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Forgoing antiretroviral therapy to evade stigma among people living with HIV, Cape Town, South Africa

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Abstract Stigma impedes HIV treatment in multiple ways, including diminished engagement in care, refusing ART, and concealing ART to evade stigma. This study disentangled the degree to which intentionally not taking ART to evade stigma influences overall non-adherence to ART. Patients receiving ART at a community clinic in Cape Town, South Africa (N = 288) completed confidential surveys of demographic and health characteristics, stigma-avoidance non-adherence, and non-stigma-related predictors of non-adherence. Results found nearly half of participants (48%) had forgone taking their ART in social settings. Hierarchically structured regression models showed that alcohol use, medication concerns, and internalized HIV stigma significantly predicted ART non-adherence, accounting for 9.9% of the variance. Stigma-avoidance non-adherence explained an additional 2.6% of the variance in ART non-adherence. The current findings extend previous research to show that stigma-avoidance independently contributes to ART non-adherence, including over and above depression and alcohol use.

Keywords HIV stigma · HIV treatment · Medication adherence · Intentional non-adherence

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Introduction

The global scale-up of antiretroviral therapy (ART) has reduced HIV-related morbidity and has brought new hope to ending HIV epidemics. The promise of HIV treatment, however, is thwarted by sub-optimal ART adherence. In South Africa, a country with one of the world's largest ART programs, more than one in five persons receiving ART are not virally suppressed (Avert, 2018). Multiple factors contribute to ART non-adherence including depression, alcohol use, and medication necessity and concerns beliefs (Horne et al., 2013). Behavioral interventions that aim to improve ART adherence focus on addressing these sources of non-adherence by building behavioral skills, using medication reminders, regulating mood, and providing social support (de Bruin et al., 2010; Simoni et al., 2006). HIV stigma, however, also contributes to poor ART adherence (Croome et al., 2017; Hoffman et al., 2017; Ramlagan et al., 2018; Sweeney & Vanable, 2016). In sub-Saharan Africa, HIV stigma overshadows side-effects and even forgetfulness as a barrier to ART adherence (Ammon et al., 2018).

One way in which HIV stigma impacts adherence occurs when ART is concealed and doses are skipped to evade HIV stigma (Croome et al., 2017; Heestermans et al., 2016). Strategies for managing medications aimed toward avoiding stigma, including hiding medications, storing them in secret places, and removing labels from medication bottles have been shown to impede adherence (Kalichman et al., 2019b). Nevertheless, ART adherence interventions have generally not addressed the effects of stigma-avoidance strategies on non-adherence. In fact, some of the most common adherence intervention strategies, such as keeping medications in clear view, use of pillbox organizers, pill-taking alarms, text message reminders, and social support are all contraindicated for

patients concerned with concealing their medications to avoid stigma (Kalichman et al., 2019a, b). Although stigma avoidance strategies are correlated with overall ART non-adherence, research has not yet determined the degree to which stigma-avoidance non-adherence contributes to overall ART non-adherence.

The current study was undertaken to disentangle the variance in ART non-adherence accounted for by intentionally forgoing ART to evade stigma. We have conceptualized stigma-motivated non-adherence as a contributing factor to overall medication adherence and this study is therefore not intended to test whether stigma avoidance is associated with non-adherence, but rather the magnitude of the association. To answer this question we constructed hierarchical regression models to test the independent contributions of multiple well-known sources of non-adherence followed by testing the independent contribution of evading stigma. We also examined HIV status disclosure practices and the use of stigma-avoidance medication management strategies among persons who have and have not forgone taking ART to evade stigma.

Methods

Participants and setting

Our study was conducted at a public health clinic located in an economically under-resourced township in Cape Town, South Africa. Participants were recruited while waiting to receive services at an ART dispensary. The clinic provides services to people living with HIV under the South African national guidelines for task shifting in primary care.

Measures

Measures were collected using a self-administered survey offered in English and isiXhosa, the two languages spoken by nearly all clinic patients. The measures were administered via smartphones using an automated survey system. Participants were instructed in how to complete the survey on a project-provided smartphone; less than 5% required the measures be delivered in an interview by research staff. Measures included demographic and health characteristics, ART adherence and contributing factors to ART non-adherence, specifically depression, alcohol use, medication beliefs, and HIV stigma experiences. We also assessed HIV status disclosure and use of medication management strategies to evade stigma. All measures were pilot tested with native speakers for clarity and translation/back-translation accuracy.

Demographic and health characteristics

Participants reported their age, gender, race, level of formal education, and marital status. We asked participants the number of ART tablets they take each day and how often their ART is dispensed. Participants also reported whether they knew if their HIV viral load was undetectable or detectable. In addition, we obtained participant permission to collect their most recent HIV viral load information from the clinic electronic medical records.

Barriers to ART adherence

The 10-item cognitive-affective subscale of the Centers for Epidemiological Studies Depression (CESD) scale assessed symptoms of depression (Radloff, 1977), alpha = 0.87. Items focused on how often a participant had specific depression thoughts, feelings and behaviors in the last seven days. Responses were *0 = 0 days, 1 = 1–2 days, 2 = 3–4 days, 3 = 5–7 days,* with scores ranging from 0 to 30. We also administered the three item Alcohol Use Disorder Identification Test Consumption (AUDIT-C) scale to assess quantity and frequency of alcohol use, including frequency of binge drinking (Maisto et al., 2000). Alcohol questions were expressed in terms of standard drinks using local alcohol beverage examples.

Medication beliefs were assessed with the Beliefs about Medicines Questionnaire (Horne et al., 2013). The medication necessity sub-scale consists of five items reflecting the perceived benefits of medications in direct relation to health; example items include 'My health, at present, depends on my medicine', 'My life would be impossible without my medicine', and 'My medicines protect me from becoming worse.' The five-item medication concerns sub-scale reflects the potential adverse effects and costs of medications; example items include 'Having to take my medicines worries me', 'My medicines disrupt my life', and 'I sometimes worry about the long-term effects of my medicines'. Items were responded to on a 5-point scale, 1 = *Strongly agree*, 5 = *Strongly disagree*. As was the case in the scale development, the medication necessity and concerns scales are independent in the current sample, r = 0.01, ns, and the medication necessity and concerns scales were both internally consistent, alphas = 0.78 and 0.81, respectively.

We administered an adapted version of the HIV Sigma Mechanism Measure (Earnshaw et al., 2013) to assess anticipated, internalized, and enacted stigma. We selected two items from the anticipated stigma and two items from the internalized stigma scales that represent the core constructs with high item-to-total correlations (Earnshaw & Chaudoir, 2009). Items used to index anticipated stigma were 'I worry that having HIV will cause me to lose a place to stay' and 'I worry that having HIV will cause me to lose a job'. For

internalized stigma, we included 'I feel embarrassed because I have HIV' and 'I feel my HIV is my own fault.' Scores represent the average rating on a 3-point scale, 0 = *Never*, 1 = *Sometimes*, and 2 = *Often*. To assess enacted stigma, we selected nine items to capture a broad array of overt stigma experiences. We adapted items from measures of HIV stigma (Earnshaw et al., 2013) and micro-aggressions (Woodford et al., 2013). Example items include 'Someone acted uncomfortable because of my HIV status', 'Family members have looked down on me because I have HIV', and 'I have been physically hurt or harmed by someone because I have HIV'. Items were coded to indicate whether participants had or had not experienced each enacted stigma event, alpha = 0.81.

HIV status disclosure

We adapted two items from previous research to assess participants' openness/disclosure of their HIV status. The items reflect perceived difficulty disclosing HIV and degree of openness about HIV status to family. The items are shown in the results and were responded to using a 3-point scale, 0 = *Never*, 1 = *Sometimes*, 2 = *Often*.

ART adherence

This study used the three item self-report instrument for retrospective adherence (IRA) developed and validated by Wilson et al. (2014, 2020). Items represent the number of days medications were taken over the previous 7 days, the frequency of taking medications as directed, and a self-perception rating of how well medications were taken over the previous week. We used methods suggested by Wilson et al. to convert scores for each item on a scale of 0 to 100 using linear transformations and calculating the mean to obtain a single adherence score with a range from 0 to 100, interpreted as percent adherence over the past week. Wilson et al. (2014) found that the IRA correlates 0.74 with electronically monitored ART adherence, alpha = 0.73. In the current sample, construct validity of the IRA was supported by participants' most recent HIV viral load in medical records with detectable values significantly associated with lower IRA scores, OR = 0.97, $p < 0.01$, 95% CI, 0.95–0.99.

Stigma-avoidance non-adherence

We assessed whether participants had foregone taking their ART in social contexts to evade stigma using the question, 'How often do you miss or skip taking your ARV tablets because other people are around and you do not want them to know?' Responses therefore represented whether participants had intentionally not taken ART in social contexts in a 30-day timeframe, responded to as 0 = *Never*, 1 = *Sometimes*, 2 = *Often*.

Stigma-medication management

Participants indicated whether they practiced seven behaviors associated with managing their medications to conceal their HIV status. Items were developed from previous research on HIV stigma and disclosure concerns among people treated for HIV (Sweeney & Vanable, 2016). The items asked whether participants practice behaviors related to storing, managing and taking ART with respect to their social environment. We used previous measures of medication stigma and social influences on adherence to guide the wording of the items (Blake Helms et al., 2017; Kamaradova et al., 2016; Sweeney & Vanable, 2016; Wang et al., 2016). Responses were dichotomous to indicate whether or not participants engaged in the behaviors.

Procedures

Individuals age 18 and older were approached to complete confidential surveys while waiting for clinical services. We sampled participants at all hours of clinic operations. Of the 334 potential participants approached, 296 (87%) patients completed the survey, of which 288 were currently receiving ART prior to the clinic visit. The most common reasons for declining participation were time constraints. Measures were self-administered and required approximately 15-min to complete. Participants provided written informed consent and were offered an incentive equivalent to US$2. All study protocols were approved by US and South African ethical review committees.

Data analyses

We first conducted descriptive analyses to examine the characteristics of patients who did (n = 139) and those who did not (n = 149) report stigma-avoidance non-adherence. Descriptive analyses for demographic and health characteristics, stigma scores, HIV disclosure, and medication-stigma management strategies were conducted with logistic regressions, reporting odds ratios and associated 95% confidence intervals.

For the main analyses, we tested a priori hierarchically structured regression models to estimate the amount of variance in ART adherence accounted for by forgoing medications in social contexts, over and above other factors that are known to influence non-adherence. The hierarchical linear regression models were constructed in four blocks: (a) Demographic and health characteristics: age, years since initiating ART, gender, depression scores, and alcohol use; (b) Medication beliefs: medication necessity

and concerns beliefs; (c) Stigma experiences: HIV-related anticipated, internalized, and enacted stigma; and (d) Stigma-Avoidance non-adherence: having not taken ART to evade stigma. Each subsequent model adjusted for the previously entered variables. We examined the individually adjusted contributions of variables in each model, as well as changes in variance accounted for across models. Relative proportions of variance in adherence accounted for in each model were tested for significance of change. We conducted a post-hoc power analysis entering five model predictors, resulting effect size (R^2), and error probability of 0.05 for a 2-tailed test. The results showed that we had observed power of 0.99 to detect model effects. Missing data was minimal (< 5%) and managed by casewise deletion.

Results

A total of 139 of the 288 (48%) participants had forgone taking their ART when other people were nearby to avoid stigma. Table 1 shows the demographic and health characteristics of participants who had and had not forgone taking their ART in social contexts. Descriptive analyses of participant demographic characteristics did not show any significant differences. In terms of health characteristics, results indicated that participants who had forgone taking ART were treated for a significantly longer-time, were overall less adherent to ART, had higher depression scores, reported greater alcohol consumption, had greater medication concerns, and experienced greater anticipated, internalized, and enacted stigma.

HIV status disclosure and medication-stigma management

Analyses indicated that there were no differences between participants who had and had not forgone taking ART to avoid stigma in terms of difficulty telling people they have HIV. However, participants who had forgone taking ART to avoid stigma did report a greater likelihood of keeping their HIV status a secret from family (see Table 2). With respect to employing strategies to conceal ART to avoid stigma, analyses indicated that participants who had forgone taking their ART in social situations were significantly more likely to keep their medications in a secret place, take their ART out of bottles, worry about people seeing their medications, and remove the labels from medication bottles. The difference between groups in telling people that their medications are for something other than for treating HIV was not significant.

Contribution of stigma-avoidance to ART non-adherence

We constructed four a priori hierarchically structured regression models to determine the degree to which stigma-avoidance contributes to ART non-adherence. The unadjusted association between stigma avoidance and ART adherence was significant, B = −6.70, se = 1.96, β = −0.196, t = 3.41, $p = 0.001$, $R^2 = 0.035$. Table 3 shows the results of the hierarchical regression models. Model 1 entered demographic and health characteristics including age, gender, years on ART, alcohol use and depression scores, and significantly predicted ART adherence, F(5,273) = 2.64, $p = 0.024$, accounting for 4.6% of the variance. Alcohol use was the only variable in Model 1 that significantly contributed to the explained variance. Model 2 added medication necessity and concerns beliefs, and the regression equation remained significant, F (7,271) = 3.21, $p = 0.003$, explaining 7.7% of the variance in adherence, R^2 Δ = 0.031, $p = 0.012$; alcohol use and medication concerns beliefs significantly contributed to the model.

Model 3 added anticipated stigma, internalized stigma, and enacted stigma with the resulting equation remaining significant, F (10,268) = 2.93, $p = 0.002$, accounting for 9.9% of the explained variance in adherence, R^2 Δ = 0.022, $p = 0.092$. In Model 3, internalized stigma along with alcohol use and medication concerns significantly contributed to the explained variance in adherence. Model 4, the final model, added stigma-avoidance non-adherence to the predictors, accounting for 12.5% of the explained variance in ART adherence, a significant change, R^2 Δ = 0.026, $p = 0.001$. The final regression equation remained significant, F (11,267) = 3.45, $p = 0.001$, with significant contributions from alcohol use, medication concern beliefs, internalized stigma, and stigma-avoidance.

Discussion

The current study found that nearly half of people living with HIV had forgone taking their ART in social situations to avoid stigma. There were few participant demographic and health characteristics that distinguished those who had forgone taking ART to avoid stigma from their counterparts who had not reported stigma-avoidance non-adherence. Specifically, stigma-avoidant participants reported experiencing greater medication concerns and anticipated, internalized and enacted stigma experiences. Stigma avoidance was also associated with using behavioral strategies intended to conceal ART. Extending past research (Kalichman et al., 2019b), the most common medication-stigma management strategy was keeping ART in a secret place. It was also common for participants to remove their ART from bottles. It

Table 1 Demographic and health characteristics among participants who had forgone ART compared to participants who had not foregone ART to avoid stigma

	Had forgone ART to avoid stigma N = 139		Had not forgone ART to avoid Stigma N = 149		OR	95% CI
	N	%	N	%		
Gender						
Men	52	37	46	31	1.33	0.81–2.18
Women	87	63	103	69		
Education						
Less than high school	104	75	110	74	1.05	0.81–1.38
Greater than high school	35	26	39	26		
Currently married	15	11	21	14	0.73	0.3–1.49
Self-report viral load						
Detectable	52	37	58	39	0.94	0.68–1.29
Undetectable	29	21	29	19		
Unknown	58	42	62	42		
Medical records viral load[a]						
Detectable	31	29	32	27	0.91	0.51–1.63
Undetectable	76	74	86	73		
Taking single tablet ART	125	90	125	84	1.71	0.84–3.46
ART dispensed monthly	128	92	137	92	1.12	0.46–2.68
	M	SD	M	SD		
Age	35.3	8.4	36.4	8.8	0.98	0.95–1.01
Years since testing HIV positive	4.7	5.5	4.4	4.8	1.01	0.96–1.05
Years taking ART	2.9	3.8	2.1	2.9	1.07*	1.01–1.15
ART Adherence	78.5	14.7	81.3	16.1	0.99+	0.97–1.00
CESD (depression) score	8.4	6.4	6.4	5.0	1.06**	1.01–1.10
AUDIT-Consumption (alcohol) score	5.8	4.5	4.7	4.3	1.05*	1.01–1.11
Medication Necessity Beliefs	3.7	0.4	3.7	0.5	0.98	0.61–1.51
Medication Concerns Beliefs	2.3	0.9	1.7	0.8	2.08**	1.57–2.76
Anticipated Stigma	0.2	0.4	0.1	0.2	3.15**	1.52–6.52
Internalized Stigma	0.5	0.5	0.3	0.4	1.88**	1.16–3.03
Enacted Stigma	0.5	0.4	0.3	0.3	3.06**	1.54–6.06

[a]Proportions of participants with available electronic medical records

ART = antiretroviral therapy; AUDIT = Alcohol Use Disorders Identification Test; CESD = Center for Epidemiological Studies Depression scale; $^{+}p = .06$, $^{*}p < .05$, $^{**}p < .01$

should be noted that medication-stigma management strategies were also reported by people who had not forgone taking ART to avoid stigma, demonstrating that the use of stigma-management strategies is not synonymous with stigma avoidance that leads to non-adherence. We did not find that participants who reported stigma-avoidance non-adherence differed in their general HIV status disclosure, but were more likely to keep their HIV status a secret from their family. Two out of three participants reported finding it difficult to disclose their HIV status and nearly one-third kept their HIV status a secret from their family.

We tested four a priori ordered hierarchical regression models predicting ART nonadherence, with stigma-avoidance added in the final model. The hierarchical ordering of the models allowed us to examine the degree to which stigma-avoidance contributes to overall non-adherence over and above all variables included in the prior models. Stigma-avoidance added 2.6% of the explained variance in ART adherence over and above factors well-known to contribute to ART non-adherence. It is particularly noteworthy that medication concerns beliefs and internalized stigma, as well as stigma-avoidance behaviors, were associated with medication non-adherence over and above depression scores and

Table 2 HIV status disclosure and medication-stigma management strategies among participants who had forgone ART to avoid stigma compared to participants who had not foregone taking ART in social contexts

	Had Forgone ART to Avoid Stigma N = 139		Had Not Forgone ART to Avoid Stigma N = 149			95% CI
	N	%	N	%	OR	
HIV status disclosure						
It is difficult to tell other people I have HIV	91	65	91	61	1.25	0.76–2.06
I keep my HIV a secret from my family	53	38	35	23	2.13**	1.27–3.57
Stigma-medication management strategies						
How often do you keep your ARV tablets in a secret place so others will not see them?	104	75	88	59	2.06**	1.24–3.40
How often do you take your ARV tablets out of their bottles so other people will not know what they are?	56	40	37	40	2.04**	1.23–3.37
How often do you tell people your ARV tablets are for treating something different, so they would not know your health condition?	37	26	26	17	1.72+	0.97–3.02
How often do you worry about people seeing that you have ARV tablets?	45	32	32	22	1.75*	1.03–2.96
How often do you remove the label from your bottle of ARV tablets so others won't know what you are taking?	34	24	14	9	3.12**	1.59–6.11
Stigma management index[a] (M, SD)	1.9	1.4	1.3	1.2	1.42**	1.19–1.69

ARV = antiretroviral, as used in survey

[a]Sum of items endorsed; $^{+}p < .10$, $*p < .05$, $**p < .01$

alcohol use. While interventions aimed to improve adherence typically address depression, alcohol use, and medication beliefs, stigma is less often a focus (CDC, 2017). By isolating the stigma-avoidance component of non-adherence, the current analysis demonstrates the importance of independently addressing stigma and behavioral strategies to avoid stigma, in efforts to improve ART adherence. It is also noteworthy that stigma-avoidance non-adherence predicted ART non-adherence over and above medication concerns, which are known to demotivate taking medications (Cooper et al., 2011). Results showed that medication concerns beliefs and internalized stigma were independent predictors of non-adherence. Concerns about side-effects and over-dependence on medications clearly contribute to non-adherence (Cooper et al., 2011), as does internalized stigma (Sweeney & Vanable, 2016). These models therefore show that multiple aspects of medication concerns, including stigma-avoidance, predict non-adherence and offer multiple points for intervention.

The current findings should be considered in light of their limitations. This study relied on self-report measures of stigma, alcohol use and ART adherence, all of which are sensitive to social response biases. For example, it is well-known that self-reported stigma and alcohol use tend to be under-reported (Keyes et al., 2010; Lacroix et al., 2017). Our findings should therefore be considered lower-bound estimates of stigma and alcohol use among people living with HIV who are receiving ART in South Africa. For ART adherence, self-report measures have the opposite reporting bias, resulting in over-reporting (Pearson et al., 2007). Thus, the ART adherence observed in this study should be considered an upper-bound estimate. We used a single item to assess the behavior of forgoing ART to avoid stigma. While this approach offered a face-valid operationalization of stigma-avoidance non-adherence, other social behaviors that undermine adherence were not assessed. In addition, our sample was one of convenience and cannot be considered representative of people receiving ART in South Africa. With these limitations in mind, we believe that the current study has implications for addressing stigma concerns in ART adherence interventions.

We found that stigma-avoidance contributes a modest but significant amount of variance to ART non-adherence. These findings add to the growing evidence that stigma concerns lead people to alter and even discontinue their use of ART in an effort to avoid stigma. Stigma stems from social structures.

Table 3 Hierarchical regression models predicting ART non-adherence

	Model 1				Model 2				Model 3				Model 4			
	B	se	ß	t	B	se	ß	t	B	se	ß	t	B	se	ß	t
Age	0.15	0.10	0.08	1.35	0.12	0.10	0.07	1.16	0.16	0.10	0.09	1.53	0.16	0.10	0.09	1.51
Gender	−1.29	1.89	−0.04	0.68	−1.96	1.88	−0.06	1.04	−1.22	1.91	−0.39	0.63	−1.36	1.89	−0.04	0.72
Years of ART	−0.37	0.27	−0.08	1.37	−0.32	0.27	−0.07	1.22	−0.26	0.27	−0.59	0.95	−0.17	0.27	−0.03	0.62
CES-depression	−0.25	1.50	−0.09	1.66	−0.10	0.16	−0.03	0.63	−0.19	0.16	−0.07	1.19	−0.18	1.60	−0.07	1.14
Alcohol use	−0.49	0.20	−0.14	2.39*	−0.47	0.20	−0.13	2.35*	−0.54	0.20	−0.15	2.61**	−0.48	0.20	−0.14	2.38*
Necessity beliefs					−0.69	1.57	−0.02	0.44	−0.73	1.56	−0.02	0.46	−0.53	1.54	−0.02	0.34
Concerns beliefs					−3.02	1.02	−0.18	2.96**	−3.31	1.02	−0.20	3.23**	−2.83	1.02	−0.17	2.76**
Anticipated stigma								0.85	2.72	0.02	0.31	−0.73	2.69	−0.01	0.27	0.02
Internalized stigma									4.33	1.94	0.14	2.22*	−4.42	1.92	−0.15	2.30*
Enacted stigma									0.99	3.40	0.02	0.29	−2.51	3.40	−0.05	0.73
Stigma-avoidance													−5.73	2.03	−0.17	2.81**
	F(5,273) = 2.64, $p = .024$, $R^2 = .046$				F(7,271) = 3.21, $p = .003$, $R^2 = .077$ $R^2\ \Delta = .031$, F = 4.49, $p = .012$				F(10,268) = 2.93, $p = .002$ $R^2 = .099$ $R^2\ \Delta = .022$, F = 2.16, $p = .092$				F(11,267) = 3.45, $p = .001$ $R^2 = .125$ $R^2\ \Delta = .026$, F = 7.94, $p = .001$			

Therefore, attempts to reduce stigma must acknowledge the challenges of changing societal attitudes. Improving medication adherence and the health of people living with HIV faces challenges posed by societal norms that imply people living with HIV are tainted and to be avoided. Structural interventions can harness mass media, public health messaging, and anti-discrimination laws to change social structures that propagate HIV stigma (Camlin et al., 2020). Individual-level interventions that aim to improve medication adherence can be adapted to address stigma-avoidance. Stigma concerns may be addressed by acknowledging their reality and developing strategies for managing ART that can achieve both goals; avoiding unwanted HIV disclosure and sustaining adherence. Behavioral strategies for mitigating stigma, such as those we report here, were not synonymous with non-adherence in that participants who did not forgo taking their ART also used these strategies. Medication management can therefore occur in conjunction with stigma management. Pillbox organizers, for example, are effective in improving adherence (Kalichman et al., 2005; Petersen et al., 2007) and they remove ART from its bottles. In addition, medication schedules can be adjusted to times when individuals are in a private place. Strategies such as these and others designed to manage ART-related stigma concerns while adhering to medication regimens will enhance behavioral interventions to improve ART adherence in stigmatized environments.

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Compliance with ethical standards

Conflict of interest Seth Kalichman, Catherine Mathews, Renee El-Krab, Ellen Banas and Moira Kalichman declare no conflict of interest.

Ethical statement This study was approved by the ethical review committees of the University of Connecticut and the South African Medical Research Council. All procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000.

Informed consent Verbal and written informed consent was obtained from all participants included in this study.

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