HIV-1, Insufficient Sleep and Vascular Endothelial Function

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I. Research Design and Methods (Be sure to include a statistical analyses plan and sample size justification as part of this section)

Subjects: Study subjects will be recruited through the Infectious Disease Group Practice (IDGP) located at the University of Colorado Hospital, which cares for approximately 1,700 HIV-1-infected individuals including more than 300 HIV-infected women. Subjects will be men and women of all races and ethnic backgrounds aged 40-75 years with documented HIV-1 infection. Subjects will be HIV-1-seropositive individuals on a stable Department of Health and Human Services approved Anti-retroviral (ART) regimen for at least 6 months, with documented virologic suppression (<50 copies HIV-1 RNA/mL) for at least 3 months. All subjects must have $CD4^+$ T cell counts $\geq$200 cells/mm^3 at the time of study entry. Importantly, in 2014, 42% of the patients cared for in the IDGP were 50 years of age of older, 85% of clinic patients had a viral <50 copies Ml and the mean clinic patient $CD4^+$ T cell count was 613 cells/mm^3. Thus, there should be ample potential study subjects in the clinic population. Subjects will be free of overt Cardiovascular disease (CVD) as assessed by: a) medical history; b) physical examination; c) electrocardiogram and BP at rest and maximal exercise; d) complete blood chemistries, lipid and lipoprotein (35), glucose, insulin and hematological evaluation. Subjects will be free of a recent (<3 months) acute infection or surgery. All candidates will be sedentary as determined from the Stanford Physical Activity Questionnaire (<35 kcal/wk; (89)) and will not have engaged in any program of regular physical activity for at least 6 months prior to the study. Habitual nightly sleep duration and quality will be determined by self-report using the Pittsburgh Sleep Quality Index questionnaire (PSQI) (14), Berlin Questionnaire for sleep apnea, Insomnia Severity Index (ISI), and Epworth Sleepiness Scale (ESS). A board certified sleep clinician will also conduct a clinical assessment of sleep disorder. Potential study candidates will stratified into 2 experimental groups: normal sleep ($\geq$ 7 hr/night) or short sleep ($\leq$ 6.5 hr/night) based on their habitual sleep behavior.

All of the women in the study will be postmenopausal and not receiving hormone replacement therapy (HRT) currently or in the preceding 3-year period. Our rationale for this approach is as follows. Firstly, estrogen and HRT use can markedly influence endothelial t-PA release (48) producing widely varying baseline levels within groups of women. Furthermore, these effects may persist for years after the cessation of use (65). It is also possible that women who receive HRT may differ constitutionally from non-users. Secondly, with respect to HRT use, the modes of application, the dosage of estrogen and whether or not it is combined with progesterone, the number of years of use (continual or intermittent), and many other factors can vary greatly among individual women, adding significant inter-subject variability to the data. Thirdly, if pre-, peri-, and postmenopausal women all were included in this cohort the widely varying circulating reproductive hormone levels (which may have profound effects on endothelial function) could introduce substantial variability into this group making it difficult to identify main effects of insufficient sleep (if present). Taken together, HRT use represents an important and avoidable potential confound in the interpretation of the results of the proposed studies. We realize that the exclusion of these groups will somewhat limit the

generalizability of our results. However, few women in the IDGP are taking HRT and therefore, this is not likely to significantly reduce ability to recruit subjects. Postmenopausal status will have been confirmed clinically by the subject's personal physician along with demonstration of plasma levels of follicle stimulating hormone (FSH) >40 IU/L (92).

Experimental Protocols

General Information for Vascular Protocols. All subjects will be studied in the supine position starting 1 to 2 hours after habitual morning wake time following an overnight fast with proper hydration (water drinking only). In addition, all protocols will be performed in the outpatient research protocol laboratories in the UC-Boulder CTRC providing the highest quality of technical and clinical support for the safe and successful performance of the proposed intra-arterial infusion protocols and drug trial. The UC-Boulder CTRC is located 22 miles northwest of the UC medical campus in Denver. Drs. Connick, DeSouza and Stauffer have conducted clinical studies on the Boulder campus for close to a decade involving HIV-1 populations from the medical campus without difficulties/problems involving subjects commuting between campuses.

Experimental Design. The proposed study design will consist of two separate phases to address the stated specific aims. Phase I is designed to assess endothelial vasodilator and fibrinolytic function in HIV-1-seropositive adults who habitually sleep more than 7 hours/night (normal sleep) and those who habitually sleep less than 6.5 hours night (short sleep) (Specific Aims 1 and 2). Phase II is designed to determine the effects of individualized targeted sleep interventions that increase sleep duration and improve sleep on endothelial vasodilator and fibrinolytic function in HIV-1 seropositive adults who habitually sleep less than 6.5 hours/night (Specific Aims 3). It is important to note that HIV-1 subjects who habitually sleep less than 6.5 hours/night who participate in the acute studies associated with Phase I will not necessarily be interested in participating in the intervention trial associated with Phase II, although they will be given the option of doing so. Subjects interested in Phase II will be consented during or at the end of Phase I.

Example timeline. Detailed information for each procedure provided in following sections.

PHASE I Vascular Studies: ~10.5 hours

Visit 1 CTRC: Time approximately 2 hours Physical Examination, Blood Chemistries and Risk Markers for Heart Disease, Blood Pressure, Treadmill Stress Test

Visit 2 CTRC: Time approximately 3.0 hours, Maximal Exercise Test**,** Body Composition, Heart Rate, Dietary Records, Physical Activity Questionnaire, Sleep Questionnaires

Ambulatory sleep monitoring: Days between Visits 2 and 3

Each subject will keep a sleep/wake log at home and wear a wrist activity and light exposure recorder (Actiwatch Spectrum, Philips Respironics, Bend, OR) between Visits 2 and 3 to assess their sleep. Subjects will be given an instruction sheet on how to use the Actiwatch. These ambulatory techniques have proven successful in examining the timing of sleep and obtaining an objective estimate of sleep duration and quality (e.g., awakenings during the night). We will use these data to examine associations between sleep and vascular outcomes.

Visit 3 CTRC: Time approximately 5.5 hours Forearm Blood Flow, Measure of Fibrinolytic Capacity

Compensation for Phase I is $125.00 per visit for a total compensation up to $375.

PHASE II Sleep Intervention: ~12.3 weeks Baseline sleep assessments

Visit 1 Sleep lab

- Days 1 - Initial sleep assessment
- Days 2-8 - Ambulatory monitoring

Visit 2 Sleep lab

- Day 8 - Arrive at the sleep laboratory in the afternoon and provide saliva samples each hour for 24 hours

Sleep health intervention

- 8 weeks

Follow-up sleep assessments

Visit 3 Sleep lab

- Days 1 – Follow-up sleep assessment (including sleep questionnaires)
- Days 2-8 Ambulatory monitoring

Visit 4 Sleep lab

- Day 8 Arrive at the sleep laboratory in the afternoon and provide saliva samples each hour for 24 hours

Follow-up vascular assessments

Visit 5 CTRC: Time approximately 3.0 hours, Maximal Exercise Test**,** Body Composition, Heart Rate, Dietary Records

Visit 6 CTRC: Time approximately 5.5 hours Forearm Blood Flow, Measure of Fibrinolytic Capacity

Compensation for Phase II is $125 for participating in each overnight sleep study (Visits 1 and 3), $125 per week of ambulatory monitoring (2 weeks x $125 each), $125 for each 24h sleep lab visit (Visits 2 and 4), and

$125 for CTRC Visits 5 and 6. Maximum compensation for participating in the sleep health component of this study is $1000.00. The 8 week sleep health intervention will be provided free of charge and there is no further compensation for the sleep health intervention component.

Specific Aims 1 and 2: ***To determine the influence of chronic insufficient sleep on endothelium-dependent nitric oxide-mediated vasodilation and endothelial t-PA release in ART-treated HIV-1-seropositive adults.***

Experimental Strategy. Endothelium-dependent, NO-mediated vasodilation and endothelial t-PA release will be determined using an isolated human forearm model (28, 109). This model has the advantages of allowing for the: a) measurement of whole foream blood flow (FBF; by plethysmography); b) manipulation (by intra-arterial infusion of vasoactive drugs) of regional blood flow, via different mechanisms, without producing potentially confounding systemic effects; (c) stimulation of local and rapid endothelial release of t-PA (by intra-arterial infusion of various vasoactive agents) without producing potential confounding systemic responses; (d) direct determination of endothelial t-PA release *in vivo* free of the confounding effects of hepatic clearance and shifts between free and complexed (with PAI-1) molecular forms; (e) the study of an intact viable vascular bed with preserved innervations, blood flow, and cell-to-cell interaction (12, 53, 54, 96); and (f) a peripheral measure of endothelial vasodilator and fibrinolytic function that correlates strongly and positively with coronary vascular function (63, 71).

Study Methods: Subjects: HIV-1-seropositive adults receiving ART (see above under "Subjects"). Plasma HIV-1 RNA measurements will be determined by RT PCR by the University of Colorado Hospital Virology Laboratory. $CD4^+$ T cell counts will be determined by flow cytometry by the University of Colorado AIDS Research Center Flow Cytometry Lab located at the University of Colorado at Denver. Preliminary Measurements: (separate day from main protocol): measures of body mass and composition will be determined by basic anthropometric techniques as well as by dual energy x-ray absorptiometry (58); graded exercise test to screen for underlying coronary artery disease; maximal oxygen consumption will be used as a measure of aerobic fitness to document the sedentary status of the subjects. It will be determined using a modified Balke incremental treadmill exercise protocol, as previously described by the P.I. (29). Daily energy expenditure will also be estimated using the Stanford Physical Activity Questionnaire (PAQ) (89). Because there is no consensus gold standard plasma marker of oxidative stress, oxidative stress will be assessed by a variety of standard measures; plasma total 8-epi-PGF_{2a} by gas chromotography (44), plasma oxLDL and MPO by enzyme immunoassay (Mercodia AB, Sweden) (112). Biomarkers of inflammation (CRP, TNF-a, IL-6, IL-18) and endothelial markers will be determined by enzyme immunoassay as previously described by Dr. DeSouza (107). Experimental Protocol: Assessing Endothelial Vasodilator and Fibrinolytic Function: The brachial artery of the non-dominant arm will be catheterized as we have previously described (47, 48, 93, 109). An intravenous catheter will then be placed in an antecubital vein of the same

arm. We (47, 48, 93, 109) and others (12, 53, 54, 96) have shown that brachial artery catheterization and intravenous catheterization in the antecubital region of the same arm is safe and well tolerated by adult human subjects. FBF at rest and in response to each vasoactive agent will be measured using venous occlusion plethysmography as previously described by the P.I. (30). Endothelium-dependent vasodilation will be assessed by changes in FBF in response to intra-arterial infusions of the endothelial agonists, acetylcholine and bradykinin. FBF responses to intra-arterial sodium nitroprusside will be used to assess endothelium-independent vasodilation. Acetylcholine will be infused at rates of 4.0, 8.0, 16.0 μg/100 mL of forearm tissue/min, bradykinin at 12.5, 25, 50 ng/100 mL of forearm tissue/min, and sodium nitroprusside at 1.0, 2.0, 4.0 μg/100 mL forearm tissue/min for 5 min at each dose. To avoid an order effect the sequence in which these agents will be administered will be randomized. To determine the contribution of NO to acetylcholine-mediated vasodilation, the acetylcholine dose-response curves will be repeated in the presence of the endothelial NO synthase inhibitor L-NMMA. After allowing 15 minutes for FBF to return to baseline, L-NMMA will be infused at 5 mg/min for 10 minutes intra-arterially. This dose of L-NMMA has been shown to safely and effectively inhibit endogenous NO production in adult humans (94). Bradykinin will not be co-infused with L-NMMA because bradykinin stimulated forearm vasodilation is largely mediated by endothelium-derived hyperpolarizing factor rather than NO (50).

To assess endothelial t-PA release, following the measurement of baseline FBF and at the end of each dose of bradykinin and sodium nitroprusside, blood will be drawn simultaneously from the brachial artery and antecubital vein of the experimental arm to measure local release of fibrinolytic factors. Bradykinin was selected to stimulate endothelial t-PA release based on its specificity and effectiveness at eliciting local and rapid endothelial t-PA release in adult humans (11). Fibrinolytic determinations in response to sodium nitroprusside are required to establish that any observed differences in local endothelial release of t-PA to bradykinin is not due to increased blood flow related shear stress (12, 53). Net endothelial release or uptake of t-PA and PAI-1 (both antigen and activity levels) at each dose of bradykinin and sodium nitroprusside will be calculated as the product of the arteriovenous concentration gradient and the infused forearm plasma flow (12, 54). Arteriovenous concentration gradients for both t-PA and PAI-1 antigen and activity for each subject (at each time point) will be determined by subtraction of the values measured in simultaneously collected venous and arterial blood samples. Forearm plasma flow will be calculated from FBF and arterial hematocrit corrected for 1% trapped plasma. Thus, net release (or uptake) will be calculated using the following equation (12, 54).

Net Release = $(C_V\text{-}C_A)$ X (FBF X [101-Hematocrit/100])

$(C_V\text{-}C_A)$ = arteriovenous concentration gradient; C_V = venous concentration; C_A= arterial concentration

Intra-Arterial Administration of Vitamin C: After allowing sufficient time (45 minutes) for FBF to return to baseline following the infusions of acetylcholine + L-NMMA, vitamin C will be infused at a constant rate (12 mg/100 mL tissue/min) for 5 minutes. This vitamin C concentration has been shown to both protect human plasma from free radical-mediated lipid peroxidation (37) and improve endothelium-dependent vasodilation and endothelial t-PA release in conditions associated with oxidative stress (79, 97, 101, 106). Vitamin C infusion will be maintained at the same rate while the bradykinin, sodium nitroprusside and acetylcholine dose-response curves are repeated in the same order as performed earlier. Net endothelial release rates of t-PA and PAI-1 (antigen and activity) will be determined at time 0 and 5 minutes of vitamin C infusion and after each dose of bradykinin and sodium nitroprusside in the presence of vitamin C. A 15-minute washout period will be provided between each dose (106).

Statistical Analysis for Specific Aims 1 and 2. For Aims 1 and 2 we will deploy standard linear regression techniques to evaluate whether chronic insufficient sleep is associated with (Aim 1) endothelium-dependent nitric oxide-mediated vasodilation and (Aim 2) vascular endothelium capacity to release tissue-type plasminogen activator. In both scenarios, the continuous vascular measures from Aim 1 and Aim 2 will be modeled as dependent variables in separate linear regression procedures. Independent variables for these models will include the primary variable of interest - insufficient sleep - as well as other important covariates including age, sex, race, BMI, CD4 count, and duration of ART. The primary test of the hypothesis will focus on the parameter estimate for insufficient sleep for both models under Aims 1 and 2.

Sample Size Calculations For Specific Aims 1 and 2. Sample sizes were determined based on data from Dr. DeSouza's laboratory and available published results of comparable condition-related (e.g. hypertension, obesity) group differences (<6.5h versus >7h habitual sleep) in acetylcholine-mediated endothelium dependent vasodilation and bradykinin-stimulated endothelial t-PA release (13, 77, 81, 93, 98, 99, 105, 106, 108, 109). Sample sizes were calculated based on 90% power at an alpha level of 0.05. This power value is equivalent to a probability of <0.10 of committing a type-II error. Power calculations yielded sample size of 28 subjects per sleep group based on effect sizes of 0.74. This sample size (N=56; n=28/group) should be sufficient to determine clinically and physiologically significant differences between groups if present. To account for an estimated 20% dropout rate, we will enroll 36 subjects.

Specific Aim 3: To determine if individualized targeted sleep interventions that increase sleep duration and improve sleep quality improve vascular endothelium-dependent nitric oxide-mediated vasodilation and endothelial tissue-type plasminogen activator release in ART-treated HIV-1-seropositive adults.

Experimental Strategy. Middle-aged and older ART-treated HIV-1-serpositive adults who completed Phase I and habitually sleep less than 6.5 hours/night will be offered to undergo 8 weeks of an individualized targeted sleep intervention and follow-up vascular assessments. *Given the heterogeneity of sleep problems in patients with HIV-1 infection resulting in habitual short nightly sleep duration, a "one size fits all" approach is not appropriate for optimization of sleep health in this population.* Thus, we have assembled a team of sleep and circadian specialists to provide an individualized targeted intervention. After a comprehensive sleep and circadian assessment of each subject, members of the investigative team will meet to create an individualized targeted treatment plan. Expert sleep clinicians will implement the plan and use established procedures to monitor compliance.

Study Design and Methods. Subjects. Middle-aged and older ART-treated HIV-1-serpositive adults who habitually sleep less than 6.5 hours/night. Baseline Sleep and Circadian Assessments. Baseline PSG assessment will occur in the Sleep and Chronobiology Laboratory to determine sleep EEG staging using a standard 8-hour sleep opportunity at the participant's preferred bedtime. The baseline PSG recording will also be used to confirm absence of sleep apnea, periodic limb movements, and other sleep disorders. PSG records will be interpreted by a board certified sleep clinician. Participants' sleep will then be monitored for a week using standardized sleep diaries, wrist actigraphy recorders with concurrent light exposure assessment, and call-ins of bed and wake times to a time stamped voice recorder. This will be followed by a second laboratory visit to assess circadian melatonin phase.

Individualized Targeted Sleep Intervention. Older ART-treated HIV-1 infected adults experience a variety of sleep problems. All sleep problems result in sleep deficiency and share features that contribute mechanistically to vascular disease. Our working hypothesis is that regardless of the specific sleep problem, improving sleep will improve vascular endothelial function. We will employ an 8-week individualized targeted sleep intervention. Individualized targeted interventions have the advantage of improving adherence, reducing attrition, and making the strategy personally meaningful. We chose an 8-week duration for the targeted sleep intervention since Cognitive Behavioral Therapy for Insomnia (CBT-I) is a 6-week long treatment and endothelial function has been shown to improve following 4-8 weeks of CPAP treatment for sleep apnea (52, 75, 103).

Participants will be provided all sleep treatments at no cost.

All subjects will be given three brochures published by the American Academy of Sleep Medicine and National Sleep Foundation: one on sleep and health, one on insomnia, and the other on sleep hygiene (Helping Yourself To a Good Night's Sleep). We will supplement this information with an individualized targeted sleep medicine approach to address individual sleep disturbance profiles. Sleep and circadian expert members of the investigative team will use results from baseline assessments and clinical assessment of sleep disorders to develop the individualized targeted

intervention. We will use evidence based sleep medicine best practice strategies and combined treatments to optimize sleep and treat sleep disorders. We will use the International Classification of Sleep Disorders 3rd Edition (ICSD-3) diagnostic criteria. The following strategies are not meant to be comprehensive of all possible treatment approaches rather details are provided for treatment of disorders most likely to be seen in our subject population.

Insomnia. We estimate that approximately 66% of patients with HIV studied will meet ICSD-3 criteria for insomnia. Insomnia will be treated using the approach of Morin et al. consisting of a 6-week course of CBT-I combined with 5-10mg zolpidem nightly (5mg females/5-10mg males per standard guidelines; taken at bedtime) and discontinuation of zolpidem at the end of the 6 week treatment (titrated reduction). We selected this approach as the addition of medication to CBT-I has been shown to result in a more rapid improvement in insomnia symptoms and since treatment outcome can be maintained long-term with learned CBT-I skills (68, 69). The CBT-I protocol will be conducted by the behavioral sleep medicine specialist in individual sessions for 60 min once a week for the first 4 weeks. The 1st stage of CBT-I will involve sleep restriction and stimulus control therapies (31, 32, 88). These therapies will strengthen homeostatic sleep drive, consolidate sleep by reducing time in bed, establish a regular sleep schedule, and curtail sleep-incompatible behaviors. The 2nd stage of CBT-I will utilize cognitive therapy to attenuate sleep-disruptive and mood-disturbing cognitions that exacerbate insomnia. Standard procedures will be used to identify and alter negative cognitive activity by recording automatic thoughts, Socratic dialog, constructive worry, and behavioral experiments (19, 73). After the first 4 weeks of treatment, telephone calls to each participant, once in each of weeks 5 and 6, will be used to discuss strategies to maintain improvements beyond the treatment period. Tapering of zolpidem treatment--Those on 10mg will be tapered to 5mg per night for one week, 5mg every other night during a second week, and then stop medication the third week. Those on 5mg per night will go to an every other night regimen for one week and then stop medication the second week. Those on existing sleep medication that are ineffectively treated will be tapered before beginning treatment. Subjects will continue practicing CBT-I until post-treatment assessments. Dr. Wright has conducted multicenter clinical trials on insomnia, as well as circadian rhythm sleep disorders that produce insomnia complaints, to provide understanding of the pathophysiology, daytime consequences, and effective treatment strategies for such sleep difficulties in young and older adults with primary insomnia (86) and patients with circadian sleep disorders (120).

Although there have been no clinical trials assessing the effectiveness of CBT-I for insomnia in adults with HIV-1, CBT-I has been shown to be effective in the treatment of insomnia in patients with other medical comorbidities. Thus we are confident that patients with HIV will also respond to CBT-I treatment for insomnia when combined with the other individualized treatment strategies.

Apnea. We estimate that <50% of participants will show clinically relevant sleep apnea. Sleep disordered breathing will be treated with standard of care practices. Because patients with HIV are expected to have comorbid conditions (e.g., hypertension, depression, cardiovascular disease), we will treat patients with five or more predominantly obstructive respiratory events (obstructive and mixed apneas, hypopneas, or respiratory effort related arousals [RERAs]) per hour of sleep during a PSG). In the absence of comorbidities, treatment is typical if there are > 15 events per hour. Participants will undergo a sleep medicine evaluation at Sleep Therapeutics to determine titration level for CPAP treatment of their apnea. Established behavioral sleep medicine and monitoring of CPAP usage (goal for a minimum of 5 h per night) will be performed to ensure adequate treatment of apnea (1, 2). CPAP adherence will be objectively monitored using Encore Pro SmartCard (Philips Respironics, Inc.) data. Study staff will contact participants twice within the first week after starting CPAP and then once every following week to ensure use and manage any problems. Alternative therapies, such a dental appliances, will be considered for those with <20 events per hour.

Insufficient Sleep Syndrome (ISS). We expect <25% of patients to be diagnosed with ISS. ISS will be treated with standard of care practices by increasing the duration of the sleep opportunity from <6.5 to 7h at a minimum. This includes instructing the participant to maintain a consistent sleep schedule.

Circadian intervention strategies to promote circadian alignment. Dr. Wright has shown that the severity of sleep onset latency problems are associated with sleep being attempted at suboptimal circadian times *(see preliminary data).* We will use circadian science and medicine principles to enhance the strength of environmental time cues and thus promote circadian alignment of the sleep schedule. Most participants will receive a version of the circadian intervention. Consistent bed and wake times. Participants will be instructed to maintain a consistent bedtime (plus/minus 30 min) and wake time (plus/minus 15 min) seven days per week (with exceptions allowed for special occasions and/or for CBT-I treatment). Maintenance of a consistent sleep schedule has been shown to stably align the internal circadian clock and sleep timing, likely through exposure to a consistent daily light-dark cycle. Maintaining a consistent wake time is most important as morning sunlight exposure is a strong time cue for synchronizing the circadian clock. Increased flexibility with bedtime improves adherence. We will review call-ins daily, download the actiwatch weekly, and provide participants weekly feedback about maintaining a consistent schedule. Light exposure. We will increase the strength of the environmental light-dark cycle to enhance circadian alignment (118). Participants will be instructed to increase exposure to sunlight throughout the day and especially in the morning (e.g., morning walk, morning coffee outside, open blinds and sit near windows when inside, go outside without sunglasses during work/school breaks). We will give participants a Sunsprite (GoodLux Technology), which provides feedback about the amount of light exposure obtained that day, to help encourage participants to obtain more sunlight.

We have been successful in modifying sleep duration using similar techniques, thus we are confident in increasing exposure to sunlight using our proposed techniques. Participants will also be instructed to reduce exposure to electrical light after sunset. Investigators will make an initial home visit to measure the light environment and instruct participants on how to reduce the intensity of artificial light exposure (dim TVs, computers and cell phones [e.g., programs such as Flux], home lights—we will provide dimmers and/or fixtures with lower intensity light bulbs to be used at night). When not at home at night, subjects will still be instructed to use light reducing methods on their light emitting technologies. We will use the actiwatch information to ensure subjects are being exposed to less light at night, and we will give feedback.

Other sleep issues. Other individual and co-morbid ICSD-3 sleep disorders and factors/physical symptoms commonly associated with HIV that may contribute to sleep disturbance (e.g., night sweats, cough, dyspnea, diarrhea, and pain) will be treated with best clinical practices. We recognize factors, such as ART medications, may contribute to sleep disruption (40, 74, 80) and will include these as covariates in our analysis.

After the 8-week targeted individualized sleep intervention, we will perform follow-up PSG sleep and circadian assessments to objectively quantify improvements in sleep duration and quality and to assess changes in circadian timing relative to sleep. Sleep assessment measures will include:

Sleep Disorder and Fatigue Questionnaires will be used to describe sleep and any changes pre- post- treatment: PSQI as an indicator of sleep disturbance; ESS as an indicator of excessive daytime sleepiness; ISI as an indicator of the clinical severity of insomnia; Berlin Questionnaire to pre-screen apnea. The cognitive battery of the NIH Toolbox will be used to assess mental processes involved in gaining knowledge and comprehension, such as thinking, knowing, remembering, judging, and problem-solving. The post sleep questionnaire is used after each PSG recorded sleep opportunity to assess subjective sleep latency, sleep quality, number of awakenings, sleep duration, and factors that may have disturbed sleep and a sleepiness rating; the Fatigue Severity Scale (FSS) with modification for HIV patients to quantify self-reported fatigue. These questionnaires have been selected for their sensitivity to sleep problems and treatment.

Ambulatory sleep, light exposure and activity monitoring. Wrist actigraphy with concurrent light exposure (Actiwatch Spectrum; Philips Respironics) will be recorded the baseline week prior to the circadian phase assessment, throughout the 8 week sleep intervention, and the week prior to follow-up circadian and vascular assessments. Participants will also maintain a daily sleep-wake log. All subjects (current and newly enrolled) will receive an instruction sheet on how to use the Actiwatch. Measuring light exposure is critical to assess adherence to circadian light exposure treatment strategies. We have successfully used these methods in many prior studies (8, 59, 117). Actigraphy is a valid and reliable method for estimating

sleep timing and total sleep time (TST). Sleep logs will be used to assess subjective sleep onset latency (SOL) and TST. Our standardized sleep log also includes questions on sleep quality, number of awakenings, daily caffeine and alcohol intake, factors that may have disturbed sleep and a morning sleepiness rating. For descriptive analyses, the average TST for baseline, treatment and follow-up sleep episodes will be calculated based on wrist actigraphy using Actiwatch v.5.57 software (1-minute epochs; medium sensitivity).

Sleep and Chronobiology Laboratory PSG Research Sleep Recording and Scoring. We will use Compumedics Inc Siesta digital sleep recorders to assess PSG data (F3xM2, C3xM2, F4xM2, C4xM1, and O2xM1 O1xM2). Sleep will be scored according to revised AASM guidelines and used to describe how the sleep treatments influenced sleep duration, architecture, arousal number and duration. Oronasal thermal sensor, nasal pressure, pulse oxymiter, thoracic and abdominal plethysmography, snoring MIC, body position, ECG, and bilateral bipolar anterior tibialis EMG recordings will be used to screen for sleep disorders (AHI >5/h, PLM >10/h). Apnea: A thermal sensor amplitude drop of $\geq$90% from baseline during sleep lasting for at least 10 sec will define apnea (inspiratory effort will distinguish between central, obstructive and mixed). A nasal pressure drop by $\geq$30% for at least 10 sec with $\geq$4% desaturation will define hypopnea (90% of duration must meet amplitude criterion). Periodic limb movements (PLMS): duration of movement 0.5-10 sec, min amplitude 8mV increase above resting EMG, min of 4 leg movements in a series; length between leg movements 5-90 sec; movements on separate legs within 5 sec will be counted as single movement. Standard precautions will be taken for biohazard exposure (e.g., wearing medical gloves when instrumenting participants; disinfecting equipment etc.)

Sleep Therapeutics - Sleep Center Test for Suspected Apnea. The same PSG measures noted above will be recorded to verify the diagnosis of sleep apnea and participants will undergo CPAP titration to determine pressure needed for treatment.

Sleep and Chronobiology Laboratory Circadian Melatonin Phase Assessment. Saliva samples will be taken every 30 min [at night] and 60 min [daytime] across 24h to measure melatonin, and we will determine the Dim Light Melatonin Onset (DLMO) and the Dim Light Melatonin Offset (DLMOff) as done in our prior research (linear interpolation of 25% of the fitted peak to trough amplitude. We will also calculate the timing of the midpoint between the DLMO and DLMOff. Light <10 lux, no food intake and stable posture 30 min prior to sampling. We will use RIA to assess melatonin in saliva (Alpco, Inc). We considered measuring additional circadian phase markers, such as core body temperature and cortisol, but decided on melatonin as it is the most precise and accurate marker of circadian clock in humans. Standard precautions will be taken for biohazard exposure.

Lastly, to minimize any potential independent influence of weight loss or changes in physical activity, body weight will be maintained during the sleep (and active

control) intervention at ±2 kg from baseline in all subjects. It is very difficult to maintain body weight any more stable than within this range. Body weight will be measured and daily physical activity (Stanford Physical Activity questionnaire) will be assessed every two weeks during the intervention. All subjects will return to the CTRC bi-weekly to meet with the CTRC's bionutritionist and exercise physiologist to monitor and identify any consistent changes in body weight outside the allowable range and changes habitual physical activity. If necessary CTRC staff will work with the subjects to make the necessary adjustments in caloric intake or activity level. *It is important to note that the P.I.s have employed this approach to successfully monitor changes in weight and physical activity* (30, 91, 93).

Statistical Analysis for Specific Aim 3. The outcome variables FBF responses to acetylcholine (in the absence and presence of L-NMMA and vitamin C), bradykinin (in the absence and presence of vitamin C) and sodium nitroprusside (in the absence and presence of vitamin C) as well t-PA and PAI-1 release in response to bradykinin and sodium nitroprusside (both in the absence and presence of vitamin C) will be measured at baseline and after 8 weeks. To accommodate the longitudinal collection these continuous outcomes will be modeled using linear mixed effects methods as implemented SAS (proc mixed; SAS Institute, Inc., Cary NC). Within-individual difference between measures before and after treatment condition (d_i) can be modeled as the primary outcome measure using standard linear regression techniques as described under Aims 1 and 2. Potential confounders such as age, sex, race, family history, BMI, nadir CD4 count, baseline CD4 count, duration of ART and HIV-1 RNA prior to ART will be considered. B-spline transformations to allow for a non-linear relationship will be considered for continuous predictors. Based on available data, literature and clinical input from co-investigators, other potential confounders may also be considered. Potential covariates will be identified using a univariate screen with entry criteria of $p < 0.25$ and non-significant covariates will be excluded in a backward stepwise approach. The primary analysis will be intention to treat, although a secondary as-treated analysis will be conducted as well. Given the design, treatment conditions and assessment protocols, we anticipate the missing data (due to subject dropout) to be at least missing at random (MAR), if not missing completely at random (MCAR). Assuming missing data that are MAR or MCAR, a "complete case analysis" would yield valid inference. However, this approach would come at a cost of reducing statistical efficiency. Therefore, we will also employ Schafer's multivariate normal imputation approach as implemented in SAS v9.3 (SAS Institute Inc.; *proc MI* - MCMC Method and *proc MIANALYZE*). According to a recent simulation study, this multiple imputation approach generally performed well and "generated more reliable information than complete-case analysis" (http://www.ncbi.nlm.nih.gov/pubmed/20106935). Results from the complete-case analysis will be compared with that of the multiple imputation analysis to ensure consistency of the regression parameters.

Sample Size Calculations for Specific Aim 3. Due to the lack of published data regarding the effects of enhancing sleep duration and sleep quality on vascular

endothelial vasodilator and fibrinolytic function in HIV-1-seropositive adults, sample sizes for the targeted sleep intervention were determined based on data from the P.I.s laboratory in HIV-1 adults and published studies on comparable lifestyle intervention (e.g. exercise) effects on nitric oxide-mediated endothelium-dependent vasodilation and endothelial t-PA release in middle-aged and older adults (30, 93, 109). The average magnitude of change in FBF response to acetylcholine was ~30% and a change in t-PA release of ~35%. Corresponding effect sizes were 0.71-1.25. Sample sizes were calculated based on 80% power at an alpha level of 0.05. A sample size of 23 subjects is required to detect this physiologically meaningful change. To facilitate equal gender distribution a sample size of 24 subjects should be sufficient to show significant changes if present. This sample size also allows for detection of a correlation coefficient of 0.35 with greater than 80% power at an alpha level of 0.05 (95). To account for an estimated ~20% dropout rate from the intervention, we will enroll 30 subjects.

VI. References

REFERENCES

1. Aloia MS. Understanding the problem of poor CPAP adherence. *Sleep Med Rev* 15: 341-342, 2011.
2. Aloia MS, Arnedt JT, Riggs RL, Hecht J, and Borrelli B. Clinical management of poor adherence to CPAP: motivational enhancement. *Behav Sleep Med* 2: 205-222, 2004.
3. Anderson T, Gerhard M, Meredith I, Charbonneau F, Delagrange D, Creager M, Selwyn A, and Ganz P. Systemic nature of endothelial dysfunction in atherosclerosis. *Am J Cardiol* 1995: 71B-74B, 1995.
4. Anderson T, Uehata A, Gerhard M, Meredith I, Knab S, Delagrange D, Lieberman E, Ganz P, Creager M, Yeung A, and Selwyn A. Close relation of endothelial function in the human coronary and peripheral circulations. *J Am Coll cardiol* 26: 1235-1241, 1995.
5. Ayas NT, White DP, Manson JE, Stampfer MJ, Speizer FE, Malhotra A, and Hu FB. A prospective study of sleep duration and coronary heart disease in women. *Arch Intern Med* 163: 205-209, 2003.
6. Banks S, and Dinges DF. Behavioral and physiological consequences of sleep restriction. *J Clin Sleep Med* 3: 519-528, 2007.
7. Barbaro G, Fisher SD, and Lipshultz SE. Pathogenesis of HIV-associated cardiovascular complications. *Lancet Infect Dis* 1: 115-124, 2001.
8. Barger LK, Flynn-Evans EE, Kubey A, Walsh L, Ronda JM, Wang W, Wright KP, Jr., and Czeisler CA. Prevalence of sleep deficiency and use of hypnotic drugs in astronauts before, during, and after spaceflight: an observational study. *Lancet Neurol* 13: 904-912, 2014.
9. Bayon C, Ribera E, Cabrero E, Griffa L, and Burgos A. Prevalence of depressive and other central nervous system symptoms in HIV-infected patients treated with HAART in Spain. *J Int Assoc Physicians AIDS Care* 11: 321-328, 2012.
10. Brommer E. The level of extrinsic plasminogen activator (t-PA) during clotting as a determinant of the rate of fibrinolysis: inefficiency of activators added afterwards. *Thromb Res* 34: 109-115, 1984.

11. Brown N, Gainer J, Murphy L, and Vaughan D. Bradykinin stimulates tissue plasminogen activator release from human forearm vasculature through B_2 receptor-dependent, NO synthase-independent, and cylcooxygenase-independent pathway. *Circulation* 102: 2190-2196, 2000.
12. Brown N, Gainer J, Stein C, and Vaughan D. Bradykinin stimulates tissue plasminogen activator release in human vasculature. *Hypertension* 33: 1431-1435, 1999.
13. Brown NJ. Blood pressure reduction and tissue-type plasminogen activator release. *Hypertension* 47: 648-649, 2006.
14. Buysse DJ, Reynolds CF, 3rd, Monk TH, Berman SR, and Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res* 28: 193-213, 1989.
15. Cappuccio FP, Cooper D, D'Elia L, Strazzullo P, and Miller MA. Sleep duration predicts cardiovascular outcomes: a systematic review and meta-analysis of prospective studies. *Eur Heart J* 32: 1484-1492, 2011.
16. Cardillo C, Campia U, Bryant M, and Panza J. Increased activity of endogenous endothelin in patients with type II diabetes mellitus. *Circulation* 106: 1783-1787, 2002.
17. Cardillo C, Campia U, Kilcoyne C, Bryant M, and Panza J. Improved endothelium-dependent vasodilation after blockade of endothelin receptors in patients with essential hypertension. *Circulation* 105: 452-456, 2002.
18. Cardillo C, Kilcoyne C, Cannon R, and Panza J. Increased activity of endougenous endothelin in patients with hypercholesterolemia. *J Am Coll Cardiol* 36: 1483-1488, 2000.
19. Carney CE, and Edinger JD. Identifying critical beliefs about sleep in primary insomnia. *Sleep* 29: 444-453, 2006.
20. Chobanian A, Bakris G, Black H, Cushman W, Green L, Izzo J, Jones D, Materson B, Oparil S, Wright J, and Roccella E. The seventh report of the joint national committe on prevention, detection, evaluation, and treatment of high blood pressure. The JNC 7 report. *JAMA* 289: 2560-2572, 2003.
21. Cohen R. The role of nitric oxide and other endothelium-derived vasoactive substances in vascular disease. *Progress Cardiovasc Dis* 38: 105-128, 1995.
22. Control CfD. HIV/AIDS Suveillance Report. Atlanta, GA: 2003.
23. Cooke J, and Dzau V. Nitric oxide synthase: role in the genesis of vascular disease. *Annu Rev Med* 48: 489-509, 1997.
24. Cooper L, Cooke J, and Dzau V. The vasculopathy of aging. *J Gerontol Bio Sci* 49: B191-B196, 1994.
25. Crothers K, Griffith TA, McGinnis KA, Rodriguez-Barradas MC, Leaf DA, Weissman S, Gibert CL, Butt AA, and Justice AC. The impact of cigarette smoking on mortality, quality of life, and comorbid illness among HIV-positive veterans. *J Gen Intern Med* 20: 1142-1145, 2005.
26. Currier JS, Taylor A, Boyd F, Dezii CM, Kawabata H, Burtcel B, Maa JF, and Hodder S. Coronary heart disease in HIV-infected individuals. *J Acquir Immune Defic Syndr* 33: 506-512, 2003.
27. d'Arminio A, Sabin CA, Phillips AN, Reiss P, Weber R, Kirk O, El-Sadr W, De Wit S, Mateu S, Petoumenos K, Dabis F, Pradier C, Morfeldt L, Lundgren JD, and Friis-Moller N. Cardio- and cerebrovascular events in HIV-infected persons. *AIDS* 18: 1811-1817, 2004.
28. DeSouza C, Clevenger C, Greiner J, Smith D, Hoetzer G, Shapiro L, and Stauffer B. Evidence for agonist-specific endothelial vasodilator dysfunction with ageing in healhty humans. *J Physiol* 542: 255-262, 2002.

29. DeSouza C, Jones P, and Seals D. Physical activity status and age-related differences in coagulation and fibrinolytic factors in women. *Arterioscler Thromb Vasc Biol* 18: 362-368, 1998.
30. DeSouza C, Shapiro L, Clevenger C, Dinenno F, Monahan KD, Tanaka H, and Seals D. Regular aerobic exercise prevents and restores the age-related decline in endothelium-dependent vasodilation in healthy men. *Circulation* 102: 1351-1357, 2000.
31. Edinger JD, Wohlgemuth WK, Krystal AD, and Rice JR. Behavioral insomnia therapy for fibromyalgia patients: a randomized clinical trial. *Arch Intern Med* 165: 2527-2535, 2005.
32. Edinger JD, Wohlgemuth WK, Radtke RA, Marsh GR, and Quillian RE. Cognitive behavioral therapy for treatment of chronic primary insomnia: a randomized controlled trial. *JAMA* 285: 1856-1864, 2001.
33. Eijnden-Schrauwen Yvd, Kooistra T, Vries Rd, and Emeis J. Studies on the acute release of tissue-type plasminogen activator from human endothelial cells in vitro and in rats in vitro: evidence for a dynamic stoorage pool. *Blood* 85: 3510-3517, 1995.
34. Emeis J. The control of t-PA and PAI-1 secretion from the vessel wall. *Vasc Med Rev* 6: 153-166, 1995.
35. Fedder D, koro C, and L'Italien G. New national cholesterol education program III guidleines for primary prevention lipid-lowering drug therapy. Projected impact on the size, sex, and age distribution of the treatment-eligible population. *Circulation* 105: 152-156, 2002.
36. Fox K, Robinson A, Knabb R, Rosamond T, Sobel B, and Bergman S. Prevention of coronary thrombosis with subthrombolytic doses of tissue-type plasminogen activator. *Circulation* 72: 1346-1354, 1984.
37. Frei B, England L, and Ames B. Ascorbate is an outstanding antioxidant in human blood plasma. *Proc Natl Acad Sci USA* 86: 6377-6381, 1989.
38. Friis-Moller N, Weber R, Reiss P, Thiebaut R, Kirk O, d'Arminio Monforte A, Pradier C, Morfeldt L, Mateu S, Law M, El-Sadr W, De Wit S, Sabin CA, Phillips AN, and Lundgren JD. Cardiovascular disease risk factors in HIV patients--association with antiretroviral therapy. Results from the DAD study. *AIDS* 17: 1179-1193, 2003.
39. Furchgott R, and Zawadski J. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 286: 373-376, 1980.
40. Gallego L, Barreiro P, del Rio R, Gonzalez de Requena D, Rodriguez-Albarino A, Gonzalez-Lahoz J, and Soriano V. Analyzing sleep abnormalities in HIV-infected patients treated with Efavirenz. *Clin Infect Dis* 38: 430-432, 2004.
41. Gamaldo CE, Gamaldo A, Creighton J, Salas RE, Selnes OA, David PM, Mbeo G, Parker BS, Brown A, McArthur JC, and Smith MT. Evaluating sleep and cognition in HIV. *J Acquir Immune Defic Syndr* 63: 609-616, 2013.
42. Gangwisch JE, Heymsfield SB, Boden-Albala B, Buijs RM, Kreier F, Pickering TG, Rundle AG, Zammit GK, and Malaspina D. Short sleep duration as a risk factor for hypertension: analyses of the first National Health and Nutrition Examination Survey. *Hypertension* 47: 833-839, 2006.
43. Gardner LI, Klein RS, Szczech LA, Phelps RM, Tashima K, Rompalo AM, Schuman P, Sadek RF, Tong TC, Greenberg A, and Holmberg SD. Rates and risk factors for condition-specific hospitalizations in HIV-infected and uninfected women. *J Acquir Immune Defic Syndr* 34: 320-330, 2003.

44. Gopaul N, Zacharowski K, Halliwell B, and Anggard E. Evaluation of the postprandial effects of a fast-food meal on human plasma F2-isoprostane levels. *Free Rad Biol Med* 28: 806-814, 2000.
45. Grunfeld C. Insulin resistance in HIV infection: drugs, host responses, or restoration to health? *Top HIV Med* 16: 89-93, 2008.
46. Grunfeld C, Delaney JA, Wanke C, Currier JS, Scherzer R, Biggs ML, Tien PC, Shlipak MG, Sidney S, Polak JF, O'Leary D, Bacchetti P, and Kronmal RA. Preclinical atherosclerosis due to HIV infection: carotid intima-medial thickness measurements from the FRAM study. *AIDS* 23: 1841-1849, 2009.
47. Hoetzer G, Stauffer B, Greiner J, Casas Y, Smith D, and DeSouza C. Influence of oral contraceptive use on endothelial t-PA release in healthy premenopausal women. *Am J Physiol Endocrinol Metab* 284: E90-E95, 2003.
48. Hoetzer G, Stauffer B, Irmiger H, Ng M, Smith D, and DeSouza C. Acute and chronic effects of oestrogen on endothelial tissue-type plasminogen activator release in postmenopausal women. *J Physiol* 551: 721-728, 2003.
49. Hoffmeister HM, Jur M, Ruf-Lehmann M, Helber U, Heller W, and Seipel L. Endothelial tissue-type plasminogen activator release in coronary heart disease: Transient reduction in endothelial fibrinolytic reserve in patients with unstable angina pectoris or acute myocardial infarction. *J Am Coll Cardiol* 31: 547-551, 1998.
50. Honing ML, Smits P, Morrison PJ, and Rabelink TJ. Bradykinin-induced vasodilation of human forearm resistance vessels is primarily mediated by endothelium-dependent hyperpolarization. *Hypertension* 35: 1314-1318, 2000.
51. Jablonski KL, Seals DR, Eskurza I, Monahan KD, and Donato AJ. High-dose ascorbic acid infusion abolishes chronic vasoconstriction and restores resting leg blood flow in healthy older men. *J Appl Physiol* 103: 1715-1721, 2007.
52. Jelic S, Padeletti M, Kawut SM, Higgins C, Canfield SM, Onat D, Colombo PC, Basner RC, Factor P, and LeJemtel TH. Inflammation, oxidative stress, and repair capacity of the vascular endothelium in obstructive sleep apnea. *Circulation* 117: 2270-2278, 2008.
53. Jern C, Selin L, and Jern S. In Vivo release of tissue-type plasminogen activator across the human forearm during mental stress. *Thromb Haemost* 72: 285-291, 1994.
54. Jern C, Selin L, and Jern S. Release of tissue-type plasminogen activator in response to muscarinic receptor stimulation in human forearm. *Thromb Haemost* 72: 588-594, 1994.
55. Jern S, Wall U, Bergbrant A, Selin-Sjogren L, and Jern C. Endothelium-dependent vasodilation and tissue-type plasminogen activator release in borderline hypertension. *Arterioscler Thromb Vasc Biol* 17: 3376-3383, 1997.
56. Kamin DS, and Grinspoon SK. Cardiovascular disease in HIV-positive patients. *AIDS* 19: 641-652, 2005.
57. Klein D, Hurley LB, Quesenberry CP, Jr., and Sidney S. Do protease inhibitors increase the risk for coronary heart disease in patients with HIV-1 infection? *J Acquir Immune Defic Syndr* 30: 471-477, 2002.
58. Kohrt W, Malley M, Dalsky G, and Holloszy J. Body composition of healthy sedentary and trained, young and older men and women. *Med Sci Sports Exerc* 24: 832-837, 1992.
59. LeBourgeois MK, Carskadon MA, Akacem LD, Simpkin CT, Wright KP, Jr., Achermann P, and Jenni OG. Circadian phase and its relationship to nighttime sleep in toddlers. *J Biol Rhythms* 28: 322-331, 2013.

60. Lee KA, Gay C, Portillo CJ, Coggins T, Davis H, Pullinger CR, and Aouizerat BE. Types of sleep problems in adults living with HIV/AIDS. *J Clin Sleep Med* 8: 67-75, 2012.
61. Lijnen H, and Collen D. Endothelium in hemostasis and thrombosis. *Prog Cardiovasc Dis* 36: 343-350, 1997.
62. Lloyd-Jones D, and Bloch K. The vascular biology of nitric oxide and its role in atherogenesis. *Annu Rev Med* 47: 365-375, 1996.
63. Luscher T, Tanner F, Tschudi M, and Noll G. Endothelial dysfunction in coronary artery disease. *Annu Rev Med* 44: 395-418, 1993.
64. Mano T, Masuyama T, Yamamoto K, Naito J, Kondo H, Nagano R, Tanouchi J, Hori M, Inoue M, and Kamada T. Endothelial dysfunction in the early stage of atherosclerosis precedes appearance of intimal lesions assessable with intravascular ultrasound. *Am Heart J* 131: 231-238, 1996.
65. Manolio T, Furberg C, Shemanski L, Psaty B, O'Leary D, Tracy R, and Bush T. Associations of postmenopausal estrogen use with cardiovascular disease and its risk factors in older women. *Circulation* 88: 2163-2171, 1993.
66. Moore R, Keruly J, and G L. Increasing Incidence of cardiovascualr disease in HIV-infected persons in care. *Abstract Conference of Retroviruses and Opportunistic Infection, Boston, MA 2003, Abs #132* 2003.
67. Moreau KL, DePaulis AR, Gavin KM, and Seals DR. Oxidative stress contributes to chronic leg vasoconstriction in estrogen-deficient postmenopausal women. *J Appl Physiol* 102: 890-895, 2007.
68. Morin CM, Beaulieu-Bonneau S, Ivers H, Vallieres A, Guay B, Savard J, and Merette C. Speed and trajectory of changes of insomnia symptoms during acute treatment with cognitive-behavioral therapy, singly and combined with medication. *Sleep Med* 15: 701-707, 2014.
69. Morin CM, Vallieres A, Guay B, Ivers H, Savard J, Merette C, Bastien C, and Baillargeon L. Cognitive behavioral therapy, singly and combined with medication, for persistent insomnia: a randomized controlled trial. *JAMA* 301: 2005-2015, 2009.
70. Muldowney J, and Vaughan D. Tissue-type plasminogen activator release new frontiers in endothelial function. *J Am Coll Cardiol* 40: 967-969, 2002.
71. Newby D, Witherow F, McLeod A, Uren N, Prendergast B, Flint L, Webb D, Fox K, and Boon N. Correlation between acute tissue plasminogen activator release from the human coronary and peripheral circulations. *J Am Coll Cardiol* 37(Suppl. A): 232A, 2001.
72. Newby D, Wright R, Labinjoh C, Ludlam C, Fox K, Boon N, and Webb D. Endothelial dysfunction, impaired endogenous fibrinolysis, and cigarette smoking. A mechanism for arterial thrombosis and myocardial infarction. *Circulation* 99: 1411-1415, 1999.
73. Norell-Clarke A, Jansson-Frojmark M, Tillfors M, Harvey AG, and Linton SJ. Cognitive processes and their association with persistence and remission of insomnia: findings from a longitudinal study in the general population. *Behav Res Therapy* 54: 38-48, 2014.
74. Nunez M, Gonzalez de Requena D, Gallego L, Jimenez-Nacher I, Gonzalez-Lahoz J, and Soriano V. Higher efavirenz plasma levels correlate with development of insomnia. *J Acquir Immune Defic Syndr* 28: 399-400, 2001.
75. Ohike Y, Kozaki K, Iijima K, Eto M, Kojima T, Ohga E, Santa T, Imai K, Hashimoto M, Yoshizumi M, and Ouchi Y. Amelioration of vascular endothelial dysfunction in obstructive sleep apnea syndrome by nasal continuous positive

airway pressure--possible involvement of nitric oxide and asymmetric NG, NG-dimethylarginine. *Circ J* 69: 221-226, 2005.
76. Ouimet H, and Loscalzo J. Fibrinolysis. In: *Thrombosis and Hemorrhage*, edited by Loscalzo J, and Schafer A. Boston: Blackwell Scientific Publications, 1994, p. 127-143.
77. Panza J, Casino P, Kilcoyne C, and Quyyumi A. Impaired endothelium-dependent vasodilation in patients with essential hypertension: evidence that the abnormality is not at the muscarinic recptor level. *J Am Coll Cardiol* 23: 1610-1616, 1994.
78. Parmer R, Mahata M, Mahata S, Sebald M, O'Connor D, and Miles L. Tissue plasminogen activator (t-PA) is targeted to the regulated secretory pathway. *J Biol Chem* 272: 1976-1982, 1997.
79. Perticone F, Ceravolo R, Candigliota M, Ventura G, Iacopino S, Sinopoli F, and Mattioli P. Obesity and body fat distribution induce endothelial dysfunction by oxidative stress. *Diabetes* 50: 159-165, 2001.
80. Portsmouth S, Imami N, Pires A, Stebbing J, Hand J, Nelson M, Gotch F, and Gazzard BG. Treatment of primary HIV-1 infection with nonnucleoside reverse transcriptase inhibitor-based therapy is effective and well tolerated. *HIV Med* 5: 26-29, 2004.
81. Pretorius M, Rosenbaum D, Lefebvre J, Vaughan D, and Brown N. Smoking impairs bradykinin-stimulated t-PA release. *Hypertension* 39: 767-771, 2002.
82. Qureshi AI, Giles WH, Croft JB, and Bliwise DL. Habitual sleep patterns and risk for stroke and coronary heart disease: a 10-year follow-up from NHANES I. *Neurology* 48: 904-911, 1997.
83. Quyyumi A. Endothelial function in health and disease: new insights into the genesis of cardiovascular disease. *Am J Med* 105: 32S-39S, 1998.
84. Reid S, and Dwyer J. Insomnia in HIV infection: a systematic review of prevalence, correlates, and management. *Psychosom Med* 67: 260-269, 2005.
85. Robinson SD, Ludlam CA, Boon NA, and Newby DE. Endothelial fibrinolytic capacity predicts future adverse cardiovascular events in patients with coronary heart disease. *Arterioscler Thromb Vasc Biol* 27: 1651-1656, 2007.
86. Roth T, Wright KP, Jr., and Walsh J. Effect of tiagabine on sleep in elderly subjects with primary insomnia: a randomized, double-blind, placebo-controlled study. *Sleep* 29: 335-341, 2006.
87. Rubinstein ML, and Selwyn PA. High prevalence of insomnia in an outpatient population with HIV infection. *J Acquir Immune Defic Syndr Hum Retrovirol* 19: 260-265, 1998.
88. Rumble ME, Keefe FJ, Edinger JD, Porter LS, and Garst JL. A pilot study investigating the utility of the cognitive-behavioral model of insomnia in early-stage lung cancer patients. *J Pain Symptom Manage* 30: 160-169, 2005.
89. Sallis J, Haskell W, Wood P, Fortmann S, Rogers T, and Blair S. Physical activity assessment methodology in the five-city project. *Am J Epidemiol* 121: 91-106, 1985.
90. Saves M, Chene G, Ducimetiere P, Leport C, Le Moal G, Amouyel P, Arveiler D, Ruidavets JB, Reynes J, Bingham A, and Raffi F. Risk factors for coronary heart disease in patients treated for human immunodeficiency virus infection compared with the general population. *Clin Infect Dis* 37: 292-298, 2003.
91. Seals D, Tanaka H, Clevenger C, Monahan K, Reiling M, Hiatt W, Davy K, and DeSouza C. Blood pressure reductions with exercise and sodium restriction in postmenopausal women with elevated systolic blood pressure: role of arterial stiffness. *J Am Coll Cardiol* 38: 506-513, 2001.

92. Sherman B, West J, and Lorenman S. The menopause transition: analysis of LH, FSH, estradiol, and progesterone concentrations during menstrual cycles in older women. *J Clin Endocrinol Metab* 42: 629-636, 1976.
93. Smith D, Hoetzer G, Greiner J, Stauffer B, and DeSouza C. Effects of ageing and regular aerobic exercise on endothelial fibrinolytic capacity in humans. *J Physiol* 546: 289-298, 2003.
94. Smith D, Hoetzer G, Greiner J, Stauffer B, and DeSouza C. Endothelial release of tissue-type plasminogen activator in the human forearm: role of nitric oxide. *J Cardiovasc Pharmacol* 42: 311-314, 2003.
95. Steel R, and Torrie J. *Principles and Procedures of Statistics: A Biometrical Approach*. New York, NY: McGraw-Hill, iInc., 1980.
96. Stein C, Brown N, Vaughan D, Lang C, and Wood A. Regulation of local tissue-type plasminogen activator release by endothelium-dependent and endothelium-independet agonists in human vasculature. *J Am Coll Cardiol* 32: 117-122, 1998.
97. Taddei S, Virdis A, Ghiadoni L, Salvetti G, Bernini G, Magagna A, and Salvetti A. Age-related reduction of NO availability and oxidative stress in humans. *Hypertension* 38: 274-279, 2001.
98. Taddei S, Virdis A, Mattei P, Ghiadoni L, Fasolo C, Sudano I, and Salvetti A. Hypertension causes premature aging of endothelial function in humans. *Hypertension* 29: 736-743, 1997.
99. Taddei S, Virdis A, Mattei P, Ghiadoni L, Gennari A, Fasolo C, Sudano I, and Salvetti A. Aging and endothelial function in normotensive subjects and patients with essential hypertension. *Hypertension* 91: 1981-1987, 1995.
100. Taibi DM. Sleep disturbances in persons living with HIV. *J Assoc Nurses AIDS Care* 24: S72-85, 2013.
101. Ting H, Timini T, Boles K, Creager S, Ganz P, and Creager M. Vitamin C improves endothelium-dependent vasodilation in patients with non-insulin dependent diabetes mellitus. *J Clin Invest* 97: 22-28, 1996.
102. Trachiotis GD, Alexander EP, Benator D, and Gharagozloo F. Cardiac surgery in patients infected with the human immunodeficiency virus. *Ann Thorac Surg* 76: 1114-1118; discussion 1118, 2003.
103. Trzepizur W, Gagnadoux F, Abraham P, Rousseau P, Meslier N, Saumet JL, and Racineux JL. Microvascular endothelial function in obstructive sleep apnea: Impact of continuous positive airway pressure and mandibular advancement. *Sleep Med* 10: 746-752, 2009.
104. United States Department of Health and Human Services NIoAAaA. The Physicians Guide to Helping Patients with Alcohol Problems. *NIH Publication No95-3796 National Institutes of Health* 1999.
105. Van Guilder GP, Hoetzer GL, Dengel DR, Stauffer BL, and DeSouza CA. Impaired endothelium-dependent vasodilation in normotensive and normoglycemic obese adult humans. *J Cardiovasc Pharmacol* 47: 310-313, 2006.
106. Van Guilder GP, Hoetzer GL, Greiner JJ, Stauffer BL, and DeSouza CA. Acute and chronic effects of vitamin C on endothelial fibrinolytic function in overweight and obese adult humans. *J Physiol* 586: 3525-3535, 2008.
107. Van Guilder GP, Hoetzer GL, Greiner JJ, Stauffer BL, and Desouza CA. Influence of metabolic syndrome on biomarkers of oxidative stress and inflammation in obese adults. *Obesity* 14: 2127-2131, 2006.
108. Van Guilder GP, Hoetzer GL, Greiner JJ, Stauffer BL, and DeSouza CA. Metabolic syndrome and endothelial fibrinolytic capacity in obese adults. *Am J Physiol Regul Integr Comp Physiol* 294: R39-44, 2008.

109. Van Guilder GP, Hoetzer GL, Smith DT, Irmiger HM, Greiner JJ, Stauffer BL, and DeSouza CA. Endothelial t-PA release is impaired in overweight and obese adults but can be improved with regular aerobic exercise. *Am J Physiol Endocrinol Metab* 289: E807-813, 2005.
110. Vita J, Keaney J, and Loscalzo J. Endothelial dysfunction in vascular disease. In: *Vascular Medicine A Textbook of Vascular Biology and Diseases*, edited by Loscalzo J, Creager M, and Dzau V. New York: Little Brown and Company, 1996, p. 245-265.
111. Vita J, Treasure C, Nabel E, McLenachan J, Fish R, Yeung A, Vekshtein V, Selwyn A, and Ganz P. Coronary vasomotor response to acetylcholine relates to risk factors for coronary artery disease. *Circulation* 81: 491-497, 1990.
112. Vita JA, Brennan ML, Gokce N, Mann SA, Goormastic M, Shishehbor MH, Penn MS, Keaney JF, Jr., and Hazen SL. Serum myeloperoxidase levels independently predict endothelial dysfunction in humans. *Circulation* 110: 1134-1139, 2004.
113. Vittecoq D, Escaut L, Merad M, Teicher E, Monsuez JJ, and Chironi G. Coronary heart disease in HIV-infected individuals. *Adv Cardiol* 40: 151-162, 2003.
114. Webel AR, Moore SM, Hanson JE, Patel SR, Schmotzer B, and Salata RA. Improving sleep hygiene behavior in adults living with HIV/AIDS: a randomized control pilot study of the SystemCHANGE(TM)-HIV intervention. *Appl Nurs Res* 26: 85-91, 2013.
115. Weil BR, Greiner JJ, Stauffer BL, and Desouza CA. Self-reported habitual short sleep duration is associated with endothelial fibrinolytic dysfunction in men: a preliminary report. *Sleep* 36: 183-188, 2013.
116. Weil BR, Mestek ML, Westby CM, Van Guilder GP, Greiner JJ, Stauffer BL, and DeSouza CA. Short sleep duration is associated with enhanced endothelin-1 vasoconstrictor tone. *Can J Physiol Pharmacol* 88: 777-781, 2010.
117. Wright KP, Jr., and Czeisler CA. Absence of circadian phase resetting in response to bright light behind the knees. *Science* 297: 571, 2002.
118. Wright KP, Jr., McHill AW, Birks BR, Griffin BR, Rusterholz T, and Chinoy ED. Entrainment of the human circadian clock to the natural light-dark cycle. *Curr Biol* 23: 1554-1558, 2013.
119. Yasue H, Matsuyama K, Matsuyama K, Okumura K, Morikami Y, and Ogawa H. Responses of angiographically normal human coronary arteries to intracoronary injection of acetylcholine by age and segment. Possible role of early coronary atherosclerosis. *Circulation* 81: 482-490, 1990.
120. Zee PC, Wang-Weigand S, Wright KP, Jr., Peng X, and Roth T. Effects of ramelteon on insomnia symptoms induced by rapid, eastward travel. *Sleep Med* 11: 525-533, 2010.