Title of Project

Effect of Pulsatile Pressure and Long Sleep Duration on Cerebral Vascular Function

Agency and Award Number

American Heart Association, Award Number 19IPLOI34760579

Date

Last Updated Study Protocol and Statistical Analysis Plan: April 12, 2022

Document Type

Study Protocol and Statistical Analysis Plan

Comment

Study protocol described below can also be read in the Methods sections within two published articles.

- Gonzales JU, Clark C, Anderson T. Effect of five nights of sleep extension on peripheral vascular function: a randomized crossover investigation into long sleep duration. Sleep Med., 2022, 90:145-152. doi: 10.1016/j.sleep.2022.01.021. PMID: 35180478.
- Clark C, Rivas E, Gonzales JU. Six nights of sleep extension increases regional cerebral oxygenation without modifying cognitive performance at rest or following acute aerobic exercise. J Sleep Res., 2022, e13582. doi: 10.1111/jsr.13582. PMID: 35266244.

Study Design

Relevant to Primary Outcomes

This study utilized a randomized, crossover design that consisted of five consecutive nights of 8 or 11 hours time in bed (TIB) each night that participants completed at home. Participants were randomly assigned based on a random allocation sequence generated in Microsoft Excel. The 8 hours TIB protocol served as a control sleep condition with the intent that participants would achieve the recommended 7 hours of sleep. The goal of the 11 hours TIB protocol was for participants to reach ≥9 hours of sleep. Participants were given at least one night of habitual sleep as a washout. Laboratory testing took place at the same time in the morning for each participant after an overnight fast.

Relevant to Secondary Outcomes

On the fifth day of 8 or 11 hours TIB, participants performed three 10-minute bouts of brisk walking throughout the day. On the night after exercise, participants followed the 8 or 11 hours TIB protocol resulting in six consecutive nights of 8 or 11 hours TIB. The morning after prescribed exercise, participants arrived at the laboratory for cognitive function testing.

Methods

Arterial stiffness

Resting supine brachial blood pressure was measured using a digital blood pressure monitor (HEM-907XL, Omron Healthcare). Average diastolic and mean blood pressure was derived from two measurements taken one minute apart and entered into a Sphygmocor device (PVx, AtCor Medical) prior to radial artery applanation tonometry at the right wrist. Radial pressure waves were used to generate a central aortic wave through a generalized transfer function (SphygmoCor algorithm). A third brachial BP measurement was obtained immediately after radial tonometry and was averaged with the earlier measurements to obtain the final diastolic and mean BP used to calibrate the central aortic waveform. Wave separation analysis of the central aortic waveform derived pulse transit time that was used to calculate carotid-femoral pulse wave velocity after inputting carotid-femoral path length calculated from the Weir-McCall equation. The average of two measurements with operator indices >90% were recorded.

Cerebral vascular reactivity

Middle cerebral artery blood velocity responses to transient hypercapnia was measured to assess cerebral vascular reactivity. A transient hypercapnia protocol that lasted three minutes was employed in this study. Briefly, participants were asked to take two maximal breaths in the supine position. During the second maximal breath, participants exhaled into a 5 liter bag after which they continued to rebreathe their own expired gas mixture for 3 minutes as 100% oxygen was leaked into the bag to maintain oxygen saturation measured with a finger pulse oximeter (Nonin ML320/F, ADInstruments). Blood flow velocity in the middle cerebral artery was measured using transcranial Doppler and a headband that held in place a 2 MHz probe (TOC2M Neurovision, Multigon). A robotic feature was used to assist with locating the artery and optimize the blood flow velocity signal, however, the middle cerebral artery could not be located in three participants. Blood flow velocity, end-tidal carbon dioxide (CO_2), and mean arterial pressure were continuously recorded in LabChart software. End-tidal CO_2 was measured using a CD-3A CO_2 analyzer (MOXUS, AEI Technologies). An average of 60s of data prior to hypercapnia was used to derive resting hemodynamics, while blood flow velocity was analyzed per breath (from start of inspiration to end of expiration) using the CO_2 curves measured during the rebreathe protocol. Maximal hemodynamics represent the average of the last three breaths at the end of the rebreathe test.

Nighttime Pulse Pressure

An oscillometric blood pressure device (Oscar2, SunTech Medical) was used to assess overnight changes in central aortic pulse pressure. Participants wore the device once within each TIB protocol. Participants were asked to wear the device on their dominant arm approximately one hour before they planned on going to sleep in order to obtain at least one measurement while they were awake. The device was programmed to take measurements once every 30 minutes until 10:00pm then every 45 minutes after 10:00pm throughout the night. Participants were instructed to turn off the device upon rising from bed in the morning.

Peak Reactive hyperemia

Peak forearm blood flow was assessed using venous occlusion plethysmography (EC6 system, Hokanson) during reactive hyperemia while participants rested in the supine position. A fitted mercury-in-silastic strain gauge was placed on the left forearm. The forearm was elevated approximately 10cm above heart level with a small occlusive cuff placed around the wrist, and a venous collecting cuff placed around the upper arm that inflated rapidly (E20 inflator, Hokanson). The strain gauge was placed around the widest portion of the forearm. During the forearm blood flow (FBF) measurement, an occlusive wrist cuff was inflated to 200 mmHg while the venous collecting cuff inflated intermittently to 50 mmHg to allow four measurements within one minute. Peak FBF was measured during a three minute period that followed 10 minutes of arterial occlusion (~220 mmHg) at the upper arm. The first measurement took place within 5 seconds after arterial occlusion was released.

Cognitive function

Participants completed a computerized battery of tests from Automated Neuropsychological Assessment Metrics software (VistaLife Sciences). The tests and order of presentation were as follows: Manikin test (spatial rotation ability), task switching (mental flexibility), and the Stroop color-word test (executive function). The software kept the same order of tests for each study visit but changed the presentation sequence within each test to reduce variance in performance scores due to the practice effect.

Statistical analysis

A two-tailed paired t-test was used to compare variables between TIB protocols for primary outcome variables (arterial stiffness, cerebral vascular reactivity, nighttime pulse pressure, peak reactive hyperemia), A two-way repeated measures ANOVA (TIB x Pre/Post Exercise) was used to evaluate the main effect of exercise on cognitive function. A Bonferroni post-hoc test was used to assess differences upon a significant main effect. Statistical significance was set a priori at $P<0.05$.