Elevated stress-responsive biomarkers are associated with HIV acquisition in young women in rural South Africa

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Objective: Biological markers of stress have been associated with HIV progression and pathogenesis but not with HIV incidence. We sought to determine if elevated stress-responsive biomarkers would be associated with incident HIV among adolescent girls and young women (AGYW).

Design: We conducted a case-cohort study within the HIV Prevention Trials Network (HPTN) 068 study among 949 AGYW in South Africa. Cases were AGYW who tested HIV-positive during the eight-year follow-up. Unmatched controls were randomly selected from the HIV-negative population at enrollment.

Methods: Dried blood spots from cases and controls were tested from enrollment (2011–2012) for C-reactive protein (CRP), herpes simplex virus type-1 (HSV-1) antibody titers, and cytomegalovirus (CMV) antibody titers. Cox proportional hazards models estimated the association between each biomarker and time to incident HIV.

Results: Compared to AGYW with the lowest CRP levels, those with medium and high CRP levels had a higher hazard ratio (HR) of incident HIV [HR: 1.45, 95% confidence interval (CI): 0.95, 2.21; HR: 1.50, 95% CI: 0.98, 2.30, respectively], although not statistically significant. The relative hazard of incident HIV was also higher among AGYW who were CMV seropositive vs. seronegative (low antibodies HR: 2.18, 95% CI: 1.2, 3.87; medium HR: 2.25, 95% CI: 1.28, 3.95; high HR: 1.78, 95% CI: 0.99, 3.21). Those with the highest HSV-1 antibody levels experienced an increased hazard of HIV compared to those who were HSV-1 seronegative (HR: 1.58, 95% CI: 1.03, 2.44).

Conclusions: Biological stress may increase AGYW's susceptibility to HIV acquisition through changes in immune function, viral infection, and increased biological vulnerability to disease.

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Introduction

Adolescent girls and young women (AGYW) remain at very high risk of HIV acquisition in Eastern and Southern Africa, accounting for 24% of new infections in South Africa [1,2]. HIV acquisition in AGYW has been strongly associated with social and structural factors such as violence and economic instability [3]. Research among adults in the United States has demonstrated that activation of the biological stress response by social and structural factors like economic disadvantage and violence, is a pathway by which these factors can affect health outcomes [4]. Several key questions remain to examine if biological stress is associated with HIV incidence among AGYW to inform interventions to prevent HIV acquisition in this population.

The stress response in the brain involves three systems: the sympathetic–adrenal–medullary (SAM) axis, the hypothalamic–pituitary–adrenal (HPA) axis, and the immune system [5,6]. Extreme or repeated reactivation of these systems through some of the factors mentioned above can dysregulate the immune response by disturbing the sensitive interplay among these systems and altering how the body responds to stress [7–9]. Both biological stress measured through stress-responsive biomarkers and self-reported perceived stress have been associated with progression and pathogenesis of HIV, including viral load, $CD4^+$ cell count, and AIDS clinical classification [10–13], and with other poor health outcomes associated with HIV, such as depression and posttraumatic stress disorder [7,14,15]. There is also rich evidence that chronic stressors over the life course directly influence the immune system by increasing susceptibility to viral and bacterial infections, such as the common cold, influenza, Toxoplasma, Salmonella, and through changes in immune response to vaccination [16–21]. Stress can alter immune function through direct innervation of lymphatic tissue, through release of hormones that bind to and alter the functions of immune cells, or through changes in behavior that are associated with stress such as increased alcohol use [7–9,22]. Alterations in immunity and stress hormone levels that occur with biological stress can cause latent herpesviruses to reactivate and start replicating to cause disease [16,21]. AGYW with impaired immune function or viral infection may therefore be more biologically vulnerable to HIV acquisition.

The goal of this study was to explore the hypothesis that biological stress affects risk of HIV acquisition for AGYW. We examined stress, immune function, and susceptibility to viral infection through commonly used stress-responsive immune biomarkers (hereafter called stress-responsive biomarkers), including C-reactive protein (CRP), herpes simplex virus type 1 (HSV-1), and cytomegalovirus (CMV). CRP, HSV-1, and CMV have been associated with an impaired immune response and with measures of adversity and disadvantage, including perceptions of stress [11,16,23,24]; high levels of crime or poverty [25,26]; poor mental health status [216,27]; and violence in childhood and adolescence [5,28–30]. Additionally, traumatic events like violence during adolescence can affect decision-making, leading to increased risk-taking behaviors such as substance abuse, and condomless sex [4,31]. This evidence indicates that biological stress likely affects risk of HIV acquisition through a number of pathways (e.g., changes in immune function and behavior among AGYW which increase susceptibility to HIV acquisition). Conceptually, the relationship between biological stress and incident HIV is plausible and supported by research suggesting that adversity at the population level, such as neighborhood poverty, is correlated with HIV prevalence [16,21]. However, limited research has been conducted to understand these relationships in high-HIV incidence areas, such as South Africa.

We conducted a case cohort study nested within the HIV Prevention Trials Network (HPTN) 068 study to estimate the association between stress-responsive biomarkers (CRP, CMV, and HSV-1) during adolescence with time to incident HIV diagnosis at later time points. We hypothesized that increased levels of CRP and infection or increased antibody titers for CMV and HSV-1 would be associated with HIV acquisition.

Methods

Parent study

Our study used longitudinal data from a sample of Black South African AGYW who were followed from 2011 to 2019 with seven visits in the HPTN 068 study. HPTN 068 was a phase 3, randomized controlled trial to determine whether providing cash transfers, conditional on school attendance, reduces young women's risk of HIV acquisition [32]. HPTN 068 enrolled 2533 young women aged 13–20 years, who were seen annually for up to 3 years from 2011 to 2015. Since study completion, participants have been followed for 4 more years postintervention (2015–2019; seven visits total). Each visit included an Audio Computer-Assisted Self-Interview (ACASI) assessment with self-reported information on sexual behaviors and HIV and herpes simplex virus type 2 (HSV-2) testing. In the 2018/ 2019 survey, dried blood spot (DBS) samples were tested for HSV-1, CMV, and CRP.

The study was conducted at the site of the Agincourt Health and Demographic Surveillance Survey in rural Bushbuckridge subdistrict in Mpumalanga Province, South Africa; in a former homeland with limited availability of basic public services and high levels of unemployment and poverty [33]. The apartheid system in South Africa supported a homeland policy whereby Black

South Africans could live in rural areas and be granted independent statehood [34]. The apartheid system in South Africa used four racial categories to enforce segregation: Black African, White, Colored (refers to mixed race individuals as well as descendants of indigenous Khoisan people) and Indian. However, in our setting there are only Black participants given that we work in a former homeland. As these categories have impacted on health, education and employment opportunities, we denote in analyses not to reinforce racial differences but rather to acknowledge the long-term consequences of segregation. In 2011, the prevalence of HIV in young women aged 15–19 years in this area was 5.5%, rising to 27% by age 20–24 years [35].

Case–cohort study design

To assess the association between stress-responsive biomarkers during adolescence and time to incident HIV acquisition over the study period, we conducted a case–cohort study among 949 AGYW who were HIV negative at baseline in HPTN 068. A case-cohort design was selected to increase efficiency and minimize costs associated with testing stress-responsive biomarkers [36,37]. Additionally, the hazard ratio from a case cohort design approximates the hazard ratio that one would get from a regular cohort study and allowed us to use time to event analysis which is appropriate for estimating HIV incidence [37]. In a case cohort design, controls are randomly selected at the beginning of the study whether or not they later become a case. We selected controls ($n = 891$) by taking a random sample of participants from HPTN 068 at baseline who were HIV negative, regardless of whether they later became a case (i.e., tested positive for HIV at later visits). This randomly sampled "sub-cohort" was designed to be representative of the original study population at enrollment, and included participants who both remained negative throughout the study ($n = 774$) and those who later became cases ($n = 117$) [37]. Cases were defined as participants from HPTN 068 who tested positive for HIV during the study's follow-up visits and who consented to testing of stored samples Additional cases ($N = 58$) were also included who were not in the sub-cohort for a total of 175 case ($n = 117$ in sub-cohort and $n = 58$ additional outside of sub-cohort). Our sample size was selected based on a power calculation using approximately 2 : 1 controls per case and was adjusted to maximize the number of samples that could be tested with our budget. We tested DBS cards stored from the enrollment visit (2011/2012) for stress-responsive biomarkers (HSV-1, CMV, and CRP) for the 949 participants. As the cash transfer intervention was not associated with HIV acquisition [32], we sampled controls irrespective of intervention arm but controlled for the cash transfer as a possible confounder.

Measures

Exposures of interest included CRP levels, HSV-1 [primary infection or optical density (OD) levels], and CMV (primary infection or OD levels) measured via DBS cards. Prior studies have shown that CRP, HSV-1, and CMV measurements from DBS are valid, feasible, and reliable [38,39]. OD levels for IgG tests approximately correspond to antibody titer where a seropositive low OD value indicates low antibody titer, and a seropositive high OD value indicates high antibody titer. CMV and HSV-1 OD levels were measured using Trinity Biotech Captia CMV immunoglobulin G(IgG) enzyme-linked immunosorbent assay (ELISA) and Trinity Biotech Captia HSV-1 IgG kit ELISA, respectively. For CMV and HSV-1, the OD value of each sample was divided by the calibrator cutoff for each plate to determine the immune status ratio (ISR). An ISR value of ≤ 0.90 was categorized as no detectable antibody to CMV/HSV-1. A value between 0.91 and 1.09 was categorized as equivocal and samples were retested. A value of more than or equal to 1.10 was defined as the presence of detectable antibody. CRP levels (mg/l) were measured using the CRP ELISA from Immunodiasgnostik (IDK) [40]. Similar to prior studies, we coded CMV and HSV-1 using four categories including seronegative (0) and OD levels divided into tertiles (1 = low, 2 = medium, and 3 = high) [29,30,37]. Equivocal results were coded as seropositive low OD levels to provide more conservative estimates. CRP was coded using tertiles (1 = low, 2 = medium, and 3 = high). CRP levels that were out of range were recoded as 0.015, and outliers over 40 were removed.

The primary outcome of interest was time to detection of incident HIV. This was defined as the time (in years) from each participant's HPTN 068 enrollment visit to a positive HV test during the study's follow-up period. HIV testing was done at approximately annual at each visit using two different HIV rapid tests performed in parallel [32]. If one or both of the HIV rapid tests was reactive, confirmatory testing was performed. Potential confounders included age (years), food insecurity (worry about food in the last 12 months), orphanhood (loss of both parents), HSV-2 serostatus, any previous physical intimate partner violence (yes/no), and receipt of the cash transfer intervention during the HPTN-068 study. These variables were selected a priori and have been shown to be associated with incident HIV infection and HIV-related sexual behaviors among AGYW in Southern Africa [3]. The construction of these covariates has been described in prior publications [29,30,32,37]. HSV-2 testing was done on blood samples at all visits using the HSV- 2 IgG ELISA assay (Kalon Biological Ltd, Guildford, UK) with prevalent infection an index cutoff of 1.5 [32].

Statistical analysis

The case-cohort design allowed us to incorporate time into the analysis to estimate a hazard ratio for incident HIV acquisition [38]. A cox proportional hazards model was used to estimate time to incident HIV infection and because the hazard ratio from a case-cohort study ($N = 949$) approximates the hazard ratio from the full

HIV negative HPTN 068 sample ($N = 2448$) [37]. To estimate the effect of each stress-responsive biomarker on time to detection of HIV, we used a Cox proportional hazards model with robust standard errors to calculate the crude and adjusted hazard ratios (HRs) and 95% CIs for the association between biomarkers at the first visit and time to detection of incident HIV over the entire follow-up period. Participants in the case-cohort study were censored either at the time of the event, death, loss to follow-up, or end of the study period. AGYW in the sub-cohort (i.e., controls and those who later became cases) entered the analysis at the time of their first study visit. Cases not in the sub-cohort were included as extremely late entries right before their event time ($t = 0.005$) to account for the case–cohort design [37]. Doing so allowed cases to only appear in their own risk set. In a sensitivity analysis, we used inverse probability weights to account for missing data due to loss to follow-up and observed similar results. Therefore, we present unweighted models in the results.

Results

Out of 949 HIV negative participants in the case cohort study, there were 175 incident cases during the follow-up period (58 cases and 117 controls who later became cases; 18.4%). Among the 891 controls at enrollment, the median age was 15 [interquartile range (IQR): 14, 17], 34.6% (266) were food insecure, 20.8% (160) had a mother or father die under the age of 18, and 17.0% (129) had ever experienced intimate partner violence (Table 1). The baseline characteristics of the controls were similar to those of the entire HIV negative HPTN 068 study population at enrollment ($N = 2448$), suggesting that the case–cohort sampling was successful (Appendix, Table S1, Supplemental Digital Content, http://links.lww.com/QAD/D289). When examining demographic imbalances by those who were lost to follow-up over the study period, there were only slight differences by case-control arm (52.2% vs. 47.9%) and by HIV diagnosis during the study (13% vs. 14.3%; data not shown).

Table 2 shows descriptive relationships between AGYW living with HIV at enrollment ($n = 36$) and stress biomarkers, although these AGYW were not included in the primary analysis of incident infection and sample sizes are small. HIV prevalence was associated with higher levels of CRP (58.8% with high CRP among those living with HIV vs. 32.4% in HIV negative; Table 2). Conversely, AGYW who were living with HIV were more likely to be HSV-1 negative (80.6%) compared to those who were not (53.4%).

Over the follow up period, AGYW with higher levels of CRP, CMV, and HSV-1 had a higher relative hazard of incident HIV acquisition, adjusting for other covariates (Table 3). Among AGYW with medium or high CRP levels at baseline, the relative hazard of incident HIV was 1.45 and 1.50 times that compared to AGYW with low CRP levels (medium HR: 1.45, 95% CI: 0.95, 2.21; high HR:1.50, 95% CI: 0.98, 2.30), although not statistically significant in adjusted models. The strongest associations were observed between CMV and incident HIV acquisition: AGYW who were CMV seropositive had 2.18, 2.25, and 1.78 times the relative hazard of incident HIV acquisition compared to CMV seronegative AGYW depending on whether their OD levels fell in the lowest, middle, or highest tertile, respectively (low HR: 2.18, 95% CI: 1.23, 3.87; medium HR: 2.25, 95% CI: 1.28, 3.95; high HR:1.78, 95% CI: 0.99, 3.21). High HSV-1 OD levels were also associated with HIV acquisition (high HR: 1.58, 95% CI: 1.03, 2.44) compared to those were seronegative.

Discussion

Elevated levels of stress-responsive biomarkers (i.e., CRP, CMV, and HSV-1) during adolescence were associated with an increased hazard of HIV incidence over a 9-year period among AGYW in rural South Africa, although associations with CRP were not statistically significant. In prior research, we found that these biological markers of stress were associated with intimate partner violence and with household economic shocks [29,30]. While more research is needed to understand the mechanisms by which biological stress affects HIV acquisition, our findings indicate that interventions to reduce stress or to support AGYW who have experienced stressful life events may reduce the risk of HIV acquisition during young adulthood. Interventions are particularly needed to prevent violence and to reduce the sequela of experiences of violence for young women such as mindfulness-based approaches, cognitive behavioral therapy, and life skills training.

We found that elevated stress biomarkers in adolescence (age 13–20 years) were associated with HIV incidence throughout young adulthood, highlighting the importance of intervening early to reduce the negative consequences of chronic stress throughout emerging adulthood. Adolescence and young adulthood is a critical time during which the brain is still developing and chronic stress can have long term effects on health, making it even more important to intervene early. Stress during adolescence elicits a larger and more prolonged response than similar experiences in adulthood and can lead to heightened emotional and physiological reactivity to similar stressors in the future [4,8]. Chronic stress experienced during one's developmental years, such as adverse childhood experiences, has been shown to have long-lasting neurobiological effects and to increases one's risk of later morbidity (e.g., anxiety, depression, chronic

Table 1. Baseline demographic characteristics and stress-responsive biomarker levels by case–control status among HIV negative adolescent girls and young women.

	Control ($n=774$)		Case ($n=175$)		Total ($n=949$)		
	N/median	%//IQR	*N*/median	%//IQR	*N*/median	%//IQR	*P*-value
Age, median IQR (interquartile range)	15	14, 17	16	15, 17	15	14, 17	0.003
Tertiles of C-reactive protein							
Low	268	36.0	44	25.9	312	34.1	0.041
Middle	243	32.7	63	37.1	306	33.5	
High	233	31.3	63	37.1	296	32.4	
Quartiles of cytomegalovirus							
Negative	139	18.0	16	9.1	155	16.3	0.007
Positive and low OD levels	215	27.8	55	31.4	270	28.5	
Positive and middle OD levels	202	26.1	61	34.9	263	27.7	
Positive and high OD levels	218	28.2	43	24.6	261	27.5	
Quartiles of herpes simplex virus type 1							
Negative	422	54.5	85	48.6	507	53.4	0.100
Positive and low OD levels	141	18.2	26	14.9	167	17.6	
Positive and middle OD levels	107	13.8	31	17.7	138	14.5	
Positive and high OD levels	104	13.4	33	18.9	137	14.4	
Conditional case transfer intervention							
Control	391	50.5	77	44.0	468	49.3	0.119
Intervention	383	49.5	98	56.0	481	50.7	
Grade enrolled							
9	202	26.1	40	22.9	242	25.5	0.092
10	226	29.2	39	22.3	265	27.9	
11	196	25.3	57	32.6	253	26.7	
12	150	19.4	39	22.3	189	19.9	
Ever pregnant							
No	708	91.8	141	81.5	849	89.9	0.000
Yes	63	8.2	32	18.5	95	10.1	
Alcohol more than once a month or more vs. other							
No	758	98.1	168	96.0	926	97.7	0.102
Yes	15	1.9	7	4.0	22	2.3	
If mother or father died when age <18							
No	611	79.2	134	77.9	745	79.0	0.696
Yes	160	20.8	38	22.1	198	21.0	
Ever any intimate partner violence (IPV)							
No	628	83.0	132	76.3	760	81.7	0.041
Yes	129	17.0	41	23.7	170	18.3	
IPV in the past 12 months							
No	680	89.8	146	84.4	826	88.8	0.041
Yes	77	10.2	27	15.6	104	11.2	
Child depression score ≥7							
No	612	82.8	126	75.9	738	81.5	0.038
Yes	127	17.2	40	24.1	167	18.5	
Worry about food past 12 months							
No	502	65.4	105	60.3	607	64.4	0.212
Yes	266	34.6	69	39.7	335	35.6	
Wealth quintiles (assets)							
Low	215	27.8	55	31.4	270	28.5	0.339
Middle low	218	28.2	54	30.9	272	28.7	0.483
Middle high	172	22.3	41	23.4	213	22.5	0.736
High	168	21.7	25	14.3	193	20.4	0.027

IQR, interquartile range; OD, optical density.

disease) and mortality [41,42]. Chronic stress early in life has also been associated with health behaviors such as sexual behaviors that are associated with HIV incidence (e.g. transactional sex) and increased alcohol use which may be a pathway through which chronic stress can lead to adverse health outcomes [21,42].

The markers used in our study may also indicate greater biological vulnerability overall that places AGYW are greater risk of HIV. CRP is a measure of systematic inflammation that can be indicative of other underlying infections, injury, and chronic health conditions [25,42]. HSV is associated with cold scores and increases risk of genital ulcer disease (GUD) which can increase HIV risk [24,43,44]. CMV is a highly prevalent (~80% seroprevalence in this study) and causes a persistent asymptomatic infection that requires immune activation to suppress, and over time may contribute to immune senescence or reductions in immune responsiveness with age [45,46]. Infection with and reactivation of herpesviruses (particularly HSV-2) has been associated with HIV acquisition [16,43], and is consistent with the findings from this study showing that CMV and HSV-1 biomarkers are associated with HIV incidence among AGYW. Although acute

Table 2. Stress biomarkers by HIV status at enrollment.

	HIV status at enrollment						Significance
	Negative ($n=949$)		Positive ($n=36$)		Total		
	No.	%	No.	%	No.	%	
Tertiles of CRP							
Low	312	34.1	4	11.8	316	33.3	0.003
Middle	306	33.5	10	29.4	316	33.3	
High	296	32.4	20	58.8	316	33.3	
CMV							
Negative	155	16.3	7	19.4	162	16.4	0.906
Low	270	28.5	9	25.0	279	28.3	
Middle	263	27.7	9	25.0	272	27.6	
High	261	27.5	11	30.6	272	27.6	
HSV-1							
Negative	507	53.4	29	80.6	536	54.4	0.012
Low	167	17.6	4	11.1	171	17.4	
Middle	138	14.5	1	2.8	139	14.1	
High	137	14.4	2	5.6	139	14.1	

CMV, cytomegalovirus; CRP, C-reactive protein; HSV-1, herpes simplex virus type-1.

inflammation and cell mediated response to infection is an adaptive response which protects the body after physical injury or infection, exaggerated and/or prolonged immune and consequent inflammatory response is associated with numerous adverse health outcomes [17]. Therefore, stress may increase CRP levels and infection and reactivation of CMV and HSV-1 which overall leads to a state or more biological vulnerability to disease that can increase HIV risk. Our results build on current evidence and indicate that CMV, CRP, and HSV-1 which are related to stress may be indicative of greater biological susceptibility overall, and increase risk of HIV acquisition among AGYW.

Currently, there are several widely used, evidence-based interventions to reduce stress or to improve mental wellbeing in AGYW in East and Southern Africa. However, these interventions need to be culturally adapted to specific contexts and to reflect the full scope of adolescent development (early and late) [47]. Psychological interventions, including problem solving therapy, cognitive behavioral therapy, mindfulness, life skills training and relaxation techniques have been found to effectively reduce stress in adults and adolescents [18,48]. A meta-analysis and systematic review of psychological interventions found that these interventions were also effective in improving immunity and may be good candidates to improve health among AGYW [49]. In the area where this research was conducted, resources and access to mental health services is limited given the rural setting but a study is currently ongoing to test a mobile phone app using behavioral activation which could also be a candidate intervention to reduce stress. Additionally, interventions are needed to address social and structural factors such as exposure to violence that are closely tied to stress and mental health. In prior work from this study, we found that having a high probability of IPV throughout adolescence was associated with elevated levels of CRP, and that the association between IPV and CRP was decreased among young women who received cash transfers [30]. We also found that elevated CMV levels were associated with IPV cross-sectionally [29]. Therefore, structural interventions such as cash transfers that can reduce biological stress or other negative health

Table 3. Hazard ratios (HR) and 95% confidence intervals (CIs) for the association between stress-responsive biomarkers and HIV incidence among adolescent girls and young women in rural South Africa[a,b].

	Unadjusted HR (95% CI)	Adjusted HR (95% CI)
CRP		
Medium vs. low	1.40 (1.00, 2.22)	1.45 (0.95, 2.21)
High vs. low	1.62 (1.08, 2.41)	1.50 (0.98, 2.30)
CMV		
Seropositive: low vs. seronegative	2.35 (1.34, 4.14)	2.18 (1.23, 3.87)
Seropositive: medium vs. seronegative	2.48 (1.42, 4.32)	2.25 (1.28, 3.95)
Seropositive: high vs. seronegative	1.79 (1.00, 3.20)	1.78 (0.99, 3.21)
HSV-1		
Seropositive: low vs. seronegative	0.86 (0.53, 1.40)	0.92 (0.56, 1.50)
Seropositive: medium vs. seronegative	1.27 (0.83, 1.94)	1.46 (0.94, 2.26)
Seropositive: high vs. seronegative	1.60 (1.06, 2.41)	1.58 (1.03, 2.44)

[a]Adjusted for the conditional cash transfer, age, previous intimate partner violence, orphanhood, food insecurity, and HSV-2 serostatus.
[b]CMV, cytomegalovirus; CRP, C-reactive protein; HSV-1, herpes simplex virus type-1.

consequences of violence may be a means of decreasing risk of HIV acquisition. Lastly, social support and diverse social networks have been shown to buffer the effects of stress on susceptibility to the common cold and is associated with numerous health outcomes in adolescents and young adults [19]. Increased social support could be used to reduce negative consequences of chronic stress on health in AGYW.

Our study is the first, to our knowledge, to document that CMV and HSV-1 are associated with increased HIV incidence among AGYW. However, there are several limitations to our study. Many different biomarkers are used as measures of the biological stress responses. We chose to use CRP, CMV, and HSV-1 because they could be measured using DBS and did not require multiple measurements, which is more feasible in low resource settings such as South Africa [38,39]. We used multiple measures to make sure that associations were consistent and to increase our opportunity to identify associations in this population that have yet to be demonstrated. Future studies should examine other stress biomarkers in addition to the ones we report in our study and examine biological mechanisms. Additionally, one time measurement of biomarkers may be indicative of other underlying health conditions and may change over time. Longitudinal studies with multiple measurements over time are needed to corroborate these results. Lastly, the cohort from which cases and controls were selected included only young women enrolled in school [50]. Young women in school may experience different stressors than those not in school. Similar studies are needed in other populations and settings to ensure consistent results.

In summary, elevated levels of stress-responsive biomarkers (i.e., CRP, CMV, and HSV-1) during adolescence were related to increased incident HIV acquisition over a 9-year period among AGYW in rural South Africa. Our results build on current evidence and indicate that biological stress likely increases the risk of HIV acquisition among AGYW through changes related to infection susceptibility, immune function and overall biological susceptibility to disease. While more research is needed to understand the specific mechanisms by which biological stress affects incident HIV and to examine other biomarkers, interventions to reduce stress or to support AGYW who have experienced stressful events early during adolescence are needed, especially those to reduce violence and the chronic stress associated with experiences of violence.

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Data sharing statement: Data from the HPTN 068 study is currently available through the HIV Prevention Trials Network. Code for this project is available by contacting the corresponding author.

Author contributions: M.C.D.S. and N.K.K. conducted data analysis. K.K. and A.E.P. were the principal investigators on the parent study. M.C.D.S. was the principal investigator of this study and oversaw testing of biospecimens with support from D.W. F.X.G.O., A.E.A., A.E., K.K., M.C.D.S. conceptualized the study. D.W. oversaw study implementation. M.C.D.S. led manuscript writing with support from N.K.K. and S.M. All authors have read and approved the final manuscript.

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Conflicts of interest

There are no conflicts of interest.

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