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CLINICAL PERSPECTIVE

Long-Term Effects of *In Utero* Antiretroviral Exposure: Systolic and Diastolic Function in HIV-Exposed Uninfected Youth

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Abstract

The aim of this study was to evaluate the association of *in utero* exposure to highly active antiretroviral therapy (HAART) with left ventricular (LV) function and structure in HIV-exposed uninfected (HEU) children. A prospective, multisite cohort study in HEU children was conducted by the Pediatric HIV/AIDS Cohort Study (PHACS). Echocardiographic measures of LV systolic and diastolic function and cardiac structure were obtained from HEU subjects aged ≥6 years enrolled in the PHACS Surveillance Monitoring of ART Toxicities study. Echocardiographic Z-scores were calculated using normative data from an established reference cohort. We used adjusted linear regression models to compare Z-scores for echocardiographic measures from HEU children exposed *in utero* to HAART with those exposed to non-HAART, adjusting for demographic and maternal health characteristics. One hundred seventy-four HEU subjects with echocardiograms and maternal ARV information were included (mean age 10.9 years; 48% male, 56% black non-Hispanic). Among 156 HEU youth with any ARV exposure, we observed no differences in Z-scores for LV systolic function measures between youth exposed *in utero* to HAART (39%) and HAART-unexposed youth in either unadjusted or adjusted models. In adjusted models, those exposed to HAART had significantly lower mitral late diastolic inflow velocities (adjusted mean Z-score = 0.00 vs. 0.52, $p = .04$) and significantly higher adjusted mean LV mass-to-volume ratio Z-scores (adjusted mean Z-score = 0.47 vs. 0.11, $p = .03$) than HAART-unexposed youth. Uninfected children with perinatal exposure to HAART had no difference in LV systolic function. However, small but significant differences in LV diastolic function and cardiac structure were observed, suggesting that continued monitoring for cardiac outcomes is warranted in this population.

Introduction

The effects of HIV and antiretroviral (ARV) exposure on the cardiovascular system of uninfected children born to mothers infected with HIV are not completely understood. Based on current recommendations for treatment of HIV-infected women during pregnancy, most HIV-exposed uninfected (HEU) children are now exposed *in utero* to highly active antiretroviral therapy (HAART) during a critical period of development of the cardiovascular system. Past studies have been somewhat inconsistent in identification of negative or positive cardiac effects of *in utero* ARV exposure. An early study of *in utero* zidovudine monotherapy exposure in 382 HEU infants born before 1995 found no adverse cardiac effects,[1] but another study observed an association of *in utero* exposure to two-drug nucleoside reverse transcriptase inhibitor therapy with mitochondrial dysfunction, which has the potential for negative cardiac effects.[2]

The National Heart, Lung, and Blood Institute-funded Cardiovascular Status of HAART in HIV-Exposed Infants and Children (CHAART 1) study of HEU children found that *in utero* exposure to ARVs was associated with decreased septal

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thickness and left ventricular (LV) mass, as well as increased LV contractility at the age of 2 years.[3] More recently, a cross-sectional evaluation of 30 HEU children (mean age 8 years) by Cade *et al.*[4] found reduced diastolic function and LV mass index when exposed to ARVs *in utero* compared with those born to HIV-negative mothers without ARV exposure.

With the exception of the later study, most evaluations of cardiac function in HEU children have focused on infants and very young children. However, as combination regimens become more widely used, potential long-term cardiac effects of *in utero* exposure warrant monitoring. Lipshultz *et al.*[5] evaluated cardiac function and structure in 189 HEU children (mean age = 11.0 years) and found significantly higher LV fractional shortening compared with perinatally HIV-infected (PHIV) children as well as significant differences in mean Z-scores for LV structural measures. However, that study focused on comparison of echocardiographic measures for PHIV versus HEU, and no evaluation of associations with maternal ARV exposure was conducted.

The use of HAART among pregnant women with HIV infection has increased dramatically over the last decade at the same time that changes in the natural history of the epidemic in women have resulted in a much lower percentage of illicit drug use during pregnancy.[6,7] Thus, comparison of cardiac measures by HAART exposure requires careful consideration of a number of potential confounding measures. As children transition to adolescence, the home environment may exert a stronger influence on child health outcomes.

We conducted an evaluation of cardiac function and structure in a group of older HEU youth with information on maternal ARV exposure based on echocardiograms conducted as part of our Pediatric HIV/AIDS Cohort Study (PHACS), with adjustment for a number of child, maternal, and household characteristics. Based on the prior studies noted above, we hypothesized that youth exposed *in utero* to HAART would have decreased LV diastolic function and LV mass compared with those not exposed to HAART.

Materials and Methods

Study population

The PHACS network has two ongoing studies of HIV-affected youth: the Adolescent Master Protocol (AMP) is a prospective cohort study conducted at 14 U.S. sites designed to evaluate development of children and adolescents born to mothers with HIV infection, including both PHIV and HEU youth. The study enrolled children aged 7–16 years between March 2007 and November 2009. The PHACS Surveillance Monitoring for ART Toxicities (SMARTT) study is an ongoing prospective cohort study of potential adverse outcomes of maternal ARV use on HEU infants and children conducted at 22 U.S. sites. This study enrolls HIV-infected mothers and their infants from birth to age of 12 years. HEU children aged seven or older may coenroll in both the AMP and SMARTT studies, allowing evaluation of maternal exposure on long-term outcomes in HEU adolescents.

The current analysis leveraged this opportunity by evaluating cardiac function measured in the AMP study in relation to *in utero* ARV exposure, measured primarily in the SMARTT study. Both study protocols were approved by the institutional review board of each participating site and the Harvard T. H. Chan School of Public Health. Written informed consent was obtained from the parent or legal guardian and assent was obtained from older participants as per local institutional review board rules.

At each annual AMP and SMARTT study visit, information about participants and their families was gathered through clinical interviews and chart reviews. Current health status was ascertained through physical and laboratory evaluations. For SMARTT, detailed information was collected through medical chart abstraction on maternal ARV use during pregnancy, and start and stop dates for all ARVs were used to code trimester of exposure to each agent. The AMP study also collected such maternal ARV information when available, but it was not required to be available as per eligibility criteria. HAART regimens were defined as containing three or more ARVs, including two or more drug classes.

Data on pregnancy and infant outcomes were abstracted from hospital medical records and from prior studies.[6] Data on self-reported substance use during pregnancy, including smoking, alcohol, and illicit drug use (e.g., marijuana, cocaine, opiates), were collected by interview. The latest maternal CD4+ lymphocyte counts and percentages and HIV RNA viral load levels available before labor and delivery were abstracted from medical records, as well as the earliest corresponding measures during pregnancy if available.

This analysis includes 174 children ≥6 years of age at the time of an echocardiogram performed predominantly as part of the AMP study (98%) and who had information available on maternal ARV use during pregnancy. Participants with cardiac congenital defects were excluded.

Echocardiographic assessment of cardiac structure and function

The echocardiogram was performed by PHACS-trained site staff, including LV M-mode, which was recorded under two-dimensional control. To improve reliability, all echocardiograms were centrally remeasured at the echocardiographic core laboratory at Boston Children's Hospital. The following structural and functional parameters were measured from M-mode recordings: LV end-diastolic and end-systolic diameters, end-diastolic interventricular septal, and LV posterior wall thicknesses, LV fractional shortening, and LV velocity of circumferential fiber shortening. The 5/6 × area × length algorithm was used to calculate LV end-diastolic and end-systolic LV volumes, LV mass, and the LV ejection fraction.

Mitral inflow velocities recorded using pulsed-wave Doppler with the sample volume placed at the tips of mitral leaflets from the apical four-chamber view were used to measure the following diastolic parameters: peak early transmitral flow velocity (E), peak late transmitral flow velocity (A), deceleration time of the E wave (DT), and the E/A ratio. Pulsed tissue Doppler (TDI) samples were recorded from the apical four-chamber view, with the sample volume placed within the basilar aspects of the myocardium in the LV lateral wall and interventricular septum. The TDI images were used to measure the peak early diastolic tissue velocities (E′) in the interventricular septum and lateral wall and the E/E′ ratios were calculated as LV diastolic function indices.

Echocardiographic Z-scores were calculated using normative data from an established reference cohort of healthy children from Boston Children's Hospital, with Z-scores calculated relative to age and body surface area, as previously

described.[8] This reference population was not known to be HIV infected or ARV exposed and was not matched to the study cohort for race, ethnicity, or socioeconomic status. Echocardiograms were discontinued from the study protocol in 2010 when preset targets were reached.

Statistical methods

Our primary analysis compared echocardiographic parameter Z-scores between HEU youth exposed *in utero* to HAART compared with HAART-unexposed youth (excluding those not exposed to any ART, $n=18$). We compared demographic and anthropomorphic characteristics between these two groups using Fisher's exact test for binary measures, chi-square tests for categorical variables, ANOVA for body measurement Z-scores, and Kruskal–Wallis tests for other continuous measures.

The distributions of Z-scores were compared between HAART-exposed and HAART-unexposed youth using general linear regression models, both unadjusted and adjusted for age, sex, race, ethnicity, BMI Z-score, and systolic blood pressure Z-score. Models for diastolic parameters were also adjusted for heart rate. Similar analyses were conducted to compare youth with versus without *in utero* exposure to ARVs during the first trimester, given this critical period in fetal cardiac development.

Several sensitivity analyses were conducted. First, we compared HEU youth exposed to zidovudine monotherapy, to regimens containing two or more ARV drugs, both unadjusted and adjusted for the covariates noted above (age, sex, race/ethnicity, BMI Z-score, systolic blood pressure Z-score, and for diastolic parameters, heart rate). Second, multivariable adjusted models comparing HAART-exposed with HAART-unexposed youth and comparing monotherapy to 2+ ARV drugs were repeated, adding those unexposed to any ARV ($n=18$) in the lesser-exposed group. General linear regression models were fit to the subset of youth with information available on substance use ($\sim$72%) and maternal health measures such as CD4% and HIV viral load during pregnancy (52%–74%). Each measure of maternal health and substance use was separately evaluated for association with echocardiographic Z-scores with adjustment for the aforementioned covariates. Comparisons of HAART-exposed vs. HAART-unexposed youth were then repeated among these subsets, controlling for measures for which $p<.10$.

Last of all, we attempted to account more appropriately for the inter-relationships among echocardiographic parameters

Table 1. Characteristics of HIV-Exposed Uninfected Children Born to HIV-Infected Mothers and Exposed to HAART *In Utero*, by Mother's Treatment Status, Among Subset with Completed Echocardiogram at Age Six or Older and Available Maternal ARV Information

	HAART exposure in utero				
Characteristic	*Not exposed to any ARVs* (n = *18*)	*Exposed to non-HAART ARV* (n = *89*)	*HAART exposed* (n = *67*)	*Total* (n = *174*)	p[a]
Age at time of echo (years), Mean (SD)	11.6 (2.8)	12.0 (2.6)	9.2 (1.4)	10.9 (2.6)	<.001
Male, *n* (%)	9 (50)	41 (46)	34 (51)	84 (48)	.86
Race/ethnicity, *n* (%)					
White non-Hispanic	2 (11)	3 (3)	5 (7)	10 (6)	.42
Black non-Hispanic	11 (61)	48 (54)	38 (57)	97 (56)	
Hispanic	5 (28)	32 (36)	23 (34)	60 (34)	
Other	0 (0)	6 (7)	1 (1)	7 (4)	
Gestational age <37 weeks, *n* (%)	6 (38)	18 (20)	11 (17)	35 (21)	.17
Body measurement Z-scores, mean (SD)					
Height	–0.25 (1.14)	0.33 (1.12)	0.22 (1.04)	0.22 (1.10)	.13
Weight	0.34 (1.03)	0.83 (1.41)	0.69 (1.39)	0.73 (1.37)	.37
Body mass index	0.60 (0.90)	0.77 (1.30)	0.72 (1.37)	0.73 (1.29)	.87
Body surface area	0.00 (1.02)	0.82 (1.84)	0.30 (1.57)	0.53 (1.69)	.059
Blood pressure (BP) Z-scores, mean (SD)					
Systolic BP	–0.36 (1.10)	–0.25 (1.04)	–0.45 (0.96)	–0.34 (1.01)	.49
Diastolic BP	0.11 (0.88)	0.46 (0.86)	0.31 (0.80)	0.37 (0.84)	.21
Mean BP	–0.51 (0.99)	–0.42 (0.90)	–0.53 (0.89)	–0.47 (0.90)	.78
AV heart rate (b/min), Median (IQR)	75 (70, 81)	76 (64, 84)	81 (72, 87)	78 (68, 85)	.13
Maternal health measures before CD4 < 350 cells/mm^3 delivery, *n* (%)					
CD4 < 350 cells/mm^3	2 (40)	19 (30)	21 (35)	42 (32)	.71
HIV-1 RNA VL >400 cpm	2 (100)	15 (48)	17 (30)	34 (38)	.039
Maternal substance use during pregnancy, *n* (%)					
Tobacco use	3 (33)	14 (23)	10 (18)	27 (22)	.49
Alcohol use	4 (44)	10 (17)	6 (11)	20 (16)	.05
Illicit drug use	2 (22)	5 (8)	3 (5)	10 (8)	.23

[a]p-Value by Fisher's exact test for binary characteristics, chi-square for categorical characteristics, ANOVA for comparison of z-scores, and Kruskal–Wallis test for age and heart rate. Data unavailable for maternal CD4 before delivery ($N=45$), maternal VL ($N=84$), and maternal substance use ($N=49$).

ARV, antiretroviral; AV, aortic valve; HAART, highly active antiretroviral therapy; SD, standard deviation; VL, viral load.

by fitting multivariate models, assessing the association of *in utero* ARV exposure with the echocardiographic Z-scores as a vector of outcomes grouped by type of measurement (LV systolic function, diastolic function, and structure). All analyses were conducted using SAS (Version 9.2) and were based on data submitted as of January 2013.

Results

Participant characteristics

Of the 678 children in the PHACS AMP Study, 227 were HEU. Of these 227, 192 (85%) had echocardiograms available for analysis (119 coenrolled in SMARTT) and 177 had detailed information on maternal ARV use during pregnancy. Three children were excluded due to cardiac congenital anomalies [one with ventricular septal defect, one with mitral valve prolapse, and the last with multiple cardiac defects (atrial septal defect, patent ductus arteriosus, ventricular septal defect) associated with trisomy 21]. Previous analyses indicated that the AMP children with echocardiograms were demographically similar to those without echocardiograms.[5]

Background characteristics of the 174 HEU youth included in the analysis are summarized in Table 1. Just under half (48%) were male and 56% were black non-Hispanic; 67 (39%) were exposed *in utero* to HAART, 58 (33%) to zidovudine monotherapy, 31 (18%) to other non-HAART ARV, and 18 (10%) were unexposed to any ARV. During the first trimester, 31% (54/174) were exposed to ARV: 25 to HAART, 14 to other non-HAART ARV, and 15 to zidovudine monotherapy. Their mean age at the time of the echocardiogram was 10.9 years overall, but HAART-exposed youth were significantly younger than those unexposed to HAART (9.2 years. vs. 12.0 years.), reflecting the increasing use of HAART during pregnancy over time. Other characteristics were generally similar between HAART-exposed and -unexposed youth, except that HAART-exposed youth had a lower prevalence of maternal alcohol use during pregnancy.

Echocardiographic parameters by in utero *HAART exposure*

Mean Z-scores for the 174 youth were generally close to zero or within half a standard deviation of the normative reference group from Boston Children's Hospital, both overall and within each of the ARV-unexposed, HAART-exposed, and non-HAART ARV-exposed groups (Supplementary Table S1; Supplementary Data are available online at www.liebertpub.com/aid). After excluding the 18 youth unexposed to any ARV, there were no significant differences in Z-scores comparing HAART-exposed with HAART-unexposed youth for the three LV systolic function measures in either unadjusted or adjusted models (Table 2).

In adjusted models for LV diastolic function parameters, HAART-exposed youth had significantly lower atrial velocity Z scores (0.00 vs. 0.52, $p = .04$) and higher E/A ratio Z-scores (0.24 vs. −0.15, $p = .04$) than HAART-unexposed youth (Fig. 1). Those exposed to HAART also had significantly higher adjusted mean mass-to-volume ratio Z-scores than HAART-unexposed youth.

Results were generally similar when comparing HEU youth exposed *in utero* to zidovudine monotherapy vs. those exposed to two or more ARV drugs in combination; there remained no significant difference in mean Z-scores for LV systolic function parameters, but for LV diastolic function measures, peak early deceleration time Z-score differed significantly between exposure groups (0.10 for zidovudine

Table 2. Results of Linear Regression Models Comparing HIV-Exposed Uninfected Children Born to HIV-Infected Mothers and Exposed or Unexposed *In Utero* to HAART

	Unadjusted Z-score mean (SE)			*Adjusted*[a] *Z-score mean (SE)*		
Echocardiographic Z-score	*Exposed to non-HAART ARV* (n = *89*)	*HAART exposed* (n = *67*)	P	*Exposed to non-HAART ARV* (n = *89*)	*HAART exposed* (n = *67*)	P
Functional systolic parameters						
Contractility	0.30 (0.12)	0.38 (0.13)	.67	0.35 (0.12)	0.32 (0.14)	.87
Fractional shortening	0.34 (0.10)	0.43 (0.12)	.57	0.34 (0.11)	0.43 (0.13)	.62
Ejection fraction	0.15 (0.09)	0.20 (0.11)	.75	0.19 (0.10)	0.15 (0.12)	.81
Functional diastolic parameters						
Peak early velocity	0.34 (0.10)	0.39 (0.12)	.79	0.37 (0.11)	0.35 (0.13)	.91
Peak atrial velocity	0.46 (0.15)	0.08 (0.16)	.091	0.52 (0.15)	0.00 (0.17)	.040
Peak early/atrial velocity (E/A ratio)	−0.13 (0.11)	0.20 (0.12)	.041	−0.15 (0.11)	0.24 (0.13)	.041
Peak early tissue (E′)	0.45 (0.17)	0.38 (0.20)	.80	0.51 (0.18)	0.28 (0.21)	.46
Peak early inflow/early tissue (E/E′)	0.04 (0.16)	0.11 (0.18)	.77	0.03 (0.16)	0.13 (0.19)	.71
Peak early deceleration time	−0.40 (0.11)	−0.26 (0.12)	.40	−0.41 (0.12)	−0.25 (0.14)	.44
Structural parameters						
LV mass	0.03 (0.09)	0.15 (0.11)	.39	0.02 (0.10)	0.18 (0.11)	.32
ED LV volume	−0.17 (0.11)	−0.18 (0.12)	.95	−0.11 (0.11)	−0.26 (0.12)	.40
ES LV volume	−0.33 (0.11)	−0.10 (0.12)	.16	−0.20 (0.10)	−0.26 (0.11)	.73
Mass-to-volume ratio	0.25 (0.10)	0.28 (0.12)	.89	0.11 (0.10)	0.47 (0.12)	.031

Results of linear regression modeling, excluding those unexposed to any ARVs during pregnancy ($N = 18$). Each row represents a separate linear regression model comparing youth exposed *in utero* to HAART with those unexposed to HAART.

[a]Adjusted for age, sex, race/ethnicity, BMI Z-score, and SBP Z-score; diastolic function measures also adjusted for heart rate.

BMI, body mass index; D, diastolic; ED, end-diastolic; ES, end-systolic; LV, left ventricular; S, systolic; SBP, systolic blood pressure.

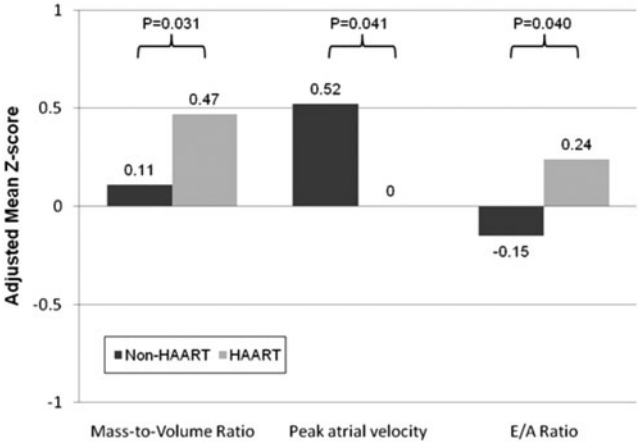

FIG. 1. Adjusted echocardiographic Z-score means by *in utero* HAART exposure.

monotherapy vs. –0.59 for combination ARVs, $p = .016$). A higher adjusted mean LV mass-to-volume ratio Z-score was also observed for combination regimens versus zidovudine monotherapy (0.46 vs. –0.08; see Supplementary Table S2).

Comparisons of mean Z-scores for echocardiographic parameters by first trimester ARV exposure are summarized in Table 3. There were no significant differences in mean Z-scores in either unadjusted or adjusted models for any of the LV systolic or diastolic function parameters.

Sensitivity analyses

In the subset of ARV-exposed youth with information on maternal substance use during pregnancy ($n = 116$ of 156, 74%), we observed few associations with echocardiographic parameters. Illicit drug use was associated with a lower mean LV mass (adjusted mean Z = –0.46 vs. 0.14, $p = .029$). When the models presented in Tables 2 and 3 were repeated within this subset with further adjustment for illicit drug use, there were essentially no changes to results. Low maternal CD4 cell count (<350 cells/mm^3) before delivery was associated with significantly higher LV mass (adjusted mean Z = 0.42 vs. –0.04, $p = .004$), higher LV volume (adjusted mean Z = 0.18 vs. –0.30, $p = .004$), and higher LV contractility (adjusted mean Z = 0.61 vs. –0.18, $p = .033$). However, the high percentage of youth missing this information precluded comparisons between HAART-exposed and HAART-unexposed subgroups adjusting for maternal health measures.

In our three sets of multivariate models, fit using sets of LV systolic functional, diastolic functional, and structural Z-scores as vectors of outcomes, we observed no association between *in utero* HAART exposure and any of these outcomes.

Discussion

We found that HIV-uninfected children born to HIV-infected women who were exposed to HAART *in utero* had significant differences in LV chamber structure compared with children not exposed to HAART, but LV systolic function was not different between the HAART-exposed and -unexposed cohorts. Specifically, there was a higher LV mass-to-volume ratio in those exposed to HAART in association with LV diastolic function differences consisting of a lower A-wave velocity and a higher E/A ratio.

Abnormalities of LV structure and function associated with antiretroviral therapy have been previously reported,[2,9] but the impact of HAART on the myocardium remains

Table 3. Results of Linear Regression Models Comparing HIV-Exposed Uninfected Children Born to HIV-Infected Mothers and Exposed or Unexposed *In Utero* to Any Antiretroviral Therapy During the First Trimester of Pregnancy

	Unadjusted Z-score mean (SE)			*Adjusted*[a] *Z-score Mean (SE)*		
Echocardiographic Z-score	*Unexposed in first trimester* (n = *102*)	*Exposed in first trimester* (n = *54*)	P	*Unexposed in first trimester* (n = *102*)	*Exposed in first trimester* (n = *54*)	P
Functional systolic parameters						
Contractility	0.28 (0.11)	0.44 (0.15)	.42	0.29 (0.10)	0.43 (0.15)	.43
Fractional shortening	0.32 (0.09)	0.48 (0.13)	.31	0.32 (0.09)	0.50 (0.13)	.26
Ejection fraction	0.18 (0.09)	0.15 (0.12)	.81	0.19 (0.09)	0.15 (0.13)	.81
Functional diastolic parameters						
Peak early velocity	0.47 (0.10)	0.16 (0.13)	.063	0.45 (0.09)	0.20 (0.13)	.13
Peak atrial velocity	0.38 (0.14)	0.13 (0.18)	.27	0.34 (0.13)	0.21 (0.18)	.57
Peak early/atrial velocity (E/A ratio)	0.02 (0.10)	0.02 (0.14)	.98	0.04 (0.10)	–0.02 (0.14)	.73
Peak early tissue (E′)	0.48 (0.16)	0.31 (0.22)	.53	0.47 (0.16)	0.30 (0.22)	.55
Peak early inflow/early tissue (E/E′)	0.13 (0.15)	–0.05 (0.20)	.47	0.12 (0.14)	–0.01 (0.20)	.61
Peak early deceleration time	–0.37 (0.10)	–0.27 (0.14)	.59	–0.37 (0.10)	–0.29 (0.14)	.69
Structural parameters						
LV mass	0.05 (0.09)	0.15 (0.12)	.52	0.05 (0.08)	0.15 (0.12)	.50
ED LV volume	–0.28 (0.10)	0.04 (0.14)	.063	–0.26 (0.09)	–0.01 (0.13)	.094
ES LV volume	–0.37 (0.10)	0.04 (0.14)	.018	–0.31 (0.08)	–0.07 (0.12)	.096
Mass-to-volume ratio	0.37 (0.09)	0.05 (0.13)	.046	0.33 (0.09)	0.13 (0.12)	.17

Results of linear regression modeling, excluding those unexposed to any ARVs during pregnancy ($N = 18$). Each row represents a separate linear regression model comparing youth exposed to any ARV during the first trimester of pregnancy with those unexposed to ARVs during the first trimester

[a]Adjusted for age, sex, race/ethnicity, BMI Z-score, and SBP Z-score; Diastolic function measures also adjusted for heart rate.

controversial. Lipshultz *et al.*[3] reported that in HIV-negative children of HIV-positive mothers, aged 0–2 years, ART exposure was associated with reduced LV mass, interventricular septal thickness, and short axis LV dimension in conjunction with increased LV fractional shortening and contractility. However, the long-term cardiac effects of fetal exposure to ART remain uncertain.

Recently, Cade *et al.*[4] reported a comparison between 30 HIV-negative children (mean age = 8 ± 2 years) born to HIV-positive women with prenatal exposure to ART vs. 30 age-matched HIV-negative children born to HIV-negative women. In their substantially smaller and somewhat younger cohort than in this study, the ART-exposed group had no differences in systolic function, but did have a lower LV mass index and lower early diastolic mitral annular velocity. These findings suggest the possibility of late myocardial effects of *in utero* ART exposure. These findings are somewhat difficult to interpret because the method used for adjusting LV mass for body size through indexing for body surface area is of questionable validity,[10] and notably, nonindexed LV mass was not significantly different between these groups who were in fact matched for age and BSA.

The two significant findings in our study of higher LV mass-to-volume ratio in conjunction with differences in LV diastolic behavior (lower late mitral inflow velocity that results in a higher E/A) in the ART-exposed cohort may be causally related since a higher LV mass-to-volume ratio is typically associated with lower ventricular compliance. Alterations in LV diastolic function can be secondary to changes in diastolic relaxation, compliance, or both and are not intrinsically related to systolic properties such as LV ejection fraction. Abnormalities of ventricular compliance and relaxation can be demonstrated by characteristic changes in mitral inflow, tissue Doppler velocity, and pulmonary venous Doppler flow patterns.[11]

The association between LV hypertrophy and LV diastolic dysfunction has been extensively described in LV hypertrophy models.[12] In HIV-infected adults, Grandi[13] showed in 60 asymptomatic patients that chronic HAART was associated with an increased LV mass-to-volume ratio and preclinical LV diastolic dysfunction, independent of blood pressure values, findings that parallel the LV remodeling pattern and LV diastolic function differences found in this study.

The fact that the alteration in LV diastolic function in our study cohort may be related to altered LV mass-to-volume ratio is important insofar as it calls into question whether this therapy has a direct adverse effect on diastolic myocardial properties, particularly since no differences were seen in E′ values, which are thought to be a more direct measure of myocardial properties. Although the potential implications of these minor variations from normal are unknown, seemingly mild abnormalities in LV structure have been associated with adverse late outcomes in other situations. For example, LV mass values that were slightly but insignificantly different from normal early after completion of therapy were associated with progressive decline over the ensuing 10 years.[14]

Because of the observational design of our study, there are some clear limitations. We were unable to fully control for maternal disease severity given the lack of information on maternal CD4 count and viral load for a large percentage of participants, which could have resulted in residual confounding. The two subgroups compared in our primary analyses defined by HAART exposure had some differences in age distributions, although we accounted for such differences through the use of echocardiographic Z-scores calculated relative to age and body size, as well as adjustment for age in our analyses. Despite these limitations, our study is the largest evaluation of long-term cardiac functioning in youth and adolescents who were exposed perinatally to HAART or other ARV regimens. Our consistent standards for echocardiogram measurement and ability to adjust for a number of potential demographic and maternal confounders are additional strengths of our study.

Altered LV systolic function in children with HIV infection is well described predominantly in studies that precede the introduction of ART.[15] Lipshultz *et al.*[16] found LV dysfunction and increased LV wall thickness as risk factors for mortality in HIV-infected children (median age 2.1 years). Cunha *et al.*[17] evaluated 93 infected children (average age 3.07 years) exposed and nonexposed to HAART and reported a positive association between the absence of combined ART and LV systolic dysfunction. In the current cohort, there were no significant differences in LV systolic function associated with HAART exposure (Table 2). Preserved LV systolic function associated with impaired LV diastolic function secondary to abnormal LV stiffness and impaired relaxation is a well-known cause of heart failure in adults[18] that has to date remained relatively recalcitrant to therapy.[19]

Conclusions

Taken together, our findings point toward the conclusion that prenatal ART exposure is associated with mild direct cardiac effects that are independent of other factors commonly seen in conjunction with the *in utero* environment of HIV-infected women. Whether the increase in the LV mass-to-volume ratio and LV diastolic dysfunction with preserved LV systolic function is of long-term importance requires longer follow-up of these vulnerable children. Further studies will be required to confirm this finding and the outcome in adult age and its clinical relevance.

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Disclaimer

The conclusions and opinions expressed in this article are those of the authors and do not necessarily reflect those of the U.S. National Institutes of Health or the U.S. Department of Health and Human Services.

Author Disclosure Statement

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