INSOMNIA AND MECHANISTIC PATHWAYS TO ATHEROSCLEROTIC CVD IN HIV

by

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Abstract

Background: Insomnia may be a risk factor for cardiovascular disease in HIV (HIV-CVD); however, mechanisms have yet to be elucidated. **Methods:** We examined cross-sectional associations of insomnia symptoms with biological mechanisms of HIV-CVD (immune

activation, systemic inflammation, and coagulation) among 1,542 people living with HIV from the Veterans Aging Cohort Study (VACS) Biomarker Cohort. Past-month insomnia symptoms were assessed by the item, "Difficulty falling or staying asleep?," with the following response options: "I do not have this symptom" or "I have this symptom and…" "it doesn't bother me," "it bothers me a little," "it bothers me," "it bothers me a lot." Circulating levels of the monocyte activation marker soluble CD14 (sCD14), inflammatory marker interleukin-6 (IL-6), and coagulation marker D-dimer were determined from blood specimens. Demographic- and fully-adjusted (CVD risk factors, potential confounders, HIV-related factors) regression models were constructed, with log-transformed biomarker variables as the outcomes. We present the exponentiated regression coefficient (exp[b]) and its 95% confidence interval (*CI*). **Results:** For sCD14 and D-dimer, we observed no significant associations. For IL-6, veterans in the "bothers a lot" group had 15% higher IL-6 than veterans in the "I do not have this symptom" group in the demographic-adjusted model (exp[b]=1.15, 95%*CI*=1.02-1.29, *p*=.03). This association was nonsignificant in the fully-adjusted model (exp[b]=1.07, 95%*CI*=0.95-1.19, *p*=.25). **Conclusion:** We observed little evidence of relationships between insomnia symptoms and markers of biological mechanisms of HIV-CVD. Other mechanisms may be responsible for the insomnia-CVD relationship in HIV; however, future studies with comprehensive assessments of insomnia symptoms are warranted.

Introduction

Although still below that of the general population, the expected lifespan of people living with human immunodeficiency virus (PLWH) has increased considerably with antiretroviral therapy (ART) (1). However, this increased lifespan has been accompanied by a rise in non-communicable diseases – most notably cardiovascular disease (CVD), a leading cause of death in PLWH (2-4). In fact, PLWH are at nearly double the risk of CVD compared to those without HIV (5), and this elevated risk persists independent of the risk attributed to HIV, ART, and traditional and non-traditional CVD risk factors (6-8). Thus, there is a need to identify novel risk factors for CVD in HIV (HIV-CVD) with the potential to serve as future CVD primary prevention targets.

Insomnia is one such possible risk factor for CVD that has been largely ignored in the HIV population. Sleep disturbance is a commonly reported experience among PLWH, with an

estimated prevalence of 58% (9). In addition, PLWH may exhibit an increased risk of developing sleep disturbance compared to the general population (10). Our recent work using the Veterans Aging Cohort Study (VACS) Survey Cohort was the first to examine insomnia as a predictor of HIV-CVD. We found that PLWH bothered a lot by difficulty falling or staying asleep exhibited a 66% greater risk of incident CVD than those without these symptoms, independent of demographics, CVD risk factors, additional potential confounders, and HIV-specific factors (11).

Insomnia has received greater attention as a possible CVD risk factor in the general population, with substantial evidence supporting an insomnia-CVD relationship. Meta-analytic evidence suggests that elevated insomnia symptoms are associated with an increased risk of CVD events (risk ratios [*RR*s] = 1.28-1.55) (12-14). Furthermore, insomnia has been associated with putative biological mechanisms of HIV-CVD (15), including altered immune function (16), increased systemic inflammation (17), and heightened coagulation (18-24) in non-HIV samples. Most striking are findings from the largest randomized control trial of behavioral interventions for insomnia in older adults suggesting a causal link between insomnia and CVD risk. Specifically, Irwin et al. (25) found that older adults receiving cognitive-behavioral therapy for insomnia (CBT-I), versus a sleep education seminar, exhibited a 74% lower risk of elevated C-reactive protein (CRP; $\geq$ 3.0 mg/L), an inflammatory marker implicated in and predictive of the development of CVD (26), at the 16-month follow-up.

Given our previous work indicating a link between insomnia and incident CVD in PLWH and the complementary supportive evidence in the general population, it is now important to examine the relationships between insomnia and putative biological mechanisms of HIV-CVD. Thus, the aim of this study was to determine the associations of insomnia symptoms with biomarkers of immune activation, systemic inflammation, and coagulation among PLWH. We hypothesized that insomnia symptoms would be positively associated with circulating levels of the monocyte activation marker soluble CD14 (sCD14), the inflammatory marker interleukin-6 (IL-6), and the coagulation marker D-dimer.

Methods

Study design, setting, and participants

Data for the present study came from the VACS Biomarker Cohort, a cross-sectional subsample of the VACS-9 parent study consisting of participants who provided a blood sample between 2005-2006 (27, 28). VACS-9 is a prospective, multisite, cohort study of HIV-positive veterans and age, sex, race/ethnicity, and clinical site-matched HIV-negative veterans from nine Department of Veterans Affairs (VA) medical centers across the U.S. (29, 30). From the total VACS Biomarker Cohort sample (N = 2,386), we excluded veterans without HIV (n = 837) and those missing a follow-up date (n = 7). Thus, our final sample consisted of 1,542 HIV-positive veterans (see Table 1.1 for participant characteristics).

Measures and procedures

Exposure variable. Insomnia symptoms were assessed by the insomnia item of the VACS HIV Symptom Index – a 20-item, self-report questionnaire assessing the frequency and bother of common symptoms in HIV-positive adults exposed to multidrug ART and protease inhibitors (31). The VACS HIV Symptom Index asks participants to indicate what response best describes their experience of each symptom over the past four weeks using the following options: 0 = "I do not have this symptom" or "I have this symptom and…" 1 = "it doesn't bother me," 2 = "it bothers me a little," 3 = "it bothers me," or 4 = "it bothers me a lot." We used responses to the insomnia item – "Difficulty falling or staying asleep?" – to create a 5-level insomnia symptoms variable. From this variable, four dummy coded variables were created with the "No Difficulty Falling or Staying Asleep" group as the reference category (i.e., "I do not have this symptom") (11).

Outcome variables. Three outcomes were examined: the monocyte activation marker sCD14 (ng/mL), the inflammatory marker IL-6 (pg/mL), and the coagulation marker D-dimer (μg/mL). As is described elsewhere (27, 28, 32), participant blood samples were collected at one time point between 2005-2006. Serum samples were collected using serum separator and EDTA tubes and were shipped to a central repository at the Massachusetts Veterans Epidemiology Research and Information Center. Assays were performed at the University of Vermont's Laboratory for Clinical Biochemistry Research. All biomarker measurements used four controls

per sample to assess interassay coefficients of variability (CVs). sCD14 was measured with an enzyme-linked immunsorbent assay (Quantikine sCD14 Immunoassay, R&D Systems, Minneapolis, MN) with a detectable range of 40-3,200 ng/mL; CVs ranged from 7.2-8.1%. IL-6 was measured using a chemiluminescent immunoassay (QuantiGlo IL-6 immunoassay, R&D Systems, Minneapolis, MN) with a detectable range of 0.4-10,000 pg/mL; CVs ranged from 7.7-12.3%. D-dimer was measured by a STAR automated coagulation analyzer (Diagnostica Stago) using an immunoturbidometric assay (Liatest D-DI; Diagnostica Stago, Parsippany, NJ) with a detectable range of 0.01-20 ug/mL; CVs ranged from 2.8-14.8%. Because of their positively skewed distributions, all three biomarkers were log-transformed to approximate normal distributions.

Covariates. Similar to our previous work, all covariates were determined using self-report measure data or routine clinical care data in the electronic medical record obtained closest to the blood collection date (27). The following covariates were included, given their prior associations with insomnia, HIV-CVD, or both.

Demographic factors. Demographic factors were age, sex (male, female), and race/ethnicity (White, African American, Hispanic, Other).

CVD risk factors. CVD risk factors were prevalent CVD, hypertension, diabetes, body mass index (BMI), smoking, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, and statin use. Prevalent CVD was defined by the presence of a *International Classification of Diseases, Ninth Revision* (ICD-9) or a *Current Procedural Terminology (CPT)* code prior to the blood collection date for myocardial infarction, unstable angina, coronary heart disease, stroke, congestive heart failure, coronary artery bypass graft, or percutaneous coronary intervention. Hypertension was defined by the average of the three routine outpatient blood pressure values obtained closest to the blood collection date. We created two dummy variables to categorize hypertension based on Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure thresholds as no hypertension (blood pressure <140/90 mmHg and no antihypertensive medication [reference category]), controlled hypertension (<140/90 mmHg with antihypertensive medication), or uncontrolled hypertension (≥140/90 mmHg) (33). Diabetes (yes/no) was defined by a validated metric incorporating glucose measurements, diabetes medication use, and/or at least one inpatient or two outpatient ICD-9 codes for diabetes (34, 35). BMI (kg/m^2) was defined by one

outpatient measurement collected during routine clinical care and was modeled continuously. Smoking (never [reference category], current, or former smoker) was determined from the VA Health Factors data, a system utilizing electronic medical record data (36). Measurements of the lipids (LDL cholesterol, HDL cholesterol, and triglycerides) were obtained from the VA Corporate Data Warehouse and modeled continuously. Statin use (yes/no) was defined as a filled prescription receipt for a 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitor at time of the blood collection.

Additional potential confounders. Additional biomedical and behavioral potential confounders were hepatitis C infection, renal function, anemia, alcohol use, and cocaine use. Hepatitis C infection (yes/no) was defined by a positive hepatitis C virus antibody test or at least one inpatient or two outpatient ICD-9 codes for hepatitis C infection (37). Renal function was defined by estimated glomerular filtration rate (eGFR). Anemia was defined by hemoglobin. Both eGFR and hemoglobin were extracted from the VA Corporate Data Warehouse and modeled continuously. Alcohol use was determined by the Alcohol Use Disorders Identification Test (AUDIT-C) administered closest to the blood collection date and by the presence of alcohol abuse/dependence ICD-9 codes in the electronic medical record prior to the blood collection date. We combined these two data sources into one dichotomous variable as no current use or non-hazardous use versus hazardous use (AUDIT-C ≥ 4) or alcohol abuse/dependence disorder. Cocaine use was assessed by self-report and by the presence of cocaine use disorder ICD-9 codes in the electronic medical record prior to the blood collection date. We combined these two data sources into one dichotomous variable as never tried or no use in past year versus use in the past year or cocaine abuse/dependence disorder.

HIV-related factors. The HIV-related factors were HIV-1 RNA level, $CD4_+$ T cell count, and ART use. HIV-1 RNA level and $CD4_+$ T-cell count, measured during routine outpatient visits, were determined from the VA Corporate Data Warehouse data obtained closest to the blood collection date and modeled continuously. ART use (yes/no) was defined as a filled prescription receipt for any ART closest to the blood collection date (-180 days to +7 days of date).

Insomnia-related factors. The insomnia-related factors were depressive symptoms, non-benzodiazepine sleep medication use, and antidepressant medication use. Given the overlap between insomnia and depression, depression symptoms were included in order to examine

insomnia's independent associations with the biomarkers. Antidepressant medication use was included, as these medications are commonly prescribed for sleep disturbance (38, 39) and have been associated with systemic inflammation among PLWH (40). Depressive symptoms were measured by the Patient Health Questionnaire-9 (PHQ-9). We removed item 3 ("Trouble falling or staying asleep, or sleeping too much") from the PHQ-9 total score calculation and subsequently refer to it as PHQ-9 (no sleep item). Non-benzodiazepine sleep medication use (yes/no) was defined as a filled prescription receipt closest to the blood collection date (-ever to +180 days of date) for the following medications: zolpidem, zaleplon, eszopiclone, and indiplon. Antidepressant medication use was defined as a filled prescription receipt for an antidepressant medication closest to the blood collection date (-ever to +180 days of date). We computed three dichotomous variables (yes/no) based on the antidepressant medication type – serotonin reuptake inhibitor (SSRI), tricyclic antidepressant (TCA), and miscellaneous other antidepressant. Of note, the treatment indications for non-benzodiazepine and antidepressant medications are not known.

Statistical analysis

Descriptive statistics – i.e., median (first quartile, third quartile) and frequency count (%) – for the participant characteristics and the biomarker levels across insomnia symptom categories were computed.

Multivariate linear regression models were constructed to estimate the associations between insomnia symptoms and monocyte activation, inflammatory, and coagulation markers in HIV-positive veterans. Two models were constructed for each outcome variable (log-transformed sCD14, IL-6, and D-dimer). Model 1 (demographics-adjusted) consisted of the four insomnia symptom dummy coded variables, age, sex, and race/ethnicity. Model 2 (fully-adjusted), which was our primary model, included the Model 1 variables plus the CVD risk factors (prevalent CVD, hypertension, diabetes, BMI, smoking, LDL cholesterol, HDL cholesterol, triglycerides, and statin use), the additional potential confounders (hepatitis C infection, renal function [eGFR], anemia [hemoglobin], alcohol use, and cocaine use), and the HIV-related factors (HIV-1 RNA level, $CD4_+$ T cell count, and ART use). Due to the use of log-transformed outcome variables, we present the exponentiated regression coefficient [exp(b)] and its 95% confidence interval (*CI*) for each association of interest. We interpret the percent change

in each biomarker per 1-unit increase in the insomnia symptoms dummy coded variables (i.e., switching from the reference category "No Difficulty Falling or Staying Asleep" to the respective insomnia symptom category) using the following equation: [exp(b)-1] x 100.

Three sensitivity analyses were conducted to individually examine the potential influence of the insomnia-related variables on the associations of interest. These three models were constructed for each outcome variable as follows: Model 3: Model 2 plus PHQ-9 total score (no sleep item), Model 4: Model 2 plus non-benzodiazepine sleep medication use, and Model 5: Model 2 plus antidepressant medication use (SSRI, TCA, and miscellaneous other use).

All analyses were performed using R software (version 3.5.2; www.r-project.org). To address missingness, data examine in regression models underwent multiple imputations using chained equations (MICE) with five separate imputed datasets generated based on predictive mean matching using the 'mice' library of R programming language. Regression models were fit in each imputed dataset and finally combined to obtain pooled effect sizes and standard errors using Rubin's rule (41).

Results

Participant characteristics

The characteristics of participants are presented in Table 1.1. The median age of our sample of 1,542 HIV-positive veterans was 52 years, with the majority being male (97%) and African American (69%). Participants exhibited high CVD risk factor burden, particularly for hypertension (72%) and current smoking (50%). Our sample had a high prevalence of hepatitis C co-infection at 47%, well above the predicted estimate in the general population of PLWH at 2.4% (42). Also of note, our sample had a high prevalence of substance misuse/abuse (42% and 21% for alcohol and cocaine, respectively). Regarding insomnia-related variables, participants exhibited lower use of non-benzodiazepine medications (10%) but higher use of antidepressant medications (26-43%), with a median PHQ-9 (no sleep item) score of 3.0. The distribution of participants across the insomnia symptom categories is presented in Table 1.2, with each category's respective original unit median (first quartile, third quartile) for sCD14, IL-6, and D-dimer. Visual inspection of Table 1.2 suggests a large proportion of participants denied having insomnia symptoms (40%), with the remaining participants distributed relatively evenly across

the other insomnia symptom categories. Approximately 50% of our sample endorsed some level of bother with insomnia symptoms.

Insomnia symptoms and sCD14

As is shown in Table 1.3, the dummy coded variables comparing "Doesn't Bother," "Bothers a Little," "Bothers," and "Bothers a Lot" categories to the "No Difficulty Falling or Staying Asleep" reference category were not significant in Model 1 (demographic-adjusted; *p*-value range: 0.09-0.53) or Model 2 (fully-adjusted; *p*-value range: 0.13-0.93). Results remained consistent in the sensitivity analyses individually adjusting for PHQ-9 (no sleep item; Model 3), non-benzodiazepine sleep medication use (Model 4), or antidepressant medication use (Model 5).

Insomnia symptoms and IL-6

The dummy coded variables comparing "Doesn't Bother," "Bothers a Little," and "Bothers" categories to the "No Difficulty Falling or Staying Asleep" reference category were not significant in Model 1 (*p*-value range: 0.28-1.00) or Model 2 (*p*-value range: 0.19-0.89; see Table 1.3). In contrast, the dummy coded variable comparing the "Bothers a Lot" category to the "No Difficulty Falling or Staying Asleep" reference category was significant in Model 1 (exp[b] = 1.15, 95% *CI*: 1.02-1.29, $p = 0.03$) adjusting for demographic factors. The exp(b) of 1.15 indicates that a 1-unit change in insomnia symptoms category (i.e., moving from "No Difficulty Falling or Staying Asleep" to "Bothers a Lot") was associated with a 15% increase in IL-6 on average [(1.15 – 1) x 100] while controlling for demographic factors. However, this association was attenuated and not significant in Model 2 (exp[b] = 1.07, 95%*CI*: 0.95-1.19, $p = 0.25$) adjusting for additional potential confounders. Results remained consistent with those of Model 2 in the sensitivity analyses individually adjusting for the insomnia-related factors (Models 3-5).

Insomnia symptoms and D-dimer

For Models 1 and 2, all of the insomnia symptom dummy coded variables were not significant in Model 1 (*p*-value range: 0.18-0.91) or Model 2 (*p*-value range: 0.34-0.75; see Table 1.3). Results remained consistent in the sensitivity analyses (Models 3-5).

Discussion

Our examination of the VACS Biomarker Cohort data did not support our hypotheses that greater insomnia symptoms would be associated with higher circulating levels of markers of monocyte activation, systemic inflammation, and coagulation in PLWH. All but one of the tested associations between insomnia symptom categories and sCD14, IL-6, and D-dimer were not significant. Furthermore, the one significant association observed in a demographics-adjusted model – i.e., higher IL-6 among veterans bothered a lot by difficulty falling or staying asleep – was no longer significant in our primary model further adjusting for CVD risk factors, additional potential confounders, and HIV-related factors. Taken together, the present results suggest that insomnia symptoms may not be associated with the putative biological mechanisms of HIV-CVD examined in the present study.

To our knowledge, this is the first study to investigate associations between insomnia symptoms and immune activation or coagulation markers among PLWH. Others have examined the relationship between sleep parameters or sleep disturbance and systemic inflammation, reporting mixed results. Of the four available studies, two examined group differences, one using self-reported sleep onset latency (SOL) ≤ 30 or > 30 minutes (43) and one using objectively measured SOL, wake-after-sleep-onset (WASO), time of sleep onset, total sleep time (TST), and sleep efficiency (SE) split at the sample median for each variable (44). Tests of group differences between these two studies yielded conflicting results. Gay et al. (43) found no group differences in various inflammatory markers (i.e., CRP, IL-1beta, IL-2, IL-6, IL-10, IL-13, and tumor necrosis factor [TNF]-alpha) between those with SOL ≤ 30 and > 30 minutes. Wirth et al. (44) found significantly higher or trending higher CRP and IL-6 among those with indicators of poorer sleep quantity and quality (i.e., lower TST, later sleep onset time, lower SE, higher SOL, and higher WASO). In addition, three of the four studies examined linear or correlational associations. Gay et al. (43) observed significant or trending positive associations between IL-13 and SOL > 30 minutes (a 1-*SD* increase in IL-13 was associated with a 34% increased odds of SOL > 30 minutes, $p = .024$) and IL-10 (a 1-*SD* increase in IL-10 was associated with a 27% increased odds of SOL > 30 minutes, $p = .068$), controlling for genomic estimates of ancestry, race/ethnicity, and viral load. SOL > 30 minutes was not associated with IL-1beta, IL-2, IL-6, TNF-alpha, and CRP. Lee et al. (45) observed positive correlations between objectively measured WASO and CRP (rho = .135, $p = .023$) and TNF-alpha (rho = .121, $p = .042$), a

trending negative association between WASO and IL-13 (rho = -.111, p = .061), and null associations between WASO and IL-1beta, IL-2, IL-6, and IL-10. Moore et al. (46) observed negative correlations between self-reported sleep disturbance and TNF-gamma (rho = -.697, p = .017) and TNF-alpha (rho = -.697, p = .017) but not for IL-6 among women, with no significant associations observed among men. Differences in results between the four available studies and our own may be due to differences in the definition of the sleep disturbance variables (e.g., subjective versus objective measurement) and/or sample characteristics (e.g., percentage with undetectable versus detectable viral loads, veteran versus non-veteran). Of note, because Gay et al. (43) and Lee et al. (45) utilized similar samples from The Symptoms and Genetic Study, our current knowledge of the insomnia-inflammation relationship, including the present study, is based on only four samples of PLWH.

The lack of associations between insomnia and putative biological mechanisms of HIV-CVD in the present study raises two possibilities. The first possibility is our null results reflect the state of nature. If accurate, this would suggest that other mechanisms underlie the previously observed insomnia-CVD relationship among PLWH. Other potential mechanisms include hypertension and overweight/obesity, which consist of both biological and behavioral components. Regarding hypertension, meta-analytic evidence in the general population suggests that adults with individual symptoms of insomnia have a 14-21% increased risk of hypertension (47). To our knowledge, there are no published studies examining longitudinal associations between sleep variables and hypertension or blood pressure among PLWH. Elucidating insomnia's role in hypertension in the HIV population could be of great importance, given hypertension's high prevalence (19-52%) and its ties to pathological consequences of the HIV virus (e.g., chronic inflammation) and ART treatment (e.g., mitochondrial toxicity and insulin resistance) (48). Concerning overweight/obesity, general population data suggests that sleep restriction is positively related to subjective hunger, caloric intake, and weight gain and negatively related to insulin sensitivity (49). A few existing studies have observed a positive association between sleep disturbance and weight variables among PLWH (i.e., obesity and increased waist size) (50, 51), although it has not been a focus of the HIV literature thus far. Other potential behavioral mechanisms identified in the general population that may translate to the HIV population includes decreased physical activity (52), smoking (53), and poor diet (54).

The second possibility is our null results are due to methodological factors and may or may not reflect the state of nature. For instance, our assessment of insomnia relied on a single item that assessed only difficulty falling or staying asleep within the past four weeks, missing symptoms such as early morning awakening, insomnia-related impairment and/or distress, and insomnia symptom frequency and duration. This reduced content validity could contribute to the misclassification of cases (e.g., participants experiencing symptoms of insomnia are inappropriately classified as not experiencing insomnia). Future research endeavors on insomnia in the HIV population should consider incorporating comprehensive and validated assessments of insomnia symptoms and/or insomnia disorder. To assess insomnia symptoms, we recommend the Insomnia Severity Index (ISI), which is sensitive to treatment response and can be administered online (55, 56). To assess insomnia disorder, we recommend either the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) Sleep Disorders (SCISD) interview (57) or the Diagnostic Interview for Sleep Patterns and Disorders (DISP) based on The International Classification of Sleep Disorders, second edition (ICSD-2) criteria (58).

In addition to our study's strengths (large sample, extensive adjustment for potential confounders, and inclusion of monocyte activation and coagulation biomarkers), there are important limitations worth considering. First, the cross-sectional, observational study design prevents examination of the directionality of associations between insomnia symptoms and putative biological mechanisms of HIV-CVD. It is plausible that immune activation and/or systemic inflammation may predict insomnia symptoms among PLWH, although human research examining this direction is limited (59). Second, as previously mentioned, our insomnia variable was limited to a single item and did not comprehensively capture insomnia symptoms. Third, the examined biomarkers may not adequately capture the intricate and interconnected nature of the immune and coagulation processes involved in the development of HIV-CVD. However, the three biomarkers examined are among the most studied in relation to CVD risk (60), increasing our ability to make comparisons with past studies. Fourth, we were unable to adjust for some factors that may confound the relationship between insomnia symptoms and putative biological mechanisms of HIV-CVD (e.g., physical inactivity, obstructive sleep apnea). Fifth, although not inherently a limitation, the present study examined a predominantly male and entirely veteran sample. This may reduce the generalizability of our results to other demographic

groups, such as women and non-veterans. Future studies examining the mechanisms underlying the insomnia-CVD relationship among PLWH should ideally utilize a prospective design with a comprehensive assessment of insomnia (e.g., ISI, SCISD, DISP); multiple indicators of immune activation, systemic inflammation, and coagulation; and more inclusive samples of PLWH.

In summary, we did not observe significant associations between insomnia symptoms and markers of immune activation, systemic inflammation, or coagulation. Our results raise the possibility that other mechanisms (e.g., hypertension or overweight/obesity) may be responsible for observed associations between insomnia symptoms and incident HIV-CVD. However, further research into the insomnia-biological mechanism relationships is still warranted as our study, and others in the extant literature, did not comprehensively assess insomnia. Ultimately, elucidating mechanistic pathways of the insomnia-CVD relationship in people with HIV could determine the viability of insomnia as a potential behavioral treatment target for the management of chronic immune activation and the subsequent risk of CVD among PLWH.

References

1. Wandeler G, Johnson LF, Egger M: Trends in life expectancy of HIV-positive adults on antiretroviral therapy across the globe: comparisons with general population. *Curr Opin HIV AIDS.* 2016, *11:*492-500.

2. Rodger AJ, Lodwick R, Schechter M, et al.: Mortality in well controlled HIV in the continuous antiretroviral therapy arms of the SMART and ESPRIT trials compared with the general population. *Aids.* 2013, *27:*973-979.

3. Trickey A, May MT, Vehreschild J, et al.: Cause-Specific Mortality in HIV-Positive Patients Who Survived Ten Years after Starting Antiretroviral Therapy. *PLoS One.* 2016, *11:*e0160460.

4. Palella FJ, Jr., Baker RK, Moorman AC, et al.: Mortality in the highly active antiretroviral therapy era: changing causes of death and disease in the HIV outpatient study. *J Acquir Immune Defic Syndr.* 2006, *43:*27-34.

5. Islam FM, Wu J, Jansson J, Wilson DP: Relative risk of cardiovascular disease among people living with HIV: a systematic review and meta-analysis. *Hiv Medicine.* 2012, *13:*453-468.

6. Freiberg MS, Chang CC, Kuller LH, et al.: HIV infection and the risk of acute myocardial infarction. *JAMA Intern Med.* 2013, *173:*614-622.

7. Paisible AL, Chang CC, So-Armah KA, et al.: HIV Infection, Cardiovascular Disease Risk Factor Profile, and Risk for Acute Myocardial Infarction. *J Acquir Immune Defic Syndr.* 2015, *68:*209-216.

8. Womack JA, Chang CC, So-Armah KA, et al.: HIV infection and cardiovascular disease in women. *J Am Heart Assoc.* 2014, *3:*e001035.

9. Wu J, Wu H, Lu CY, Guo L, Li PS: Self-reported sleep disturbances in HIV-infected people: a meta-analysis of prevalence and moderators. *Sleep Medicine.* 2015, *16:*901-907.

10. Chen YC, Lin CY, Strong C, et al.: Sleep disturbances at the time of a new diagnosis: a comparative study of human immunodeficiency virus patients, cancer patients, and general population controls. *Sleep Med.* 2017, *36:*38-43.

11. Polanka BM, Kundu S, So-Armah KA, et al.: Insomnia as an Independent Predictor of Incident Cardiovascular Disease in HIV: Data from the Veterans Aging Cohort Study. *J Acquir Immune Defic Syndr.* 2019.

12. Sofi F, Cesari F, Casini A, et al.: Insomnia and risk of cardiovascular disease: a meta-analysis. *European Journal of Preventive Cardiology.* 2014, *21:*57-64.

13. Li M, Zhang X-W, Hou W-S, Tang Z-Y: Insomnia and risk of cardiovascular disease: A meta-analysis of cohort studies. *International Journal of Cardiology.* 2014, *176:*1044-1047.

14. He Q, Zhang P, Li G, Dai H, Shi J: The association between insomnia symptoms and risk of cardio-cerebral vascular events: A meta-analysis of prospective cohort studies. *Eur J Prev Cardiol.* 2017, *24:*1071-1082.

15. Vachiat A, McCutcheon K, Tsabedze N, Zachariah D, Manga P: HIV and Ischemic Heart Disease. *J Am Coll Cardiol.* 2017, *69:*73-82.

16. Besedovsky L, Lange T, Haack M: The Sleep-Immune Crosstalk in Health and Disease. *Physiol Rev.* 2019, *99:*1325-1380.

17. Irwin MR, Olmstead R, Carroll JE: Sleep Disturbance, Sleep Duration, And Inflammation: A Systematic Review And Meta-Analysis Of Cohort Studies And Experimental Sleep Deprivation. *Biol Psychiatry.* 2015.

18. Jackowska M, Steptoe A: Sleep and future cardiovascular risk: prospective analysis from the English Longitudinal Study of Ageing. *Sleep Medicine.* 2015, *16:*768-774.

19. Mausbach BT, Ancoli-Israel S, von Kanel R, et al.: Sleep disturbance, norepinephrine, and D-Dimer are all related in elderly caregivers of people with Alzheimer disease. *Sleep.* 2006, *29:*1347-1352.

20. von Kanel R, Ancoli-Israel S, Dimsdale JE, et al.: Sleep and Biomarkers of Atherosclerosis in Elderly Alzheimer Caregivers and Controls. *Gerontology.* 2010, *56:*41-50.

21. von Känel R, Dimsdale JE, Ancoli-Israel S, et al.: Poor Sleep Is Associated with Higher Plasma Proinflammatory Cytokine Interleukin-6 and Procoagulant Marker Fibrin D-Dimer in Older Caregivers of People with Alzheimer's Disease. *Journal of the American Geriatrics Society.* 2006, *54:*431-437.

22. Matthews KA, Zheng HY, Kravitz HM, et al.: Are Inflammatory and Coagulation Biomarkers Related to Sleep Characteristics in Mid-Life Women?: Study of Women's Health Across the Nation Sleep Study. *Sleep.* 2010, *33:*1649-1655.

23. Prather AA, Epel ES, Cohen BE, Neylan TC, Whooley MA: Gender differences in the prospective associations of self-reported sleep quality with biomarkers of systemic inflammation and coagulation: Findings from the Heart and Soul Study. *Journal of Psychiatric Research.* 2013, *47:*1228-1235.

24. Suarez EC: Self-reported symptoms of sleep disturbance and inflammation, coagulation, insulin resistance and psychosocial distress: Evidence for gender disparity. *Brain Behavior and Immunity.* 2008, *22:*960-968.

25. Irwin MR, Olmstead R, Carrillo C, et al.: Cognitive behavioral therapy vs. Tai Chi for late life insomnia and inflammatory risk: a randomized controlled comparative efficacy trial. *Sleep.* 2014, *37:*1543-1552.

26. Helfand M, Buckley D, Fleming C, et al.: U.S. Preventive Services Task Force Evidence Syntheses, formerly Systematic Evidence Reviews. *Screening for Intermediate Risk Factors for Coronary Heart Disease*. Rockville (MD): Agency for Healthcare Research and Quality (US), 2009.

27. Armah KA, McGinnis K, Baker J, et al.: HIV Status, Burden of Comorbid Disease, and Biomarkers of Inflammation, Altered Coagulation, and Monocyte Activation. *Clinical Infectious Diseases.* 2012, *55:*126-136.

28. So-Armah KA, Tate JP, Chang CH, et al.: Do Biomarkers of Inflammation, Monocyte Activation, and Altered Coagulation Explain Excess Mortality Between HIV Infected and Uninfected People? *J Acquir Immune Defic Syndr.* 2016, *72:*206-213.

29. Justice AC, Dombrowski E, Conigliaro J, et al.: Veterans Aging Cohort Study (VACS): Overview and description. *Med Care.* 2006, *44:*S13-24.

30. Fultz SL, Skanderson M, Mole LA, et al.: Development and verification of a "virtual" cohort using the National VA Health Information System. *Med Care.* 2006, *44:*S25-30.

31. Justice AC, Holmes W, Gifford AL, et al.: Development and validation of a self-completed HIV symptom index. *J Clin Epidemiol.* 2001, *54 Suppl 1:*S77-90.

32. Taylor BS, So-Armah K, Tate JP, et al.: HIV and Obesity Comorbidity Increase Interleukin 6 but Not Soluble CD14 or D-Dimer. *J Acquir Immune Defic Syndr.* 2017, *75:*500-508.

33. Chobanian AV, Bakris GL, Black HR, et al.: Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension.* 2003, *42:*1206-1252.

34. Butt AA, Fultz SL, Kwoh CK, et al.: Risk of diabetes in HIV infected veterans pre- and Post-HAART and the role of HCV coinfection. *Hepatology.* 2004, *40:*115-119.

35. Butt AA, McGinnis K, Rodriguez-Barradas MC, et al.: HIV infection and the risk of diabetes mellitus. *Aids.* 2009, *23:*1227-1234.

36. McGinnis KA, Brandt CA, Skanderson M, et al.: Validating smoking data from the Veteran's Affairs Health Factors dataset, an electronic data source. *Nicotine Tob Res.* 2011, *13:*1233-1239.

37. Butt AA, Xiaoqiang W, Budoff M, et al.: Hepatitis C virus infection and the risk of coronary disease. *Clin Infect Dis.* 2009, *49:*225-232.

38. Everitt H, McDermott L, Leydon G, et al.: GPs' management strategies for patients with insomnia: a survey and qualitative interview study. *Br J Gen Pract.* 2014, *64:*e112-119.

39. Morlock RJ, Tan M, Mitchell DY: Patient characteristics and patterns of drug use for sleep complaints in the United States: analysis of National Ambulatory Medical Survey data, 1997-2002. *Clin Ther.* 2006, *28:*1044-1053.

40. Stewart JC, Polanka BM, So-Armah KA, et al.: Associations of Total, Cognitive/Affective, and Somatic Depressive Symptoms and Antidepressant Use with Cardiovascular Disease-Relevant Biomarkers in HIV: Veterans Aging Cohort Study. *Psychosom Med.* 2020.

41. Barnard J, Rubin DB: Small-Sample Degrees of Freedom with Multiple Imputation. *Biometrika.* 1999, *86:*948-955.

42. Platt L, Easterbrook P, Gower E, et al.: Prevalence and burden of HCV co-infection in people living with HIV: a global systematic review and meta-analysis. *Lancet Infect Dis.* 2016, *16:*797-808.

43. Gay CL, Zak RS, Lerdal A, et al.: Cytokine polymorphisms and plasma levels are associated with sleep onset insomnia in adults living with HIV/AIDS. *Brain Behav Immun.* 2015, *47:*58-65.

44. Wirth MD, Jaggers JR, Dudgeon WD, et al.: Association of Markers of Inflammation with Sleep and Physical Activity Among People Living with HIV or AIDS. *AIDS Behav.* 2015, *19:*1098-1107.

45. Lee KA, Gay C, Pullinger CR, et al.: Cytokine polymorphisms are associated with poor sleep maintenance in adults living with human immunodeficiency virus/acquired immunodeficiency syndrome. *Sleep.* 2014, *37:*453-463.

46. Moore SE, Voss JG, Webel AR: Sex-Based Differences in Plasma Cytokine Concentrations and Sleep Disturbance Relationships Among People Living With HIV. *J Assoc Nurses AIDS Care.* 2020, *31:*249-254.

47. Meng L, Zheng Y, Hui R: The relationship of sleep duration and insomnia to risk of hypertension incidence: a meta-analysis of prospective cohort studies. *Hypertens Res.* 2013, *36:*985-995.

48. Kent ST, Burkholder GA, Tajeu GS, Overton ET, Muntner P: Mechanisms Influencing Circadian Blood Pressure Patterns Among Individuals with HIV. *Curr Hypertens Rep.* 2015, *17:*88.

49. Zhu B, Shi C, Park CG, Zhao X, Reutrakul S: Effects of sleep restriction on metabolism-related parameters in healthy adults: A comprehensive review and meta-analysis of randomized controlled trials. *Sleep Med Rev.* 2019, *45:*18-30.

50. Gomez-Olive FX, Rohr JK, Roden LC, Rae DE, von Schantz M: Associations between sleep parameters, non-communicable diseases, HIV status and medications in older, rural South Africans. *Sci Rep.* 2018, *8:*17321.

51. Crum-Cianflone NF, Roediger MP, Moore DJ, et al.: Prevalence and factors associated with sleep disturbances among early-treated HIV-infected persons. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2012, *54:*1485-1494.

52. Kredlow MA, Capozzoli MC, Hearon BA, Calkins AW, Otto MW: The effects of physical activity on sleep: a meta-analytic review. *J Behav Med.* 2015, *38:*427-449.

53. Liao Y, Xie L, Chen X, et al.: Sleep quality in cigarette smokers and nonsmokers: findings from the general population in central China. *BMC Public Health.* 2019, *19:*808.

54. Chaput JP: Sleep patterns, diet quality and energy balance. *Physiol Behav.* 2014, *134:*86-91.

55. Thorndike FP, Ritterband LM, Saylor DK, et al.: Validation of the Insomnia Severity Index as a Web-Based Measure. *Behavioral Sleep Medicine.* 2011, *9:*216-223.

56. Morin CM, Belleville G, Belanger L, Ivers H: The Insomnia Severity Index: Psychometric indicators to detect insomnia cases and evaluate treatment response. *Sleep.* 2011, *34:*601-608.

57. Taylor DJ, Wilkerson AK, Pruiksma KE, et al.: Reliability of the Structured Clinical Interview for DSM-5 Sleep Disorders Module. *Journal of clinical sleep medicine : JCSM : official publication of the American Academy of Sleep Medicine.* 2018, *14:*459-464.

58. Merikangas KR, Zhang J, Emsellem H, et al.: The structured diagnostic interview for sleep patterns and disorders: rationale and initial evaluation. *Sleep Med.* 2014, *15:*530-535.

59. Motivala SJ: Sleep and inflammation: psychoneuroimmunology in the context of cardiovascular disease. *Ann Behav Med.* 2011, *42:*141-152.

60. Vos AG, Idris NS, Barth RE, Klipstein-Grobusch K, Grobbee DE: Pro-Inflammatory Markers in Relation to Cardiovascular Disease in HIV Infection. A Systematic Review. *PLoS One.* 2016, *11:*e0147484.

Table 1.1
Characteristics of Veterans with HIV in the VACS Biomarker Cohort (*N = 1,542*)

Demographic Factors	
Age, years	52.1 [46.8, 57.5]
Sex, male	1,500 (97)
Race/Ethnicity	
White	292 (19)
African American	1,065 (69)
Hispanic	127 (8)
Other	58 (4)
CVD Risk Factors	
Prevalent CVD	269 (17)
Hypertension	
None	438 (28)
Controlled	748 (49)
Uncontrolled	355 (23)
Diabetes	290 (19)
BMI, kg/m^2	25.2 [22.7, 28.3]
Smoking	
Never	370 (24)
Current	772 (50)
Former	398 (26)
LDL Cholesterol, mg/dL	97.3 [75.0, 122.0]
HDL Cholesterol, mg/dL	42.0 [33.0, 52.0]
Triglycerides, mg/dL	140.0 [90.0, 209.0]
Statin Use	463 (30)
Additional Potential Confounders	
Hepatitis C Infection	724 (47)
eGFR, $mL/min/1.73m^2$	95.7 [79.8, 114.1]
Hemoglobin, g/dL	14.0 [12.9, 15.0]
Alcohol Use, hazardous use or abuse/dependence	653 (42)
Cocaine Use, past-year use or abuse/dependence	324 (21)
Insomnia-Related Factors	
PHQ-9 (no sleep item)	3.0 [0.0, 9.0]
Non-benzodiazepine Sleep Medication Use	146 (10)
Antidepressant Medication Use	
SSRI Use	665 (43)
TCA Use	393 (26)
Miscellaneous Other Use	652 (42)
HIV-Related Factors	
HIV-1 RNA Level, copies/mL	75.0 [50.0, 3634.0]
$CD4^+$ T Cell Count, mm^3	399.0 [250.0, 586.3]
ART regimen	1,302 (84)

Table 1.1 Continued

Outcome Variables	
sCD14, ng/mL	1,719 [1,448, 2,085]
IL-6, pg/mL	2.08 [1.42, 3.38]
D-dimer, μg/mL	0.26 [0.15, 0.49]

Note. Continuous variables are presented as median [first quartile, third quartile]. Outcome variables are presented in their original, untransformed units. Categorical variables are presented as *n* (%).

The following variables include fewer than 1,542 participants because of missing data (*n*, % missing): hypertension (3, 0.2%), BMI (5, 0.3%), smoking (2, 0.1%), LDL cholesterol (42, 2.7%), HDL cholesterol (39, 2.5%), triglycerides (17, 1.1%), eGFR (1, 0.1%), hemoglobin (1, 0.1%), alcohol use (5, 0.3%), cocaine use (76, 4.9%), PHQ-9 (79, 5.1%), HIV-1 RNA (2, 0.1%), and CD4+ cell count (2, 0.1%).

HIV = human immunodeficiency virus; VACS = Veterans Aging Cohort Study; CVD = cardiovascular disease; BMI = body mass index; LDL = low-density lipoprotein; HDL = high-density lipoprotein; eGFR = estimated glomerular filtration rate; PHQ-9 = Patient Health Questionnaire-9; SSRI = selective serotonin reuptake inhibitor; TCA = tricyclic antidepressant; ART = antiretroviral therapy; sCD14 = soluble CD14; IL-6 = interleukin-6.

Table 1.2
Biomarker Levels across Insomnia Symptom Categories of Veterans with HIV in the VACS Biomarker Cohort

Insomnia Symptoms	sCD14 (ng/mL)	IL-6 (pg/mL)	D-dimer (μg/mL)
No Difficulty Falling or Staying Asleep (*n* = 611)	1678.78 [1408.06, 2101.91]	2.01 [1.37, 3.34]	0.28 [0.16, 0.49]
Doesn't Bother (*n* = 147)	1746.91 [1480.16, 2193.70]	2.08 [1.43, 3.58]	0.26 [0.16, 0.50]
Bothers a Little (*n* = 330)	1731.93 [1446.65, 2061.89]	1.98 [1.39, 3.20]	0.26 [0.15, 0.47]
Bothers (*n* = 217)	1750.63 [1492.02, 2029.61]	2.14 [1.55, 3.42]	0.29 [0.16, 0.51]
Bothers a Lot (*n* = 211)	1728.03 [1492.17, 2039.50]	2.40 [1.54, 3.62]	0.28 [0.18, 0.49]

Note. Outcome variables are presented as median [first quartile, third quartile] in their original units. A total of 26 cases are not included in Table 2, as they were imputed due to missing data on the insomnia symptoms item of the VACS HIV Symptom Index.
HIV = human immunodeficiency virus; VACS = Veterans Aging Cohort Study; sCD14 = soluble CD14; IL-6 = interleukin-6.

Table 1.3

Associations of Insomnia Symptoms Categories with sCD14, IL-6, and D-dimer levels in Veterans with HIV in the VACS Biomarker Cohort *(*N = *1,542)*

Model	sCD14			IL-6			D-dimer		
	exp(b)	**95%*CI***	***p*-value**	**exp(b)**	**95%*CI***	***p*-value**	**exp(b)**	**95%*CI***	***p*-value**
Model 1: Demographic-adjusted									
No Difficulty Falling or Staying Asleep	Ref.	---	---	Ref.	---	---	Ref.	---	---
Doesn't Bother	1.05	(0.99-1.10)	0.09	1.00	(0.87-1.15)	1.00	1.07	(0.89-1.28)	0.48
Bothers a Little	1.02	(0.98-1.06)	0.38	0.98	(0.89-1.09)	0.73	1.01	(0.88-1.15)	0.91
Bothers	1.02	(0.98-1.07)	0.31	1.07	(0.95-1.21)	0.28	1.10	(0.94-1.29)	0.22
Bothers a Lot	1.01	(0.97-1.06)	0.53	**1.15***	**(1.02-1.29)**	**0.03**	1.11	(0.95-1.30)	0.18
Model 2: Fully-adjusted									
No Difficulty Falling or Staying Asleep	Ref.	---	---	Ref.	---	---	Ref.	---	---
Doesn't Bother	1.04	(0.99-1.09)	0.13	0.99	(0.87-1.13)	0.87	1.06	(0.90-1.26)	0.49
Bothers a Little	1.00	(0.96-1.03)	0.93	0.94	(0.85-1.03)	0.19	0.98	(0.86-1.11)	0.75
Bothers	1.00	(0.96-1.05)	0.85	1.01	(0.90-1.14)	0.89	1.08	(0.92-1.26)	0.34
Bothers a Lot	1.01	(0.97-1.05)	0.70	1.07	(0.95-1.19)	0.25	1.06	(0.91-1.23)	0.47
Model 3: PHQ-9 (no sleep item)									
No Difficulty Falling or Staying Asleep	Ref.	---	---	Ref.	---	---	Ref.	---	---
Doesn't Bother	1.04	(0.99-1.09)	0.15	1.00	(0.88-1.14)	0.97	1.05	(0.89-1.25)	0.55
Bothers a Little	1.00	(0.96-1.03)	0.85	0.95	(0.86-1.04)	0.28	0.97	(0.85-1.10)	0.65
Bothers	1.00	(0.96-1.05)	0.97	1.03	(0.91-1.16)	0.68	1.06	(0.90-1.25)	0.47
Bothers a Lot	1.00	(0.96-1.05)	0.86	1.09	(0.97-1.23)	0.16	1.03	(0.88-1.22)	0.68
Model 4: Non-Benzodiazepine Sleep Medication Use									
No Difficulty Falling or Staying Asleep	Ref.	---	---	Ref.	---	---	Ref.	---	---
Doesn't Bother	1.04	(0.99-1.09)	0.13	0.99	(0.87-1.13)	0.88	1.06	(0.90-1.26)	0.49
Bothers a Little	1.00	(0.96-1.03)	0.92	0.94	(0.85-1.03)	0.20	0.98	(0.86-1.11)	0.75
Bothers	1.00	(0.96-1.05)	0.85	1.01	0.90-1.14	0.87	1.08	(0.92-1.26)	0.34
Bothers a Lot	1.01	(0.96-1.05)	0.71	1.07	0.96-1.20	0.23	1.06	(0.91-1.23)	0.46
Model 5: Antidepressant Medication Use									
No Difficulty Falling or Staying Asleep	Ref.	---	---	Ref.	---	---	Ref.	---	---
Doesn't Bother	1.04	(0.99-1.09)	0.13	0.99	(0.87-1.13)	0.91	1.05	(0.89-1.25)	0.55
Bothers a Little	1.00	(0.96-1.03)	0.87	0.94	(0.85-1.03)	0.20	0.96	(0.85-1.09)	0.54
Bothers	1.00	(0.96-1.05)	0.98	1.00	(0.89-1.13)	0.96	1.05	(0.90-1.23)	0.53
Bothers a Lot	1.01	(0.96-1.05)	0.75	1.07	(0.96-1.21)	0.23	1.01	(0.87-1.19)	0.86

Table 1.3 Continued

Note. For the results presented in Table 3, the outcome variables are log-transformed.
Model 1: Demographic-adjusted (age, sex, race/ethnicity)
Model 2: Fully-adjusted (Model 1 + CVD Risk Factors: prevalent CVD, hypertension, diabetes, BMI, smoking, total cholesterol, statin use; Additional Potential Confounders: hepatitis C infection, renal function, anemia, alcohol use, cocaine use; HIV-Related Factors: HIV-1 RNA viral load, $CD4_+$ T cell count, ART regimen)
Model 3: PHQ-9 Total Score-adjusted (Model 2 + PHQ-9 [no sleep item])
Model 4: Non-Benzodiazepine Sleep Medication Use (Model 2 + non-benzodiazepine sleep medication use)
Model 5: Antidepressant Medication Use (Model 4 + SSRI use, TCA use, and miscellaneous other antidepressant use)
*$p < 0.05$
sCD14 = soluble CD14; IL-6 = interleukin-6; HIV = human immunodeficiency virus; VACS = Veterans Aging Cohort Study; PHQ-9 = Patient Health Questionnaire-9; CVD = cardiovascular disease; BMI = body mass index; ART = antiretroviral therapy; SSRI = serotonin selective reuptake inhibitor; TCA = tricyclic antidepressant; Ref. = reference category

ASSOCIATIONS OF SLEEP DISTURBANCE WITH MARKERS OF INFLAMMATION, COAGULATION, AND ENDOTHELIAL DYSFUNCTION OVER TIME IN HIV

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Abstract

Background: While insomnia has been identified as a potential risk factor for cardiovascular disease in HIV (HIV-CVD), research on the underlying pathophysiological mechanisms is scarce. **Methods:** We examined associations between 0-to-12-week changes in sleep disturbance and the concurrent 0-to-12-week changes and the subsequent 12-to-24-week changes in markers of systemic inflammation, coagulation, and endothelial dysfunction among people living with HIV (n = 33-38) enrolled in a depression clinical trial. Sleep disturbance was measured using the Pittsburgh Sleep Quality Index. Inflammatory markers interleukin-6 (IL-6) and C-reactive protein (CRP) and coagulation marker D-dimer were determined from blood specimens; endothelial dysfunction marker brachial flow-mediated dilation (FMD) was determined by ultrasound. 0-to-12-week variables were calculated as 12-week visit minus baseline, and 12-to-24-week variables were calculated as 24-week minus 12-week. We constructed multivariate linear regression models for each outcome adjusting for age, sex, race/ethnicity, Framingham risk score, and baseline depressive symptoms. **Results:** We did not observe statistically significant associations between 0-to-12-week changes in sleep disturbance and 0-to-12-week or 12-to-24-week changes in IL-6, CRP, D-dimer, or FMD. However, we did observe potentially meaningful associations, likely undetected due to low power. For 0-to-12-weeks, every 1-standard deviation (*SD*) increase, or worsening, in the sleep disturbance change score was

associated with a 0.41 pg/mL and 80 ng/mL decease in IL-6 and D-dimer, respectively. For 12-to-24-weeks, every 1-*SD* increase in sleep disturbance change score was associated with a 0.63 mg/L, 111 ng/mL, and 0.82% increase in CRP, D-dimer, and FMD, respectively. **Conclusion:** We observed potentially meaningful, though not statistically significant, associations between changes in sleep disturbance and changes in biological mechanisms underlying HIV-CVD over time. Some associations were in the expected direction, but others were not. Additional studies are needed that utilize larger samples and validated, comprehensive assessments of insomnia.

Introduction

With the reduction in human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS)-related mortality due to effective antiretroviral therapy (ART), the HIV population is now facing new health challenges. One such challenge is that of sleep disturbance, including insomnia, among people living with HIV (PLWH). The HIV population has a high prevalence of self-reported sleep disturbance at 58% (1) and may have a higher risk of developing sleep disturbance than the general population or other immunocompromised groups (i.e., cancer patients) (2). When compared to other symptoms experienced by PLWH, sleep disturbance and its consequences (e.g., fatigue) are among the most commonly reported distressing symptoms (3).

Sleep disturbance has been associated with both general and HIV-specific factors in PLWH. Regarding general factors, sleep disturbance has been linked to psychological symptoms (e.g., depression, anxiety, and fatigue), substance use, pain, and cognitive impairment (4-8). Interestingly, the opposite relationship between sleep disturbance and age emerges among PLWH. While subjective sleep disturbance is positively associated with age in the general population (9), it is negatively associated with age in PLWH, as younger (i.e., 18-44 years) versus older (i.e., 45-80 years) adults exhibit higher rates of sleep disturbance (10). Regarding HIV-specific factors, sleep disturbance has been linked to advanced HIV disease/AIDS, longer duration of HIV infection, and particular ART regimens (e.g., efavirenz) (4). Research is mixed on whether or not poorly managed HIV (i.e., high viral load and/or low CD4 count) is related to sleep disturbance outside the context of advanced HIV disease/AIDS.

Sleep disturbance is of particular importance, given its potential association with another medical challenge being faced by the HIV population – namely, cardiovascular disease (CVD).

PLWH are over twice as likely to develop CVD than those without HIV (11). The increased risk of CVD in HIV (HIV-CVD) has been attributed to a several factors, including HIV infection, ART treatment, and an increased prevalence of traditional and non-traditional CVD risk factors (12, 13). However, the increased CVD risk in PLWH persists among those with optimal CVD risk profiles (14), suggesting the need to identify novel contributors to HIV-CVD. Sleep disturbance may be one such novel risk factor for HIV-CVD, given that insomnia symptoms have been associated with incident CVD in both general population (15-17) and HIV population (18) research.

The putative biological mechanisms underlying HIV-CVD include systemic inflammation, altered coagulation, and endothelial dysfunction (19, 20). HIV population research has only recently begun to examine potential relationships between sleep disturbance and biological mechanisms of HIV-CVD. Specifically, the cross-sectional literature examining associations between sleep disturbance or objective sleep parameters and inflammatory markers among PLWH is small in size and has yielded mixed results (21-24). To our knowledge, no longitudinal studies of the sleep disturbance-inflammation relationship exist, nor do studies examining the sleep disturbance-coagulation or sleep disturbance-endothelial function relationships in PLWH. Thus, the purpose of the present study is to examine associations between changes in sleep disturbance and changes in inflammatory, coagulation, and endothelial dysfunction markers over time among PLWH.

Methods

Study design, setting, and participants

We utilized data from a 24-week randomized controlled pilot trial comparing computerized cognitive-behavioral therapy (CBT) for depression to usual care. HIV-positive, ART-treated, virologically suppressed adults with a positive depression screen were randomized in a 1:1 fashion. Following a screening visit, participants completed two entry visits (1-15 days apart), a 12-week follow-up visit, and a 24-week follow-up visit. Participants completed a blood draw and an ultrasound assessment of brachial artery flow-mediated dilation (FMD) at each visit and self-report questionnaires at the second entry, 12-week, and 24-week follow-up visits. Participants were required to fast and abstain from smoking for at least 8 hours prior to each

study visit. Randomization was completed at the second entry visit using a computerized algorithm stratified by baseline antidepressant use (yes/no).

Briefly, participants randomized to the intervention arm received an internet CBT program, *Beating the Blues US*TM (BtB), to be completed between the second entry and 12-week follow-up visits at a location of their choosing (e.g., at home or at one of the investigator's laboratory). BtB is an 8-session, online, stand-alone depression treatment program (see http://www.beatingthebluesus.com/ for video tutorial). BtB is empirically supported, with effect sizes similar to face-to-face CBT for depression (25-27). Participants randomized to the usual care arm were informed of their positive depression screen and were encouraged to follow-up with their primary care or HIV provider regarding their depression. In addition, providers of participants were sent a letter indicating their patient had screened positive for depression and was randomized to usual care; this letter also included a list of local mental health services.

Participants were recruited from the HIV clinics of the Indiana University Medical Center and Eskenazi Health. At the screening visit, participants were screened for the following inclusion criteria: HIV-positive, at least 18 years of age, receipt of ART for at least 1 year prior to screening, HIV-1 RNA viral load of < 75 copies/mL, and a Patient Health Quesionnaire-9 (PHQ-9) score of ≥ 10 (i.e., a clinically significant level of depressive symptoms) (28). Participants were also screened for the following exclusion criteria: active suicidality; history of psychotic or bipolar disorders based on chart review; pre-existing cardiovascular or inflammatory disease (with the exception of hepatitis B or C co-infection); treatment of malignancy in the last 6 months; elevated blood pressure (> 160/110 mmHg), fasting blood glucose or hemoglobin A1c (≥ 140 mg/dL or > 8.0%), or total cholesterol (> 240 mg/dL); reduced estimated glomerular filtration rate (< 50 mL/min/1.73m$_2$); or current pregnancy or breastfeeding during the study period. Participants were allowed to engage in other depression treatments while enrolled in the study.

Missing data across the blood-based biomarkers was among the same participants, while the missing data for FMD was among different participants than those with missing biomarker data. Thus, to maximize our sample size, we applied different exclusions to the study sample depending on the outcome (biomarkers versus FMD) and timeframe (0-to-12 weeks versus 12-to-24 weeks) creating 4 separate analytic cohorts. For the 0-to-12-week biomarker analyses, from the total pilot trial sample (N = 54), we excluded participants who did not attend both study visits

(*n* = 4), were missing data on the exposure variable (*n* = 9) or outcome variables (*n* = 3), or were suspected of an infection producing drastic changes in biomarker levels over time (*n* = 1), resulting in a sample of 38 PLWH. For the 0-to-12-week FMD analysis, we excluded participants who did not attend both study visits (*n* = 4), were missing data on the exposure variable (*n* = 9) or outcome variable (*n* = 2), or were suspected of an infection producing drastic changes in biomarker levels over time (*n* = 1), resulting in a sample of 37 PLWH. For the 12-to-24-week biomarker analyses, we excluded participants who did not attend both study visits (*n* = 5), were missing data on the exposure variable (*n* = 9) or outcome variables (*n* = 6), or were suspected of an infection producing drastic changes in biomarker levels over time (*n* = 1), resulting in a sample of 33 PLWH. For the 12-to-24-week FMD analysis, we excluded participants who did not attend both study visits (*n* = 5), were missing data on the exposure variable (*n* = 9) or outcome variable (*n* = 6), or were suspected of an infection producing drastic changes in biomarker levels over time (*n* = 1), resulting in a sample of 33 PLWH.

The study was conducted in accordance with the Helsinki Declaration, approved by the Indiana University Institutional Review Board, and registered with ClinicalTrials.gov (NCT02309372).

Measures and procedures

Exposure variable. Sleep disturbance was measured at the second entry, 12-week follow-up, and 24-week follow-up visits using the Pittsburgh Sleep Quality Index (PSQI). The PSQI is a 19-item self-report scale with research supporting its reliability and validity (29, 30). Meta-analytic evidence supports the PSQI's specificity in assessing insomnia versus sleep-disordered breathing, with strong associations observed with the Insomnia Severity Index (*r* = 0.80) and sleep efficiency scores from sleep diaries (*r* = -0.76) and weak associations observed with the Apnea Hypopnea Index (*r* = 0.11-0.30) and number of oxygen desaturation events (*r* = 0.21) (31). The PSQI measures sleep disturbance across seven components – sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbance, use of sleeping medications, and daytime dysfunction – with component scores ranging from 0-3. The seven component scores are summed to compute a global score ranging from 0-21. The 0-to-12-week sleep disturbance change score was calculated as 12-week minus second entry. This change score

reflects treatment phase changes in sleep disturbance, with more negative values indicating greater improvement.

Outcome variables. Circulating levels of inflammatory markers interleukin-6 (IL-6) and C-reactive protein (CRP) and coagulation marker D-dimer were assessed at each study visit via blood draw. IL-6, CRP, and D-dimer were measured by standard enzyme-linked immunosorbent assay (ELISA) kits per instructions provided by the manufacturer (IL-6 and CRP: R&D Systems; D-dimer: Thermo Scientific). Assays were performed by the Center for Diabetes and Metabolic Diseases Translational Core at the Indiana University School of Medicine. In accordance with guidelines set by the International Brachial Artery Reactivity Task Force (32), endothelial dysfunction marker brachial FMD was assessed at each visit using a GE Logic e high resolution ultrasound machine with a 15MHz vascular transducer. FMD measurements were made using AccessPoint 2011 software (Freeland Systems, Version 8.2). Specifically, participants laid supine with a blood pressure cuff placed on the forearm, which was inflated to 250 mmHg for 5 minutes. The brachial artery dilation was measured in response to reactive hyperemia at 60 and 90 seconds post-cuff deflation, and the maximum value was retained. FMD was calculated as the percent increase in the diameter of the brachial artery.

Because participants underwent two entry visits, the IL-6, CRP, D-dimer, and FMD values for these visits were averaged to increase the stability of the baseline estimates. The 0-to-12-week and 12-to-24-week change scores for each outcome variable were calculated as 12-week minus baseline and 24-week minus 12-week, respectively. The 0-to-12-week change scores reflect treatment phase changes in the outcome variables, while the 12-to-24-week change scores reflect follow-up phase changes. More negative values indicate greater improvement for IL-6, CRP, and D-dimer, whereas more positive values indicate greater improvement for FMD. Of note, no variable transformations were necessary as all outcome variables were approximately normally distributed.

Covariates. The included covariates were age, sex, race/ethnicity, Framingham risk score (FRS), and baseline depressive symptoms. Age (years), sex at birth (0 = female, 1 = male), and race/ethnicity were assessed via chart review at the screening visit. Due to low counts in some racial and ethnic categories, we re-coded race/ethnicity into a dichotomous variable. Specifically, we collapsed the race (White, Black/African American, Asian, Native Hawaiian/Pacific Islander, American Indian, Other, Declined) and ethnicity (non-Hispanic/Latino, Hispanic/Latino,

unknown) variables into race/ethnicity (0 = non-Hispanic Black, 1 = Other). FRS was calculated using a validated formula consisting of age (via chart review at screening visit), smoking status (via self-report at second entry visit), diabetes status (via chart review at second entry visit), and objectively measured systolic blood pressure, total cholesterol, and high-density lipoprotein cholesterol at the second entry visit (33). Of note, the FRS was chosen because evidence suggests that this tool more accurately captures CVD risk among PLWH than the ACC/AHA Pooled Cohort Equations (PCEs), the Systematic COronary Risk Evaluation (SCORE), and the Data Collection on Adverse Effects of Anti-HIV Drugs (D:A:D) study equation (34). However, it is acknowledged that, overall, the existing CVD risk prediction models for the general population underestimate CVD risk in the HIV population (35). Baseline depressive symptoms were measured using the Hopkins Symptom Checklist Depression Scale (SCL-20) (36-39) at the second entry visit. The three sleep-related items (i.e., "trouble falling asleep," "awakening in the early morning," and "sleep that is restless or disturbed") were not included in the total depression score calculation, given their overlap with the exposure variable. Thus, we subsequently refer to this variable as SCL-17. The two variables used in the sensitivity analyses described below are 0-to-12-week changes in depressive symptoms and randomization status. The 0-to-12-week SCL-17 change score was calculated as 12-week minus second entry. Randomization status (0 = usual care, 1 = BtB) was based on participant randomization to either BtB or usual care at the second entry visit.

Statistical analysis

All analyses were performed using IBM SPSS software (v. 26). Participant characteristics were summarized using descriptive statistics. Specifically, continuous variables were summarized as mean (standard deviation; *SD*), and categorical variables were summarized as frequency (%). Within-person mean imputation was used for self-report questionnaires (i.e., PSQI and SCL-17) if ≤ 25% of items were missing. We used component scores for the PSQI and items for the SCL-17 when assessing missingness and imputing missing values.

To examine associations between 0-to-12-week changes in sleep disturbance and 0-to-12-week and 12-to-24-week changes in inflammatory, coagulation, and endothelial dysfunction markers, we constructed eight multivariate linear regression models (see Figure 1). Four models examined the adjusted association between 0-to-12-week changes in sleep disturbance and 0-to-

12-week changes in each outcome variable (IL-6, CRP, D-dimer, and FMD). Four models examined the adjusted associations between 0-to-12-week changes in sleep disturbance and 12-to-24-week changes in each outcome variable. All models included the following covariates: age, sex, race/ethnicity, FRS, and baseline depressive symptoms. Due to the small sample sizes, it is highly likely that these models are underpowered to detect the associations of interest. Thus, we made the decision to proceed with a tentative interpretation of standardized regression coefficients (βs) ≥ 0.10 or ≤ -0.10 in order to characterize these associations in the variables' original units and assess whether they may be clinically meaningful.

We additionally conducted two types of sensitivity analyses. First, to examine the influence of changes in depressive symptoms on the associations of interest, we replaced the baseline depressive symptoms variable with the 0-to-12-week changes in depressive symptoms variable in each of the eight models. Second, to examine the influence of trial arm on the associations of interest, we added the randomization status variable to each of the eight models.

Results

Participant characteristics

Participant characteristics by analytic cohort are displayed in Table 2.1. Of note, due to the eligibility criteria of the parent study, all participants have high ART adherence, are relatively healthy (e.g., did not have pre-existing cardiovascular conditions), and screened positive for depression. The average age was approximately 45 years, the vast majority of participants were male, and roughly half were non-Hispanic Black. Overall, the 10-year CVD risk was in the low range (~10%) based on the Framingham risk score, although this may underestimate the CVD risk of PLWH. The mean SCL-17 total score was 2.2 (possible range 0-4), which falls in the severe depression range. The mean PSQI global score was 10-11 (possible range 0-21), above the recommended cut-off of 5 to identify "poor sleepers." This appears higher than other HIV samples observing mean PSQI global scores of approximately 6 (40, 41).

Changes over time in the exposure and outcome variables

On average, participants displayed an improvement in sleep disturbance as evidenced by a mean decrease of 1.2-1.6 PSQI points between the second entry and 12-week visits. We

observed good variability in the PSQI change score, with an average *SD* of approximately 3 points (see Table 2.2 for biomarker analytic cohorts and Table 2.3 for FMD analytic cohorts).

For the 0-to-12-week period, we observed an increase (worsening) in inflammatory markers IL-6 and CRP of 0.69 pg/mL and 0.45 mg/L, respectively, but a decrease (improvement) in coagulation marker D-dimer of 24 ng/mL (see Table 2.2). We also observed an increase (improvement) in endothelial dysfunction marker FMD of 0.28% (see Table 2.3). The 0-to-12-week change scores for IL-6, CRP, D-dimer, and FMD all displayed good variability, with *SD*'s of 2.88 pg/mL, 2.92 mg/L, 554 ng/mL, and 2.76%, respectively.

For the 12-to-24-week period, we observed a similar pattern for CRP and D-dimer, as both maintained the same direction in their change scores. However, we observed the opposite direction in change scores for IL-6 and FMD, with a decrease (improvement) in IL-6 and a decrease (worsening) in FMD. The 12-to-24-week change scores for IL-6, CRP, D-dimer, and FMD showed good variability, with *SD*'s of 3.03 pg/mL, 4.63 mg/L, 680 ng/mL, and 2.86% respectively.

0-to-12-week changes

There were no statistically significant associations between 0-to-12-week changes in sleep disturbance and 0-to-12-week changes in IL-6, CRP, D-dimer, or FMD (see Table 2.4). However, we continued with interpretation for βs ≥ 0.10 or ≤ -0.10 in order to characterize these associations in the variables' original units and assess whether they may be clinically meaningful but missed due to type II error. Two of the four examined associations had a β beyond our cut points, specifically IL-6 (β = -0.141) and D-dimer (β = -0.144). For every 1-*SD* (2.9 PSQI points) increase (worsening) in the sleep disturbance change score, the IL-6 change score decreased (improved) by 0.41 pg/mL (-0.141 x 2.88), and the D-dimer change score decreased (improved) by 80 ng/mL (-0.144 x 554). Interestingly, the observed negative associations are in the opposite of the expected direction, with increases in sleep disturbance potentially associated with decreases, rather than increases, in these biomarkers implicated in HIV-CVD.

12-to-24-week changes

There were no statistically significant associations between 0-to-12-week changes in sleep disturbance and 12-to-24-week changes in IL-6, CRP, D-dimer, or FMD (see Table 2.4). Three of the four examined associations had a β beyond our cut points, specifically CRP (β = 0.137), D-dimer (β = 0.163), and FMD (β = 0.287). Among the biomarker analytic cohort, for every 1-*SD* (3.0 PSQI points) increase (worsening) in the sleep disturbance change score, the CRP change score increased (worsened) by 0.63 mg/L (0.137 x 4.63), and the D-dimer change score increased (worsened) by 111 ng/mL (0.163 x 680). Among the FMD analytic cohort, for every 1-*SD* (2.9 PSQI points) increase (worsening) in sleep disturbance change score, the FMD change score increased (improved) by 0.82% (0.287 x 2.86). In contrast to the 0-to-12-week results, we observed the expected positive associations, with increases in sleep disturbance potentially associated with increases in CRP and D-dimer. However, we observed the opposite of the expected negative association between changes in sleep disturbance and FMD, with increases in sleep disturbance potentially associated with increases, rather than decreases, in FMD.

Sensitivity analyses

Results of the two sensitivity analyses are presented in Table 2.5. Sensitivity analysis #1, in which we replaced baseline depressive symptoms with 0-to-12-week changes in depressive symptoms, yielded nearly identical results to the primary results. For both the 0-to-12-week changes and the 12-to-24-week changes in the outcome variables, there were no statistically significant associations. However, for the 0-to-12-week results, all four of the examined associations had βs ≥ 0.10 or ≤ -0.10, with the βs for CRP and FMD now beyond the cut points despite not appearing to be much different in magnitude from the primary results (βs = 0.128 and -0.115 versus 0.092 and -0.093, respectively). Thus, we now observed the expected positive association between changes in sleep disturbance and changes in CRP and the expected negative association between changes in sleep disturbance and changes in FMD.

Sensitivity analysis #2, in which we added randomization status, also yielded nearly identical results. For both the 0-to-12-week changes and the 12-to-24-week changes in the outcome variables, there were no statistically significant associations. However, for the 0-to-12-week results, three of the four examined associations had βs ≥ 0.10 or ≤ -0.10, with the β for

CRP now beyond the cut point despite having a similar magnitude to the primary results (β = .103 versus .092). Thus, we again observed the expected positive association between changes in sleep disturbance and changes in CRP. For the 12-to-24-week results, two of the four examined associations had βs ≥ 0.10 or ≤ -0.10, with the β for CRP now falling short of the cut point despite having a similar magnitude to the primary results (β = .090 versus .137).

Discussion

Our study adds to the scarce literature on associations of sleep disturbance with markers of systemic inflammation among PLWH and, to our knowledge, is the first to examine associations of sleep disturbance with markers of coagulation and endothelial dysfunction in the HIV population. Overall, we did not detect any statistically significant relationships between changes in sleep disturbance and changes in IL-6, CRP, D-dimer, or FMD over time in this analysis of data from our 24-week pilot randomized controlled trial examining the effect of computerized CBT for depression on CVD risk markers in PLWH. Given the novelty of our research questions and the low statistical power due to our small sample size, we further examined all relationships with βs ≥ 0.10 or ≤ -0.10 to assess whether they may be clinically meaningful. Five of the eight examined associations were potentially meaningful based on these cut points. Specifically, we observed a potential signal for a negative relationship between changes in sleep disturbance and concurrent changes in IL-6 and D-dimer. These were in the opposite of the expected direction, with each 1-*SD* increase in sleep disturbance (worsening) potentially associated with decreases (improvements) in IL-6 and D-dimer of 0.41 pg/mL and 80 ng/mL, respectively. Furthermore, we observed a potential signal for a positive relationship between changes in sleep disturbance and subsequent changes in CRP, D-dimer, and FMD. These were in the expected direction for CRP and D-dimer but the opposite direction for FMD, with each 1-*SD* increase in sleep disturbance (worsening) potentially associated with increases (worsening) in CRP and D-dimer of 0.63 mg/L and 111 ng/mL, respectively, and with an increase (improvement) in FMD of 0.82%.

Evaluating the clinical meaningfulness of these relationships is difficult due to the lack of standard norms for the examined biomarkers and FMD among PLWH. The Strategies for Management of Antiretroviral Therapy (SMART) study observed that 1-SD increases (values not reported) in IL-6, CRP, and D-dimer were associated with a 39%, 43%, and 40% greater risk of

CVD, respectively (42). In the present study, the changes in these biomarkers associated with a 1-*SD* increase in sleep disturbance were much smaller than the *SD*s for these outcomes at baseline (see Table 2), suggesting that these associations may not be clinically meaningful. To our knowledge, no study has examined the value of FMD in predicting CVD risk among PLWH, although general population research suggests that a 1% increase in FMD is related to a 12% decreased risk of future CVD events (43). When compared to this, our finding that a 3-point increase in PSQI total score may be associated with a 0.82% increase in FMD appears to be potentially meaningful, although in the opposite of the expected direction. The reasons for this unexpected association are currently unclear, and future studies are needed to assess the reproducibility of this finding and to explore potential underlying causes.

Only four studies, all cross-sectional, have examined associations between various measures of sleep disturbance and inflammatory markers among PLWH, and the results have been mixed. Two studies utilized actigraphy-derived sleep parameters indicative of sleep disturbance. Lee et al. (23) found positive correlations with wake after sleep onset for TNF-alpha (rho = .121, p = .042) and CRP (rho = .135, p = .023) but not for IL-6. In that study, no associations were observed between total sleep time and the inflammatory markers. In contrast, Wirth et al. (21) found higher CRP and IL-6 in people with lower total sleep time but did not observe group differences in inflammatory markers for wake after sleep onset, sleep latency, or sleep efficiency. The other two studies utilized self-reported sleep disturbance. Gay et al. (22) did not find group differences in inflammatory markers IL-6, TNF-alpha, or CRP between people with higher versus lower self-reported sleep onset latency. Moore et al. (24) did observe associations but in the opposite of the expected direction. Specifically, they observed negative correlations with global sleep disturbance for TNF-gamma (rho = -.697, p = .017) and TNF-alpha (rho = -.697, p = .017) but not for IL-6 among women living with HIV. No significant associations were observed between sleep disturbance and the inflammatory markers among men living with HIV. Of note, this study utilized a very small sample (N = 20, 11 women and 9 men).

In contrast to these prior studies, we examined changes over time in sleep disturbance. However, we may still observe consistencies and inconsistencies with the existing literature. Our CRP results suggest that changes in sleep disturbance may be unrelated to concurrent changes in systemic inflammation, consistent with the null results observed by Gay et al. (22), but related to subsequent changes. However, our results are inconsistent with the positive, cross-sectional

associations between sleep disturbance and CRP found by Wirth et al. (21) and Lee et al. (23). We observed a negative association between changes in sleep disturbance and concurrent changes in IL-6, inconsistent with all four studies that found either positive or null cross-sectional relationships (21-24). These inconsistent results may be due to differences in the assessments of sleep disturbance and/or differences in HIV-related sample characteristics (e.g., percentage of participants with suppressed viral loads). As this area grows, future research will be able to explore potential moderators that may help account for these mixed results.

There are several potential explanations for our results. First, we observed potentially meaningful associations in the expected direction between changes in sleep disturbance and subsequent changes in CRP and D-dimer. This suggests that sleep disturbance may influence some of the pathophysiological mechanisms underlying HIV-CVD. The mechanisms by which sleep disturbance may be related to systemic inflammation and coagulation are poorly understood, with existing evidence largely based on intentional sleep restriction or deprivation studies. One hypothesis linking sleep deprivation to inflammation proposes that the reduced opportunity for nighttime blood pressure dipping leads to the activation of vascular endothelium, which results in the production of cellular adhesion molecules and inflammatory cytokines (e.g., IL-6) (44). Second, we also observed potentially meaningful results in the opposite of the expected direction for changes in sleep disturbance and concurrent changes in IL-6 and D-dimer and subsequent changes in FMD. It is possible that the present study's methodology played a role in these unexpected findings. Our results need to be considered within the context of a depression clinical trial – our sample consisted entirely of depressed adults living with HIV, half of whom received CBT for depression. While we attempted to statistically control for the potential effects of depressive symptoms and randomization study group on our outcomes, this may have been insufficient. It is unknown whether the relationships between sleep disturbance and putative biological mechanisms of HIV-CVD differ based on depression status. Third, we observed null results, suggesting that there may be no meaningful relationships between changes in sleep disturbance and concurrent changes in CRP and FMD and subsequent changes in IL-6. One possible explanation for our null results is the limitation of our sleep assessment. While the PSQI is correlated with measures specifically assessing insomnia symptoms (31), its components go beyond diagnostic criteria (e.g., sleep medication use). This reduced content validity may have negatively impacted our ability accurately capture participant experiences of insomnia

symptoms. Another potential explanation for our null results is that behavioral, rather than biological, pathways are responsible for the insomnia-CVD relationship among PLWH. For example, others have found that insomnia is related to suboptimal ART adherence (45, 46), potentially leading to inconsistently managed HIV and thus increasing the risk for CVD.

The present study has limitations worthy of consideration. First, our small sample size resulted in underpowered analyses limiting our ability to detect potentially meaningful associations. Second, we utilized a global measure of sleep disturbance, which does not specifically capture insomnia symptoms. Third, our analyses were within the context of a depression clinical trial, with participants screening positive for clinically significant depression at study entry. Despite these limitations, examining data from the parent trial offered a unique opportunity to examine associations between changes in sleep disturbance and changes in markers of systemic inflammation, coagulation, and endothelial dysfunction over time in a sample of adults with well-managed HIV.

In sum, we did not detect any statistically significant associations between changes in sleep disturbance and changes in IL-6, CRP, D-dimer, and FMD over time. However, we did observe potentially meaningful associations based on the magnitude of the standard regression coefficients, although some associations were in the opposite of the expected direction. Due to the dearth of research in this area and the limitations of the current studies, there is a need for additional investigations examining associations between insomnia and the putative biological mechanisms underlying HIV-CVD. Future studies should be adequately powered, utilize comprehensive insomnia assessments based on diagnostic criteria (e.g., Insomnia Severity Index), and assess and control for depressive symptoms to determine insomnia's independent association. Ultimately, identifying the mechanisms underlying the insomnia-CVD relationship in PLWH could inform the development or selection of interventions designed to help lower the risk of HIV-CVD.

References

1. Wu J, Wu H, Lu CY, Guo L, Li PS: Self-reported sleep disturbances in HIV-infected people: a meta-analysis of prevalence and moderators. *Sleep Medicine.* 2015, *16:*901-907.

2. Chen YC, Lin CY, Strong C, et al.: Sleep disturbances at the time of a new diagnosis: a comparative study of human immunodeficiency virus patients, cancer patients, and general population controls. *Sleep Med.* 2017, *36:*38-43.

3. Sabranski M, Erdbeer G, Sonntag I, et al.: Physical and mental health in HIV-infected patients with virological success and long-term exposure to antiretroviral therapy. *AIDS Care.* 2020*:*1-9.

4. Low Y, Goforth H, Preud'homme X, Edinger J, Krystal A: Insomnia in HIV-infected patients: pathophysiologic implications. *AIDS Rev.* 2014, *16:*3-13.

5. Sun-Suslow N, Saloner R, Serrano V, et al.: Lifetime Methamphetamine Use Disorder and Reported Sleep Quality in Adults Living with HIV. *AIDS Behav.* 2020.

6. Vosvick M, Gore-Felton C, Ashton E, et al.: Sleep disturbances among HIV-positive adults: the role of pain, stress, and social support. *J Psychosom Res.* 2004, *57:*459-463.

7. Byun E, Gay CL, Portillo CJ, et al.: Cytokine polymorphisms are associated with daytime napping in adults living with HIV. *Sleep Med.* 2017, *32:*162-170.

8. Mahmood Z, Hammond A, Nunez RA, Irwin MR, Thames AD: Effects of Sleep Health on Cognitive Function in HIV+ and HIV- Adults. *J Int Neuropsychol Soc.* 2018, *24:*1038-1046.

9. Brewster GS, Riegel B, Gehrman PR: Insomnia in the Older Adult. *Sleep Med Clin.* 2018, *13:*13-19.

10. Ning C, Lin H, Chen X, et al.: Cross-sectional comparison of various sleep disturbances among sex- and age-matched HIV-infected versus HIV-uninfected individuals in China. *Sleep Med.* 2020, *65:*18-25.

11. Shah ASV, Stelzle D, Lee KK, et al.: Global Burden of Atherosclerotic Cardiovascular Disease in People Living With HIV. *Circulation.* 2018, *138:*1100-1112.

12. Triant VA, Grinspoon SK: Epidemiology of ischemic heart disease in HIV. *Curr Opin HIV AIDS.* 2017.

13. Freiberg MS, Chang CC, Kuller LH, et al.: HIV infection and the risk of acute myocardial infarction. *JAMA Intern Med.* 2013, *173:*614-622.

14. Paisible AL, Chang CC, So-Armah KA, et al.: HIV Infection, Cardiovascular Disease Risk Factor Profile, and Risk for Acute Myocardial Infarction. *J Acquir Immune Defic Syndr.* 2015, *68:*209-216.

15. Sofi F, Cesari F, Casini A, et al.: Insomnia and risk of cardiovascular disease: a meta-analysis. *European Journal of Preventive Cardiology.* 2014, *21:*57-64.

16. Li M, Zhang X-W, Hou W-S, Tang Z-Y: Insomnia and risk of cardiovascular disease: A meta-analysis of cohort studies. *International Journal of Cardiology.* 2014, *176:*1044-1047.

17. He Q, Zhang P, Li G, Dai H, Shi J: The association between insomnia symptoms and risk of cardio-cerebral vascular events: A meta-analysis of prospective cohort studies. *Eur J Prev Cardiol.* 2017, *24:*1071-1082.

18. Polanka BM, Kundu S, So-Armah KA, et al.: Insomnia as an Independent Predictor of Incident Cardiovascular Disease in HIV: Data from the Veterans Aging Cohort Study. *J Acquir Immune Defic Syndr.* 2019.

19. Beltran LM, Rubio-Navarro A, Amaro-Villalobos JM, et al.: Influence of immune activation and inflammatory response on cardiovascular risk associated with the human immunodeficiency virus. *Vasc Health Risk Manag.* 2015, *11:*35-48.

20. Vachiat A, McCutcheon K, Tsabedze N, Zachariah D, Manga P: HIV and Ischemic Heart Disease. *J Am Coll Cardiol.* 2017, *69:*73-82.

21. Wirth MD, Jaggers JR, Dudgeon WD, et al.: Association of Markers of Inflammation with Sleep and Physical Activity Among People Living with HIV or AIDS. *AIDS Behav.* 2015, *19:*1098-1107.

22. Gay CL, Zak RS, Lerdal A, et al.: Cytokine polymorphisms and plasma levels are associated with sleep onset insomnia in adults living with HIV/AIDS. *Brain Behav Immun.* 2015, *47:*58-65.

23. Lee KA, Gay C, Pullinger CR, et al.: Cytokine polymorphisms are associated with poor sleep maintenance in adults living with human immunodeficiency virus/acquired immunodeficiency syndrome. *Sleep.* 2014, *37:*453-463.

24. Moore SE, Voss JG, Webel AR: Sex-Based Differences in Plasma Cytokine Concentrations and Sleep Disturbance Relationships Among People Living With HIV. *J Assoc Nurses AIDS Care.* 2020, *31:*249-254.

25. Rollman BL, Herbeck Belnap B, Abebe KZ, et al.: Effectiveness of Online Collaborative Care for Treating Mood and Anxiety Disorders in Primary Care: A Randomized Clinical Trial. *JAMA Psychiatry.* 2018, *75:*56-64.

26. Proudfoot J, Ryden C, Everitt B, et al.: Clinical efficacy of computerised cognitive-behavioural therapy for anxiety and depression in primary care: randomised controlled trial. *Br J Psychiatry.* 2004, *185:*46-54.

27. Jonassaint CR, Gibbs P, Belnap BH, et al.: Engagement and outcomes for a computerised cognitive-behavioural therapy intervention for anxiety and depression in African Americans. *BJPsych Open.* 2017, *3:*1-5.

28. Kroenke K, Spitzer RL, Williams JB: The PHQ-9: validity of a brief depression severity measure. *J Gen Intern Med.* 2001, *16:*606-613.

29. Carpenter JS, Andrykowski MA: Psychometric evaluation of the Pittsburgh Sleep Quality Index. *J Psychosom Res.* 1998, *45:*5-13.

30. Buysse DJ, Reynolds CF, 3rd, Monk TH, Berman SR, Kupfer DJ: The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res.* 1989, *28:*193-213.

31. Mollayeva T, Thurairajah P, Burton K, et al.: The Pittsburgh sleep quality index as a screening tool for sleep dysfunction in clinical and non-clinical samples: A systematic review and meta-analysis. *Sleep Med Rev.* 2016, *25:*52-73.

32. Corretti MC, Anderson TJ, Benjamin EJ, et al.: Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol.* 2002, *39:*257-265.

33. D'Agostino RB, Sr., Vasan RS, Pencina MJ, et al.: General cardiovascular risk profile for use in primary care: the Framingham Heart Study. *Circulation.* 2008, *117:*743-753.

34. Thompson-Paul AM, Lichtenstein KA, Armon C, et al.: Cardiovascular Disease Risk Prediction in the HIV Outpatient Study. *Clin Infect Dis.* 2016.

35. Triant VA, Perez J, Regan S, et al.: Cardiovascular Risk Prediction Functions Underestimate Risk in HIV Infection. *Circulation.* 2018, *137:*2203-2214.

36. Katon W, Von Korff M, Lin E, et al.: Stepped collaborative care for primary care patients with persistent symptoms of depression: a randomized trial. *Arch Gen Psychiatry.* 1999, *56:*1109-1115.

37. Katon W, Robinson P, Von Korff M, et al.: A multifaceted intervention to improve treatment of depression in primary care. *Arch Gen Psychiatry.* 1996, *53:*924-932.

38. Katon W, Von Korff M, Lin E, et al.: Collaborative management to achieve treatment guidelines. Impact on depression in primary care. *Jama.* 1995, *273:*1026-1031.

39. Derogatis LR, Lipman RS, Rickels K, Uhlenhuth EH, Covi L: The Hopkins Symptom Checklist (HSCL). A measure of primary symptom dimensions. *Mod Probl Pharmacopsychiatry.* 1974, *7:*79-110.

40. Crum-Cianflone NF, Roediger MP, Moore DJ, et al.: Prevalence and factors associated with sleep disturbances among early-treated HIV-infected persons. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2012, *54:*1485-1494.

41. Seay JS, McIntosh R, Fekete EM, et al.: Self-reported sleep disturbance is associated with lower CD4 count and 24-h urinary dopamine levels in ethnic minority women living with HIV. *Psychoneuroendocrinology.* 2013, *38:*2647-2653.

42. Duprez DA, Neuhaus J, Kuller LH, et al.: Inflammation, coagulation and cardiovascular disease in HIV-infected individuals. *PLoS One.* 2012, *7:*e44454.

43. Matsuzawa Y, Kwon TG, Lennon RJ, Lerman LO, Lerman A: Prognostic Value of Flow-Mediated Vasodilation in Brachial Artery and Fingertip Artery for Cardiovascular Events: A Systematic Review and Meta-Analysis. *J Am Heart Assoc.* 2015, *4*.

44. Mullington JM, Simpson NS, Meier-Ewert HK, Haack M: Sleep loss and inflammation. *Best Pract Res Clin Endocrinol Metab.* 2010, *24:*775-784.

45. Saberi P, Neilands TB, Johnson MO: Quality of sleep: associations with antiretroviral nonadherence. *AIDS Patient Care STDS.* 2011, *25:*517-524.

46. Downing MJ, Millar BM, Hirshfield S: Changes in Sleep Quality and Associated Health Outcomes among Gay and Bisexual Men Living with HIV. *Behav Sleep Med.* 2020, *18:*406-419.

Table 2.1
Participant Characteristics at Study Entry by Analytic Cohort

	0-to-12-week biomarker (*n* = 37)	0-to-12-week FMD (*n* = 38)	12-to-24-week biomarker (*n* = 33)	12-to-24-week FMD (*n* = 33)
Age, years	45.3 (11.7)	44.6 (11.8)	45.7 (11.1)	45.6 (11.1)
Sex, % female	4 (10.8)	3 (7.9)	3 (9.1)	3 (9.1)
Race/ethnicity, % non-Hispanic Black	20 (54.1)	22 (57.9)	17 (51.5)	20 (60.6)
FRS, 10-year CVD risk %	9.5 (5.06)	9.7 (5.1)	9.5 (4.7)	10.2 (5.1)
HDL cholesterol, mg/dL	20.9 (5.10)	20.9 (5.1)	21.1 (5.3)	10.6 (5.1)
Total cholesterol, mg/dL	86.4 (15.3)	86.5 (15.4)	87.1 (15.4)	86.9 (15.7)
SBP, mmHg	126.4 (11.9)	127.1 (12.0)	126.3 (11.4)	128.3 (11.3)
Antihypertensive medication, % yes	10 (27.0)	10 (26.3)	9 (27.3)	9 (27.3)
Current smoker, % yes	14 (37.8)	16 (42.1)	11 (33.3)	14 (42.4)
Diabetes, % yes	1 (2.7)	2 (5.3)	1 (3.0)	2 (6.1)
SCL-17 total score (possible range: 0-4)	2.2 (0.8)	2.2 (0.8)	2.2 (0.8)	2.2 (0.8)

Note. Continuous variables are presented as mean (standard deviation). Categorical variables are presented as *n* (%). The four analytic cohorts are non-exclusive with overlap of participants between cohorts (see Study design, setting, and participants).
FMD = flow-mediated dilation; FRS = Framingham risk score; CVD = Cardiovascular disease; HDL = high-density lipoprotein; SBP = systolic blood pressure; SCL-17 = Hopkins Symptom Checklist Depression Scale without the 3 sleep-related items (see Methods).

Table 2 2

Sleep Disturbance and Biomarker Values at Each Time Point by Analytic Cohort

	0-to-12-week biomarker (*n* = 37)	12-to-24-week biomarker (*n* = 33)
PSQI (possible range: 0-21)		
Entry	11.0 (3.9)	10.9 (4.0)
12-week	9.4 (4.5)	9.4 (4.7)
24-week	---	---
Change score	-1.6 (2.9)	-1.5 (3.0)
IL-6, pg/mL		
Entry	2.56 (1.97)	---
12-week	3.24 (3.11)	3.12 (3.16)
24-week	---	2.73 (2.37)
Change score	0.69 (2.88)	-0.39 (3.03)
CRP, mg/L		
Entry	4.59 (4.61)	---
12-week	5.03 (5.65)	4.85 (6.63)
24-week	---	5.00 (5.51)
Change score	0.45 (2.92)	0.15 (4.63)
D-dimer, ng/mL		
Entry	2444 (831)	---
12-week	2420 (513)	2406 (514)
24-week	---	2400 (786)
Change score	-24 (554)	-6 (680)

Note. Variables are presented as mean (standard deviation). Change scores were calculated as 12-week minus second entry and 24-week minus 12-week. The two analytic cohorts are non-exclusive with overlap of participants between cohorts (see Study design, setting, and participants).

PSQI = Pittsburgh Sleep Quality Index; IL-6 = interleukin-6; CRP = C-reactive protein.

Table 2.3

Sleep Disturbance and FMD Values at Each Time Point by Analytic Cohort

	0-to-12-week FMD (*n* = 38)	12-to-24-week FMD (*n* = 33)
PSQI (possible range 0-21)		
Entry	11.5 (3.9)	11.3 (4.0)
12-week	9.9 (4.5)	10.1 (4.5)
24-week	---	---
Change score	-1.6 (2.9)	-1.2 (2.9)
FMD, %		
Entry	2.90 (1.72)	---
12-week	3.18 (2.71)	3.34 (2.82)
24-week	---	2.12 (2.12)
Change score	0.28 (2.76)	-1.22 (2.86)

Note. Variables are presented as mean (standard deviation). Change scores were calculated as 12-week minus second entry and 24-week minus 12-week. The two analytic cohorts are non-exclusive with overlap of participants between cohorts (see Study design, setting, and participants).

FMD = flow-mediated dilation; PSQI = Pittsburgh Sleep Quality Index.

Table 2.4

Multivariate Linear Regression Models Examining Associations of 0-to-12-Week Changes in Sleep Disturbance with 0-to-12-Week and 12-to-24-Week Changes in Biomarkers and FMD

	B	SE	β	*p*-value
0-to-12-week				
Changes in IL-6	-.138	.184	-.141	.458
Changes in CRP	.092	.186	.092	.625
Changes in D-dimer	-27.06	31.73	-.144	.401
Change in FMD	-.088	.176	-.093	.620
12-to-24-week				
Changes in IL-6	.050	.213	.050	.817
Changes in CRP	.210	.307	.137	.500
Changes in D-dimer	36.56	45.95	.163	.433
Change in FMD	.283	.175	.287	.117

Note. Models adjusted for age, sex, race/ethnicity, Framingham risk score, and baseline depressive symptoms.

FMD = flow-mediated dilation; B = unstandardized regression coefficient; SE = standard error; β = standardized regression coefficient; IL-6 = interleukin-6; CRP = C-reactive protein.

Table 2.5

Sensitivity Analyses of Multivariate Linear Regression Models Examining the Association Between Changes in 0-to-12-Week Insomnia Symptoms and Changes in Biomarkers and FMD

	B	SE	β	*p*-value
Sensitivity analysis #1				
0-to-12-week				
Changes in IL-6	-.103	.184	-.104	.581
Changes in CRP	.127	.185	.128	.498
Changes in D-dimer	-25.09	31.63	-.133	.434
Change in FMD	-.109	.174	-.115	.535
12-to-24-week				
Changes in IL-6	.034	.211	.034	.874
Changes in CRP	.167	.303	.109	.585
Changes in D-dimer	.289	45.78	.171	.408
Change in FMD	.289	.174	.293	.108
Sensitivity analysis #2				
0-to-12-week				
Changes in IL-6	-.129	.189	-.131	.500
Changes in CRP	.102	.190	.103	.596
Changes in D-dimer	-22.96	32.21	-.122	.482
Change in FMD	-.092	.179	-.097	.611
12-to-24-week				
Changes in IL-6	-.047	.214	-.047	.830
Changes in CRP	.138	.321	.090	.671
Changes in D-dimer	31.44	48.47	.140	.522
Change in FMD	.284	.171	.288	.109

Note. Sensitivity analysis #1 adjusted for age, sex, race/ethnicity, Framingham risk score, and 0-to-12-week changes in depressive symptoms. Sensitivity analysis #2 adjusted for age, sex, race/ethnicity, Framingham risk score, baseline depressive symptoms, and randomization status.

FMD = flow-mediated dilation; B = unstandardized regression coefficient; SE = standard error; β = standardized regression coefficient; IL-6 = interleukin-6; CRP = C-reactive protein

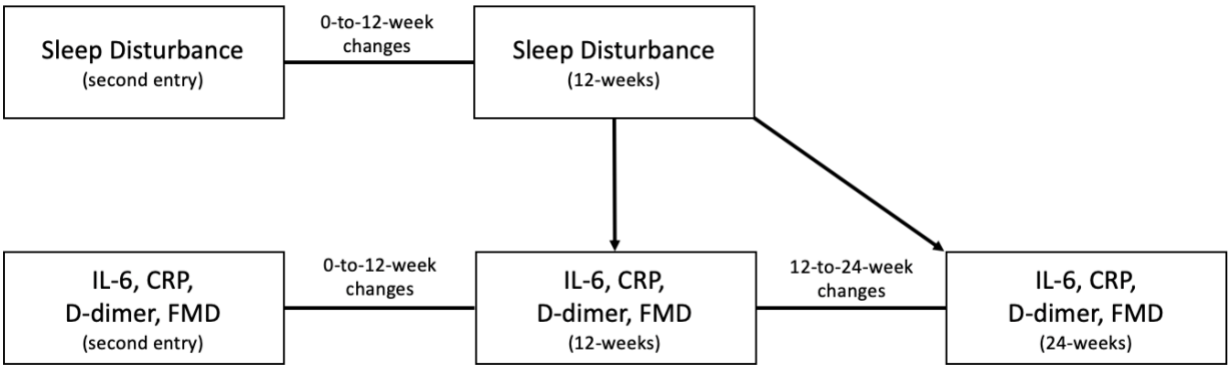


Figure 2.1. Visual representation of the examined associations between 0-to-12-week changes in sleep disturbance and 0-to-12-week and 12-to-24-week changes in interleukin-6 (IL-6), C-reactive protein (CRP), D-dimer, and brachial flow-mediated dilation (FMD).

APPENDIX

DISSERTATION PROPOSAL

Cardiovascular disease (CVD) is now one of the leading causes of death in people with human immunodeficiency virus (HIV).[1-3] Meta-analytic findings indicate that HIV+ adults on antiretroviral therapy (ART) have a 1.6-2.0 times greater risk of CVD than uninfected adults.[4] Leading candidate mechanisms underlying HIV-CVD include immune activation, systemic inflammation, altered coagulation, and endothelial dysfunction.[5] However, immune activation, inflammatory, coagulatory, and endothelial dysfunction markers are only partially improved by ART,[6-9] and HIV+ adults on ART achieving lower viral loads continue to exhibit a 39% greater risk of acute myocardial infarction (MI).[10] Thus, there is a current need to identify novel, modifiable risk factors for HIV-CVD that can be targeted by primary CVD prevention approaches.

Insomnia is a potential risk factor for CVD in HIV that has been ignored thus far. In the general population, evidence indicates that insomnia is a predictor of future CVD events,[11-13] – independent of traditional CVD risk factors and depression. Leading candidate mechanisms underlying the insomnia-CVD relationship mirror those underlying the HIV-CVD relationship – namely, systemic inflammation, altered coagulation, and endothelial dysfunction.[14-16] Though the relationship between insomnia and immune activation has not been studied in the general population, immune activation is closely related to systemic inflammation and an important feature of HIV pathophysiology.[17] Importantly, the largest RCT of behavioral interventions for insomnia in older adults (*N*=123) found that cognitive-behavioral therapy for insomnia (CBT-I), versus a sleep seminar, significantly reduced the risk of high C-reactive protein (CRP) at 1-year follow-up (*OR*=0.26).[18] CRP is an inflammatory marker implicated in atherosclerosis and predictive of future CVD events.[19] An additional analysis of this RCT indicated that CBT-I significantly reduced proinflammatory cytokine gene expression at post-treatment, suggesting a genomic mechanism through which insomnia treatment reduces systemic inflammation.[20] At present, little is known about insomnia's potential contribution to HIV-CVD. It is now important to evaluate insomnia in this regard because: a) insomnia is a common complaint in the HIV population (58%)[21] and b) initial evidence suggests that greater sleep disturbance may be related to higher levels of inflammatory markers implicated in CVD in HIV+ samples.[22-24]

My long-term goal is to direct a program of research aimed at preventing CVD in HIV by identifying and intervening on psychosocial risk factors for CVD in HIV. In line with this goal, my Master's thesis examined insomnia symptoms as a predictor of atherosclerotic CVD in HIV+ veterans. In further pursuit of this goal, the proposal seeks to examine associations of insomnia and insomnia changes over time with the putative mechanisms of HIV-CVD. I am in a unique position to complete the proposal and accelerate my trajectory as an independent scholar because: (a) my sponsor and co-sponsors were the first to examine psychosocial contributors (i.e., depression) to CVD in HIV,[25,26] and (b) through my sponsor and co-sponsors, I will be able to leverage two unique data sources: the Veterans Aging Cohort Study (VACS) Biomarker Cohort and a 24-week RCT (R01HL126557). The specific aims of this application are:

Aim 1: **To determine the associations of insomnia symptoms with circulating levels of immune activation, systemic inflammation, and coagulation markers in HIV+ adults.**
We hypothesize that, among HIV+ veterans in the VACS Biomarker Cohort, insomnia symptoms (self-report) will be positively associated with circulating levels of the immune activation marker soluble CD14 (sCD14), systemic inflammation marker interleukin-6 (IL-6), and coagulation marker D-dimer – the latter two of which are predictive of future CVD events in HIV.[27] The VACS Biomarker Cohort, an observational study of HIV+ veterans (*N*=1,542), is a subsample of the VACS 8 site study. Existing data was merged with new queries of the VA electronic medical record system.

Aim 2: **To determine the associations of changes in insomnia symptoms over time with changes in systemic inflammation, coagulation, and endothelial dysfunction markers over time in HIV+ adults.**
We hypothesize that, in a 24-week RCT of HIV+ adults on effective, continuous ART (*N*=51; R01HL126557), changes in insomnia symptoms (self-report) from Week 0 to 12, will predict changes in the systemic inflammation markers IL-6 and CRP, the coagulation marker D-dimer, and the endothelial dysfunction marker brachial flow-mediated dilation (FMD) from Week 12 to 24. Brachial FMD is related to inflammatory and coagulatory markers in adults with HIV[28] and is predictive of future CVD events in the general population.[29]

Support for these hypotheses would provide preliminary evidence that insomnia may be a modifiable contributor to immune activation, inflammation, coagulation, and endothelial dysfunction in people with HIV. In turn, this new knowledge would provide the mechanistic rationale for a future RCT testing whether treating insomnia in people with HIV helps to prevent clinical CVD onset. Ultimately, this line of research could identify novel targets for primary CVD prevention approaches in people with HIV.

RESEARCH STRATEGY

A. Significance

(A1) CVD: A Leading Health Concern in the HIV-Infected Population

CVD is now one of the leading causes of death in people with HIV.[1-3] HIV-CVD has been attributed to the virus itself,[30-32] ART treatment, [33,34] and both traditional [35] and non-traditional (e.g., renal disease and hepatitis C co-infection)[36,37] CVD risk factors. However, excess CVD risk remains among HIV+ adults after controlling for HIV viral load, ART use, and CVD risk factors,[10] and among HIV+ adults on newer ART regimens free of the dysmetabolic effects.[33] Furthermore, when comparing HIV+ and uninfected adults free of traditional CVD risk factors, HIV+ adults continue to exhibit twice the risk of MI.[38] These findings indicate that HIV+ adults are at a disproportionately higher risk of CVD than uninfected adults independent from HIV infection, ART, and traditional CVD risk factors, prompting the examination of other potential, and treatable, etiologies of HIV-CVD.

(A2) Biological Etiologies of HIV-CVD: Immune Activation, Systemic Inflammation, Altered Coagulation, and Endothelial Dysfunction

Numerous reviews suggests immune activation, systemic inflammation, altered coagulation, and endothelial dysfunction as putative mechanisms of HIV-CVD.[5,39-41] HIV+ adults exhibit elevated markers of immune activation (e.g., sCD14, soluble CD163), systemic inflammation (e.g., IL-6, high sensitivity CRP), and altered coagulation (e.g., D-dimer), and increased endothelial dysfunction (e.g., reduced brachial FMD and elevated soluble VCAM-1).[41] In turn, markers of these mechanisms have been associated with measures of subclinical CVD or clinical CVD. Specifically, immune activation marker sCD14 and T-cell activation markers are positively associated with coronary artery calcification (CAC)[42] and carotid artery plaque.[43] A recent systematic review of the relationships between inflammatory and coagulatory markers and CVD in HIV found that IL-6, CRP, and D-dimer were associated with the occurrence of

future CVD in three out of four studies.[27] While studies examining brachial FMD as a predictor of future CVD in HIV+ samples are lacking, evidence suggests that endothelial dysfunction is associated with HIV-related altered coagulation.[28] Moreover, in the general population, brachial FMD is an independent predictor of incident CVD,[29] and intervention-related improvements in FMD predicts a reduced incidence of CVD events.[44-46]

While ART has been successful in reducing HIV viral loads and improving some markers of immune function (e.g., CD4+ T-cell counts), it does not entirely eradicate reservoirs of viral replication[47] or fully normalize markers of immune activation, inflammation, or coagulation.[6-9] Likewise, ART does not normalize markers of endothelial functioning.[9,48] Thus, there is a need to continue the search for other novel, modifiable risk factors of HIV-CVD with the potential to effect these mechanisms.

(A3) Insomnia as an independent, clinically meaningful risk factor for CVD in the general population.

A potential, modifiable risk factor of CVD in the general population is insomnia. Insomnia symptoms include (a) dissatisfaction with sleep quantity and/or quality associated with difficulties initiating, maintaining, or returning to sleep, (b) clinically significant distress or impairment in functioning, and (c) frequency of sleep difficulties ≥3 nights/week for ≥3 months, despite the opportunity for sleep.[49,50] Current diagnostic manuals conceptualize insomnia as an independent clinical problem, regardless of its occurrence with or without co-morbid psychiatric conditions.[49,50] In support of this notion, previous research indicates that 10-54% of chronic insomnia cases occur without the presence of depressive symptoms.[51-55] Additionally, insomnia is a more consistent predictor of future depression[56,57] than depression is of future insomnia.[58-62] Taken together, this evidence challenges the common assumption that insomnia occurs solely or most often as a symptom of depression. This view is supported by recent diagnostic manual revisions that identify insomnia as an independent condition warranting clinical attention regardless of its manifestation as primary or co-morbid with other conditions.[49,50,63]

First, existing research indicates that insomnia is a predictor of CVD in the general population, independent of traditional CVD risk factors. To date, three meta-analyses have examined insomnia as a predictor of CVD events, all of which have observed significant positive associations.[11-13] The updated 2014 meta-analysis (17 prospective studies, *N*=311,260 adults) found that those with insomnia symptoms have a 55% greater risk of stroke, 28% greater risk of coronary heart disease, 41% greater risk of MI, and 33% greater risk of CVD-related mortality than those without insomnia symptoms.[12] The subsequent 2017 meta-analysis examining individual insomnia symptoms (23 studies, *N*=160,867) found that difficulty initiating sleep, difficulty maintaining sleep, and non-restorative sleep significantly predicted a 27%, 11%, and 18% increased risk of CVD events, respectively.[13] While these meta-analyses do not specifically examine the insomnia-CVD relationship independent from traditional CVD risk factors, the majority of the studies included in these meta-analyses control for traditional CVD risk factors. For instance, in the meta-analysis conducted by Li et al.[12] only three out of the seventeen included effect sizes did not control for traditional CVD risk factors. Thus, the meta-analytic findings are largely based on traditional CVD risk factor adjusted effects.

Second, sufficient evidence exists to support insomnia as a predictor of CVD in the general population, independent of depression. In the updated 2014 meta-analysis, the majority of studies controlling for depressive symptoms (four out of six) observed that significant positive relationships persisted between insomnia symptoms and CVD.[12] Furthermore, a large prospective cohort study (*N*=44,080) found that adults with insomnia disorder and without a depressive disorder were at 75% greater risk of incident acute MI and 65% greater risk of incident stroke than adults without either condition.[64]

Third, the CVD risk conferred by insomnia is clinically meaningful. To illustrate, the risk ratios from the insomnia-CVD meta-analyses (*RR*=1.28-1.55)[11-13] are approaching those of traditional CVD risk factors, such as diabetes and insufficient levels of high-density lipoprotein.[65]

(A4) The behavioral and biological plausibility of the insomnia-CVD relationship

Briefly, as the current proposal will focus on potential biological mechanisms, general population research has examined the association between insomnia symptoms and a number of behavioral factors that may potentially underlie the insomnia-CVD relationship. Research supports a significant positive association between insomnia symptoms and physical inactivity,[66,67] excessive alcohol use,[66] smoking,[68,69] and poor diet.[67] In addition, research indicates that insomnia symptoms increase the risk of incident diabetes[70] and incident hypertension,[71] which have both behavioral and biological aspects.

General population research has also examined the association between insomnia symptoms and several biological factors that could underlie the insomnia-CVD relationship, including the HIV-CVD mechanisms of inflammation, coagulation, and endothelial dysfunction. There's currently no research in the general population examining the relationship between insomnia and immune activation. With respect to inflammation, a large body of research supports a significant positive association between insomnia symptoms and inflammation. To illustrate, a meta-analysis of 72 studies involving >50,000 adults found that insomnia symptoms are associated with higher levels of IL-6 and CRP.[14] Regarding altered coagulation, less is known, but individual study data suggests a significant positive association between sleep disturbance and a number of coagulatory factors, including elevated D-dimer,[72-74] fibrinogen,[15,75-77] and von Willebrand factor.[78] Concerning endothelial dysfunction, individual study data suggests a significant inverse association between insomnia symptoms and brachial FMD.[79,80]

Going beyond associations, the strongest evidence indicating that insomnia may play a causal role in the development of CVD is provided by the largest (*N*=123) RCT of behavioral interventions for insomnia in older adults.[18] This trial found that, among adults with primary insomnia (i.e., insomnia without psychiatric co-morbidities), those treated with CBT-I had a significantly lower risk (*OR*=0.26) of elevated CRP (>3.0 mg/L), an inflammatory marker predictive of incident CVD,[19] than those treated with an educational sleep seminar at 16-months follow-up. The observed effect size was similar to that of exercise[81] and weight loss.[82] Furthermore, adults whose insomnia remitted during the trial had significantly lower CRP levels than those whose insomnia persisted at 16-months follow-up.[18] The authors concluded that CBT-I is a viable option for modifying an inflammatory marker of CVD risk.[18] An additional analysis of this RCT indicated that CBT-I, versus the educational sleep seminar, significantly reduced proinflammatory cytokine gene expression at 4-montha follow-up, suggesting a genomic mechanism through which insomnia treatment reduces inflammation.[20]

(A5) Insomnia as a prevention target for HIV-CVD

Insomnia and sleep disturbance are receiving increasing attention in people with HIV, as evidenced by two qualitative reviews and a meta-analysis.[21,83,84] The qualitative reviews reported a wide range for the occurrence of sleep disturbance (29-97%),[83,84] and the meta-analysis (27 studies) estimated the occurrence to be 58%.[21] However, all three acknowledged that the lack of insomnia assessments based on validated measures or diagnostic criteria limited their ability to make claims regarding the prevalence of insomnia. Rather, the existing literature on sleep disturbance in adults with HIV have largely utilized measures of "informal criteria," the Pittsburgh Sleep Quality Index (PSQI), and the General Sleep Disturbance Scale (GSDS). In addition, the majority of the included studies did not exclude or control for depressive symptoms.[21] As a result, the prevalence of insomnia symptoms in the HIV+ population is unknown, but the number of adults with HIV experiencing subjective sleep complaints appears to be high. Thus, should insomnia be implicated in HIV-CVD, it would be a primary CVD prevention target applicable to a large portion of the HIV+ population.

Among the HIV+ population, no published research has examined insomnia as a predictor of future CVD. However, preliminary evidence from my completed Master's thesis project suggests that highly bothersome insomnia symptoms are an independent predictor of atherosclerotic CVD among HIV+ veterans (see Approach C1 for details). In addition, initial

evidence exists indicating that sleep disturbance is cross-sectionally associated with higher levels of IL-6, CRP, and inflammation-related single nucleotide polymorphisms in people with HIV.[22-24] However, these studies failed to control for depressive symptoms and are limited by small samples sizes. No studies thus far have examined the insomnia-immune activation relationship. Likewise, no studies thus far have examined the longitudinal associations between insomnia symptoms and immune activation, systemic inflammation, altered coagulation, or endothelial dysfunction. Thus, an investigation of the relationship between insomnia and these putative mechanisms of HIV-CVD is warranted.

(A6) Scientific Premise of and Current Need for the Proposed Research

The foundation on which the proposed research is built is strong. In the general population, substantial evidence indicates that insomnia is a clinically meaningful predictor of CVD, independent of traditional CVD risk factors and depression, and that inflammation, coagulation, and endothelial dysfunction may, in part, explain the excess CVD risk in people with insomnia. In the HIV+ population, sleep disturbance is a common complaint, and preliminary evidence suggests that insomnia is independently associated with atherosclerotic CVD risk and inflammatory markers predictive of CVD. Despite this, there remains a large gap in our knowledge regarding HIV+ adults that will be addressed by the proposed projects, including the (1) cross-sectional associations between insomnia and immune activation, altered coagulation, and endothelial dysfunction, (2) the longitudinal associations between insomnia and systemic inflammation, altered coagulation, and endothelial dysfunction, and (3) the potential for insomnia to serve as a novel target for CVD primary prevention efforts.

If the proposed projects' hypotheses are supported, it would provide preliminary evidence that insomnia may be a modifiable driver of immune activation, systemic inflammation, altered coagulation, and endothelial dysfunction in people with HIV. In turn, this new knowledge would provide the mechanistic rationale for a future RCT testing whether treating insomnia in people with HIV could help to prevent the onset of clinical CVD. Ultimately, this line of research could identify novel targets for CVD primary prevention approaches in people with HIV that may lead to reduced HIV-CVD comorbidity, mortality, and associated costs.

B. Innovation

The proposed projects will be the first to examine associations of insomnia and insomnia changes over time with mechanisms thought to underlie CVD in HIV+ adults. We will leverage two unique sources of data from HIV+ participants. First, the Aim 1 project will utilize the VACS Biomarker Cohort, a large HIV+ cohort that includes VA laboratory, clinical, pharmacy, and administrative data. Second, the Aim 2 project will utilize data from a prospective RCT, which will allow us to examine associations between changes in insomnia symptoms and changes in the putative mechanisms of HIV-CVD.

C. Approach

(C1) Preliminary Data

In developing my program of research investigating psychosocial risk factors for cardiometabolic diseases in vulnerable populations, I completed a Master's thesis examining insomnia symptoms as an independent risk factor for atherosclerotic CVD in HIV+ veterans in the VACS Survey Cohort (*N*=6,148). My novel results indicated that HIV+ veterans bothered a lot by difficulty falling or staying asleep were at a 64-77% increased risk of incident CVD events (acute MI, coronary artery revascularization, or stroke over an 8-year follow-up period) than HIV+ veterans without these symptoms) – independent of demographics, CVD risk

Table 1. Characteristics of VACS Biomarker Cohort HIV+ Participants (*N*=1,542)

Age, years (median [Q1, Q3])	52.1 [46.8, 57.5]
Sex (% male)	97.3
Race/Ethnicity (% African American)	69.1
CD4 cell count, mm_3 (% ≥ 500)	34.8
HIV-1 RNA copies/mL (% < 500)	75.9
sCD14 pg/mL (median [Q1, Q3])	1718 [1448, 2085]
IL-6 pg/mL (median [Q1, Q3])	2.08 [1.42, 3.38]
D-dimer ug/mL (median [Q1, Q3])	0.26 [0.15, 0.49]

factors, HIV-specific factors, depressive symptoms, and sleep medication use (*p*s = .010-.045). The aims of this application are a direct extension of my thesis project, as it proposes to examine underlying candidate mechanisms of HIV-CVD.

Table 2. Frequencies of insomnia symptoms without and with non-benzodiazepine use in the VACS Survey Cohort (*N*=6,148)

	0	1	2	3	4
Without	2,527 (44.0)	491 (8.6)	1,149 (20.0)	832 (14.5)	743 (12.9)
With	71 (17.4)	22 (5.4)	82 (20.0)	100 (24.9)	131 (32.3)

(C2) Methods

(C2.1) *Aim 1*: To determine the associations of insomnia symptoms with circulating levels of immune activation, systemic inflammation, and coagulation markers in HIV+ adults.

Hypotheses. We hypothesize that insomnia symptoms will be positively associated with sCD14 (H1), IL-6 (H2) and D-dimer (H3).

Study Design and Sample. To test these hypotheses, we will conduct an analysis of the VACS Biomarker Cohort (*N*=1,542), an observational study consisting of a subsample of VACS participants. VACS is a prospective cohort study launched in 1998 at 8 VA Medical Centers continuously enrolling HIV+ patients seen in infectious disease clinics and age-, race-, and clinical site-matched uninfected patients seen in general clinics. The VACS Biomarker Cohort consists of participants who consented to give blood specimens at an annual exam between 2005 and 2007.[85] All HIV+ participants in the VACS Biomarker Cohort will be included in the proposed analyses (see Table 1 for sample characteristics). The VACS Biomarker Cohort utilizes data from a number of sources, including a self-report battery administered at enrollment and VA laboratory, pharmacy, administrative, and immunology case registry data.

Statistical Considerations. Independent variable. For H1-H3, we will use the insomnia symptoms item on the HIV Symptom Checklist[86] completed at enrollment. Participants were asked if they had "difficulty falling or staying asleep" during the past 4 weeks, with response options of "I do not have this symptom" (0), or "I have this symptoms and it..." "Doesn't Bother Me" (1), "Bothers Me a Little" (2), "Bothers Me" (3), or "Bothers Me a Lot" (4). Responses will be used to create a 5-level variable, which will then be used to compute four dummy-coded variables with "No Symptoms" as the reference group. This variable is strongly associated, in the expected direction, with non-benzodiazepine sleep medication (χ^2=192.93, *p*<.001; see Table 2) in the VACS Survey Cohort.

Dependent variables. The dependent variables are sCD14 (H1), IL-6 (H2), and D-dimer (H3; assessment details[87]). These variables will be analyzed as continuous variables and will be logged transformed if significant skew is observed. A recent systematic review reported that IL-6 and D-dimer are the most commonly assessed biomarkers examining CVD in HIV, with both exhibiting significant associations with future CVD events.[4]

Covariates. *Demographic Variables*: age (years), sex (0 = male, 1 = female), and race/ethnicity (White [reference], African American, other). *Biomedical and Behavioral Confounders*: clinical CVD (ICD-9 codes for acute MI, unstable angina, revascularization, stroke or transient ischemic attack, peripheral vascular disease, or heart failure),[10,88] hypertension (no hypertension [<140/90 mmHg and no antihypertensive medication - reference], controlled hypertension [<140/90 mmHg with antihypertensive medication], uncontrolled hypertension [≥140/90 mmHg]), diabetes (validated metric incorporating glucose measurements, diabetes medication use, and/or at least one inpatient or two outpatient ICD-9 codes),[89] body mass index (BMI; kg/m$_2$), smoking (never [reference], current, former), low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, statin use (prescription receipt), renal function (estimated glomerular filtration rate [eGFR]), hemoglobin, alcohol use (ICD-9 codes and Alcohol Use Disorders Identification Test [AUDIT]≥4), cocaine abuse/dependence (ICD-9 codes and self-report). *HIV-Related Factors*: HIV-1 RNA levels (<500 or ≥500 copies/mL), CD4 cell count (≥ 500 [reference], 200-499, or <200 mm$_3$), and ART use (yes/no; prescription receipt). *Depressive*

Symptoms and Medications: Patient Health Questionnaire-9 (PHQ-9) total score (without item 3), non-benzodiazepine sleep medication use (yes/no; prescription receipt), and three dichotomous antidepressant medication use variables (prescription receipt): selective serotonin reuptake inhibitor (SSRI) antidepressant use, tricyclic antidepressant use (TCA), and other antidepressant use.

Data Analysis. Prior to running hypothesis-testing models, the associations between insomnia symptoms and the covariates will be tested using *t* tests for continuous variables and χ^2 tests for categorical variables. Next, we will conduct separate linear regression analyses with the insomnia symptoms dummy variables as the predictor variables and sCD14 (H1), IL-6 (H2), and D-dimer (H3) as the outcome variables. For each hypothesis, we will run six models. *Model 1* (demographic-adjusted): age, sex, race/ethnicity; *Model 2* (fully-adjusted): Model 1 + biomedical and behavioral confounders + HIV-specific factors; *Model 3*: Model 2 + non-benzodiazepine medication use; *Model 4*: Model 2 + PHQ-9 total score (without item 3); *Model 5*: Model 2 + antidepressant medication use (SSRI, TCA, other); and *Model 6*: Model 2 + non-benzodiazepine medication use, PHQ-9 total score (without item 3), and antidepressant medication use.

Rigorous nature of the research. The Aim 1 methodology ensures a rigorous and transparent approach. First, this cohort includes a large number of HIV+ veterans, providing excellent power to detect clinically meaningful differences. Second, because of the multiple data sources, we can adjust for numerous potential confounders. Third, we propose the use of immune activation, systemic inflammation, and coagulation markers most often examined in the HIV-CVD research. This provides the ability to connect our findings to existing literature and to facilitate future meta-analytic examinations. Fourth, our hierarchical linear regression approach assesses the relationships of interest with increasing certainty that any observed associations are not due to confounders. **Anticipated Problems, Alternative Approaches, and Limitations.** We do not anticipate major problems as we have worked with the insomnia symptoms variable in my Master's thesis and others have worked with the biomarker data. The first limitation of the Aim 1 project is that results may not generalize to women, as the cohort is predominately male. The second limitation of the Aim 1 project is that the cross-sectional data cannot determine directionality of associations between insomnia and the biomarkers of interest. These limitations will be addressed in our Aim 2 project, as it will include both sexes and utilize a prospective design.

(C2.2) *Aim 2*: To determine the associations of changes in insomnia symptoms over time with changes in systemic inflammation, coagulation, and endothelial dysfunction markers over time in HIV+ adults.

Table 3. Inclusion and Exclusion Criteria

Inclusions
• Screening HIV-1 RNA level <75 copies/mL while on ART for ≥1 year
• Screening PHQ-9 depression score ≥10
Exclusions
• History of clinical CVD
• Treatment for malignancy in last 6 months
• Uncontrolled diabetes (HbA1c >8.0%) or newly diagnosed glucose intolerance (glucose ≥140 mg/dL)
• Uncontrolled hypertension (>160/110 mmHg)
• Screening eGFR <60mL/min/1.73$_2$
• Screening total cholesterol >240 mg/dL
• Pro-inflammatory conditions besides HIV infection (hepatitis B or C co-infection is allowed)

Hypotheses. As is displayed in Figure 1, we hypothesize that 0 to 12-week changes in insomnia symptoms will be positively associated with 0 to 12 week changes in IL-6, CRP, D-dimer, and brachial FMD (H3). We also hypothesize that 0 to 12-week changes in insomnia symptoms will positively predict 12 to 24 week changes in IL-6, CRP, D-dimer, and brachial FMD (H4).

Study Design and Sample. We will use data from a recently completed 24-week RCT (R01 HL126557; co-PIs: Gupta, Stewart, and Freiberg) comparing computerized cognitive-behavioral therapy (CBT) for depression to usual care. 51 HIV+, ART-treated, virologically

suppressed adults with a positive depression screen were randomized in a 1:1 fashion (see Table 3 for inclusion and exclusion criteria). The primary outcome is brachial FMD, and the secondary outcomes are IL-6, CRP, and D-dimer. The 0 to 12-week period is the treatment phase, and the 12 to 24-week period is the follow-up phase. The schedule of events is as follows: screening visit, two entry visits, computerized CBT (intervention arm only), 12-week post-treatment visit, and 24-week follow-up visit. Once eligibility was established at the screening visit, participants completed two entry visits, the second of which randomized participants. Relevant to the proposed project, insomnia symptoms, inflammation and coagulation biomarkers, and brachial FMD were assessed at both entry visits, the 12-week visit, and the 24-week visit. The CTSI's Imaging Core, directed by Dr. Gupta, performed to brachial FMD as outlined in consensus guideline recommendations,[90] as demonstrated by our previous peer-reviewed publications.[91-95] The RCT's recruitment end date was 08/31/2018. The scoring of questionnaires and FMD ultrasound scans is ongoing, and standard assays for the inflammation and coagulation biomarkers will be completed under the direction of Dr. Russell Tracy at the University of Vermont shortly following the completion of recruitment. Thus, the RCT data for the Aim 2 project will be available fall of 2018.

Statistical Considerations. Independent variables. The independent variable for H3 and H4 will be the 0 to 12-week change score for the PSQI global score (12-week PSQI adjusted for 0-Week PSQI). This variable will reflect treatment phase changes in insomnia symptoms. The PSQI is a 19-item scale that yields a global score and seven component scores (sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbance, use of sleeping medications, and daytime dysfunction), with research supporting its reliability and validity.[96] A meta-analysis reported that the PSQI has strong associations with the Insomnia Severity Index (r=0.80) and sleep efficiency scores from sleep diaries (r = -0.76), and nonsignificant to small associations with the Apnea Hypopnea Index (r=0.11-0.30) and number of oxygen desaturation events (r=0.21).[97] Moreover, the PSQI measures core symptoms of insomnia disorder as defined by the DSM-5[50]: dissatisfaction with sleep quality (sleep quality), difficulty initiating sleep (sleep latency), difficulty sustaining sleep (sleep duration, habitual sleep efficiency, sleep disturbance), and significant distress or impairment (daytime dysfunction).

Dependent variables. The dependent variables for H3 are the 0 to 12-week change scores for IL-6, CRP, D-dimer, or brachial FMD (12-week marker value adjusted for baseline value). These variables will reflect treatment phase changes in each marker. The dependent variables for H4 are the 12 to 24-week change scores for the four mechanism markers (24-week marker value adjusted for 12-week value), which will reflect follow-up phase changes in each marker.

Covariates. Models will include covariates with the potential to confound associations between changes in insomnia symptoms and changes in the markers under investigation. Models will be adjusted for the following baseline factors: age (years), sex (0=male, 1=female), race/ethnicity (0=White, 1=non-White), Framingham general cardiovascular risk score (FRS; continuous),[98] depressive symptoms (Hopkins Symptom Checklist-20 [SCL-20] without items 12, 16, and 17), and randomization status (0=usual care, 1=intervention). A sensitivity analysis will further adjust for changes in depressive symptoms. The FRS was chosen because recent evidence suggests that this tool most accurately captures risk of CVD events among HIV+ adults, while the ACC/AHA Pooled Cohort equations (PCEs), the Systematic COronary Risk Evaluation (SCORE), and the Data Collection on Adverse Effects of Anti-HIV Drugs (D:A:D) study equation.[99]

Data analysis. We will conduct a series of measured-variable path analyses using Mplus. For each dependent variable, two separate analyses will be performed. The first analysis will test H3 and the second will test H4, each adjusting for the baseline factors detailed above. A subsequent sensitivity analyses will be performed with the addition of changes in depressive symptoms. As is shown in Figure 1, the structural paths between 0 to 12-week changes in insomnia symptoms and 0 to 12-week changes in mechanisms markers will be the tests of H3.

The structural paths between 0 to 12-week changes in insomnia symptoms and 12 to 24-week changes in mechanism markers will be the tests of H4. If these coefficients of these paths are significant and positive, it would indicate that as insomnia symptoms decrease so, too, do levels of candidate mechanisms underlying HIV-CVD. Although both sets of analyses will provide useful knowledge, the tests of H4 will provide a stronger test, as the insomnia changes precede, and thus cannot be influenced, by changes in the markers of interest.

Path analysis is a particularly good fit, as it will allow us to simultaneously create the insomnia and marker change variables and use them as independent and dependent variables to test H3 and H4. To assess model fit, we will use two goodness-of-fit statistics.[100] The χ^2 statistic measures the absolute fit between the hypothesized model and the observed pattern of relationships among measured variables. Because a nonsignificant χ^2 statistic demonstrates that there is no difference between the hypothesized and observed patterns of relationships, it indicates that the hypothesized model is acceptable. The root mean square error of approximation (RMSEA) statistic adjusts the estimate of absolute fit for the complexity of the hypothesized model. Smaller values of RMSEA indicate better model fit, with values less than 0.06 representing acceptable model fit.[101] Parameters will be estimated by full information maximum likelihood, which uses all of the observed data and is superior to traditional methods of handling missing data.[102]

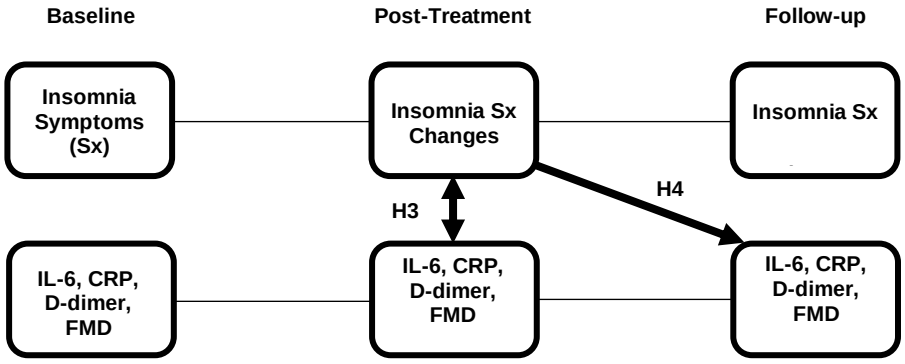
Rigorous nature of the research. The Aim 2 methodology ensures a rigorous and transparent approach. First, the prospective nature of the data enables an examination of the directionality of associations between insomnia and putative mechanisms of HIV-CVD. Second, the sample cohort is well characterized and excludes potential confounders, which reduces the number of competing explanations for observed relationships. Third, the data source provides repeated measures of insomnia symptoms and multiple mechanisms of HIV-CVD. Fourth, we apply the appropriate use of measured-variable path analyses to test associations between changes in insomnia symptoms and changes in HIV-CVD candidate mechanisms over time.

Anticipated Problems, Limitations, Alternative Approaches. In the unanticipated event that our hypotheses from Aim 1 and 2 are not supported, we would change our focus to other potential mechanisms to which insomnia may contribute, such as poor health behaviors. The first limitation of the Aim 2 project is that all participants in the RCT will have screened positive for depression. Thus, we view this as a first test of these hypotheses. New data will need to be collected to test these hypotheses in a cohort not selected based on depression status, as we are not aware of any data sources with repeated assessments of insomnia and candidate mechanisms underlying HIV-CVD. The second limitation of the Aim 2 project is a small sample size.

Project Benchmarks

Aim 1	**FA 18**	**SP 19**	**SU 19**	**FA 19**	**SP 20**	**SU 20**
Prepare dataset for analysis	→					
Analyze and interpret data		→				
Present results at conference			→			
Submit manuscript for publication				→		
Aim 2	**FA 18**	**SP 19**	**SU 19**	**FA 19**	**SP 20**	**SU 20**
Assist in data collection	→	→				
Prepare dataset for analysis			→			
Analyze and interpret data				→		
Present results at conference					→	→
Submit manuscript for publication						→

Figure 1



PROTECTION OF HUMAN SUBJECTS

A. Protection of Human Participants (For Observational Study in Aim 1)

The Human Subjects Research under Aim 1 meets the definition of Human Subjects Research.

(A1) Risk to Human Subjects

Human subjects involvement, characteristics, and design. The Veterans Aging Cohort Study (VACS) Biomarker Cohort is a cross-sectional biospecimen subsample of the larger VACS 8-site study, an ongoing longitudinal, prospective multisite observational cohort of HIV+ and uninfected veterans beginning in 1999. The VACS Biomarker Cohort subsample was collected between 2005-2007 and was designed to examine the associations of HIV-related biomarkers, HIV treatment, and comorbid conditions with morbidity and mortality. Aim 1 will utilize data from the 1,542 HIV+ participants in the VACS Biomarker Cohort. The majority of the VACS Biomarker Cohort participants are men (97%) of a racial/ethnic minority (69% African American) and has one or more traditional or non-traditional cardiovascular disease risk factors.
Sources of materials. The proposed data analysis for Aim 1 will utilize previously collected data merged from a number of sources, including VACS study collected blood biospecimens and annual survey instruments, clinical and administrative data from VA sources (i.e., immunology case registry, corporate data warehouse, and pharmacy benefits management databases), and non-VA sources (i.e., Medicare, Medicaid, and National Death Index data).
Potential Risks. The risks to participants inherent to the proposed study are minimal as the proposed study will be analyzing previously collected data from the various sources listed above. The primary risk to participants is a loss of confidentiality (i.e., private patient information could become known to persons not involved in the research project). The data protection practices in place for the VACS data sources minimizes the likelihood of this.

(A2) Adequacy of Protection Against Risk

Recruitment and informed consent. The proposed study for Aim 1 will not involve active recruitment or enrollment of any new participants. All of the VACS Biomarker participants are a part of the larger VACS 8-site parent study and have consented to the use of medical records and blood biospecimens in future clinical research efforts.
Protections against risk. The potential risk of loss of confidentiality has been minimized by a number of measures. First, all data files and computer workstations are password protected. Second, data management will follow protocols established by the VACS Coordinating Center for access to VACS data and integration of additional data elements with VACS data. Specifically, an experienced VACS programmer will create a patient level analytic dataset using scrambled social security numbers that links existing VACS data (e.g., immunology case

registry, annual survey instruments), VACS blood biospecimen analysis, and other databases (e.g., Medicare/Medicaid databases). The created analytic database will be de-identified and uploaded into a SQL server database for storage, queries, manipulation, and analyses by the study analyst. Third, key linking participant identifiers, including scrambled social security numbers and participant level study data, will be kept in a password-protected file and accessed only by the VACS programmer. Fourth, all VA data will be maintained behind the VA firewall at all times and accessed via password-protected folders. Fifth, no data will be transferred electronically in order to maximize protection of the data.

(A3) Potential Benefits of the Proposed Research to Participants and Others
Due to the observational nature of the study of Aim 1, participants are unlikely to directly benefit from the proposed study. However, participants and other HIV+ patients may benefit from the scientific knowledge to be gained from this work.

(A4) Importance of the Knowledge to be Gained
There is important knowledge to be gained through the completion of Aim 1. No other studies currently exist that examine insomnia disorder in relation to inflammation and coagulation and insomnia symptoms in relation to coagulation in HIV+ individuals. Results from this Aim could suggest an independent association of insomnia symptoms with immune activation, systemic inflammation, and coagulation in a large, HIV+ cohort, prompting a need to further investigate insomnia's role in mechanisms of atherosclerotic CVD.

Again, given the risks to participants are minimal, the value of the knowledge to be gained and it's possible impact on the improvement of care for HIV+ individuals significantly outweigh potential risks. Even in the event of negative findings from the current proposal, the results of these investigations will substantially add to our current gap in knowledge regarding insomnia as a psychosocial risk factor of CVD among individuals with HIV.

(A5) Data Safety and Monitoring Plan
Although Aim 1 does not involve a clinical trial, Dr. Freiberg and the VACS study team have a data safety and monitoring plan in place. Experienced personnel from the University of Pittsburgh and VACS are responsible for the confidentiality, security, and integrity of the potentially identifiable data for the study. Access to specific data elements will be granted only after Dr. Freiberg verifies that the individual (a) has a need to access the particular data, (b) is approved to do so by IRB, (c) is fully compliant with VHA Privacy Policy, Human Subjects Protection, and Good Clinical Practices training requirements, and (d) has signed any applicable data use agreements. In the event of unauthorized access to study data or other unanticipated adverse events the IRB will be notified in a timely fashion.

To assure the protection of human subjects and to comply with federal and state laws, the University of Pittsburgh IRB and the West Haven VA (site of the VACS Coordinating Center) will review and approve all aspects of the research project prior to project initiation. All study personnel will (a) document completion of the institution-required human subjects training and Health Insurance Portability and Accountability Act (HIPPA) privacy training, and (b) abide by established policies and procedures (consistent with HIPPA Privacy and Security Rules) for the protection of personal health information. All data analyzed in the course of the proposed study will be confidential and secure.

Data center architecture, backup strategies, and physical data storage that will support the protection of the data are detailed below:
Data Center Architecture. The databases for the Coordinating Center are housed on a networked array of Dell servers, running Microsoft Server 2008 as the OS and SQL Server 2008 for database management. The servers are connected to the West Haven campus network of gigabit fiber Ethernet backbone and 100-megabit 10-base-T network switches. The network is

protected by VA firewalls and monitored for physical and remote intrusions. The network systems and data center completed a VA-mandated recertification and an inspection by the Inspector General. Security is maintained by a full-time Information Security Officer in coordination with VACT IT staff.

a. Coordinating Center personnel access the servers using Remote Desktop Connections and SAS 9.2 or higher using personal computers running Microsoft Windows SP Service Pack 3 or higher. The user desktop computers are kept current with existing VA requirements for automated updating or virus protection definition files and Microsoft Windows XP security patches. Existing VA standards for composition and frequencies of changing of passwords for network logins are followed.
b. The SQL database server resides in a secure server room in the sub-ground level of Building 1 at the VA Connecticut Healthcare System's West Haven campus. The server room is fully environmentally controlled and is manned and access-controlled 365 days/year, 24 hours/day. It is also guarded by combination lock and video surveillance systems. The server power is protected by a facility-based uninterruptible power supply with a minimum of 6 hours of runtime, with automated clean shutdown capabilities. In addition, the power to the entire server room is backed up with diesel generator, which powers critical areas of the campus in the event of power failure. The room is further protected by inert gas flooding in the event of a fire.

Backup Strategy. Backups of data will be to a mirror database on Dell PowerEdge servers housed in the protected, secure computer room at the VA Connecticut Healthcare System's West Haven Campus. To protect subject and data privacy, all VA-mandated security procedures will be maintained on the mirror server as well. Mirroring operations will be performed in concert with operations on the main server, as network bandwidth allows. In the event of failure of or lack of electrical power or physical access to the main server, operations can continue using the mirror server.

Physical Data Storage. The physical data storage mechanism will be a combination of Direct Attached Storage (DAS) and Network Area Storage (NAS). Both DAS and NAS can be configured to provide highly redundant and fault-tolerant storage. The study data will be organized into a redundant series of time delineated 'Builds'. Each Build will be stored on several storage devices as backup. These Data Center Builds will be placed on the aforementioned DAS and NAS devices, each operating under RAID 5 protocols. All hardcopy cohort data received and analysis datasets used will be archived in accordance with NIH requirements and HIPAA standards.

B. Protection of Human Subjects (For RCT Study in Aim 2)

The Human Subjects Research under Aim 2 meets the definition of a phase II clinical trial. The parent R01 (R01HL126557) clinical trial is currently registered in ClinicalTrials.gov (Identifier: NCT02309372).

(B1) Risks to Human Subjects

Human subjects involvement, characteristics, and design. The RCT randomized 51 HIV+ virologically-suppressed (HIV-1 RNA <75 copies/mL), depressed (Patient Health Questionnaire score ≥10) adults. Specific inclusion and exclusion criteria for the RCT are listed below. There were no exclusions based on ethnicity, race, sex, or CD4 cell count. The Indiana University Institutional Review Board has approved this study. Participants were recruited from the HIV outpatient clinics of Indiana University
Health-University Hospital, Indiana University Health-Methodist Hospital, Eskenazi Health Hospital, and the Roudebush VA Medical Center. All study assessment visits were conducted in the Indiana University Health-University Hospital's Clinical Research Center. For participants randomized to the intervention arm, the computerized cognitive behavioral therapy was

administered at a private location chosen by the participant that allows Internet access (e.g., from participant's home, from participant's family/friend's home, Dr. Stewart's laboratory offices).

Inclusions
• ≥18 years of age • Documented HIV infection • Screening HIV-1 RNA level <75 copies/mL while on ART for ≥1 year • Screening PHQ-9 score ≥10
Exclusions
• Current CVD or congestive heart failure • Treatment for malignancy (besides localized skin cancers) within 6 months of screening • Uncontrolled diabetes (defined as screening Hgb A1c >8.0%) or newly diagnosed glucose intolerance (defined as screening glucose ≥140 mg/dL) • Uncontrolled hypertension (systolic BP >160 mmHg or diastolic BP >110 mmHg) • Screening eGFR <60mL/min/1.73$_2$ (using the 2009 CKD-EPI creatinine formula) • Screening total cholesterol >240 mg/dL • Pro-inflammatory conditions besides HIV infection (e.g. autoimmune diseases, but allowing hepatitis B or C co-infection)

Sources of materials. Data was obtained through existing medical records, blood samples, urine samples, ultrasound images, and self-report questionnaires. Medical records were reviewed manually for demographics, medical diagnoses, and medications by Dr. Gupta. Blood samples were obtained via peripheral venipuncture for testing of chemistries, cell counts, HIV-1 RNA levels, CD4 cell counts, inflammatory/coagulatory markers, and metabolic markers. Urine samples were obtained via standard clean-catch technique for pregnancy testing. Ultrasound images of the brachial artery were measured using high frequency ultrasound images downloaded to a secure, encrypted electronic database for testing of endothelial function. Self-report questionnaires were administered to participants for the assessment of depression, sleep quality, anxiety, anger/aggression, positive/negative affect, medication adherence, tobacco use, and physical activity. Data is stored in a password-protected computerized database via REDCap that includes only the participants' study identification number (names and other identifiable information will not be included). Only the R01 principal investigators, co-investigators, and research personnel who will directly obtain the necessary data will have access to the participant identities. All data obtained for this study was obtained only after written, informed consent is provided by each participant.

Potential risks. The risks to participants inherent to the proposed study are minimal. First, there is the possibility of loss of confidentiality. Second, there are risks associated with the blood draw/needle stick, such pain, bruising, infection, and phlebitis. Third, there are risks associated with the nitroglycerin administration as part of the brachial artery testing, including headache, transient hypotension, sensation of flushing of the skin and rash, and pain associated with the inflation of the forearm cuff as part of the brachial reactivity testing. Fourth, there is a risk that participants may feel uncomfortable completing the questionnaires. There are no known risks related to the use of the computerized cognitive behavioral therapy *Beating the Blues*. The principle alternative to these procedures would be to not participate in the research study.

(B2) Adequacy of Protection Against Risks

Recruitment and informed consent. The Indiana University Institutional Review Board has approved this study (protocol # 1409114254) Participants were recruited from the HIV outpatient clinics of Indiana University
Health-University Hospital, Indiana University Health-Methodist Hospital, Eskenazi Health Hospital, and the Roudebush VA Medical Center. If the primary provider for the patient believes a patient is eligible for the study and allows the patient to be approached for screening, one of the study investigators or a study nurse approached each potential participant during the

patient's regularly scheduled clinic visit. If eligibility was confirmed, then the purpose, procedures, and risks and benefits of the study were discussed with the participant. Participants had ample opportunity to ask questions and to have all concerns addressed. If the participant wished to pursue screening, then written informed consent was obtained (and a copy given to the participant). All consent forms are stored in a locked file cabinet.

Protections against risk.

a) Confidentiality. In order to minimize the risk to participant's loss of confidentiality, identifying information will be removed from all participant data once it is abstracted and recorded. We will only use the random study identification number (generated when consent is provided). All hard copies of study data will be kept in a secure and locked cabinet. All electronic copies of study data will be kept in a password protected computer database (i.e., REDCap). The master linking list between the patient identifiers and the random study identification number will be kept in a separate secure and locked cabinet than the hard copy data. Identifiers will never be used in the analysis or presentation of study results.
b) Blood draws. In order to minimize the risk to participants from the blood draw/needle stick, only trained medical personnel will perform the procedure. The amount of blood to be collected falls within the safety standards for blood donation.
c) Nitroglycerin. All brachial artery reactivity testing will be performed in a controlled setting at the Indiana University Health-University Hospital's Clinical Research Center. In order to minimize the risk to participants of the nitroglycerin administration, registered CRC nurses will monitor the participant's blood pressure throughout the brachial reactivity testing. Participants with inherent resting hypotension will be excluded from the NTG-mediated flow dilation portion of the brachial reactivity testing. If the participant becomes symptomatic (e.g. dizzy, perspiring, weak or nauseated, or if the systolic blood pressure falls below 80mmHg or more then 30mmHg below their baseline systolic blood pressure, the study will be stopped immediately), the patient will be placed in Trendelenberg position. If the blood pressure does not recover spontaneously, fluids will be given through the previously placed IV heplock. If the patient develops a headache, an analgesic will be recommended. In addition, participants with recent use of phosphodiesterase inhibitors (e.g. erectile dysfunction medications) prior to NTG administration or planned use after NTG administration will be excluded with the study visit to be rescheduled.
d) Questionnaires. In order to minimize the risk to participants as a result of becoming uncomfortable during questionnaire administration, participants will complete questionnaires in a private setting. In addition, a trained study team psychology personnel will be available to address any questions regarding completion of the questionnaires.
e) Suicidality. Although not an inherent risk to participation in the study, eligible participants have a probable depressive disorder as indicated from completion of the PHQ-9. As such, these participants may report suicidal ideation at screening or develop suicidal ideation during the course of the study. We have already put into place a protection protocol for just this event in our previous studies in HIV-uninfected depressed patients. If a participant reports having thoughts of being better off dead or of hurting him/herself (i.e., responds with a 1, 2, or 3 to PHQ-9 Item #9 or spontaneously reports suicidal ideation), the study visit will stop and the Suicidal Ideation Protection Protocol of the parent RCT will be initiated. This protocol requires the interviewer to complete the Patient Suicidality Form, which asks the participant if s/he (1) has a suicide plan, (2) has been struggling against thoughts of suicide, and (3) has attempted suicide in the past. If the participant answers "no" to all three suicide questions, the visit will proceed as normal and the completed Patient Suicidality Form will be given to the principal

investigator. If the participant answers “yes” to any of the three questions, the research team member will escort the participant to the Crisis Intervention Unit at the Eskenazi Health Hospital Emergency Department (located one block away from the Indiana Clinical Research Center) for a formal mental health evaluation. Participants who require a formal mental health evaluation will be withdrawn from the study. If an enrolled participant reports having thoughts of being better off dead or of hurting him/herself during any telephone calls (e.g., a scheduling call or a call to the study team initiated by the participant), the participant will be given three local crisis intervention numbers and strongly encouraged to call.

(B3) Potential Benefits of the Proposed Research to Participants and Others

There are a number of potential benefits to participants in the clinical trial we propose to use. First, participants will receive an evaluation of their cardiovascular and immunologic status. Second, participants randomized to the intervention arm may derive short-term benefits from the *Beating the Blues* depression treatment program, although this is not guaranteed. Third, participants may benefit from knowing that their participation will accrue potentially beneficial knowledge to other HIV+ patients.

The standard of care for the usual care arm will remain unaltered and the *Beating the Blues* depression treatment program used in the intervention arm is safe and has the potential to provide clinical benefits. In addition, the impact of the potential risks on participants has been minimized. Thus, the ancillary benefits to the participants in the clinical trial significantly outweigh the minimal risks in this study.

(B4) Importance of the Knowledge to be Gained

There is important knowledge to be gained through the completion of Aim 2. No studies currently exist that examine changes in sleep quality and changes in inflammation, coagulation, and FMD in HIV+ individuals. Results from this study could suggest that insomnia is a potential novel target for future efforts to reduce CVD in HIV. In fact, positive results from the proposed research project would serve as preliminary evidence that insomnia may be a causal and modifiable driver of inflammation, coagulation, and endothelial dysfunction in people with HIV and, thus, would support the mechanistic rationale for a future RCT testing whether treating insomnia in people with HIV helps to prevent clinical CVD onset.

Again, given the risks to participants are minimal, the value of the knowledge to be gained and it’s possible impact on the improvement of care for HIV+ individuals significantly outweigh potential risks. Even in the event of negative findings from the current proposal, the results of these investigations will substantially add to our current gap in knowledge regarding insomnia as psychosocial risk factor of CVD among individuals with HIV.

(B5) Data Safety and Monitoring Plan

Due to the active R01’s phase II clinical trial status, a formal Data and Safety Monitoring Board is not required; however, a data and safety monitoring plan (DSMP) has been implemented. The DSMP consists of study progress reports every 6 months monitoring data security, participant enrollment, protocol deviations, and all serious adverse events (SAE). The progress report is reviewed by a panel including the R01 PI’s (Drs. Gupta, Stewart, and Freiberg), the study biostatistician (Dr. Ziyue Liu; Department of Biostatistics, Indiana University School of Medicine and School of Public Health), and an expert investigator at Indiana University not directly connected with the study (Dr. Allon Friedman; Division of Nephrology, Indiana University School of Medicine). Any study participants prematurely discontinued due to a SAE will be reviewed immediately. Standard procedures for reporting study protocol deviations will be implemented for Indiana University Clinical Research Center, IRB, and NHLBI. SAEs will be reported to the IRB within 30 business days and subsequently forwarded to the NHLBI as required.

INCLUSION OF CHILDREN

The VACS Biomarker Cohort being utilized in Aim 1 does not include individuals under the age of 18. Those under the age of 18 were excluded from participation in VACS data collection as recruitment targeted active duty or veteran status military employees utilizing Veterans Affair (VA) services, which only includes those over the age of 18.

The active RCT utilized in Aim 2 will not include individuals under the age of 18. Individuals under the age of 18 are excluded on the basis of potential confounding of hormonal fluctuations during adolescence on the proposed endpoints of inflammation, coagulation, and endothelial function.

INCLUSION OF WOMEN AND MINORITIES

The epidemiologic study proposed in Aim 1 and the RCT proposed for use in Aim 2 do not have exclusion criteria based on gender, race, or ethnicity.

Based on the R01's (R01HL126557) co-investigators cumulative experience and the HIV+ population cared for at the proposed recruitment sites (i.e., Indiana University Health-University Hospital, Indiana University Health-Methodist Hospital, Eskenazi Health Hospital, and the Roudebush VA Medical Center), the study subjects are anticipated to be approximately 25% women, 40% African American, and 10% Hispanic. American Indians, Alaskan Natives, Asians, and Native Hawaiians or Other Pacific Islanders are not expected to be represented in the clinical trial due to extremely low representation of these groups within the Indiana University health Hospitals HIV outpatient clinics and the general Indianapolis area.

REFERENCES

1. Palella FJ, Jr., Baker RK, Moorman AC, et al. Mortality in the highly active antiretroviral therapy era: changing causes of death and disease in the HIV outpatient study. *Journal of acquired immune deficiency syndromes (1999).* 2006;43(1):27-34.
2. Trickey A, May MT, Vehreschild J, et al. Cause-Specific Mortality in HIV-Positive Patients Who Survived Ten Years after Starting Antiretroviral Therapy. *PloS one.* 2016;11(8):e0160460.
3. Rodger AJ, Lodwick R, Schechter M, et al. Mortality in well controlled HIV in the continuous antiretroviral therapy arms of the SMART and ESPRIT trials compared with the general population. *AIDS (London, England).* 2013;27(6):973-979.
4. Islam FM, Wu J, Jansson J, Wilson DP. Relative risk of cardiovascular disease among people living with HIV: a systematic review and meta-analysis. *HIV Med.* 2012;13(8):453-468.
5. Beltran LM, Rubio-Navarro A, Amaro-Villalobos JM, Egido J, Garcia-Puig J, Moreno JA. Influence of immune activation and inflammatory response on cardiovascular risk associated with the human immunodeficiency virus. *Vascular health and risk management.* 2015;11:35-48.
6. Wolf K, Tsakiris DA, Weber R, Erb P, Battegay M. Antiretroviral therapy reduces markers of endothelial and coagulation activation in patients infected with human immunodeficiency virus type 1. *The Journal of infectious diseases.* 2002;185(4):456-462.
7. Torriani FJ, Komarow L, Cotter BR, et al. Control of HIV viral replication is associated with rapid improvement in endothelial function sustained over 24 weeks: A5152s, a substudy of A5142. *Antiviral therapy.* 2007;12(6):L15-L15.
8. Baker JV, Neuhaus J, Duprez D, et al. Changes in inflammatory and coagulation biomarkers: a randomized comparison of immediate versus deferred antiretroviral therapy in patients with HIV infection. *Journal of acquired immune deficiency syndromes (1999).* 2011;56(1):36-43.
9. Beltran LM, Munoz Hernandez R, de Pablo Bernal RS, et al. Reduced sTWEAK and increased sCD163 levels in HIV-infected patients: modulation by antiretroviral treatment, HIV replication and HCV co-infection. *PloS one.* 2014;9(3):e90541.
10. Freiberg MS, Chang CC, Kuller LH, et al. HIV infection and the risk of acute myocardial infarction. *JAMA internal medicine.* 2013;173(8):614-622.
11. Sofi F, Cesari F, Casini A, Macchi C, Abbate R, Gensini GF. Insomnia and risk of cardiovascular disease: a meta-analysis. *Eur J Prev Cardiol.* 2014;21(1):57-64.
12. Li M, Zhang X-W, Hou W-S, Tang Z-Y. Insomnia and risk of cardiovascular disease: A meta-analysis of cohort studies. *International Journal of Cardiology.* 2014;176(3):1044-1047.
13. He Q, Zhang P, Li G, Dai H, Shi J. The association between insomnia symptoms and risk of cardio-cerebral vascular events: A meta-analysis of prospective cohort studies. *European journal of preventive cardiology.* 2017;24(10):1071-1082.
14. Irwin MR, Olmstead R, Carroll JE. Sleep Disturbance, Sleep Duration, And Inflammation: A Systematic Review And Meta-Analysis Of Cohort Studies And Experimental Sleep Deprivation. *Biological Psychiatry.* 2015(0).
15. Jackowska M, Steptoe A. Sleep and future cardiovascular risk: prospective analysis from the English Longitudinal Study of Ageing. *Sleep Med.* 2015;16(6):768-774.
16. Kohansieh M, Makaryus AN. Sleep Deficiency and Deprivation Leading to Cardiovascular Disease. *Int J Hypertens.* 2015:5.
17. Ipp H, Zemlin AE, Erasmus RT, Glashoff RH. Role of inflammation in HIV-1 disease progression and prognosis. *Critical reviews in clinical laboratory sciences.* 2014;51(2):98-111.

18. Irwin MR, Olmstead R, Carrillo C, et al. Cognitive behavioral therapy vs. Tai Chi for late life insomnia and inflammatory risk: a randomized controlled comparative efficacy trial. *Sleep.* 2014;37(9):1543-1552.
19. Helfand M, Buckley D, Fleming C, et al. U.S. Preventive Services Task Force Evidence Syntheses, formerly Systematic Evidence Reviews. *Screening for Intermediate Risk Factors for Coronary Heart Disease*. Rockville (MD): Agency for Healthcare Research and Quality (US); 2009.
20. Irwin MR, Olmstead R, Breen EC, et al. Cognitive behavioral therapy and tai chi reverse cellular and genomic markers of inflammation in late-life insomnia: a randomized controlled trial. *Biological psychiatry.* 2015;78(10):721-729.
21. Wu J, Wu H, Lu CY, Guo L, Li PS. Self-reported sleep disturbances in HIV-infected people: a meta-analysis of prevalence and moderators. *Sleep Med.* 2015;16(8):901-907.
22. Gay CL, Zak RS, Lerdal A, Pullinger CR, Aouizerat BE, Lee KA. Cytokine polymorphisms and plasma levels are associated with sleep onset insomnia in adults living with HIV/AIDS. *Brain, behavior, and immunity.* 2015;47:58-65.
23. Wirth MD, Jaggers JR, Dudgeon WD, et al. Association of Markers of Inflammation with Sleep and Physical Activity Among People Living with HIV or AIDS. *AIDS and behavior.* 2015;19(6):1098-1107.
24. Lee KA, Gay C, Pullinger CR, Hennessy MD, Zak RS, Aouizerat BE. Cytokine polymorphisms are associated with poor sleep maintenance in adults living with human immunodeficiency virus/acquired immunodeficiency syndrome. *Sleep.* 2014;37(3):453-463.
25. Khambaty T, Stewart JC, Gupta SK, et al. Depressive disorders are associated with incident acute myocardial infarction in HIV-infected adults: Veterans Aging Cohort Study. *JAMA cardiology.* 2016.
26. White JR, Chang CC, So-Armah KA, et al. Depression and human immunodeficiency virus infection are risk factors for incident heart failure among veterans: Veterans Aging Cohort Study. *Circulation.* 2015;132(17):1630-1638.
27. Vos AG, Idris NS, Barth RE, Klipstein-Grobusch K, Grobbee DE. Pro-Inflammatory Markers in Relation to Cardiovascular Disease in HIV Infection. A Systematic Review. *PloS one.* 2016;11(1):e0147484.
28. Hileman CO, Longenecker CT, Carman TL, et al. Elevated D-dimer is independently associated with endothelial dysfunction: a cross-sectional study in HIV-infected adults on antiretroviral therapy. *Antiviral therapy.* 2012;17(7):1345-1349.
29. Ras RT, Streppel MT, Draijer R, Zock PL. Flow-mediated dilation and cardiovascular risk prediction: a systematic review with meta-analysis. *Int J Cardiol.* 2013;168(1):344-351.
30. Lang S, Mary-Krause M, Simon A, et al. HIV Replication and Immune Status Are Independent Predictors of the Risk of Myocardial Infarction in HIV-Infected Individuals. *Clin Infect Dis.* 2012;55(4):600-607.
31. Sico JJ, Chang CC, So-Armah K, et al. HIV status and the risk of ischemic stroke among men. *Neurology.* 2015;84(19):1933-1940.
32. Triant VA, Regan S, Lee H, Sax PE, Meigs JB, Grinspoon SK. Association of immunologic and virologic factors with myocardial infarction rates in a US healthcare system. *Journal of acquired immune deficiency syndromes (1999).* 2010;55(5):615-619.
33. Friis-Moller N, Reiss P, Sabin CA, et al. Class of antiretroviral drugs and the risk of myocardial infarction. *The New England journal of medicine.* 2007;356(17):1723-1735.
34. Friis-Moller N, Sabin CA, Weber R, et al. Combination antiretroviral therapy and the risk of myocardial infarction. *The New England journal of medicine.* 2003;349(21):1993-2003.

35. Currier JS, Lundgren JD, Carr A, et al. Epidemiological evidence for cardiovascular disease in HIV-infected patients and relationship to highly active antiretroviral therapy. *Circulation.* 2008;118(2):e29-35.
36. George E, Lucas GM, Nadkarni GN, Fine DM, Moore R, Atta MG. Kidney function and the risk of cardiovascular events in HIV-1-infected patients. *AIDS (London, England).* 2010;24(3):387-394.
37. Osibogun O, Ogunmoroti O, Michos ED, et al. HIV/HCV coinfection and the risk of cardiovascular disease: A meta-analysis. *Journal of viral hepatitis.* 2017;24(11):998-1004.
38. Paisible AL, Chang CC, So-Armah KA, et al. HIV Infection, Cardiovascular Disease Risk Factor Profile, and Risk for Acute Myocardial Infarction. *Journal of acquired immune deficiency syndromes (1999).* 2015;68(2):209-216.
39. Triant VA, Grinspoon SK. Epidemiology of ischemic heart disease in HIV. *Current opinion in HIV and AIDS.* 2017.
40. Lacson JC, Barnes RP, Bahrami H. Coronary Artery Disease in HIV-Infected Patients: Downside of Living Longer. *Current atherosclerosis reports.* 2017;19(4):18.
41. Nou E, Lo J, Grinspoon SK. Inflammation, immune activation, and cardiovascular disease in HIV. *AIDS (London, England).* 2016;30(10):1495-1509.
42. Longenecker CT, Jiang Y, Orringer CE, et al. Soluble CD14 is independently associated with coronary calcification and extent of subclinical vascular disease in treated HIV infection. *AIDS (London, England).* 2014;28(7):969-977.
43. Kaplan RC, Sinclair E, Landay AL, et al. T cell activation and senescence predict subclinical carotid artery disease in HIV-infected women. *The Journal of infectious diseases.* 2011;203(4):452-463.
44. Modena MG, Bonetti L, Coppi F, Bursi F, Rossi R. Prognostic role of reversible endothelial dysfunction in hypertensive postmenopausal women. *Journal of the American College of Cardiology.* 2002;40(3):505-510.
45. Suessenbacher A, Frick M, Alber HF, Barbieri V, Pachinger O, Weidinger F. Association of improvement of brachial artery flow-mediated vasodilation with cardiovascular events. *Vascular medicine (London, England).* 2006;11(4):239-244.
46. Kitta Y, Obata JE, Nakamura T, et al. Persistent impairment of endothelial vasomotor function has a negative impact on outcome in patients with coronary artery disease. *Journal of the American College of Cardiology.* 2009;53(4):323-330.
47. Kulpa DA, Chomont N. HIV persistence in the setting of antiretroviral therapy: when, where and how does HIV hide? *Journal of virus eradication.* 2015;1(2):59-66.
48. Ross AC, Rizk N, O'Riordan MA, et al. Relationship between inflammatory markers, endothelial activation markers, and carotid intima-media thickness in HIV-infected patients receiving antiretroviral therapy. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2009;49(7):1119-1127.
49. American Academy of Sleep Medicine. *The International Classification of Sleep Disorders, (ICSD-3).* Westchester, IL: American Academy of Sleep Medicine; 2014.
50. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5).* 5 ed. Arlington, VA: American Psychiatric Association; 2013.
51. Ford DE, Kamerow DB. Epidemiologic study of sleep disturbances and psychiatric disorders. An opportunity for prevention? *Jama.* 1989;262(11):1479-1484.
52. Vollrath M, Wicki W, Angst J. The Zurich study. VIII. Insomnia: association with depression, anxiety, somatic syndromes, and course of insomnia. *European archives of psychiatry and neurological sciences.* 1989;239(2):113-124.
53. Zorick FJ, Roth T, Hartze KM, Piccione PM, Stepanski EJ. Evaluation and diagnosis of persistent insomnia. *The American journal of psychiatry.* 1981;138(6):769-773.

54. Morin CM, Stone J, McDonald K, Jones S. Psychological management of insomnia: A clinical replication series with 100 patients. *Behavior Therapy.* 1994;25(2):291-309.
55. Buysse DJ, Reynolds CF, 3rd, Hauri PJ, et al. Diagnostic concordance for DSM-IV sleep disorders: a report from the APA/NIMH DSM-IV field trial. *The American journal of psychiatry.* 1994;151(9):1351-1360.
56. Baglioni C, Battagliese G, Feige B, et al. Insomnia as a predictor of depression: A meta-analytic evaluation of longitudinal epidemiological studies. *Journal of affective disorders.* 2011;135(1):10-19.
57. Li L, Wu C, Gan Y, Qu X, Lu Z. Insomnia and the risk of depression: a meta-analysis of prospective cohort studies. *BMC psychiatry.* 2016;16(1):375.
58. Jansson-Fröjmark M, Lindblom K. A bidirectional relationship between anxiety and depression, and insomnia? A prospective study in the general population. *Journal of Psychosomatic Research.* 2008;64(4):443-449.
59. Jansson M, Linton SJ. The role of anxiety and depression in the development of insomnia: cross-sectional and prospective analyses. *Psychology and Health.* 2006;21(3):383-397.
60. Johnson EO, Roth T, Breslau N. The association of insomnia with anxiety disorders and depression: exploration of the direction of risk. *J Psychiatr Res.* 2006;40(8):700-708.
61. Ohayon MM, Roth T. Place of chronic insomnia in the course of depressive and anxiety disorders. *J Psychiatr Res.* 2003;37(1):9-15.
62. Morphy H, Dunn KM, Lewis M, Boardman HF, Croft PR. Epidemiology of insomnia: a longitudinal study in a UK population. *Sleep.* 2007;30(3):274-280.
63. Buysse DJ. Insomnia. *Jama.* 2013;309(7):706-716.
64. Hsu CY, Chen YT, Chen MF, et al. The association between insomnia and increased future cardoiovascular events: A nationwide population-based study. *Psychosomatic Medicine.* 2015;77(7):743-751.
65. Rozanski A, Blumenthal JA, Davidson KW, Saab PG, Kubzansky L. The epidemiology, pathophysiology, and management of psychosocial risk factors in cardiac practice. *Journal of the American College of Cardiology.* 2005;45(5):637-651.
66. Haario P, Rahkonen O, Laaksonen M, Lahelma E, Lallukka T. Bidirectional associations between insomnia symptoms and unhealthy behaviours. *Journal of Sleep Research.* 2013;22(1):89-95.
67. Hoefelmann LP, Lopes AD, da Silva KS, da Silva SG, Cabral LGA, Nahas MV. Lifestyle, self-reported morbidities, and poor sleep quality among Brazilian workers. *Sleep medicine.* 2012;13(9):1198-1201.
68. Htoo A, Talwar A, Feinsilver SH, Greenberg H. Smoking and sleep disorders. *Medical Clinics of North America.* 2004;88(6):1575-+.
69. Blank M, Zhang J, Lamers F, Taylor AD, Hickie IB, Merikangas KR. Health correlates of insomnia symptoms and comorbid mental disorders in a nationally representative sample of US adolescents. *Sleep.* 2015;38(2):197-204.
70. Vgontzas AN, Calhoun S, Liao DP, Karataraki M, Pejovic S, Bixler EO. Insomnia with Objective Short Sleep Duration Is Associated With Type 2 Diabetes A population-based study. *Diabetes Care.* 2009;32(11):1980-1985.
71. Meng L, Zheng Y, Hui R. The relationship of sleep duration and insomnia to risk of hypertension incidence: a meta-analysis of prospective cohort studies. *Hypertension research : official journal of the Japanese Society of Hypertension.* 2013;36(11):985-995.
72. Mausbach BT, Ancoli-Israel S, von Kanel R, et al. Sleep disturbance, norepinephrine, and D-Dimer are all related in elderly caregivers of people with Alzheimer disease. *Sleep.* 2006;29(10):1347-1352.

73. von Kanel R, Ancoli-Israel S, Dimsdale JE, et al. Sleep and Biomarkers of Atherosclerosis in Elderly Alzheimer Caregivers and Controls. *Gerontology.* 2010;56(1):41-50.
74. von Känel R, Dimsdale JE, Ancoli-Israel S, et al. Poor Sleep Is Associated with Higher Plasma Proinflammatory Cytokine Interleukin-6 and Procoagulant Marker Fibrin D-Dimer in Older Caregivers of People with Alzheimer's Disease. *Journal of the American Geriatrics Society.* 2006;54(3):431-437.
75. Matthews KA, Zheng HY, Kravitz HM, et al. Are Inflammatory and Coagulation Biomarkers Related to Sleep Characteristics in Mid-Life Women?: Study of Women's Health Across the Nation Sleep Study. *Sleep.* 2010;33(12):1649-1655.
76. Prather AA, Epel ES, Cohen BE, Neylan TC, Whooley MA. Gender differences in the prospective associations of self-reported sleep quality with biomarkers of systemic inflammation and coagulation: Findings from the Heart and Soul Study. *J Psychiatr Res.* 2013;47(9):1228-1235.
77. Suarez EC. Self-reported symptoms of sleep disturbance and inflammation, coagulation, insulin resistance and psychosocial distress: Evidence for gender disparity. *Brain Behav Immun.* 2008;22(6):960-968.
78. Miller MA, Kandala NB, Kumari M, Marmot MG, Cappuccio FP. Relationships between sleep duration and von Willebrand factor, factor VII, and fibrinogen: Whitehall II study. *Arteriosclerosis, thrombosis, and vascular biology.* 2010;30(10):2032-2038.
79. Routledge FS, Dunbar SB, Higgins M, et al. Insomnia Symptoms Are Associated With Abnormal Endothelial Function. *The Journal of cardiovascular nursing.* 2015.
80. Cooper DC, Ziegler MG, Milic MS, et al. Endothelial function and sleep: associations of flow-mediated dilation with perceived sleep quality and rapid eye movement (REM) sleep. *J Sleep Res.* 2014;23(1):84-93.
81. Ford ES. Does exercise reduce inflammation? Physical activity and C-reactive protein among U.S. adults. *Epidemiology (Cambridge, Mass).* 2002;13(5):561-568.
82. Esposito K, Pontillo A, Di Palo C, et al. Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women: a randomized trial. *Jama.* 2003;289(14):1799-1804.
83. Reid S, Dwyer J. Insomnia in HIV infection: A systematic review of prevalence, correlates, and management. *Psychosomatic Medicine.* 2005;67(2):260-269.
84. Low Y, Goforth H, Preud'homme X, Edinger J, Krystal A. Insomnia in HIV-infected patients: pathophysiologic implications. *AIDS reviews.* 2014;16(1):3-13.
85. Justice AC, Dombrowski E, Conigliaro J, et al. Veterans Aging Cohort Study (VACS): Overview and description. *Medical care.* 2006;44(8 Suppl 2):S13-24.
86. Justice AC, Holmes W, Gifford AL, et al. Development and validation of a self-completed HIV symptom index. *Journal of clinical epidemiology.* 2001;54 Suppl 1:S77-90.
87. Armah KA, McGinnis K, Baker J, et al. HIV Status, Burden of Comorbid Disease, and Biomarkers of Inflammation, Altered Coagulation, and Monocyte Activation. *Clin Infect Dis.* 2012;55(1):126-136.
88. Armah KA, Chang CC, Baker JV, et al. Prehypertension, hypertension, and the risk of acute myocardial infarction in HIV-infected and -uninfected veterans. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2014;58(1):121-129.
89. Butt AA, Fultz SL, Kwoh CK, Kelley D, Skanderson M, Justice AC. Risk of diabetes in HIV infected veterans pre- and Post-HAART and the role of HCV coinfection. *Hepatology (Baltimore, Md).* 2004;40(1):115-119.
90. Corretti MC, Anderson TJ, Benjamin EJ, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the

International Brachial Artery Reactivity Task Force. *Journal of the American College of Cardiology.* 2002;39(2):257-265.
91. Gupta SK, Johnson RM, Mather KJ, et al. Anti-inflammatory treatment with pentoxifylline improves HIV-related endothelial dysfunction: a pilot study. *AIDS (London, England).* 2010;24(9):1377-1380.
92. Gupta SK, Johnson RM, Saha C, et al. Improvement in HIV-related endothelial dysfunction using the anti-inflammatory agent salsalate: a pilot study. *AIDS (London, England).* 2008;22(5):653-655.
93. Gupta SK, Mi D, Dube MP, et al. Pentoxifylline, inflammation, and endothelial function in HIV-infected persons: a randomized, placebo-controlled trial. *PloS one.* 2013;8(4):e60852.
94. Gupta SK, Shen C, Mather KJ, Agarwal R, Dube MP. Neither proteinuria nor albuminuria is associated with endothelial dysfunction in HIV-infected patients without diabetes or hypertension. *The Journal of infectious diseases.* 2011;204(12):1946-1950.
95. Dube MP, Shen C, Mather KJ, Waltz J, Greenwald M, Gupta SK. Relationship of body composition, metabolic status, antiretroviral use, and HIV disease factors to endothelial dysfunction in HIV-infected subjects. *AIDS research and human retroviruses.* 2010;26(8):847-854.
96. Carpenter JS, Andrykowski MA. Psychometric evaluation of the Pittsburgh Sleep Quality Index. *J Psychosom Res.* 1998;45(1):5-13.
97. Mollayeva T, Thurairajah P, Burton K, Mollayeva S, Shapiro CM, Colantonio A. The Pittsburgh sleep quality index as a screening tool for sleep dysfunction in clinical and non-clinical samples: A systematic review and meta-analysis. *Sleep Med Rev.* 2016;25:52-73.
98. D'Agostino RB, Sr., Vasan RS, Pencina MJ, et al. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. *Circulation.* 2008;117(6):743-753.
99. Thompson-Paul AM, Lichtenstein KA, Armon C, et al. Cardiovascular Disease Risk Prediction in the HIV Outpatient Study. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2016.
100. Steiger JH. Structural Model Evaluation and Modification: An Interval Estimation Approach. *Multivariate behavioral research.* 1990;25(2):173-180.
101. Hu Lt, Bentler PM. Cutoff criteria for fit indexes in covariance structure analysis: Conventional criteria versus new alternatives. *Structural Equation Modeling: A Multidisciplinary Journal.* 1999;6(1):1-55.
102. Enders CK. The Performance of the Full Information Maximum Likelihood Estimator in Multiple Regression Models with Missing Data. *Educational and Psychological Measurement.* 2001;61(5):713-740.

Hopkins Symptom Checklist-20 (SCL-20)

Below is a list of problems and complaints that people sometimes have. Please read each one carefully. After you have done so, please check one of the spaces to the right that best describes HOW MUCH THAT PROBLEM HAS BOTHERED OR DISTRESSED YOU *DURING THE PAST WEEK*, INCLUDING TODAY. Mark only one space for each problem and do not skip any.

How much were you bothered by:

	Not at all	A little bit	Moderately	Quite a bit	Extremely
1. Loss of sexual interest or pleasure	☐	☐	☐	☐	☐
2. Feeling low in energy or slowed down	☐	☐	☐	☐	☐
3. Thoughts of ending your life	☐	☐	☐	☐	☐
4. Poor appetite	☐	☐	☐	☐	☐
5. Crying easily	☐	☐	☐	☐	☐
6. Feeling of being trapped or caught	☐	☐	☐	☐	☐
7. Blaming yourself for things	☐	☐	☐	☐	☐
8. Feeling lonely	☐	☐	☐	☐	☐
9. Feeling blue	☐	☐	☐	☐	☐
10. Worrying too much about things	☐	☐	☐	☐	☐
11. Feeling no interest in things	☐	☐	☐	☐	☐
12. Trouble falling asleep	☐	☐	☐	☐	☐
13. Feeling hopeless about the future	☐	☐	☐	☐	☐
14. Thoughts of death or dying	☐	☐	☐	☐	☐
15. Overeating	☐	☐	☐	☐	☐
16. Awakening in the early morning	☐	☐	☐	☐	☐
17. Sleep that is restless or disturbed	☐	☐	☐	☐	☐
18. Feeling everything is an effort	☐	☐	☐	☐	☐
19. Feelings of worthlessness	☐	☐	☐	☐	☐
20. Feelings of guilt	☐	☐	☐	☐	☐

Pittsburgh Sleep Quality Index (PSQI)

The following questions relate to your usual sleep habits during *the past month only*. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions.

1. During the past month, what time have you usually gone to bed at night?

   BED TIME _____

2. During the past month, how long (in minutes) has it usually taken you to fall asleep each night?

   NUMBER OF MINUTES _____

3. During the past month, what time have you usually gotten up in the morning?

   GETTING UP TIME _____

4. During the past month, how many hours of actual sleep did you get at night? (This may be different than the number of hours you spent in bed.)

   HOURS OF SLEEP PER NIGHT _____

For each of the remaining questions, check the one best response. Please answer all questions.

5. During the past month, how often have you had trouble sleeping because you…

	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
a. Cannot get to sleep within 30 minutes	☐	☐	☐	☐
b. Wake up in the middle of the night or early morning	☐	☐	☐	☐
c. Have to get up to use the bathroom	☐	☐	☐	☐
d. Cannot breathe comfortably	☐	☐	☐	☐
e. Cough or snore loudly	☐	☐	☐	☐
f. Feel too cold	☐	☐	☐	☐
g. Feel too hot	☐	☐	☐	☐
h. Had bad dreams	☐	☐	☐	☐
i. Have pain	☐	☐	☐	☐
j. Other reason	☐	☐	☐	☐

6. During the past month, how would you rate your sleep quality overall?

   ☐ Very Good
   ☐ Fairly Good
   ☐ Fairly Bad
   ☐ Very Bad

7. During the past month, how often have you taken medicine (prescribed or “over the count”) to help you sleep?

☐ Not during the past month	☐ Less than once a week	☐ Once or twice a week	☐ Three or more times a week

8. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?

☐ Not during the past month	☐ Less than once a week	☐ Once or twice a week	☐ Three or more times a week

9. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?

   ☐ No problem at all
   ☐ Only a very slight problem
   ☐ Somewhat of a problem
   ☐ A very big problem

Framingham Risk Score

Risk Factor		Risk Points			
		Men		Women	
Age					
30-34		0		0	
35-39		2		2	
40-44		5		4	
45-49		6		5	
50-54		8		7	
55-59		10		8	
60-64		11		9	
65-69		12		10	
70-74		14		11	
75+		15		12	
HDL-C					
60+		-2		-2	
50-59		-1		-1	
45-49		0		0	
35-44		1		1	
<35		2		2	
Total Cholesterol					
<160		0		0	
160-199		1		1	
200-239		2		3	
240-279		3		4	
280+		4		5	
Systolic Blood Pressure (mmHG)		**Not treated**	**Treated**	**Not treated**	**Treated**
<120		-2	0	-3	-1
120-129		0	2	0	2
130-139		1	3	1	3
140-149		2	4	2	5
150-159		2	4	4	6
160+		3	5	5	7
Smoker	**No**	0		0	
	Yes	4		3	
Diabetes	**No**	0		0	
	Yes	3		4	
Total Points:					

Adapted from:
D'Agostino, RB, Vasan, RS, Pencina, MJ, et al. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. *Circulation*. 2008; 117(6): 743-53.

HIV-Depression Trial

CONSORT 2018 Flow Diagram