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Original research

The impact of acute stress on the HIV reservoir: a prospective interventional trial

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ABSTRACT

The persistence of latently infected $CD4^+$ T cells is the major barrier to cure of people with HIV (PWH) on antiretroviral therapy (ART). While most latently infected cells are transcriptionally silent, some express low levels of cell associated (CA) HIV RNA. In this prospective controlled interventional study, we tested the hypothesis that acute psychological stress could drive HIV transcription in PWH on ART. PWH on suppressive ART underwent the Trier Social Stress Test (TSST) and comparisons were made to a similar period of time without an intervention (control).

During the test, physiological markers of acute psychological stress including pre-ejection period and cardiac output changed in all participants, as anticipated. Compared to the control day, the TSST led to a significant increase in CA HIV RNA with no change in the level of cell associated HIV DNA, indicating an increase in HIV transcription in response to stress. Change in HIV transcription was associated with physiological markers of stress but not with changes in immune cells. These data demonstrate that HIV transcription is increased following acute stress and have implications on the impact of stress on the HIV reservoir and the design of cure strategies for PWH.

1. Introduction

Antiretroviral therapy (ART) for people living with HIV (PWH) has dramatically improved clinical outcomes but treatment is life long and there is no cure. The major barrier to cure is the long-term persistence of latently infected cells that can contain an integrated copy of intact virus capable of contributing to viral rebound upon ART cessation [reviewed in Ref. 1]. These cells have a range of transcriptional activity and are either fully quiescent or express low levels of cell associated (CA) HIV RNA.[2,3] One strategy being examined to eliminate latently infected cells is activation of transcription to induce virus protein expression allowing for immune-mediated or virus-induced cytolysis.[4] We and others have reported significant variation in CA unspliced (US) HIV RNA in PWH on ART[2,5,6] and a direct correlation between CA-US HIV RNA and markers of acute stress, including cortisol and thyroid stimulating hormone.[7] We therefore tested the hypothesis that stress alters basal levels of HIV

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transcription and the HIV reservoir.

We performed a controlled prospective clinical trial comparing the effects of the Trier Social Stress Test (TSST)[8] to no intervention in PWH on suppressive ART. We found that stress resulted in a significant increase in CA US HIV RNA, which was sustained post the stress intervention. Furthermore, there was a strong relationship between the fold increase in CA US HIV RNA from baseline and sympathetic nervous system activity. Together these data show that HIV transcription is increased and sustained following acute stress and have implications on the impact of stress on the HIV reservoir and the design of cure strategies in PWH.

2. Methods

Ethics Statement: Written informed consent was received from participants prior to study inclusion. Ethics approval was obtained from review boards of the University of Melbourne (ID 1544739), the University of San Francisco California (IRB 15–17652), and the Medical College of Wisconsin (ID PRO00024359).

Study design: This was a prospective interventional study (NCT02895087) where participants were assessed at a control visit when measurements were taken but without an active intervention, and blood collected at baseline (following a 10 min rest period) and after 30 and 65 min. At the following visit (within 7 days of the control visit), participants similarly had a baseline blood draw after a 10 min rest period, but then completed the stress intervention before the 30 and 65 min blood draws (with sample collection times matched to the control visit, which served as an internal control[9]) (Fig. 1A). We used a well-established laboratory stress challenge, the Trier Social Stress Test (TSST),[8] to evaluate the effect of stress responses on the HIV reservoir and immune subsets. The TSST induces social evaluative threat, which leads to a several fold increase in cortisol in most individuals (see **Supplementary Methods**). It is used to assess acute physiological responses from moderate stress.[8] We also performed physiological monitoring to assess autonomic nervous system responses including respiratory sinus arrhythmia (RSA), pre-ejection period (PEP) and cardiac output (CO) and obtained salivary samples to measure cortisol during the TSST procedure (see **Supplementary Methods**). Salivary samples were also obtained on the control day, but physiological monitoring was not performed as our assumption was that the rapid changes in autonomic nervous system responses would allow us to use measures during a rest period prior to the TSST as a baseline for physiologic monitoring data.

Participants: Eligible participants were male PWH who had been on suppressive ART with plasma HIV-RNA <50 copies/ml for at least 3 years. Most participants were recruited from one of two HIV research cohorts, the UCSF Options and SCOPE cohorts. We focused on male participants because the majority of available research volunteers in San Francisco with HIV infection were male, and we wanted to limit possible sex differences in neuroendocrine responses in this initial study.

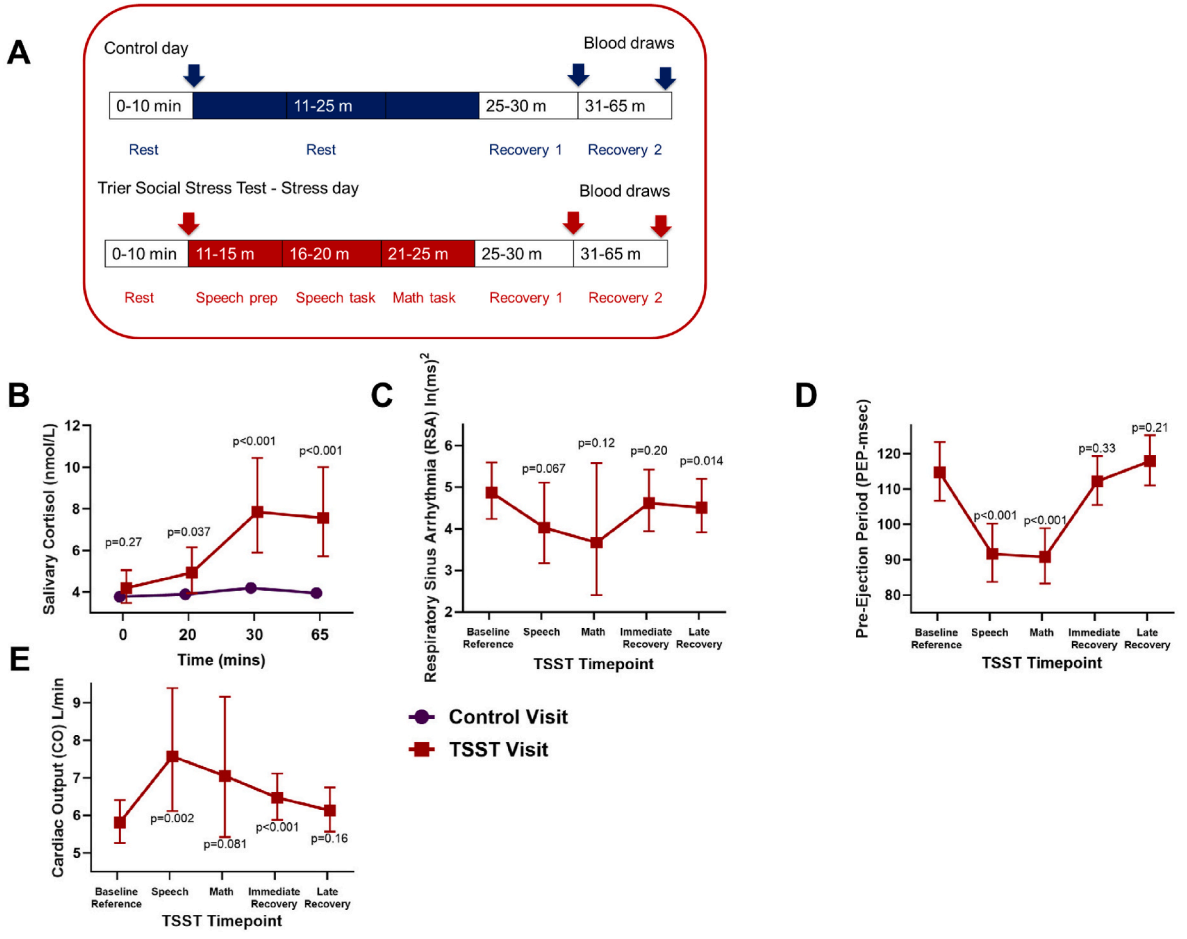

Fig. 1. Changes in markers of psychological stress post TSST. A. Biomarkers associated with physiological stress were measured during, prior to and following TSST activities **B.** Changes in cortisol on TSST (red) and control days (purple) are represented as fitted estimates of fold-change in cortisol relative to the amount at the matched control visit time derived from a linear mixed model. The fitted values were multiplied by the geometric mean of cortisol at the matched control time point to convert from fold-change over the reference to indicated concentration, p-values for comparisons with control times are shown; **C.** same as above for changes in pre-ejection period; **D.** same as above for changes in respiratory sinus arrhythmia; and **E.** same as above for changes in cardiac output; p-values for comparisons with the baseline reference time are shown. Data is shown as mean estimates with vertical bars representing 95 % confidence intervals.

Inclusion criteria included being on a once-daily ART regimen. Exclusion criteria included having a known sleep disorder, Addison's disease, thyroid/pituitary/adrenal/splenic disorders, diabetes, depression, anxiety, frequent recreational drug or systemic glucocorticoid use, or transcontinental travel in the last month.

Virological assessment: Quantification of CA-US HIV RNA and HIV DNA were performed as previously described.[10,11] CA-US HIV RNA was normalized to number of copies of the host 18S ribosomal RNA; HIV DNA was normalized to total number of $CD4^+$ T cells via host CCR5 copies. In a smaller subset of samples, we performed HIV transcriptional profiling to quantify HIV RNA transcription initiation, elongation, and splicing, on remaining RNA as we have previously described.[12]

Immunological assessment: In a subset of n = 16 participants with sufficient sample for testing, PBMC were stained with two separate panels for markers of T cell subsets, exhaustion, and activation (Supplementary Table 1, **Supplementary Methods**).

***In vitro* latency reversal:** In vitro latency reversal experiments using stress-associated compounds is described in the **Supplementary Methods**.

Statistical Methods: Supplementary Methods.<a name = "Line_manuscript_27">

3. Results

3.1. The Trier Social Stress Test induced acute psychological stress in PWH

The 25 participants were male with mean age 47 years, diagnosed an average of 9 years earlier, and were on suppressive ART with HIV RNA <50 copies/ml in plasma for greater than 3 years (Table 1). The participants underwent observation with no intervention at the first visit (control visit, Fig. 1A). As expected, we observed no increase in salivary cortisol during the control visit indicating no outward stress response during this time (Fig. 1B). The following visit, when the participants underwent the TSST, took place on average 3.9 days after the control visit (range: 1–7 days). A significant increase in salivary cortisol was observed at minutes 10, 20 and 55 after initiating the TSST (Fig. 1B). We observed a significant decrease in pre-ejection period (PEP) during the speech and math tasks, indicating a significant increase in sympathetic nervous system activation, which returned to resting levels during the immediate and late recovery timepoints (Fig. 1C). A decrease in respiratory sinus arrhythmia (RSA) was observed during the speech and math tasks although the decreases were not statistically significant (Fig. 1D). Finally, cardiac output (CO) significantly increased during the speech and math tasks, only returning to baseline during the late recovery period (Fig. 1E). Taken together, participants undergoing the TSST displayed clear changes in physiological responses consistent with an acute stress response.

Table 1
Participant characteristics.

Characteristic	% (n/total)
Race/ethnicity	
Black/African American	4.0 % (1/25)
White, not Hispanic origin	60.0 % (15/25)
Hispanic/Latino	16.0 % (4/25)
Asian/Pacific Islander	4.0 % (1/25)
Other/Mixed	16.0 % (4/25)
Gender, self-report	
Male	100.0 % (25/25)
HIV risk:	
MSM contact	92.0 % (23/25)
Heterosexual contact	0.0 % (0/25)
Injection drug use	0.0 % (0/25)
Needle stick injury	4.0 % (1/25)
Unknown	4.0 % (1/25)
Age, years; median [IQR] (range)[a]	47 [41–53] (30–55)
Years since HIV diagnosis (calculated); median [IQR] (range)[a]	9 [7–16] (4–31)

[a] Median [Interquartile range, IQR] (full range) where described.

3.2. CA-US HIV RNA but not DNA is upregulated in peripheral blood $CD4^+$ T cells during a stress response

HIV RNA was quantifiable in 24 of 25 participants across both the control and TSST visit, with a median of 8.8 copies CA US HIV RNA/10^6 18S rRNA copies (range 0–399, Supplementary Fig. 1A) at baseline. HIV DNA was quantifiable in 22 of 25 participants with a median of 167 HIV DNA copies/10^6 $CD4^+$ T cells (range 0–1004, Supplementary Fig. 1B) at baseline. During the control visit, the level of CA US HIV RNA remained consistent across the three timepoints measured (Fig. 2A).

During the TSST, we observed a 1.57-fold (95 %CI: 1.02-fold–2.42-fold) increase in CA US HIV RNA at the 65 min recovery timepoint (p = 0.04) compared to the corresponding timepoint on the control visit (Fig. 2A). These differences became more pronounced following a sensitivity analysis, where we removed one participant with very high levels of CA US HIV RNA at the 65 min timepoint of the control visit (2424.5 copies/10^6 18S rRNA; Fig. 2B). When controlling for reactivity in PEP, RSA, CO and cortisol as dichotomized variables (i.e. above and below the median), CA US HIV RNA at visit 2 was a mean 1.61-fold greater than visit 1 (95 %CI: 1.25 to 2.07, p = 0.0002). Results were identical when controlling for reactivity using a continuous variable. When comparing the TSST visits to the control visits, the CA US HIV RNA was higher at both the 10 min timepoint (p = 0.1) and the 30 min timepoint (p = 0.09) which may be reflective of anticipatory stress, but this did not reach statistical significance (Fig. 2A; Supplementary Fig. 1C). Importantly, we detected no difference in the level of HIV DNA across all timepoints and between the control visit and TSST visit (Fig. 2C; Supplementary Fig. 1D). Confirming our observation of increased HIV RNA, we observed a 2.0-fold (95 %CI: 1.3-fold–3.08-fold) increase in the HIV RNA/HIV DNA ratio at the 65 min timepoint (p = 0.0015) (Supplementary Fig. 1E and F).

Our a priori planned analysis was to compare results from timepoints on the TSST visit with the corresponding control visit timepoint, as shown (Fig. 2A and B). In additional post hoc analyses we assessed changes in CA US HIV RNA relative to baseline at the TSST visit and the control visit separately. When we did this analysis, we observed no increase in CA US HIV RNA at 30 min and 65 min relative to baseline at the TSST or the control visit (Supplementary Fig. 1G).

We hypothesized that these findings may be due to the influence of anticipatory stress present at baseline on the TSST day, which would have reduced the ability of the TSST to induce acute stress. Indeed, we saw a high variation in the fold increase in cortisol at 30 min compared to baseline on the TSST day (Supplementary Fig. 1H). We divided participants into two groups, based on being above or below the median change in cortisol on the TSST day (Supplementary Fig. 1H). In participants with a 'high' change in cortisol, CA US HIV RNA at the baseline TSST visit was similar to the control visit (1.15-fold, 95 % CI: 0.66 to 2.01, p = 0.61), while in participants with a 'low' change in cortisol the CA US HIV RNA level was higher at the baseline TSST visit compared to the control visit (1.83-fold, 95 % CI: 1.11 to 3.34, p = 0.05) (Supplementary Fig. 1I). During the TSST, participants with a 'high' compared to 'low' change in cortisol had a 7.44-fold (95 % CI: 1.19 to 46.41, p = 0.032) increase in CA US HIV RNA (Supplementary Fig. 1J). These data demonstrate that in participants with a higher change in cortisol during the TSST, there was a significant increase in CA US HIV RNA.

For a small subset of participants with remaining RNA, we performed HIV Transcriptional Profiling,[12] to better understand the induction of HIV RNA and whether the increase in CA US HIV RNA was indicative of transcript elongation, completion or multiple splicing (Supplementary Fig. 2A). Given the low number of samples available to analyse, we did not perform a similar grouped analysis of changes over time following

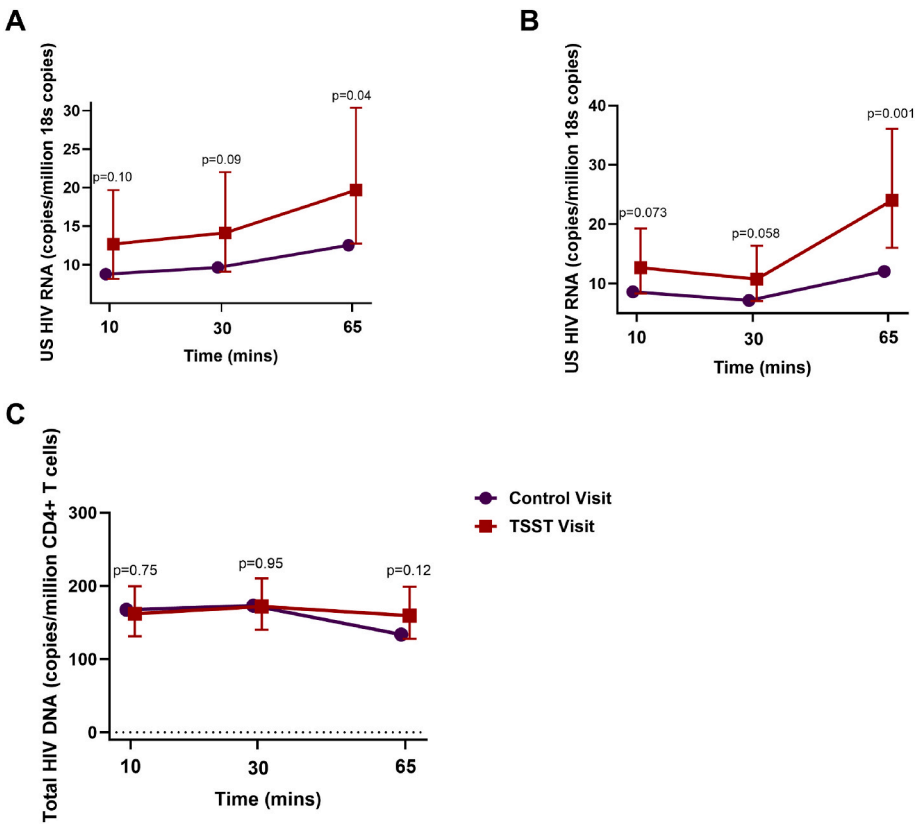

Fig. 2. The TSST led to an increase in cell associated (CA) unspliced (US) HIV RNA but no change in HIV DNA. A. CA US HIV RNA was normalized to a house keeping gene 18S RNA and is shown as CA US HIV RNA per million 18S copies at each visit and time point, with vertical bars representing 95 % confidence intervals around the estimates comparing the TSST visit (red) to the matched times at the control visit (purple). Fitted estimates of fold-change in CA US HIV RNA relative to the amount at the matched control visit time were derived from a mixed effects negative binomial regression model. The fitted values were multiplied by the median of CA US HIV RNA at the matched control time points to convert from fold-change over the reference, to concentration. **B** Same as figure A, but with removal of a single outlier with extremely high baseline US HIV RNA**. C**. Changes in HIV DNA at the TSST and control visits using the same analysis as described in A. Wald test p-values for comparisons at each time point between the TSST visit and the control visit are shown above each time point.

the intervention compared to placebo as done for the primary endpoint, CA US HIV RNA, however, the change in CA US HIV RNA did not correlate significantly with changes in proximal elongation (Long LTR), elongation past *pol* (Pol) or completion (PolyA) (Supplementary Fig. 2B). Taken together, these data support the finding that HIV transcription initiation changes during a stress response in the peripheral blood $CD4^+$ T cells of PWH.

3.3. Immunological changes associated with psychological stress

Given that the level of both HIV DNA and CA US HIV RNA are not uniformly distributed amongst $CD4^+$ T cell subsets in PWH on ART,[13–15] we sought to determine if cell trafficking between blood and tissue could explain the increase in CA US HIV RNA seen during a stress response. To address this, we analyzed $CD4^+$ and $CD8^+$ T cell subsets and markers of cellular activation or exhaustion on a subset of n = 16 participants. We observed a 2.73 % increase in $CD4^+$ effector memory (TEM) ($p = 0.02$) and 0.67 % increase in CD45RA + effector memory (TEMRA) ($p < 0.01$) subsets at the 30 min timepoint of the TSST, with a corresponding 4.49 % reduction in the level of the naïve (TN) population ($p = 0.04$) (Fig. 3A). These changes returned to the baseline level at the 65 min timepoint of the TSST. We observed no changes in the proportion of cells expressing activation markers, CD38 or HLA-DR (Fig. 3B), CCR5, CD25 or CD69 (Fig. 3C); or cells expressing exhaustion markers CD57 or PD1 (Fig. 3D).

Similar to the $CD4^+$ T cells, at the 30 min TSST timepoint, we observed a 2.57 % increase in the TEM ($p = 0.02$) and 5.87 % increase in the TEMRA ($p < 0.01$) subsets with a proportionate 8.02 % decrease in TN $CD8^+$ T cells ($p < 0.01$) (Fig. 4A). Again, similar to the $CD4^+$ T cells, these differences returned to baseline at the 65 min timepoint of the TSST. $CD8^+$ T cell activation remained consistent with no changes in the proportion of cells expressing CD38 or HLA-DR (Fig. 4B), CCR5, CD25, or CD69 (Fig. 4C). Assessing immune exhaustion of $CD8^+$ T cells demonstrated only a significant 3.82 % decrease in the proportion of $CD57^-PD1^-CD8^+$ T cells at the 30 min timepoint of the TSST ($p = 0.03$), which returned to baseline at 65 min ($p = 0.60$) (Fig. 4D). Together these results demonstrate changes in circulation of $CD4^+$ and $CD8^+$ T cell memory subsets in response to psychological stress, with more marked changes in $CD8^+$ compared to $CD4^+$ T-cells. These changes were not sustained and returned to baseline by the 65 min timepoint.

To further investigate the influence of immunological markers on the observed changes in HIV transcription during the TSST, we performed Spearman correlations between changes in T cell subset frequency and markers of T cell activation with the viral reservoir (Supplementary Fig. 3). We observed a moderately strong positive correlation between changes in HIV DNA and the proportion of $CD4^+$ T cells that were $CD38^+HLA\text{-}DR^-$ ($rho = 0.52$, $p = 0.048$); and a negative association between changes in CA US HIV RNA and the proportion of $CD8^+$ T cells expressing CD69 ($rho = -0.52$, $p = 0.05$) but no relationship between the frequency of $CD4^+$ or $CD8^+$ T-cell subsets and either CA US HIV RNA or HIV DNA.

3.4. Physiological markers of acute stress are associated with changes in HIV transcription

To further investigate the mechanism behind increased CA US HIV

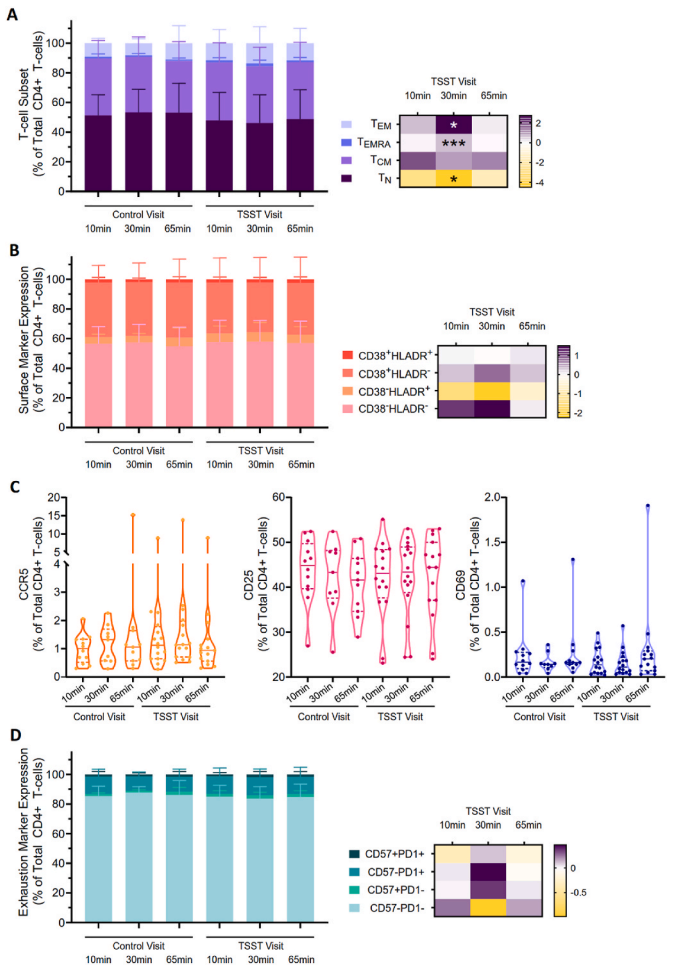

Fig. 3. Change in CD4$^+$ T cell distribution and phenotype during TSST. **A.** Percentage of naïve, central memory, effector memory, and terminally differentiated CD4$^+$ T-cell subsets at each timepoint as defined by CCR7 and CD45RA co-expression (left) and absolute changes in subsets at each TSST timepoint compared to the average of all control visit timepoints using a linear mixed effects model with random intercepts (right). **B.** Percentage of CD38 and HLA-DR co-expression on total CD4$^+$ T-cells at each timepoint (left) and absolute changes in subsets at each TSST timepoint compared to the average of all control visit timepoints using a linear mixed effects model with random intercepts (right). **C.** Expression of total CD4$^+$ T-cell activation markers, CCR5 (left), CD25 (middle) and CD69 (right); violin plots represent minimum and maximum values, with a solid line at the median and dashed lines at the 1st and 3rd quartiles. **D.** Percentage of PD-1 and CD57 marker co-expression on total CD4$^+$ T-cells at each timepoint (left) and absolute changes in subsets at each TSST timepoint compared to the average of all control visit timepoints using a linear mixed effects model with random intercepts (right). *p < 0.05, **p < 0.01, ***p < 0.001.

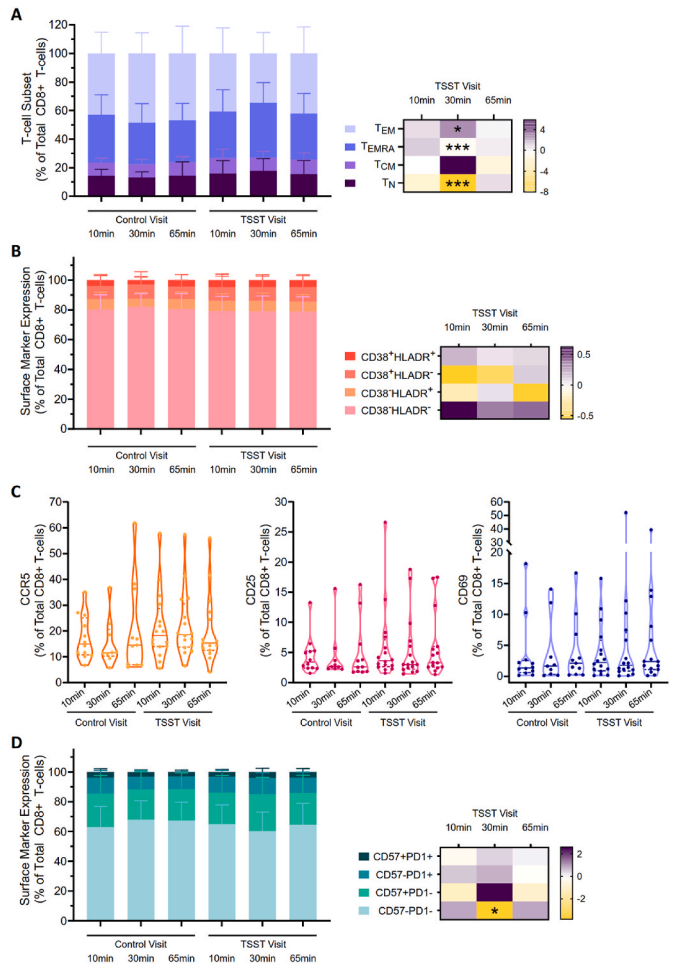

Fig. 4. Change in CD8$^+$ T cell distribution and phenotype during TSST. **A.** Percentage of naïve, central memory, effector memory, and terminally differentiated CD8$^+$ T-cell subsets at each timepoint as defined by CCR7 and CD85RA co-expression (left) and absolute changes in subsets at each TSST timepoint compared to the average of all control visit timepoints using a linear mixed effects model with random intercepts (right). **B.** Percentage of CD38 and HLA-DR co-expression on total CD8$^+$ T-cells at each timepoint (left) and absolute changes in subsets at each TSST timepoint compared to the average of all control visit timepoints using a linear mixed effects model with random intercepts (right). **C.** Expression of total CD8$^+$ T-cell activation markers, CCR5 (left), CD25 (middle) and CD69 (right); violin plots represent minimum and maximum values, with a solid line at the median and dashed lines at the 1st and 3rd quartiles. **D.** Percentage of PD1 and CD57 marker co-expression on total CD8$^+$ T-cells at each timepoint (left) and absolute changes in subsets at each TSST timepoint compared to the average of all control visit timepoints using a linear mixed effects model with random intercepts (right). *p < 0.05, **p < 0.01, ***p < 0.001.

RNA in response to acute psychological stress, we assessed the relationship between the change in CA US HIV RNA and HIV DNA and changes in physiological markers of stress including cortisol, PEP, RSA, and CO. We observed a significant inverse relationship between change in CA US HIV RNA and PEP (rho = −0.59, p < 0.01), and between HIV RNA and CO (rho = 0.6, p < 0.01) (Table 2). There was no relationship between physiologic responses and HIV DNA. Some minor associations were observed between T cell subset and activation markers and markers of stress (Supplementary Fig. 4). These data suggest an association between an increase in HIV transcription and activation of the autonomic nervous system.

3.5. *Modulation of HIV transcription by compounds associated with a stress response*

Given the association we observed between physiological responses to acute stress and increased HIV transcription, we next determined whether compounds known to be released or upregulated during a stress response were capable of modulating HIV transcription in cell line models of HIV latency. We assessed the glucocorticoid receptor agonists, hydrocortisone and dexamethasone; catecholamines, epinephrine and norepinephrine; the protein kinase A agonist, forskolin; the pituitary hormone, prolactin; and the thyroid hormone, Triiodothyronine (T3). None of the tested compounds increased HIV LTR-mediated expression in two latently infected cell lines, either TZM-bl (Supplementary Fig. 5) or J-Lat A2 (Supplementary Fig. 6) cells at concentrations between

Table 2
Association of changes in autonomic nervous system measures with cell associated HIV RNA and DNA.

ANS Measure	US RNA		DNA	
	Spearman rho[a]	P-value	Spearman rho[a]	P-value
Pre-ejection Period (PEP)	−0.59	0.002	−0.09	0.68
Respiratory Sinus Arrhythmia (RSA)	−0.05	0.81	−0.01	0.96
Cardiac Output (CO)	0.60	0.003	−0.02	0.94
Salivary cortisol	0.37	0.072	0.17	0.42

[a] Spearman rank correlations between change in ANS measures and change in US RNA or DNA. Change in ANS measures was calculated as observed value at the start of the speech task (minute 16) minus the value at the end of the resting baseline period (minute 10). Change in cortisol was calculated as observed value at the recovery timepoint (minute 65) minus the value of the baseline timepoint (minute 0). The difference in US RNA and DNA (65 min timepoint TSST visit verses control visit) was calculated as a difference in logs, which translates to fold-change.

10^{-1}nM and 10^{5}nM. Rather, hydrocortisone at any concentration and 2.55×10^{4} nM dexamethasone ($p = 0.0045$) suppressed LTR-mediated expression in TZM-bl cells (Supplementary Fig. 5A and B) but not J-Lat A2 cells (Supplementary Fig. 6A and B).

4. Discussion

This is the first prospective controlled interventional study to evaluate the influence of psychological stress on the HIV reservoir in virally suppressed male PWH on ART. Using an established laboratory stress challenge, the TSST, we observed that acute stress (as measured by changes in physiological responses) increased levels of CA US HIV RNA but not HIV DNA and these increases were sustained 40 min into the recovery period, after the end of the stressor period of the intervention. Furthermore, changes in responses to psychological stress including a reduced PEP (increased SNS activation) and increased CO were associated with an increase in CA US HIV RNA, consistent with a direct impact of acute stress on the HIV reservoir. We observed some transient changes in $CD4^+$ and $CD8^+$ T cell subsets during the TSST, although none were significantly associated with an increase in CA US HIV RNA. Together, these data show that HIV transcription is increased and sustained following acute stress and these findings have implications on the impact of stress on the HIV reservoir and future cure strategies in PWH.

The increase in CA US HIV RNA in the setting of acute stress supports our previous findings in CD4+ T cells isolated from PWH on ART prior to administration of an intervention to reverse HIV latency.[7,10] We believe our results point to an increase in HIV transcription for a number of reasons. First, although the proportion of $CD4^+$ TEMs and TEMRAs, increased after psychological stress and these $CD4^+$ T-cells harbor higher amounts of HIV DNA,[13,16] the HIV DNA did not vary during the TSST, indicating that the number of infected cells in the blood remained constant. Therefore, redistribution of cells alone could not explain the increase in CA US HIV RNA. Second, although we observed changes in $CD4^+$ and $CD8^+$ T cell subsets including an increase in EM $CD4^+$ T-cells at the 30 min timepoint of the TSST, these changes were not evident at the 65 min timepoint, where the greatest increase in CA US HIV RNA was observed. Changes in CA US HIV RNA during the TSST are therefore likely to not be due to increased numbers of memory subsets known to harbor more transcriptionally active proviruses such as effector memory cells.[13,17,18] Third, this study was designed such that the control visit and TSST visit were conducted at similar times-of-day, thus eliminating the possibility that the increases in HIV transcription arose due to circadian rhythms as we have observed to occur *in vivo*.[9] Given our findings reported here and our previous study demonstrating diurnal rhythm of HIV transcription,[9] external factors such as time and stress need to be considered in the design and interpretation of latency reversal studies. This includes the timing of baseline samples, the need to collect multiple baseline samples, and consideration of the psychological state of participants.

Although our study only focused on the induction of acute psychological stress, our findings raise the possibility that chronic stress or repeated episodes of acute stress in PWH on ART may also impact reservoir activity, immune activation, and inflammation. It is important to highlight that the changes in the HIV reservoir in our study persisted beyond the stress intervention and into the recovery period. Stress and its manifestations including depression and post-traumatic stress disorder are known to be more prevalent in PWH.[19–26] Chronic stress is known to dysregulate the immune system,[27] and a Conserved Transcriptional Response to Adversity (CTRA) has been identified in immune cells characterized by increased inflammatory gene expression.[28,29] Given that viral proteins such as p24 can also be expressed in PWH on ART in blood[30] and tissue[31]; the expression of CA HIV RNA and viral proteins is associated with expanded HIV-specific $CD8^+$ T-cells[32] and persisting immune activation/exhaustion[33–35]; and abortive HIV transcripts (likely making up the majority of the increase in CA HIV RNA seen in this study) themselves can induce potent immune activation,[36,37] there is a need for greater understanding about the effect of chronic stress on the HIV reservoir and immune activation on long-term ART.

We screened compounds known to be associated with a psychological stress response and did not find any that reversed latency in two latently infected cell lines. In contrast, we observed repression of HIV transcription with glucocorticoids, consistent with other reports.[38,39] It is possible that an increase in glucocorticoids mediated by stress, has an indirect effect on HIV transcription, through activation of other common latent viruses, such as HSV-1 and Epstein-Barr Virus reactivation,[40–45] but these viruses were not measured in our study. Others have shown that increased cAMP levels, a consequence of stress, can reverse HIV latency.[46] In productive HIV infection, catecholamines have been shown to have both positive[47] and negative effects[48] on HIV transcription, but no prior studies have directly assessed effects on latent virus. Considering that CA-US HIV RNA increased in this study only after the TSST and salivary cortisol normalized, these data suggest that the role of psychological stress in inducing HIV transcription is likely more complex than endocrine/paracrine signaling or a direct effect of these molecules on the HIV LTR.

Our study is the first prospective interventional study to determine the impact of stress on the HIV reservoir, however there were a number of limitations. Firstly, for the majority of samples we were only able to measure CA US HIV RNA, which is primarily a marker of transcription initiation.[49] Second, given the short sampling period and the observation that HIV transcription was highest at the last timepoint, we are unable to determine if these changes in HIV transcription continued to increase after observation and for how long they persisted. Third, given that mental health conditions (e.g. depression and anxiety) and sleep disorders were exclusion criteria for this study, we cannot comment as to whether the relationship between acute stress and HIV transcription is attenuated or exacerbated amongst PWH with psychological comorbidities. Similarly, our study exclusively enrolled cisgender males and is limited in the applicability of our findings to females and trans/gender diverse people.[50] We did not measure changes in host transcripts so can not say if the effect of acute stress is specific to HIV transcription, although this is unlikely as others have shown transcriptome changes in response to acute stress.[51] Finally, we only quantified total HIV DNA and not intact DNA and therefore are unable to conclude if there was a change in the replication competent viral reservoir.

Conclusions

In summary, our results demonstrate that induction of acute psychological stress in PWH on ART is associated with an increase in HIV transcription. These findings have significant implications for the impact of recurrent episodes of acute stress on the HIV reservoir and

needs to be considered in the design of clinical trials assessing HIV cure interventions.

CRediT authorship contribution statement

Jared Stern: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation. **Michael Roche:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation. **Rory Shepherd:** Writing – review & editing, Visualization, Investigation. **Wendy Hartogensis:** Writing – review & editing, Writing – original draft, Visualization, Methodology. **Patricia Moran:** Writing – review & editing, Methodology. **Leslie Cockerham:** Writing – review & editing, Methodology. **Nadia Saraya:** Writing – review & editing, Investigation, Conceptualization. **Ajantha Rhodes:** Writing – review & editing, Visualization, Investigation. **Paul U. Cameron:** Writing – review & editing, Methodology. **Judy J. Chang:** Writing – review & editing, Methodology. **Nitasha Kumar:** Writing – review & editing, Visualization, Investigation. **Wendy B. Mendes:** Writing – review & editing, Methodology. **Steven G. Deeks:** Writing – review & editing, Methodology. **Frederick M. Hecht:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Conceptualization. **Sharon R. Lewin:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Conceptualization.

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Declaration of competing interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jve.2025.100613.

Data availability

Data will be made available on request.

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