are not known. Another limitation is that randomization was performed in the larger, parent study (ACTG 5142) prior to voluntary enrollment in our sub-study. While we believe this minimized treatment bias, this study was not, in the strictest sense, a randomized clinical trial. This study was designed to detect differences in FMD from baseline through 24 weeks within the treatment arms, not to detect between-group differences. The absence of differences between groups should be interpreted cautiously, especially in regard to FMD. Although this study was relatively large in regard to the longitudinal assessment of changes in FMD, it was underpowered to detect correlations <0.35 between changes in the parameters studied and changes in FMD.

This study did not have a control group, so the possibility that regression to the mean contributed to some of the improvement in FMD cannot be excluded. Because it would be unethical to withhold ART from individuals who require treatment, it would not have been possible to have had a HIV-infected, untreated control group. Using a group of HIV-infected patients who do not require ART would not be appropriate due to differences in the stage of

HIV disease. It also would not be ethical to treat HIV-negative individuals with ART for 24 weeks, given the possible toxicities of these medications. To the extent that measurement variability can contribute to regression to the mean, we have demonstrated highly reproducible measurements of FMD and NTGMD at all time points. Biological variability cannot be excluded; however the consistent, incremental improvements in FMD seen in each arm, at each time point, and their magnitude suggest that the observations in this study were related to ART.

Conclusions

Patients with HIV-infection who met criteria for starting ART had impaired endothelial function. Use of 3 different ART regimens rapidly improved endothelial function in treatment-naïve patients with HIV infection. Improvements were similar for all ART regimens, appeared quickly, and persisted over 24 weeks. It is unclear if the improvements were due to ART, suppression of viremia, or changes in factors not measured in this study, such as immune activation or biological variation. Larger, prospective, controlled studies of longer duration are needed to determine the long-term effects of ART on endothelial function and ultimately on atherosclerosis and CVD events.

Abbreviations

ACTG, AIDS Clinical Trials Group; ART, antiretroviral therapy; CVD, cardiovascular disease; FMD, flow-mediated dilation; HIV, human immunodeficiency virus; NNRTI, non-nucleoside reverse transcriptase inhibitors; NRTI, nucleoside reverse transcriptase inhibitors; NTGMD, nitroglycerin-mediated vasodilation; PI, protease inhibitor.

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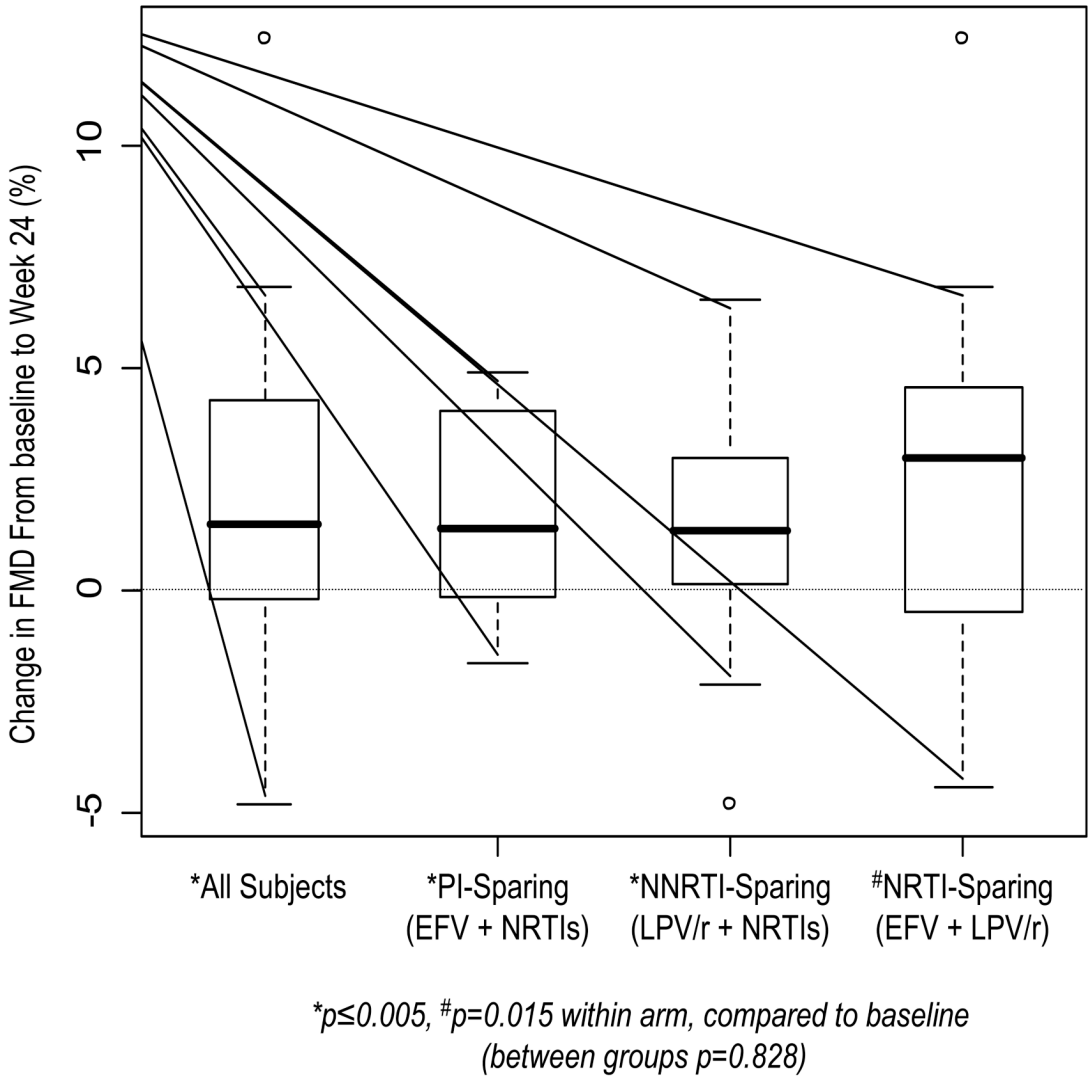

Figure 1.
Changes in Brachial Artery Flow-Mediated Vasodilation from Baseline to Week 24
Thick bar = medians
Box edges = 25th and 75th percentiles
Error bars = 95% confidence intervals

Table 1

Baseline Subject Characteristics

	All	PI-Sparing	NNRTI-Sparing	NRTI-Sparing	P_{KW}
N	82	23	31	28	
Age, years	35 (30 – 40)	35 (31 – 40)	36 (30 – 41)	35 (29 – 42)	0.664
Male, %	91	91	94	89	0.887*
Current Smoker, %	44	43	39	50	0.694*
Heart rate, bpm	69 (62 – 75)	72 (68 – 75)	70 (61 – 80)	67 (61 – 72)	0.238
Body-mass index, kg/m^2	25.1 (22.8 – 27.7)	23.9 (22.0 – 26.7)	26.0 (23.8 – 28.5)	25.0 (22.7 – 27.7)	0.157
Systolic blood pressure, mmHg	119 (112 – 130)	115 (108 – 121)	120 (112 – 137)	120 (113 – 137)	0.119
Diastolic blood pressure, mmHg	74 (66 – 82)	71 (66 – 78)	74 (67 – 84)	78 (66 – 85)	0.459
CD4, cells/μL	245 (119 – 356)	237 (97 – 413)	239 (150 – 333)	251 (127 – 369)	0.958
HIV RNA, log_{10}copies/mL	4.82 (4.49 – 5.32)	4.72 (4.43 – 5.33)	4.89 (4.57 – 5.33)	4.83 (4.44 – 5.32)	0.684

All values are median (interquartile ranges), unless otherwise noted

*Fisher's Exact test

KW = Kruskal-Wallis test: comparison between arms, unless otherwise noted

NNRTI = non-nucleoside reverse transcriptase inhibitor

NRTI = nucleoside reverse transcriptase inhibitor

PI = protease inhibitor

Table 2

Baseline Laboratory Values

	All	PI-Sparing	NNRTI-Sparing	NRTI-Sparing	P_{KW}
Total cholesterol, mg/dL	144 (128 – 163)	145 (130 – 155)	144 (121 – 169)	144 (132 – 163)	0.979
HDL cholesterol, mg/dL	31 (26 – 40)	29 (25 – 39)	32 (28 – 41)	36 (27 – 42)	0.565
Direct LDL cholesterol, mg/dL	89 (75 – 103)	93 (75 – 100)	88 (75 – 111)	86 (73 – 106)	0.936
Triglycerides, mg/dL	113 (90 – 178)	138 (89 – 240)	108 (88 – 145)	114 (90 – 187)	0.562
Total/HDL cholesterol ratio	4.42 (3.79 – 6.90)	4.84 (3.60 – 5.76)	4.35 (3.79 – 5.60)	4.34 (3.89 – 5.26)	0.645
Lipoprotein (a), mg/dL	18 (12 – 74)	24 (13 – 79)	22 (13 – 50)	16 (12 – 38)	0.603
Glucose, mg/mL	86 (80 – 94)	88 (79 – 94)	84 (80 – 94)	86 (80 – 92)	0.981
Insulin, μU/mL	6 (4 – 10)	7 (5 – 10)	6 (3 – 11)	7 (5 – 13)	0.541
Adiponectin, mg/dL	4.2 (3.5 – 5.1)	4.0 (3.4 – 5.2)	4.3 (3.2 – 4.8)	4.1 (3.6 – 5.4)	0.849
High-sensitivity C-reactive protein, mg/L	1.3 (0.7 – 2.9)	1.2 (0.6 – 2.2)	1.6 (0.7 – 5.2)	1.3 (0.5 – 2.8)	0.351

All values are median (interquartile ranges)

HDL = high-density lipoprotein

LDL = low-density lipoprotein

KW = Kruskal-Wallis test: comparison between arms

NNRTI = non-nucleoside reverse transcriptase inhibitor

NRTI = nucleoside reverse transcriptase inhibitor

PI = protease inhibitor

Table 3

Changes in Laboratory Tests after 4 and 24 weeks of Antiretroviral Therapy

	All	PI-Sparing	NNRTI-Sparing	NRTI-Sparing	P_{KW}
HIV RNA, log_{10}copies/mL					
At week 4	−2.0* (−2.4 – −1.7)	−2.0* (−2.4 – −1.8)	−1.9* (−2.3 – −1.7)	−2.0* (−2.5 – −1.6)	0.635
At week 24	−3.0* (−3.5 – −2.7)	−2.8* (−3.3 – −2.7)	−3.2* (−3.5 – −2.7)	−3.0* (−3.6 – −2.6)	0.733
CD4+ counts, cells/mm^3					
At week 24	155*> (95 – 198)	152* (106 – 185)	168* (93 – 208)	154* (81 – 220)	0.828
Total cholesterol, mg/dL					
At week 4	30* (6 – 58)	19* (3 – 32)	15# (−9 – +41)	61* (35 – 102)	<0.001
At week 24	27* (8 – 67)	18* (3 – 29)	21* (6 – 57)	65* (32 – 108)	<0.001
HDL-C cholesterol, mg/dL					
At week 4	4* (0 – 7)	4# (0 – 10)	2 (−2 – +8)	5* (2 – 6)	0.344
At week 24	9* (2 – 14	9* (5 – 15))	3# (−2 – +13)	11* (7 – 17)	0.053
Direct LDL Cholesterol, mg/dL					
At week 4	8* (−4 – +23)	7# (−5 – +19)	1 (−12 – +8)	23* (10 – 33)	<0.001
At week 24	10* (−3 – +31)	6 (−5 – +24)	7 (8 – 19)	26* (11 – 54)	<0.001
Triglycerides, mg/dL					
At week 4	46* (7 – 141)	15 (−36 – 38)	60* (9 – 126)	117* (46 – 280)	0.003
At week 24	44* (−4 – +126)	22 (−49 – +79)	72* (−2 – +186)	83* (11 – 164)	0.051
Total/HDL cholesterol ratio					
At week 4	0.35# (−0.51 – +1.19)	−0.34 (−0.93 – +0.15)	0.29 (−0.68 – +1.19)	1.00* (0.35 – 2.34)	<0.001
At week 24	−0.28 (−0.75 – +0.88)	−0.58* (−1.64 – −0.02)	0.02 (−0.99 – +1.29)	0.01 (−0.51 – +1.43)	0.017
Lipoprotein (a), mg/dL					
At week 4	2* (0–30)	1# (0–39)	2* (0 – 28)	5* (1 – 31)	0.680
At week 24	5* (0–33)	3# (0–20)	4* (0 – 28)	7* (2 – 41)	0.309
Glucose, mg/mL					
At week 4	3 (−3 – +9)	5# (1 – 11)	1 (−9 – +9)	3 (−2 – 8)	0.360
At week 24	2 (−4 – +8)	4# (0 – 9)	−1 (−6 – +3)	5# (−3 – +12)	0.040
Insulin, μU/mL					
At week 4	0 (−3 – +2)	0 (−2 – +5)	1 (−1 – +2)	−1# (−3 – 0)	0.010
At week 24	0 (−2 – +3)	1 (−2 – +3)	1 (−1 – +3)	−1 (−2 – +2)	0.236
Adiponectin, mg/dL					
At week 4	−0.2	−0.4	−0.2	0	0.992

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	All	PI-Sparing	NNRTI-Sparing	NRTI-Sparing	P_{KW}
	(−1.2 – +0.8)	(−1.1 – +0.6)	(−1.0 – +0.3)	(−1.8 – +1.3)	
At week 24	−0.4	−0.2	−0.1	−0.7#	0.442
	(−1.4 – +0.8)	(−2.2 – +0.7)	(−1.0 – +1.2)	(−1.4 – +0.1)	
High-sensitivity C-reactive protein, mg/L					
At week 4	0.8*	1.0#	0	1.6*	0.138
	(−0.4 – +2.7)	(−0.4 – +2.3)	(−0.7 – +2.2)	(0.1 – 4.0)	
At week 24	−0.1	0	−0.6	0.1	0.234
	(−1.4 – +1.1)	(−0.4 – +0.5)	(−2.0 – +1.0)	(−0.8 – +2.5)	

All values are median (interquartile ranges)

*p<0.01 compared to baseline, Wilcoxon signed rank probability test

#0.01≤p<0.05 compared to baseline, Wilcoxon signed rank probability test

HDL = high-density lipoprotein

KW = Kruskal-Wallis test: comparison between arms

LDL = low-density lipoprotein

NNRTI = non-nucleoside reverse transcriptase inhibitor

NRTI = nucleoside reverse transcriptase inhibitor

PI = protease inhibitor

Table 4

Ultrasound Measurements at Baseline and After 4 and 24 weeks of Antiretroviral Therapy

	All	PI-Sparing	NNRTI-Sparing	NRTI-Sparing	P_{KW}
Baseline					
N_{FMD}	75	21	27	27	-
Brachial artery diameter (cm)	0.44 (0.39–0.46)	0.42 (0.38–0.45)	0.45 (0.41–0.46)	0.45 (0.38–0.48)	0.186
Reactive hyperemia velocitytime integral (cm)	72.6 (57.8–82.4)	72.3 (59.1–75.5)	74.0 (54.7–83.9)	73.7 (55.4–83.4)	0.537
FMD (%)	3.68 (1.98–5.51)	3.33 (2.11–5.10)	3.68 (1.91–6.11)	4.02 (2.37–6.20)	0.815
NTGMD (%)	14.71 (11.51–17.57)	15.46 (14.53–18.33)	14.48 (10.88–17.59)	13.02 (10.94–16.85)	0.132
Week 4					
N_{FMD}	65	20	21	24	-
Brachial artery diameter (cm)	0.45 (0.39–0.47)	0.43 (0.38–0.46)	0.46 (0.40–0.48)	0.45 (0.41–0.47)	0.291
Reactive hyperemia velocity time integral (cm)	76.1 (62.3–90.2)	71.7 (60.0–89.1)	76.3 (62.2–89.2)	75.9 (67.8–95.5)	0.517
FMD (%)	4.69 (2.87–6.12)	4.95 (2.10–5.82)	4.69 (2.34–7.37)	4.49 (3.12–7.03)	0.759
NTGMD (%)	15.32 (11.50–19.44)	15.95 (14.06–19.19)	13.72 (10.93–22.90)	15.32 (11.60–19.21)	0.833
Week 24					
N_{FMD}	67	20	24	23	-
Brachial artery diameter (cm)	0.45 (0.41–0.47)	0.44 (0.41–0.46)	0.45 (0.40–0.49)	0.46 (0.43–0.47)	0.317
Reactive hyperemia velocity time integral (cm)	77.0 (60.5–90.2)	70.7 (55.7–79.3)	72.2 (49.9–88.3)	81.0 (66.4–98.9)	0.137
FMD (%)	5.10 (3.35–6.78)	5.50 (3.60–5.98)	4.99 (4.19–6.86)	3.78 (2.71–8.17)	0.586
NTGMD (%)	15.49 (11.69–17.37)	15.23 (12.16–17.82)	15.86 (11.80–20.84)	14.50 (9.74–18.00)	0.781

All values are median (interquartile ranges)

FMD = flow-mediated dilation

KW = Kruskal-Wallis test: comparison between arms

NNRTI = non-nucleoside reverse transcriptase inhibitor

NRTI = nucleoside reverse transcriptase inhibitor

NTGMD = nitroglycerin-mediated dialtion

PI = protease inhibitor