Effect of antiretroviral therapy on allele-associated Lp(a) level in women with HIV in the Women's Interagency HIV Study[S]

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Abstract We previously demonstrated an association between lipoprotein (a) [Lp(a)] levels and atherosclerosis in human immunodeficiency virus (HIV)-seropositive women. The effects of antiretroviral therapy (ART) on Lp(a) levels in relation to apo(a) size polymorphism remain unclear. ART effects on allele-specific apo(a) level (ASL), an Lp(a) level associated with individual apo(a) alleles within each allele-pair, were determined in 126 HIV-seropositive women. ART effects were tested by a mixed-effects model across pre-ART and post-ART first and third visits. Data from 120 HIV-seronegative women were used. The mean age was 38 years; most were African-American (~70%). Pre-ART ASLs associated with the larger (4.6 mg/dl vs. 8.0 mg/dl, $P = 0.024$) or smaller (13 mg/dl vs. 19 mg/dl, $P = 0.041$) apo(a) sizes were lower in the HIV-seropositive versus HIV-seronegative group, as was the prevalence of a high Lp(a) level ($P = 0.013$). Post-ART ASL and prevalence of high Lp(a) or apo(a) sizes and frequency of small size apo(a) (≤22 kringles) did not differ between the two groups. ART increased Lp(a) level (from 18 to 24 mg/dl, $P < 0.0001$) and both ASLs ($P < 0.001$). In conclusion, regardless of genetic control, Lp(a) can be modulated by HIV and its treatment. ART initiation abrogates HIV-induced suppression of Lp(a) levels and ASLs, contributing to promote CVD risk in HIV-seropositive individuals.—Enkhmaa, B., E. Anuurad, W. Zhang, C-S. Li, R. Kaplan, J. Lazar, D. Merenstein, R. Karim, B. Aouizerat, M. Cohen, K. Butler, S. Pahwa, I. Ofotokun, A. A. Adimora, E. Golub, and L. Berglund. **Effect of antiretroviral therapy on allele-associated Lp(a) level in women with HIV in the Women's Interagency HIV Study. *J. Lipid Res.* 2018.** 59: **1967–1976.**

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Abbreviations: ART, antiretroviral therapy; ASL, allele-specific apo(a) level; BP, blood pressure; cIMT, carotid intima media thickness; HAART, highly active antiretroviral therapy; HCV, hepatitis C virus; HIV, human immunodeficiency virus; K, kringle; Lp(a), lipoprotein (a); NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; WIHS, Women's Interagency HIV Study.

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An elevated level of plasma lipoprotein (a) [Lp(a)] is established as an independent causal risk factor for CVD and recognized in clinical guidelines (1). Human immunodeficiency virus (HIV) infection is associated with a 50–100% greater risk of CVD beyond that explained by traditional CVD risk factors (2), and even among those without traditional CVD risk factors, the risk is 2-fold higher (3). Furthermore, some studies have shown an association between antiretroviral therapy (ART) and CVD risk in HIV-seropositive individuals (4–6). A few previous studies have investigated Lp(a) levels in HIV-seropositive individuals, yielding inconsistent results (7–9). Of note, data on the role of apo(a) size polymorphism, a major genetic regulator of Lp(a) level, and its interactions with HIV and/or ART are significantly lacking. In general, there is an inverse association between the apo(a) size polymorphism and Lp(a) level (10, 11), and smaller apo(a) isoforms are associated with a 2-fold greater risk of coronary heart disease or ischemic stroke (12); although there is a need to further explore whether apo(a) isoforms exert their effects independently of Lp(a) levels. Furthermore, recent studies have identified multiple SNPs in the *LPA* (13–15) and non-*LPA* (16, 17) genes as potential regulators of Lp(a) level. For the majority of cases, however, the effect size of these SNPs on Lp(a) level is small and varies across populations, leaving the apo(a) size polymorphism as the major single genetic regulator of Lp(a) level (18).

In our previous studies, we determined the Lp(a) level associated with the larger or smaller apo(a) allele/isoform within a given allele-pair [i.e., allele-specific apo(a) level (ASL)] and showed that these levels can inform CVD risk assessment (19). By employing this approach to HIV-seropositive cohorts, we demonstrated a higher ASL carried by atherogenic smaller apo(a) sizes in HIV-seropositive individuals with a higher CD4+ T-cell count (20), and a significant positive association of ASL with carotid intima media thickness (cIMT), a measure of subclinical atherosclerosis (21). These findings suggested that HIV infection and/or ART may modulate Lp(a) level and associated atherogenic properties. However, the effects of ART initiation on Lp(a) level over time and any modulation of this relationship by the apo(a) size polymorphism under HIV conditions remain unclear.

In the present study, we investigated the effects of ART initiation on Lp(a) levels and ASLs, in relation to apo(a) sizes, over three time points in HIV-seropositive women enrolled in the Women's Interagency HIV Study (WIHS). We hypothesized that ART initiation would increase allele-associated Lp(a) level from its pretreatment level independently of apo(a) phenotypes in HIV-seropositive women. We further tested to determine whether HIV infection reduces, or ART increases, genetically regulated Lp(a) levels. To test the latter, we compared the levels at pre- or post-ART visits in the HIV-seropositive women, respectively, to those in HIV-seronegative control women. The findings of the current study help to improve our understanding of HIV-associated elevated CVD risk in this contemporary era of ART by specifically focusing on a highly heritable CVD risk trait, Lp(a).

MATERIALS AND METHODS

Study design and cohort

Details of the WIHS have been described previously (22–25). Briefly, the WIHS is a prospective multicenter study designed to investigate the characteristics and course of HIV infection in US women. A total of 3,766 HIV-seropositive and HIV-seronegative women were enrolled at six different sites in two waves (first wave: October 1994 and November 1995; second wave: October 2001 and September 2002). Written informed consent was obtained from study participants, and the study was approved by the Institutional Review Boards at each site. All WIHS participants are invited to complete study visits every six months for collection of biological specimens, questionnaire data, and clinical measurements. Treatment was defined as use of highly active ART (HAART), combination therapy, or monotherapy at the time of visit concurrent with cIMT. The definition of HAART was guided by the DHHS/Kaiser Panel guidelines and is defined as: the reported use of three or more antiretroviral medications, one of which has to be a protease inhibitor (PI), a nonnucleoside reverse transcriptase inhibitor (NNRTI), and a nucleoside reverse transcriptase inhibitor (NRTI), one of the NRTIs (abacavir or tenofovir), an integrase inhibitor (e.g., raltegravir), or an entry inhibitor (e.g., maraviroc or enfuvirtide). The combination therapy was defined as: *a*) only two NRTIs; *b*) three or more NRTIs without abacavir or tenofovir and in the absence of PIs and NNRTIs; and *c*) at least one PI and at least one NNRTI in the absence of NRTI. The most frequent case of monotherapy in the WIHS was of one NRTI. Furthermore, the current study was approved by the Institutional Review Board at the University of California, Davis.

Laboratory assays

Plasma HIV RNA levels were quantified using nucleic acid sequence-based amplification commercial assays, and total peripheral CD4+ T-cell counts were measured with standard flow cytometric methods. Concentrations of fasting total cholesterol, HDL cholesterol, triglyceride, and LDL cholesterol were measured centrally (23).

Total plasma Lp(a) level was assessed by an apo(a) size-insensitive sandwich ELISA (Mercodia Inc., Uppsala, Sweden) (26) and apo(a) isoform size was determined by immunoblotting (27) in a cohort of 126 HIV-seropositive and 120 HIV-seronegative women. HIV-seropositive and HIV-seronegative groups were matched by age and race/ethnicity. A major inclusion criterion for the HIV-seropositive group was availability of appropriate specimens for three required visits (see below). With regard to lipid-lowering therapy, participants were not excluded if they were receiving statins due to their negligible effect on Lp(a) levels (28, 29), but were excluded if they were receiving niacin due to its considerable effect on Lp(a) levels (30, 31).

To assess the effects of ART initiation in the HIV-seropositive group, plasma Lp(a) levels and apo(a) phenotypic characteristics were determined at three time points: pre-ART visit (up to 6 months before ART initiation), post-ART first visit (up to 6 months after ART initiation), and post-ART third visit (up to 18 months after ART initiation). In the HIV-seronegative group, Lp(a) levels and apo(a) phenotypic characteristics were determined at a single time point. ASLs were determined based on computerized scanning

of apo(a) protein bands as previously described (19, 32–34). Briefly, protein dominance was determined by optical analysis of the apo(a) protein bands on a Western blot in samples with double expressed apo(a) isoforms, followed by a validation by computerized scanning. The relative intensity of each apo(a) isoform was multiplied by the total plasma Lp(a) level to compute the Lp(a) level associated with each apo(a) isoform, i.e., ASL (19, 32). For individuals with a single expressed apo(a) band, it was classified as a smaller apo(a) [kringle (K)] band. As a smaller apo(a) band does not always correspond to the dominant plasma apo(a) protein isoform (32), we assessed and classified apo(a) dominance pattern as larger-dominating, smaller-dominating, or co-dominating and compared frequency by HIV status.

To account for the contribution of Lp(a) cholesterol to total and LDL cholesterol levels, we first calculated levels of total and LDL cholesterol corrected for the Lp(a) contribution as in previous studies (35). Thus, the level of Lp(a) mass (milligrams per deciliter) multiplied by 0.3 was subtracted from total and LDL cholesterol values, respectively. ART-induced changes in these corrected levels were assessed and the coefficients of correlation with changes in Lp(a) levels and ASLs were determined.

Clinical variables

Major cardiovascular and HIV-related covariates were obtained by self-report and direct measurements. Seated blood pressure (BP) was measured using a standardized protocol. Hypertension was defined as systolic BP ⩾140 mmHg, diastolic BP ⩾90 mmHg, or self-reported physician's diagnosis of hypertension. BMI was calculated as weight in kilograms divided by the square of height in meters.

Statistics

All analyses were performed with SAS Version 9.4 (SAS Institute Inc., Cary, NC). Demographic, clinical, and laboratory values for the HIV-seronegative and HIV-seropositive (pre-ART, post-ART first, or post-ART third values, respectively) groups were compared using a two-sided *t*-test, Wilcoxon rank-sum test, Chi-square test, or Fisher's exact test where appropriate. Within the HIV-seropositive group, the relationship of changes in Lp(a) levels and ASLs from the pre-ART visit to the post-ART first or third visit, respectively, with the HIV therapy status (with and without therapy) was assessed by a two-sided *t*-test or the Wilcoxon rank-sum test. To compare ART-induced changes in laboratory measurements between the post-ART first and third visits, a two-sided paired *t*-test or the Wilcoxon signed-rank test was used. ART-induced changes in Lp(a) levels and ASLs at the post-ART first or third visit, respectively, across single and double apo(a) protein isoform groups were compared by a two-sided *t*-test or the Wilcoxon rank-sum test. Further, within the single or double isoform group, ART-induced changes in Lp(a) levels and ASLs at the post-ART first versus third visit were compared with a two-sided paired *t*-test or the Wilcoxon signed-rank test. The effects of ART initiation on HIV-related and other laboratory values, including Lp(a) levels and ASLs, were tested by a mixed-effects model across the three time points (pre-ART and post-ART first and third visits). Correlations of changes in Lp(a) levels and ASLs with changes in other clinical and laboratory values from the pre-ART visit to the post-ART first visit were estimated by the Pearson's correlation coefficient or the Spearman's rank correlation coefficient as appropriate. A P value of <0.05 was considered statistically significant.

RESULTS

Characteristics of study population across HIV status

Comparisons of pre-ART characteristics in the HIV-seropositive group to those in the HIV-seronegative group are shown in **Table 1**. HIV-seropositive and HIV-seronegative women were similar with regard to demographic and anthropometric characteristics. The mean age of the cohort was 38 ± 8 years (range: 20–66 years); most were African-Americans (∼70%). Among the HIV-seropositive women, 58% had a CD4+ T-cell count of <350 cells/mm^3 and 67% had an HIV RNA viral load of ⩾10,000 copies/ml. The average duration of diagnosed HIV infection was 6.7 ± 3.7 years. The use of heart and hypertension medication did not differ by HIV status. There was no statin user in the HIV-seronegative group and only five occasions of statin use (∼1%) were reported in the HIV-seropositive group across the pre- and post-ART visits. Approximately half of the women in each group were current smokers, and most were free of hepatitis C virus (HCV) coinfection (72%) or premenopausal (84%). HIV-seropositive women had significantly lower levels of total cholesterol ($P < 0.0001$) and HDL cholesterol ($P < 0.0001$) and a significantly higher level of triglyceride ($P < 0.0001$) compared with HIV-seronegative women (Table 1).

Lp(a)- and apo(a)-related variables across HIV status

First, we compared pre-ART values of the HIV-seropositive group to those of the HIV-seronegative group (**Table 2**). We found significantly lower values of ASLs associated with the larger (median: 5 mg/dl vs. 8 mg/dl, $P = 0.024$) or smaller (median: 13 mg/dl vs. 19 mg/dl, $P = 0.041$) apo(a) sizes in the HIV-seropositive versus the seronegative group (**Fig. 1**). Consistent with this finding, pre-ART Lp(a) levels in the HIV-seropositive group were borderline lower compared with those in the HIV-seronegative group ($P = 0.087$). Furthermore, pre-ART frequency of high Lp(a) levels (⩾30 mg/dl) in the HIV-seropositive group was significantly lower compared with those in the HIV-seronegative group (30% vs. 46%, $P = 0.013$).

The median sizes for the larger or smaller apo(a) isoforms were similar across HIV status (Table 2). Approximately one-quarter of the women in both groups had at least one small (⩽22 K) size apo(a). The distribution pattern of apo(a) phenotypes (i.e., single and double isoforms) did not differ significantly by HIV status. As expected, most women had two detectable apo(a) protein bands (68% and 74% in the HIV-seronegative and HIV-seropositive groups, respectively). Among these women (i.e., carriers of double protein isoforms), a smaller dominating (∼60%) or a co-dominating (∼35%) apo(a) pattern was more common, and the overall distribution pattern of apo(a) dominance did not differ significantly by HIV status.

Next, we compared post-ART values in the HIV-seropositive group to those in the HIV-seronegative group. There were no significant differences in either of the ASLs (larger and smaller) at the post-ART first and third visits (Table 2). Similarly, post-ART Lp(a) levels in the HIV-seropositive group did not differ significantly from those in the HIV-seronegative group.

Effects of ART-initiation on Lp(a) levels and ASLs over time in the HIV-seropositive group

We examined the effects of ART initiation on Lp(a) levels and ASLs across the three time points. Total Lp(a) level

TABLE 1. Cohort characteristics by HIV status

	HIV-Seronegative[a]	HIV-Seropositive	*P*
Number	120	126	
Age (years)	38 ± 9	38 ± 8	0.728
Race [n (%)]	—	—	0.202
Whites	25 (21)	28 (22)	—
African-Americans	84 (70)	77 (61)	—
Others	11 (9)	21 (17)	—
BMI (kg/m^2)	29 ± 7	28 ± 8	0.202
Systolic BP (mmHg)	125 ± 20	120 ± 15	0.154
Diastolic BP (mmHg)	74 ± 15	72 ± 11	0.812
HIV RNA (copies/ml)	—	31,000 (6,900; 120,000)	—
≤80 (lower detection limit) [n (%)]	—	3 (2)	—
81–999 [n (%)]	—	7 (6)	—
1,000–9,999 [n (%)]	—	27 (22)	—
≥10,000 [n (%)]	—	85 (67)	—
Missing	—	4 (3)	—
Duration of HIV infection (years)	—	6.7 ± 3.7	—
CD4+ T-cell nadir (cells/mm^3)	823 (699; 998)	230 (131; 348)	<0.0001
CD4+ T cell count (cells/mm^3)	999 (806; 1236)	311 (176; 443)	<0.0001
Heart and high BP medication [n (%)]	25 (26)	17 (19)	0.293
Diabetic medication [n (%)]	5 (4)	3 (2)	0.491
Current smoke [n (%)]	55 (47)	66 (53)	0.370
HCV coinfection, negative [n (%)]	86 (72)	90 (71)	0.655
Premenopausal [n (%)]	101 (84)	104 (83)	0.651
Total cholesterol (mg/dl)	185 ± 36	161 ± 36	<0.0001
LDL cholesterol, direct (mg/dl)	104 ± 37	94 ± 29	0.116
HDL cholesterol (mg/dl)	62 ± 20	46 ± 13	<0.0001
Triglycerides (mg/dl)	89 (60; 126)	121 (88; 189)	<0.0001

Data are expressed as mean ± standard deviation, median (25th; 75th percentiles), or number (%). For the HIV-seropositive group, pre-ART visit data is shown.

[a]Data for lipid profile are based on a fewer number of subjects: n = 85 for total cholesterol, HDL cholesterol, and triglycerides; n = 64 for directly measured LDL cholesterol.

($P < 0.0001$ for overall test) and ASL associated with the larger ($P < 0.001$ for overall test) or smaller ($P < 0.0001$ for overall test) apo(a) sizes increased significantly from the pre-ART levels. Further analyses indicated that Lp(a) levels ($P < 0.0001$) and ASLs associated with the larger ($P < 0.001$) or smaller ($P < 0.001$) apo(a) sizes were significantly higher at both post-ART visits compared with the pre-ART visit, respectively (Fig. 1). There were no significant differences between the two post-ART visits for either Lp(a) levels or ASLs.

Change and percent change in Lp(a) levels and ASLs and impact by nontreated HIV infection

Change (milligrams per deciliter) and percent change from pre-ART to post-ART first or third visit, respectively, in Lp(a) levels and ASLs in all HIV-seropositive women and by HIV therapy status are shown in **Table 3**. In all HIV-seropositive women, both the median change (milligrams per deciliter) and percent change in Lp(a) levels and ASLs were positive and comparable between the two post-ART visits ($P > 0.05$).

A total of 15 (12%) women at the post-ART first visit reported that they were not taking ART. Among those taking ART (n = 110), the majority (n = 101, 92%) were on HAART; only six (5%) and three (3%) women were receiving combination therapy or monotherapy, respectively. At the post-ART third visit, 26 (20%) women reported that they were not taking ART; while the majority (n = 90) of treated HIV-seropositive women were on HAART. We tested the impact of nontreated HIV infection on Lp(a) levels and ASLs over time within each post-ART visit. At the post-ART first visit, percent (not unit) changes in ASLs associated with the smaller apo(a) sizes were significantly higher among women receiving ART versus those not receiving ART ($P = 0.027$). At the post-ART third visit, changes in Lp(a) levels (median: 3 mg/dl vs. −1.4 mg/dl, $P < 0.001$) and ASLs associated with the smaller apo(a) sizes (median: 3 mg/dl vs. −2 mg/dl, $P < 0.001$) were significantly greater among women receiving ART versus those not receiving ART. Similar findings were observed for percent changes in Lp(a) levels and ASLs (**Fig. 2**, Table 3).

Impact of apo(a) phenotypes or presence of HCV coinfection on changes and percent changes in Lp(a) levels

We further investigated whether ART-induced changes in Lp(a) levels differ by apo(a) phenotypes, i.e., across single and double apo(a) protein isoform groups (supplemental Table S1). A total of 93 (74%) HIV-seropositive women had double protein isoforms. Neither change (milligrams per deciliter) nor percent change in Lp(a) levels differed significantly across single and double apo(a) isoform groups at post-ART visits.

As apo(a) is hepatically derived, we tested the impact of the presence of HCV coinfection on changes in Lp(a) levels and ASLs. At the pre-ART visit, a total of 27 (22%) HIV-seropositive women were tested positive for the presence of HCV coinfection. Neither change (milligrams per deciliter) nor percent change differed significantly between the HIV-seropositive women with and without HCV coinfection at post-ART visits.

TABLE 2. Comparisons of Lp(a)- and apo(a)-related variables in the HIV-seronegative women to those in the HIV-seropositive women initiating ART

	HIV-seronegative	HIV-seropositive			*P* for Differences between HIV-Seronegative versus HIV-Seropositive pre-ART Visit	*P* for Differences between HIV-Seronegative versus HIV-Seropositive post-ART First Visit	*P* for Differences between HIV-Seronegative versus HIV-Seropositive post-ART Third Visit
		Pre-ART Visit	Post-ART First Visit	Post-ART Third Visit			
Lp(a) (mg/dl)	23 (9; 43)	18 (7; 33)	24 (9; 45)	24 (9; 47)	0.087	0.794	0.991
Lp(a) (≥30 mg/dl) [n (%)]	55 (46)	38 (30)	50 (40)	50 (40)	0.013	0.368	0.369
ASL, larger (mg/dl)	8 (3; 15)	5 (1; 12)	6 (1; 16)	6 (1; 14)	0.024	0.178	0.256
ASL, smaller[a] (mg/dl)	19 (7; 36)	13 (6; 25)	16 (7; 35)	16 (7; 32)	0.041	0.612	0.662
Apo(a) size, larger (K)	31 (28; 33)	31 (28; 33)	—	—	0.322	—	—
Apo(a) size, smaller[a] (K)	26 (23; 29)	27 (22; 29)	—	—	0.795	—	—
Presence of a small size apo(a) (≤22 K) [n (%)]	29 (24)	35 (28)	—	—	0.562	—	—
Apo(a) expression			—	—	0.660	—	—
Single isoform [n (%)]	38 (32)	32 (25)					
Double isoforms [n (%)]	81 (68)	93 (74)					
No isoform [n (%)]	1 (1)	1 (1)					

Data are expressed as median (25th; 75th percentiles) or number (%).

[a]Given the evidence that the vast majority of individuals are heterozygotes for apo(a) alleles, it is likely that the apo(a) bands on Western blots are products of the smaller rather than the larger apo(a) allele within a given individual. For this reason, for subjects with a single expressed apo(a) band, data are entered as smaller apo(a) band [kringles (K)] and accounted for the size of smaller apo(a), and thus also for the ASL, smaller.

Fig. 1. Effect of ART initiation on Lp(a) levels (upper panel) and ASLs (lower panel) in the HIV-seropositive women in comparison to those in the HIV-seronegative control women. Box and whisker plots represent median and interquartile ranges with upper and lower extremes. *$P < 0.05$ for differences between the pre-ART values in the HIV-seropositive group and those in the HIV-seronegative group. **$P < 0.001$ for differences between the pre-ART and post-ART values in the HIV-seropositive group. ***$P < 0.0001$ for differences between the pre-ART and post-ART values in the HIV-seropositive group. HIV(−), HIV-seronegative; HIV(+), HIV-seropositive.

Effects of ART-initiation on HIV-related and other clinical variables over time in HIV-seropositive women

As expected, ART initiation reduced HIV RNA viral load (median: 31,000 copies/ml at pre-ART visit to 80 copies/ml at both post-ART visits, $P < 0.0001$ for differences between visits) and increased CD4+ T-cell counts (median: 311 cells/mm^3 at pre-ART visit to 417 cells/mm^3 and 451 cells/mm^3, at post-ART first and third visit, respectively, $P < 0.0001$ for differences between visits) (supplemental Table S2). Accordingly, the distribution pattern of categorized HIV RNA viral load or CD4+ T-cell counts changed significantly over time, with more women having a lower HIV RNA viral load and a higher CD4+ T-cell count at post-ART versus pre-ART visits. Furthermore, body weight and BMI were higher at both post-ART visits versus pre-ART visit ($P < 0.005$ for trend). Compared with their respective pre-ART values, total cholesterol, LDL cholesterol, and HDL cholesterol levels were significantly higher at both post-ART visits ($P < 0.0001$ for overall trend and for differences between pre-ART versus post-ART first or post-ART third visits) (supplemental Table S2).

Correlations between ART-induced changes in Lp(a) and other clinical and laboratory measurements

We subsequently estimated correlation coefficients between changes from pre-ART visit to post-ART first visit in

TABLE 3. Change and percent change in Lp(a) levels and ASLs from pre-ART to post-ART visits in all HIV-seropositive women and by HIV therapy status

	Post-ART First Visit				Post-ART Third Visit				
	All	Therapy (−)	Therapy (+)	*P* for Differences between Therapy (−) and Therapy (+)	All	Therapy (−)	Therapy (+)	*P* for Differences between Therapy (−) and Therapy (+)	*P* for Differences between First and Third post-ART Visits (All)
Lp(a) level	n = 125	n = 15	n = 110		n = 125	n = 26	n = 99		
Change (mg/dl)	3 (−0.6; 10)	1.4 (−4; 8)	4 (0; 11)	0.254	2 (−0.9; 11)	−1.4 (−7; 3)	3 (−0.1; 14)	<0.001	0.796
Percent change	23 (−3; 54)	10 (−32; 32)	24 (0; 62)	0.079	19 (−5; 64)	−6 (−32; 15)	29 (−0.5; 74)	<0.001	0.966
ASL, larger	n = 93	n = 11	n = 82		n = 92	n = 20	n = 72		
Change (mg/dl)	0.7 (−0.7; 4)	−0.3 (−3; 10)	0.8 (−0.3; 4)	0.328	0.6 (−0.5; 3)	0.3 (−0.5; 1)	0.8 (−0.5; 3)	0.458	0.942
Percent change	23 (−18; 64)	−6 (−40; 47)	24 (−15; 70)	0.146	22 (−8; 74)	20 (−15; 57)	23 (−7; 97)	0.418	0.518
ASL, smaller	n = 125	n = 15	n = 110		n = 124	n = 26	n = 98		
Change (mg/dl)	2 (−0.1; 8)	−0.1 (−7; 7)	2 (0; 8)	0.093	2 (−0.9; 9)	−2 (−7; 2)	3 (0; 10)	<0.001	0.635
Percent change	21 (−2; 50)	−2 (−32; 29)	23 (0; 55)	0.027	20 (−8; 60)	−10 (−37;7)	28 (0; 74)	<0.001	0.945

Data are expressed as median (25th; 75th percentiles).

Fig. 2. Distributions of percent changes in Lp(a) level (upper panel) and ASL associated with the smaller apo(a) sizes (lower panel) at the post-ART third visit in all HIV-seropositive women and by HIV therapy status. Box and whisker plots represent median and interquartile ranges with upper and lower extremes. Horizontal dashed lines mark zero (0). HIV(+), HIV-seropositive.

Lp(a) levels or ASLs and other clinical and laboratory measurements (**Table 4**). ART-induced changes (milligrams per deciliter) in Lp(a) levels were significantly and positively correlated with changes (milligrams per deciliter) in total cholesterol ($r = 0.316$, $P < 0.001$) and LDL cholesterol ($r = 0.304$, $P < 0.001$), respectively. Similarly, percent changes in Lp(a) levels were significantly and positively correlated with percent changes in total cholesterol ($r = 0.318$, $P < 0.001$) and LDL cholesterol ($r = 0.342$, $P < 0.001$) levels. However, after taking the contribution of Lp(a) to these levels into account, the significant correlations of changes in Lp(a) with changes in total cholesterol ($r = 0.055$, $P = 0.606$ for milligrams per deciliter change; $r = -0.007$, $P = 0.950$ for percent change) or LDL cholesterol ($r = -0.125$, $P = 0.240$ for milligrams per deciliter change; $r = -0.007$, $P = 0.950$ for percent change) were abolished. There was a significant negative correlation between changes in triglyceride and Lp(a) levels ($r = -0.222$, $P = 0.014$ for milligrams per deciliter change; $r = -0.256$, $p = 0.004$ for percent change). Moreover, changes in both smaller and larger ASLs were significantly and positively correlated with changes in total cholesterol and LDL cholesterol levels, respectively. In line with Lp(a) findings, there was a significant and negative correlation between changes in triglyceride and ASLs (Table 4). Finally, although no significant

TABLE 4. Correlations of changes in Lp(a) levels and ASLs with changes in other clinical variables from pre-ART visit to post-ART first visit

	Change in Lp(a)			Change in ASL, Larger			Change in ASL, Smaller		
	Number	Correlation Coefficient	*P*	Number	Correlation Coefficient	*P*	Number	Correlation Coefficient	*P*
Unit change[a]									
BMI (kg/m^2)	118	0.017	0.852	86	0.023	0.831	118	0.036	0.700
CD4+ T-cell count (cells/mm^3)	122	−0.034	0.707	91	−0.094	0.374	122	0.029	0.747
Triglycerides (mg/dl)	122	−0.222	0.014	90	−0.303	0.004	122	−0.180	0.048
Cholesterol (mg/dl)	121	0.316	<0.001	89	0.253	0.017	121	0.329	<0.001
LDL cholesterol (mg/dl)	123	0.304	<0.001	91	0.323	0.002	123	0.289	0.001
HDL cholesterol (mg/dl)	121	0.158	0.083	89	0.112	0.295	121	0.212	0.019
Percent change[b]									
BMI (kg/m^2)	118	0.039	0.671	87	−0.021	0.844	118	0.060	0.518
CD4+ T-cell count (cells/mm^3)	121	0.176	0.054	92	0.035	0.741	121	0.217	0.017
Triglycerides (mg/dl)	122	−0.256	0.004	91	−0.269	0.010	122	−0.228	0.012
Cholesterol (mg/dl)	121	0.318	<0.001	90	0.266	0.011	121	0.306	<0.001
LDL cholesterol (mg/dl)	121	0.342	<0.001	91	0.319	0.002	121	0.279	0.002
HDL cholesterol (mg/dl)	121	0.082	0.369	90	0.056	0.599	121	0.123	0.180

[a]Changes expressed as milligrams per deciliter in Lp(a) levels and ASLs were used to estimate correlation coefficients.
[b]Changes expressed as percent in Lp(a) levels and ASLs are used to estimate correlation coefficients.

correlation was seen for the unit changes, there was a significant positive correlation between percent changes in CD4+ T-cell count and percent changes in ASLs for the smaller apo(a) sizes ($r = 0.217$, $P = 0.017$).

DISCUSSION

This is the first report on the effects of ART initiation on Lp(a) levels and ASLs, in relation to apo(a) size polymorphism among people with HIV. The major novel findings in this longitudinal study of HIV-seropositive and HIV-seronegative control women are: *a*) a significantly lower level of allele-specific apo(a) for both the larger and smaller apo(a) isoform sizes in the HIV-seropositive versus the HIV-seronegative group, when comparing pre-ART levels of the HIV-seropositive group to those of the HIV-seronegative group; *b*) a significant increase from pre-ART to post-ART visits in Lp(a) levels and ASLs in the HIV-seropositive group; and *c*) no significant difference in either ASLs (larger and smaller), when comparing post-ART levels of the HIV-seropositive group to those of the HIV-seronegative group. Moreover, HIV-seropositive and HIV-seronegative women had similar distribution patterns of apo(a) size, apo(a) expression, apo(a) dominance, and small (≤22 K repeats) size apo(a). These findings suggest that HIV-infection downregulated, while ART upregulated, genetically controlled Lp(a) levels.

HIV-seropositive individuals are now being increasingly treated with ART and living longer with the infection. Compared with HIV-seronegative individuals, HIV-seropositive individuals have a higher risk of CVD as well as an early onset of the disease (2, 3, 36–38). CVD in HIV is multifactorial in nature, resulting from a complex interplay between diverse components, such as genetic and environmental factors, as well as heightened state of systemic inflammation and immune activation intrinsic to HIV infection. In line with our previous study (21) and studies by others (39), HIV was associated with an atherogenic lipid profile (low HDL cholesterol and high triglyceride) in the current study. The impact of ART on traditional CVD risk factors, including lipid profile, has been extensively investigated in various HIV-seropositive population groups such as children and adults (across the lifespan) or males and females (40–42). These studies have found increases in routinely assessed atherogenic lipid and lipoprotein levels with ART use. In agreement with these findings, in the current study, post-ART levels of total cholesterol and LDL cholesterol were significantly elevated compared with their respective pre-ART levels. A meta-analysis of 51 observational studies in HIV-seropositive adults also found significantly higher concentrations of total cholesterol, LDL cholesterol, and triglycerides in ART-exposed versus ART-naïve patients (43). In this meta-analysis, ART-exposure was associated with a 3.8-fold greater risk for hypercholesterolemia and a 2.2-fold greater risk for hypertriglyceridemia (43).

In contrast to the situation in the general population where abundant evidence is available to support the risk factor role of Lp(a) and apo(a) size polymorphism in CVD development, data in HIV-seropositive populations are significantly lacking. Only a few previous studies have focused on Lp(a) in HIV-seropositive individuals. A notable limitation in these studies is a lack of consideration of apo(a) size despite its major regulatory role and potential impact on the findings. In the Swiss HIV Cohort Study, enrolling predominantly HIV-seropositive males (up to 80%), various PI-based ART regimens increased Lp(a) from its pretreatment level during a study period of ~15–17 months (7). In this study, the median Lp(a) level at baseline was very low (presumably due to white race), ranging from 1.8 to 2.9 mg/dl, and the PI-induced Lp(a)-increasing effect was pronounced in HIV-seropositive patients with a higher baseline Lp(a) level (48% increase in 11 PI-treated patients with a pretreatment level of >20 mg/dl). In our HIV-seropositive cohort, reflecting its ethnic composition (61% African-Americans), the median Lp(a) level was relatively high, i.e., 18 mg/dl at the pre-ART visit, which further rose to 24 mg/dl at the post-ART first visit and remained elevated at the post-ART third visit. The extent of percent

increase in Lp(a) levels in our HIV-seropositive cohort were comparable between the two post-ART visits (38% versus 41%). Further, Périard et al. (7), found no significant association between changes in Lp(a) level and changes in other lipids and lipoproteins. We found a significant positive association between changes in Lp(a) and total cholesterol levels at the post-ART first and third visits. We also found a significant positive association between changes in Lp(a) and changes in directly measured LDL cholesterol and a significant negative association between changes in Lp(a) and changes in triglyceride at the post-ART first visit. The disappearance of significant correlations between changes in Lp(a) and total cholesterol (or LDL cholesterol) after correction of these levels for the contribution of Lp(a) cholesterol suggests a Lp(a) change-driven relationship. Overall, these heterogeneous findings between our study and the study by Périard et al. (7) could be due to differences in cohort demographics (Whites versus Blacks; males versus females) and/or geographical regions (Swiss versus US).

In a small longitudinal study, enrolling 15 HIV-seropositive individuals (93% males), the Lp(a) level increased in parallel with progression of HIV disease (9). An early study reported no difference in mean Lp(a) level across different CD4+ T-cell count groups in HIV-seropositive individuals (~70% males) (8); and in the same cohort, the median Lp(a) level was higher in the HIV-seropositive versus HIV-seronegative group (44). In contrast, in our study, the median Lp(a) level at the pre-ART visit in HIV-seropositive women was considerably lower compared with those in HIV-seronegative women. Analyses based on ASLs supported this observation as pre-ART ASLs were significantly lower in the HIV-seropositive versus HIV-seronegative group. As we were able to characterize the entire cohort with regard to apo(a) phenotypes and confirmed a similar distribution pattern for apo(a) sizes, phenotypes, and dominance across HIV status, it is expected that the observed differences between the two groups were not due to differences in apo(a)-related traits.

In addition, our HIV-seropositive and HIV-seronegative cohorts were similar with regard to ethnic/racial composition, a major contributor to Lp(a) variability (45, 46). Of note, differences between the HIV-seropositive and HIV-seronegative groups became no longer significant when assessed at the post-ART visits due to ART-induced increases in Lp(a) levels and ASLs. These findings suggest that HIV-infection acted as a reducer, while ART acted as an enhancer and/or normalizer, of Lp(a) levels, independently of apo(a) genetic variability. Furthermore, the presence of a small group of HIV-seropositive women, who were not taking ART at the post-ART visits, provided an opportunity to gather more information on the longitudinal effects of nontreated HIV-infection on Lp(a) levels. Overall, changes in Lp(a) levels and ASLs in women taking ART were positive (i.e., an increase), while small but negative (i.e., a decrease) changes were observed in women not taking ART at both post-ART visits. These findings further support an Lp(a)-reducing effect of HIV infection over time. Although we acknowledge the need to confirm these findings in other studies. Due to the limited number of women who were not taking ART at the post-ART visits, we did not assess the impact of the length of untreated HIV infection on Lp(a) levels. Nonetheless, taken together, these findings indicate that, although being considered as one of the most heritable quantitative human traits (11), Lp(a) levels can be modulated by HIV infection and its treatment, clinically relevant nongenetic factors.

In our previous cross-sectional study in HIV-seropositive men and women, we found that Lp(a) levels carried by the smaller more atherogenic apo(a) sizes were significantly elevated in HIV-seropositive individuals with a higher versus lower CD4+ T-cell count (20). In a subsequent cross-sectional study among HIV-seropositive young women enrolled in the WIHS (a different subcohort), we demonstrated a significant positive association of cIMT with both Lp(a) level and ASL determined by the smaller apo(a) size (21). To the best of our knowledge, the latter study was the first of its kind to establish a link between Lp(a) and a cardiovascular outcome in the HIV setting. In this study, Lp(a) levels and ASLs were also lower in the HIV-seropositive versus HIV-seronegative women. We postulated that the high frequency of untreated HIV-seropositive women (~50%) contributed to the overall lower Lp(a) level seen in the HIV-seropositive group in the latter study. The findings of the current study support this hypothesis, as HIV-infection suppressed Lp(a) levels/ASLs. The biological underpinnings of these effects remain to be explored as well as any relationship to the immune system.

As a risk factor with the potential to remain informative over the course of lifespan and/or HIV disease due to its strong genetic control (11), Lp(a) presents a unique avenue for both risk assessment and intervention in HIV-seropositive populations. It is of particular interest in African populations, as these populations endure a greater burden of both elevated Lp(a) level and HIV infection compared with any other population groups (45, 46). New developments in lipid-lowering drugs, such as proprotein convertase subtilisin/kexin 9 (PCSK9) inhibitors, open possibilities for a targeted intervention, as monoclonal antibodies directed against PCSK9 significantly reduced Lp(a) levels in multiple clinical trials (47). While it is generally accepted that statins have minor to no effect on Lp(a) level (48), recent evidence suggests a possibility for a dose-dependent rise in Lp(a) level with statins (49). In this context, it is important to note that the prevalence of statin use in our cohort was negligible. Overall, the findings in the current study contribute to a better understanding of ART-related increased CVD risk in HIV-seropositive individuals in this modern era of ART, and are of public health importance due to a projected scale up in the use of ART across the globe (particularly in Africa) (50).

We acknowledge limitations of our study. As our results are based on a women-only HIV-seropositive cohort, there is a need to extend this investigation to other HIV-seropositive groups, such as men and/or children. Also, our cohort consisted primarily of African-Americans; therefore, studies in other ethnic/racial groups are needed to confirm the effects of natural and treatment history of HIV-infection on

Lp(a) levels and impact by apo(a) genetics. Furthermore, long-term effects of ART (beyond 18 months) on Lp(a) levels remain to be established. Nevertheless, our longitudinal investigation provides evidence on the role of HIV-infection and its treatment as modulators of a genetically regulated CVD risk factor. We acknowledge the need for further larger investigations to assess the relationship of ART-induced changes in Lp(a) levels and ASLs with clinical outcomes, such as CVD, under HIV setting across ethnic and gender groups.

In conclusion, while HIV infection exerted an Lp(a)-reducing effect, initiation of ART induced an Lp(a)-increasing effect, independent of apo(a) phenotypic characteristics. The findings indicate that Lp(a) levels and ASLs, regardless of their highly heritable nature, can be modulated by clinically relevant nongenetic factors: HIV and ART. ART initiation abrogates HIV-induced suppression of Lp(a) levels and ASLs, contributing to promote CVD risk in HIV-seropositive individuals.

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