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Effects of acute prolonged sitting on cerebral perfusion and executive function in young adults: a randomized cross-over trial

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ABSTRACT

Exposure to acute prolonged sitting reportedly leads to decreased cerebral blood flow. However, it is unclear whether this exposure translates to decreased cerebral perfusion and executive function, or whether simple strategies to break-up sitting can maintain cerebral perfusion and executive function. This study sought to answer two questions: in young, healthy adults (i) does prolonged (3 h) sitting lead to decreased cerebral perfusion and executive function? and (ii) does breaking-up prolonged sitting, using intermittent calf raise exercises, prevent changes in cerebral perfusion and executive function? Twenty young, healthy participants (21.7 [2.5] y, 70% F, 25.5 [6.1] kg/m^2) were randomized to: 3 h sitting with 10 calf raises every 10 min (CALF), and 3 h sitting without calf intermittent calf raises (CON). Prefrontal cortex perfusion was assessed using near-infrared spectroscopy to monitor total hemoglobin (tHB) concentration and tissue saturation index (TSI, oxygenated hemoglobin). Executive function was assessed using the Stroop Word and Color Tasks. Following 3 h sitting, tHb was significantly lower in CALF vs. CON (-2.1 µ*M*, 95% CI: -3.1, -1.1). TSI was not significantly different between conditions (*P* = .667). Word (1.6 ms, 95% CI: 0.7, 2.5) and Color (1.3 ms, 95% CI: -0.2, 2.8) completion times were longer (worse) for CALF compared to CON. In conclusion, calf raises decreased both cerebral perfusion and executive function. Simple strategies, such as fidgeting or calf raises, which have been reported to preserve vascular function in the legs, appear not to be sufficient enough to benefit cerebral perfusion or executive function.

KEY WORDS

Cognition; near infra-red spectroscopy; oxygenation; sedentary; total hemoglobin; inactivity

1. INTRODUCTION

Considering there are no effective long-term pharmacological therapies for the treatment or prevention of dementias, such as Alzheimer's disease, a modifiable risk factor worthy of investigation is sedentary behavior. Sedentary behavior refers to very low intensity behaviors (≤1.5 metabolic equivalents) in a seated, reclined or supine posture.(Tremblay et al., 2017) The available evidence indicates that chronic sedentary behavior is associated with dementia risk factors, including reductions in brain white matter (Arnardottir et al., 2016), medial temporal lobe thickness (Siddarth, Burggren, Eyre, Small, & Merrill, 2018), and mild cognitive impairment.(Hamer & Stamatakis, 2014; Vancampfort et al., 2018) However, the mechanism(s) linking repeated bouts of acute sedentary behavior exposure to chronic cerebrovascular complications, including cognitive decline, is less clear. A potential pathway linking repeated prolonged (>30 min) sitting exposure to peripheral and central cardiovascular complications, is blood flow. (Credeur et al., 2018; Dempsey, Larsen, Dunstan, Owen, & Kingwell, 2018; R M Restaino, Holwerda, Credeur, Fadel, & Padilla, 2015; R M Restaino et al., 2016; Wheeler et al., 2017)

No known studies have investigated whether repeated acute exposure to prolonged sitting leads to chronic cardiovascular complications. However, a series of acute studies have demonstrated mechanistic plausibility. With exposure to prolonged sitting, particularly in the absence of leg muscle activity, blood pools in the lower extremities, leading to decreased venous return, a decline in cardiac output, and a subsequent decrease in leg blood flow-induced shear stress.(Morishima et al., 2016; Robert M. Restaino et al., 2016) The decreased leg blood flow-induced shear stress has been reported to lead to decreased nitric oxide bioavailability, endothelial dysfunction, and subsequent arterial stiffening.(Jenkins et al., 2013; Laughlin, Newcomer, & Bender, 2008) Conversely, if subjects remain seated but strategies are used to maintain leg blood flow, including local heating,(Robert M. Restaino et al., 2016) fidgeting

(Morishima et al., 2016) and calf raises,(Vranish et al., 2018) vascular function is maintained in the legs.

Normal brain function is dependent on adequate oxygen delivery and glucose disposal, which in turn are regulated by cerebral blood flow.(Marshall et al., 2001; Wheeler et al., 2017) Blood pooling in the lower extremities and the subsequent decreased venous return may also impair cerebral blood flow and perfusion (blood volume in microvasculature), leading to impaired cognitive functions, including executive functions. While the term executive function is broad, it generally refers to the hypothesized processes that control other brain process which are critical to everyday functioning.(Hughes, 2013) The limited available literature does indicate that prolonged sitting decreases cerebral blood flow by 5%,(Carter et al., 2018) cerebral vascular conductance by 6%,(Carter et al., 2018) increases perceived fatigue by 25%,(Wennberg et al., 2016) and increases problem solving errors by 56%,(Baker, Coenen, Howie, Williamson, & Straker, 2018) Conversely, regular walking breaks prevent the decline in cerebral blood flow associated with prolonged sitting.(Carter et al., 2018) However, it should be noted that the aforementioned study (Carter et al., 2018) investigated the middle cerebral artery, it did not assess whether the prefrontal cortex, a key structure for performing executive functions,(Funahashi & Mario, 2013) was adequately perfused. The reverse has been examined; that is acute exercise has been shown to simultaneously increase both cerebral perfusion and executive function.(Faulkner, Lambrick, Kaufmann, & Stoner, 2016; Faulkner et al., 2017; Lambrick, Stoner, Grigg, & Faulkner, 2016; Lucas et al., 2012) However, further study is warranted to determine whether acute exposure to prolonged sitting reduces prefrontal cortex perfusion and executive function.

Two research questions were addressed in this study. In young, healthy adults: (i) does exposure to acute prolonged (3 h) sitting lead to decreased cerebral perfusion and executive

function? And (ii) does breaking-up prolonged sitting, using intermittent calf raise exercises, prevent changes in cerebral perfusion and executive function? Considering this is the first study to simultaneously assess cerebral perfusion and executive function during acute prolonged sitting, a homogenous group of young, healthy adults were recruited to minimize the confounding influence of age and cardiometabolic abnormalities. In a similar population, our group previously reported a positive relationship between cerebral perfusion and executive function in young adults during cycling exercise.(Faulkner et al., 2016) A 3 h sitting duration was selected following previous work demonstrating this duration to result in negative impairments to peripheral and central vascular health.(Credeur et al., 2018) Calf raise exercise was selected because it engages the muscle pump, one of the primary mechanisms ensuring venous return.

2. METHOD

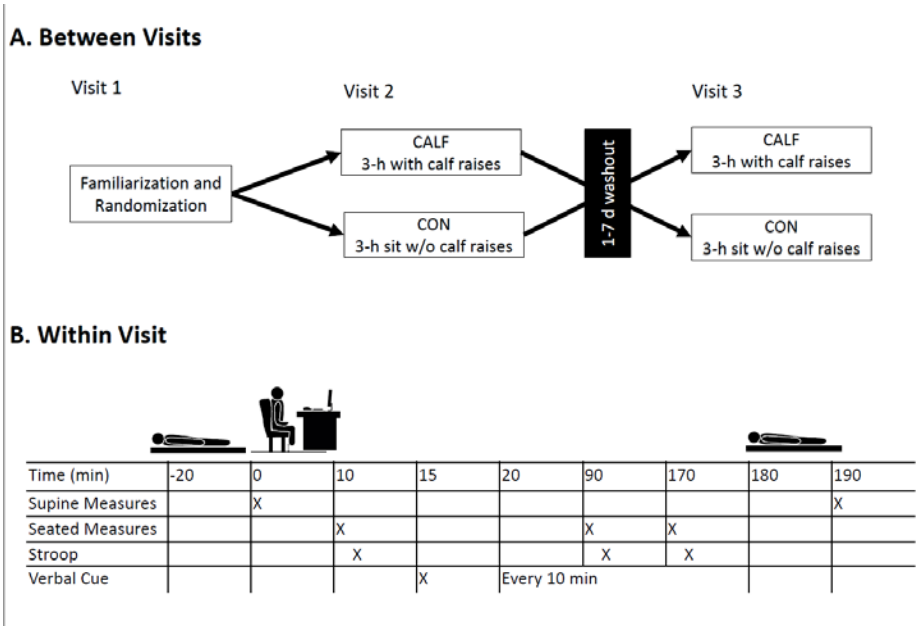
This study is reported in accordance with CONSORT (Consolidated Standards of Reporting Trials) guidelines.(Schulz, Altaian, & Moher, 2018) Ethical approval was obtained from the University of North Carolina at Chapel Hill institutional review board, and all participants provided written informed consent prior to participating in the study.

2.1 PARTICIPANTS

Twenty young (18 – 35 y) and healthy men and women were recruited from the population of a large state university. The university broadly represents the young population of the state. Exclusion criteria included pregnancy, regular engagement in moderate-to-vigorous physical activity ($\geq$ 150 min/wk), current smoker, any known cardio-metabolic disorders, or use of medications known to affect cardiovascular health. In women, because fluctuations in estrogen can affect cardiovascular measures, the first testing session was performed within the first 1-7 d of their menstrual cycle, and the second condition was performed within 7 d of the previous visit. Women reporting contraceptive use were tested during the placebo week.

2.2 EXPERIMENTAL DESIGN

This study was a randomized cross-over trial with two experimental conditions (Figure 1): prolonged (3 h) sitting (CON), and prolonged (3 h) sitting with intermittent calf raises (CALF). Pilot work was also conducted on a separate sample (n = 5) with similar characteristics to confirm that the calf raise exercise resulted in an adequate leg blood flow stimulus. All testing took place in the Applied Physiology Laboratory at the University of North Carolina. Participants reported to the laboratory on three occasions, one familiarization visit, and the two experimental visits.



Time (min)	-20	0	10	15	20	90	170	180	190
Supine Measures		X							X
Seated Measures			X			X	X		
Stroop			X			X	X		
Verbal Cue				X	Every 10 min				

FIGURE 1. Experimental protocol for CON (no calf raises) and CALF (calf rises). Participants arrived between 0600 and 1000, having eaten a protein bar 2 h prior to arrival. Participants completed a familiarization session and then two experimental condition in a randomized order. The primary outcome was prefrontal cortex perfusion (total hemoglobin). Additional outcomes included executive function (Stroop Task), calf venous pooling (total hemoglobin), peripheral hemodynamics (blood pressure and heart rate), autonomic function (heart rate variability) and movement analysis (accelerometry). The Stroop Task proceeded peripheral hemodynamic measurements. A verbal cue was given at min15, min 20 and then every 10 min until min 170. For CON the cue instructed participants to remain motionless, for CALF the cue instructed participants to perform 10 calf at a rate of 0.33 Hz

2.2.1 FAMILIARIZATION

After obtaining height and weight, participants were positioned on a three-section table (Armedica AM353 Hi-Lo, Tiger Medical, Irvington, NJ). The table head section can be repositioned upwards 78° and the foot piece downwards 90°, enabling passive maneuvering from the fully supine to seated position. Table height was adjusted to place feet flat on the ground with a thigh to calf angle at approximately 90 degrees, which was standardized between trials. To avoid a learned effect, participants practiced the Stroop Word and Stroop Color Tasks until completion time plateaued, as previously reported.(Faulkner et al., 2016, 2017; Lambrick et al., 2016) Participants also practiced several sets of 10 calf raises at a rate of 0.33 Hz (20 per min), set to a metronome (Pro Metronome Xiao Yixiang ©2016 EUMLab, Xanin Tech). After reviewing pre test guidelines, participants were given a small standardized meal (protein bar) with low glycemic index (Pure Protein, Bohemia, NY, USA) to consume 2 h before their first visit to reduce hunger distractions.

2.2.2 EXPERIMENTAL VISITS

Participants arrived between 0600 and 1000, and at the same time between-visits. Mode of transportation to the laboratory was recorded and standardized. All participants had fasted for 12h aside from the protein bar, consuming only water, and having refrained from supplement intake that morning. Additionally, participants were asked to avoid strenuous physical activity and alcohol for 24 h prior to experimentation. Experimental procedures were conducted in a quiet, environmentally controlled room (Average: 22 ˚C, 50 % humidity, 748 mmHg). After being asked to empty their bladder, participants were fitted with a near infra-red spectroscopy (NIRS, Portalite, Artinis Medical Systems, Netherlands) optodes on the calf and forehead (prefrontal cortex) for the assessment of perfusion, a 3-lead electrocardiogram for the assessment of autonomic function (Powerlab 30 series, ADInstruments, CO, USA), an accelerometer on the non-dominant ankle (wActiSleep +; ActiGraph LLC, FL, USA) for movement analysis, and a

standard blood pressure cuff on the left arm. Subsequently, in accordance with guidelines, participants were asked to rest in the supine position for 20 min, with the left arm stretched at an angle and at heart level prior to baseline assessment.(Stoner, Lambrick, Faulkner, & Young, 2013)

Following baseline [supine] perfusion and hemodynamic assessments the participants were passively maneuvered to the seated position (Figure 1). To replace predicted water loss, 500mL of water was provided for participants to consume *ad libitum*. Participants were asked to remain seated, without fidgeting, for 180 min while watching a non-stimulatory television program (*Grand Designs*, Boundless, London, UK) to maintain wakefulness. Throughout the 180 min of sitting participants were given a verbal cue at min 15, min 20 and then every 10 min until min 170. For CALF the cue instructed participants to perform 10 calf raises at a rate of 0.33 Hz, for CON the cue instructed participants to remain motionless. The motionless state was ensured by observing the participants throughout the 3 h, and confirmed using accelerometry. Calf blood volume, prefrontal cortex perfusion, executive function, hemodynamic and autonomic assessments were made pre (10 min) and post (170 min) sitting, as well as at the mid-way point (90 min). At 180 min, participants were passively returned to the supine position and calf blood volume and cerebral perfusion measurements were repeated at 190 min.

2.3 PILOT WORK: CONFIRMATION OF STIMULUS

The effects of the calf raise protocol on superficial femoral artery blood flow was assessed on a separate sub-sample (n = 5) of participants. Superficial femoral artery blood flow and shear stress was measured using duplex doppler ultrasound (Logiq P6; GE Medical systems, WI, USA). To ensure recording could be made during the calf raises, the probe was manually held in place by an experienced operator. Recordings were taken at 30 Hz using an external video capture system (AV.io HD Frame Grabber, Epiphan Video, CA, USA), and analysed offline

using specialized image analysis software (FMD Studio®, QUIPU, Italy).(Stoner & McCully, 2012) Measurements were made continuously during and for 90 sec following one set of 10 calf raises at 0.33 Hz, and during and for 90 s following a stationary control condition (Figure 2). The control condition was completed on the same leg 10 min following the calf raise condition. Peak values and 60 s area under the curve were calculated for each condition.

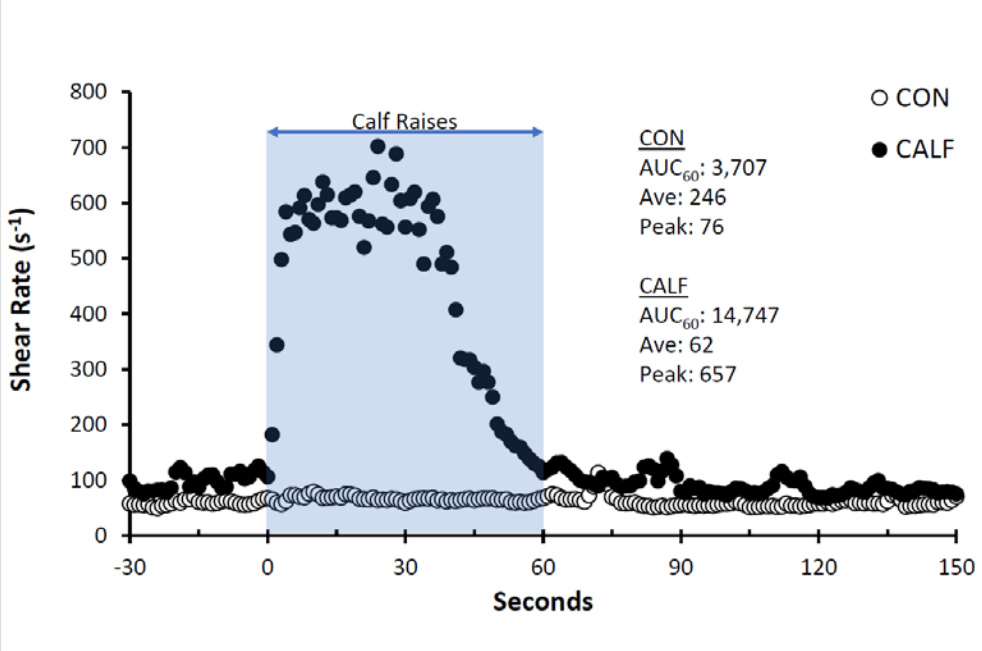


FIGURE 2. Shear stress stimulus response to 10 calf raises at 0.33 Hz

2,4 EXPERIMENTAL MEASURES

2.4.1 CALF BLOOD VOLUME

Venous pooling in the calf was monitored using a continuous wave NIRS device (PortaLite, Artinis Medical Systems BV, the Netherlands). The optode was securely fixed to the skin with bi-adhesive tape, and the location was marked to ensure identical placement on the second day. A custom stencil was used to identify the vertical plane over the central belly of the medial gastrocnemius at the position of maximum circumference. B-mode ultrasound (Logiq P6) was used to confirm the position of the optode on the calf, and to confirm the absence of light-absorbing blood vessels. A custom-made cover shielded the probe from ambient light while

allowing it to move with the skin during contractions to minimize changes in contact pressure. The Portalite emits wavelengths of 760 and 850 nm to detect relative changes in concentrations of oxygenated hemoglobin [HbO2] and deoxygenated hemoglobin [HHb], respectively, as well as total hemoglobin ([tHb] = [O2Hb] + [HHb]) and tissue saturation index (TSI). The TSI, which is an absolute measure of oxygenated hemoglobin, was calculated with manufacturer software using spatially-resolved spectroscopy. Both wavelengths were emitted from three transmitters at 3.0, 3.5, and 4.0 cm from the photodiode detector, allowing for theoretical penetration distances between 1.5–2 cm.(Chance, Dait, Zhang, Hamaoka, & Hagerman, 1992) The deepest (4.0 cm) wavelength was used for analysis. A differential path-length factor of 4.0 was used to correct for photon scattering within the tissue, and data were collected at 10 Hz (Oxysoft, Artinis Medical Systems BV, the Netherlands). The Portalite uses continuous wave NIRS; the scattering of light in tissue cannot be measured, and therefore can only relative changes in Hb concentration can be detected.(Grassi & Quaresima, 2016) For each time point (10 min, 90 min, 170 min, post) data were averaged over 30 s and expressed relative to supine baseline. Our laboratory has previously reported that the Portalite device is able to monitor changes in tHb with acceptable validity and reliability (ICC range: 0.75-0.98),(Lucero et al., 2017; Stone, Fryer, Ryan, & Stoner, 2016) and changes in TSI with acceptable reliability (ICC: 0.7-0.9).(Lucero et al., 2017)

2.4.2 PRIMARY OUTCOME: PREFRONTAL CORTEX PERFUSION

Prefrontal cortex perfusion was monitored using the NIRS device described above. Depending on individual head geometry, the probe was positioned over the participant's prefrontal cortex at Fp1 for right-side dominant participants and at Fp2 for left-side dominant participants according to the International 10-20 system of electrode placement.(Klem, Lüders, Jasper, & Elger, 1999) The NIRS device was fixed to the skin with biadhesive tape and the location was marked to ensure identical placement on the second day. The optode was covered with a dark opaque cloth to prevent signal contamination by ambient light, as per manufacturer recommendations.

As described above, for each time point (10 min, 90 min, 170 min, post) data were averaged over 30 s and expressed relative to supine baseline.

2.4.3 SECONDARY OUTCOME: EXECUTIVE FUNCTION

Executive function was assessed using the Stroop inference test (Xavier Educational Software Ltd., Bangor, UK), a classic measure of prefrontal cortex function (MacLeod, 1991) that has been widely used to assess the effects of acute exercise on cognition.(Faulkner et al., 2016, 2017; Lambrick et al., 2016; Lucas et al., 2012) The Stroop interference test involves 4 words (blue, yellow, green, and red) being randomly presented, consecutively, on a computer screen. The color that each word is presented in is either congruent or incongruent with the relevant semantic information (eg, “red” presented in the color red or the color green, respectively). Each presentation of a word constituted a sequence; each test comprised 32 sequences, and the total time to complete each test was recorded as a measure of performance. Acceptable reliability has been reported for both Word (ICC: 0.71) and Color (ICC: 0.79) Tasks in young adults.(Strauss, Allen, Jorgensen, & Cramer, 2005)

2.3.4 PERIPHERAL HEMODYNAMICS

Blood pressure and heart rate (HR) were recorded using an oscillometric device (SphygmoCor XCEL device, AtCor Medical, Sydney, Australia). An appropriately sized cuff was selected according to manufacturer guidelines and placed around the left upper arm. For each measurement cycle the arm was placed at heart level. All measurements were made in triplicate and the closest two recordings were averaged. We have previously reported that supine (ICC: 0.84) and seated (ICC: 0.74) mean arterial blood pressure (MAP) measures using this device are acceptably reliable.(Young et al., 2015)

2.3.5 AUTONOMIC FUNCTION

Autonomic nervous system (ANS) function was assessed by calculating heart rate variability (HRV), a measure which primarily reflects parasympathetic activity.(Malik et al., 1996) Participants were fitted with a 3-lead electrocardiogram (Powerlab 30 series, ADInstruments, CO, USA). Continuous R-R intervals were recorded for the duration of sitting and 5 minutes of R-R intervals were selected for the period preceding min 10, 90, and 170. LabChart (ADInstruments, CO, USA) software identified ectopic beats according to complexity and frequency and calculated the root mean square of the standard deviation of the time between heart beats (RMSSD). Owing to non-uniformity of error, data was log transformed for statistical analyses. Our group has previously reported that this device can reliably (ICC: 0.78) measure changes in RMSSD while supine and in response of orthostatic stress.(Montaño et al., 2017)

2.3.6 MOVEMENT ANALYSIS

As small stimuli such as fidgeting have been shown to restore vascular function during sitting,(Morishima et al., 2016; Robert M. Restaino et al., 2016) a subset of participants were fitted with an accelerometer (wActiSleep +; ActiGraph LLC, FL, USA) on their right ankle to verify differences in movement between conditions. The raw data were downloaded to Actilife 6 (ActiGraph LLC, FL, USA) and are expressed at total counts for each 180 min sitting period.

2.4 RANDOMIZATION

The randomization was performed by chance, where two sets of 10 unique numbers were generated from a number range of 1-20 (www.randomizer.org). The randomization procedure was performed by a research assistant. Considering the nature of the experimental conditions, we were unable to conceal conditions from the participants or the researchers collecting the data.

2.5 SAMPLE SIZE

Previously, we reported that the typical error for change in the tHb signal is 4 μ*M*.(Stone et al., 2016) To estimate the sample size required to detect the smallest detrimental (or beneficial) effect in a cross-over study,(Hopkins, Marshall, Batterham, & Hanin, 2009) with a type I error rate of 0.05 and 80% power, approximately 18 participants are required to detect a small (2.5 μ*M*) change in tHb. Twenty participants completed the experimental protocol to account for potential missing data.

2.6 STATISTICAL ANALYSIS

All statistical analyses were performed using SPSS 25 (IBM Armonk, NY, USA). The α level was set *a priori* for all statistical procedures at α = .05. Supine (Pre vs. Post) and sitting (min 10, 90 and 170) date were analyzed separately. Correlations between variables were examined using the Pearson product-moment correlation coefficient. Pre vs. post change values were assessed using paired sample *t*-tests. For the sitting data, the effects of time (pre vs. post and 10 vs. 90 vs. 170) and condition (CON vs. CALF) were analyzed using linear mixed models with fixed effects of condition and random effect of time and subject. As the NIRS data was relative to baseline, and therefore baseline was 0, the intercepts were fixed. The time-by-condition interaction term was removed when non-significant. Effect sizes were calculated as Cohen's *d*, where < 0.20 is considered to be a small, > 0.20 to < 0.50 a moderate, and > 0.60 a large effect. For the mixed models Cohen's *d* was calculated as the effect of condition (β) or time (β) from linear mixed models divided by the baseline *SD*. Raw data are presented as mean [standard deviation] and mixed model data are presented as mean [95% confidence interval].

3 RESULTS

3.1 PARTICIPANTS

Twenty participants (21.7 [2.5] y, 70% F, 25.5 [6.1] kg/m^2) were recruited, who self-identified as non-Hispanic White (n = 13), African American (n = 4), Hispanic (n = 1), Middle-eastern (n = 1),

and Asian (n = 1). All participants successfully completed both experimental trials. The Stroop Task data file was corrupted for 1 participant, and the HRV data was unreadable for a separate participant. All other measures were successfully collected on all participants. The participants with missing Stroop Task and HRV data were similar to the remainder of the populations for all other outcomes.

3.2 BASELINE DATA

Baseline data is reported in Table 1. Baseline tHb data is not reported as values as they are expressed relative to baseline. No significant differences were observed between conditions for HR (P = .140), MAP (P = .917) or for the prefrontal cortex (P = .754) or calf (P = .680) TSI signal.

3.3 STIMULUS

Pilot work confirmed that the 10 calf raises resulted in an adequate femoral artery shear rate (Figure 2) and blood flow (data not shown) stimulus. Compared to the control condition, calf raises resulted in a 4-fold increase in the shear rate AUC (ES: 2.54, P = .02), and a 9-fold increase in peak shear rate (ES: 3.2, P = .01). The stimulus was confirmed for the experimental conditions using accelerometry (Table 2); there was a 12-fold increase in step counts during CALF compared to CON (P < 0.001). The stimulus was achieved without affecting the ANS (Table 2), as evident by no condition effect for MAP (P = .109), HR (P = .466) or RMSSD (P = .543). There was a time effect for RMSSD (P = .017); for both conditions RMSSD increased, suggesting elevated parasympathetic activity with prolonged sitting.

TABLE 1. Baseline values. (n = 20 for all measures)

				NIRS-TSI	
		HR	MAP	Head	Calf
	Group	*bpm*	*mm Hg*	*%*	*%*
X	CON	66	83.5	66.8	66.8
	CALF	64	83.7	66.5	66.2
SD	*CON*	*8*	*9.9*	*3.2*	*3.1*
	CALF	*10*	*7.1*	*3.9*	*4.5*
T-test	*P*	*.140*	*.917*	*.754*	*.680*
	ES	-0.27	0.01	-0.06	-0.14

Abbreviations: CON, control condition; EXP, calf raise condition; HR, heart rate; MAP, mean arterial pressure; TSI, tissue saturation index (oxygenated hemoglobin)

3.4 SUPINE (PRE-POST) DATA

The pre versus post (change) perfusion (NIRS) data is reported in Table 2. Calf tHb was greater for CON (11.2 vs. 7.4 μ*M*) compared to CALF, but this small effect (ES: 0.34) was not significant (*P* = .215). Calf TSI did decrease more for CON vs. CALF (*P* = .004, ES: 0.97). Prefrontal cortex tHb (*P* = .483) and TSI (*P* = .440) did not significantly differ by condition.

TABLE 2. Pre vs. Post (supine) responses. (Accel *n* = 15, *n* = 20 for all other outcomes).

		Accel.	NIRS-Head		NIRS-Calf	
			tHb	TSI	tHb	TSI
		Counts	*μM*	*%*	*μM*	*μM*
X	CON	472	1.6	0.0	11.2	-8.1
	CALF	5490	0.0	-1.5	7.4	-4.1
SD	*CON*	*468*	*3.7*	*2.0*	*10.2*	*4.9*
	CALF	*3466*	*9.4*	*8.1*	*11.6*	*3.2*
T-test	*P*	<.001	.483	.440	.215	.004
	ES	2.03	-0.22	-0.27	-0.34	0.97

Abbreviations: CON, control condition; EXP, calf raise condition; Accel., Accelerometery; tHb, total hemoglobin; TSI, tissue saturation index (oxygenated hemoglobin)

3.5 SEATED DATA

All seated data is reported in Table 3. Note that the perfusion (NIRS) data is expressed as the change from supine baseline. Mixed model data indicate that the calf NIRS tHb signal was 1.9 μ*M* (95%CI: -0.4, 4.2) higher for CON vs. CALF, but the effect was not significant (*P* = .106). There was a significant condition effect for calf TSI (*P* = .015), with TSI decreasing a greater amount (-1.5 %, 95% CI: -2.5, -0.3) for CON vs. CALF (*P* = .006). There was a significant condition main effect for prefrontal cortex tHb, with decreased tHb for CALF vs. CON (-2.1 μ*M*, 95% CI: -3.1, -1.1). Prefrontal cortex TSI was not significantly different between conditions (*P* = .667).

TABLE 3. Responses during prolonged (3 h) sitting. (Stroop task *n* = 19, RMSSD *n* = 19, all other measures n = 20)

			Stimulus			**NISR-Head**		**NIRS-CALF**		**Stroop**	
			MAP	HR	RMSSD	tHb	TSI	tHb	TSI	Word	Color
			mm Hg	bpm	*ms*	*μM*	*%*	*μM*	*%*	*ms*	*ms*
X	CON	10	73	82	3.6	1.6	0.2	11.4	-4.3	32.4	39.4
		90	75	82	3.6	1.1	0.0	14.6	-7.2	32.7	38.6
		170	74	82	3.7	1.3	0.4	12.5	-7.8	31.7	36.5
	CALF	10	72	80	3.7	0.0	0.1	10.0	-3.7	34.1	39.4
		90	74	81	3.8	-1.6	1.0	12.0	-5.6	34.0	38.8
		170	74	83	3.9	-2.5	1.6	10.7	-5.9	33.1	38.3
SD	*CON*	*10*	*10*	*9*	*0.5*	*2.6*	*1.5*	*6.6*	*3.6*	*3.5*	*9.3*
		90	*11*	*8*	*0.5*	*3.7*	*2.1*	*8.5*	*4.5*	*3.9*	*6.5*
		170	*11*	*9*	*0.6*	*5.4*	*2.0*	*9.7*	*4.4*	*2.8*	*5.5*
	CALF	*10*	*10*	*8*	*0.5*	*3.3*	*1.4*	*7.0*	*2.4*	*5.0*	*6.9*
		90	*11*	*9*	*0.5*	*6.0*	*2.6*	*7.6*	*2.9*	*4.9*	*5.9*
		170	*13*	*8*	*0.6*	*7.7*	*5.7*	*8.5*	*3.0*	*3.5*	*5.3*
Interaction		*P*	*.266*	*.698*	*.874*	*.332*	*.264*	*.917*	*.646*	*.971*	*.530*
Time		*P*	*.606*	*.731*	*.017*	*.800*	*.509*	*.446*	*.006*	*.520*	*.642*
		ES	0.16	0.09	0.17	-0.10	0.13	0.07	-0.05	-0.14	-0.15
Condition		*P*	*.109*	*.466*	*.543*	*<.001*	*.667*	*.106*	*.015*	*.001*	*.083*
		ES	-0.36	0.16	0.58	-0.93	0.15	-0.37	0.56	0.85	0.41

Abbreviations: CON, control condition; EXP, calf raise condition; HR, heart rate; MAP, mean arterial pressure; RMSSD, root mean square of the standard deviation (heart rate variability); TSI, tissue saturation index (oxygenated hemoglobin)

Neither the Stroop Word (*P* = .520) or Color (*P* = .642) Tasks changed over time. There was a large (ES: 0.85, *P* = .001) condition effect for the Stoop Word Task, with a longer completion time for CALF vs. CON, (1.6 ms, 95% CI: 0.7, 2.5), indicating worse executive function for CALF. Compared to CON, CALF also resulted in a greater Color completion time (1.3 ms, 95% CI: -0.2, 2.8), albeit this effect not significant (*P* = .083).

Exploratory analysis examining the correlations between change in outcomes for the CALF condition are reported in Table 4. Prefrontal cortex perfusion and TSI was not significantly associated with any variable. The Stroop Color Task was significantly associated with calf tHb (*r* = -.60, *P* = <.01), and Stroop Word Task was associated with RMSSD (*r* = .47, *P* = <.05) and sex (*r* = -.45, *P* = <.05).

TABLE 4. Associations between change (10 vs 170 min) in outcomes for CALF condition. (Stroop task and HRV data *n* = 19, all other measures *n* = 20)

	PC_Thb	PC_TSI	C_Thb	C_TSI	Word	Color	RMSSD	MAP	HR	Sex	Accel.
PC_Thb		.00	.05	.09	-.03	.15	-.14	-.03	-.37	-.33	.11
PC_TSI	.00		-.35	-.33	.29	.10	.36	.29	-.18	-.25	.02
C_Thb	.05	-.35		.34	-.08	-.60**	-.20	-.39	.34	.06	-.45
C_TSI	.09	-.33	.34		-.22	-.25	-.16	-.46*	-.14	.28	.17
Word	-.03	.29	-.08	-.22		.11	.47*	-.23	-.11	-.45*	-.40
Color	.15	.10	-.60**	-.25	.11		.12	.08	-.32	.18	.20
RMSSD	-.14	.36	-.20	-.16	.47*	.12		-.32	.01	-.37	-.14
MAP	-.03	.29	-.39	-.46*	-.23	.08	-.32		-.21	.12	.35
HR	-.37	-.18	.34	-.14	-.11	-.32	.01	-.21		-.08	-.42
Sex	-.33	-.25	.06	.28	-.45*	.18	-.37	.12	-.08		.31
Accel	.11	.02	-.45	.17	-.40	.20	-.14	.35	-.42	.31	

Abbreviations: PC, prefrontal cortex (NIRS placement); C, calf (NIRS placement); color, Stroop color task; HR, heart rate; MAP, mean arterial pressure; RMSSD, root mean square of the standard deviation (heart rate variability); TSI, tissue saturation index (oxygenated hemoglobin); Word, Stroop word task; SEX (0 = male, 1 = female)

*Correlation is significant at the 0.05 level (2-tailed).

**Correlation is significant at the 0.01 level (2-tailed).

4 DISCUSSION

The major findings of this study are that prefrontal cortex perfusion and executive function did not change with time during prolonged uninterrupted sitting control condition, and that compared to a control condition, intermittent calf raise exercise decreased cerebral perfusion and executive function. Simple exercises, such as calf raises, during prolonged sitting may not be of benefit to the cerebral perfusion or executive function in healthy young adults

4.1 LIMITATIONS AND STRENGTHS

To better contextualize the discussion, the limitations and strengths of the current study must be stated. First, we purposefully elected to recruit a homogenous group of young, healthy adults to minimize the confounding influence of age and cardiometabolic abnormalities. As such further study is warranted to determine whether the findings are generalizable to other populations. Second, we recently reported that, compared to females, males exhibit a greater increase in aortic arterial stiffness following prolonged sitting.(Credeur et al., 2018) While we were not adequately powered to determine sex interaction effects, we cannot rule out that sex differences existed. Third, while we did recruit young, healthy participants, the health history screening questionnaire did not ask questions pertaining to cognitive disorders. As such, we cannot discount that the presence or absence of cognitive disorders confounded the findings. Lastly, we elected to use calf raise exercise to break-up sitting. We hypothesized that this activity would

engage the muscle pump, one of the primary mechanisms ensuring venous return occurred. The calf raise stimulus may not have been adequate. However, previous studies have shown that similar strategies, including fidgeting (Morishima et al., 2016; Robert M. Restaino et al., 2016) and calf raises (Vranish et al., 2018) are adequate for preserving leg endothelial function, and our pilot data indicate that the calf raises resulted in substantial increases in leg blood flow and shear stress. Further, our experimental data indicate that we increased the step counts 12-fold for CALF compared to CON without significantly engaging the ANS. Collectively, we are confident that the experimental conditions were well controlled.

4.2 COMPARISON TO LITERATURE

Prefrontal cortex perfusion did not decrease with stationary prolonged sitting. The lack of change in tHb during stationary sitting is contrary to a recent study reporting that prolonged sitting decreases middle cerebral artery blood flow by 5%.(Carter et al., 2018) Collectively, these findings indicate that while blood flow may decrease through the middle cerebral artery during prolonged sitting,(Carter et al., 2018) the prefrontal cortex microvasculature may auto-regulate to ensure adequate oxygen delivery,(Attwell et al., 2010; Willie, Tzeng, Fisher, & Ainslie, 2014) at least in young healthy adults.

We also found that intermittent calf raise exercise, to activate the muscle pump, resulted in a small decrease in prefrontal cortex tHb (perfusion). This finding is contrary to a recent study by Carter et al. (Carter et al., 2018) which reported that interrupting prolonged sitting with walking breaks prevents a decline in middle cerebral artery blood flow. It may be rationalized that, as opposed to the Carter et al study (Carter et al., 2018) which likely activated greater muscle mass, the calf raise exercise used in the current study was not sufficient enough for activating the muscle pump, preventing venous pooling and subsequently ensuring adequate venous return. Indeed, while there was a small effect size (ES: 0.37) greater increase in calf tHb for the

control compared to calf raise condition, this effect was not significant (*P* = .106). However, this outcome may be spurious as there was also a smaller decrease in calf TSI (oxygen saturation) for CALF compared to CON, indicating that the arterial side of the muscle may have been maintained while the venous side was decreased to a greater extent for the calf raise condition.(Grassi & Quaresima, 2016) Coupled with the pilot data indicating a large (ES: 2.54) increase in femoral artery blood flow during calf raises, we believe the calf raises served the intended purpose in aiding adequate venous return. Additionally, it should be stated that the decrease in prefrontal cortex tHb may not be meaningful. There is no known data with which to establish a clinically meaningful change in prefrontal cortex tHb. However, CALF resulted in a 2.5 μM decrease in prefrontal cortex tHb, compared to the ~23 μM increase we previously reported, with the same NIRS device, during 30 min moderate intensity cycling exercise.(Faulkner et al., 2017)

Contrary to expected, the Stroop Task (executive function) completion times were shorter (improve) with prolonged sitting, and longer (worse) completion times were recorded for CALF. The worse Stroop Word scores for CALF may be related to the decrease in cerebral perfusion. We did not find a significant association between change in prefrontal cortex perfusion and Stroop scores, but it should be acknowledged that we were not sufficiently powered to perform such analysis. The lack of change in executive function over time may also be the result of permitting the participants to practice during familiarization, and administering the test too frequently, which may have provided some mental stimulation. Additionally, the lack of change in estimated executive function over time may reflect the lack of sensitivity in the Stroop Task with this population. We elected to use the Stroop Task because it is relatively short, which would minimize mental stimulation during sitting, and it is a classic measure of prefrontal cortex function (MacLeod, 1991) that has been widely used to assess the effects of acute exercise on

cognition.(Faulkner et al., 2016, 2017; Lambrick et al., 2016) Nonetheless, the young healthy participants in the current study may not have found the Stroop Tasks sufficiently challenging.

4.3 IMPLICATIONS

While previous studies have associated chronic sedentary behavior with cognitive function decline, (Hamer & Stamatakis, 2014; Vancampfort et al., 2018) the mechanism(s) are unclear. A potential pathway is blood flow, and a recent study did report that prolonged sitting decreases middle cerebral artery blood flow by 5%.(Carter et al., 2018) However, it was unclear as to whether the decrease in middle cerebral artery blood flow leads to impaired prefrontal cortex oxygen delivery. Here we report that prolonged sitting may not impair the ability to deliver oxygen, at least acutely. However, it should be acknowledged that this preliminary study was conducted on young, healthy participants, and the findings may not be generalizable.

Findings from this study also indicate calf raises to interrupt prolonged sitting do not improve prefrontal cortex perfusion or executive function. These findings are contrary to studies showing that simple perturbations to maintain leg blood flow, including local heating,(Robert M. Restaino et al., 2016) fidgeting (Morishima et al., 2016; Robert M. Restaino et al., 2016) and calf raises,(Vranish et al., 2018) are sufficient for maintaining leg endothelial function. However, our findings are in line with a study reporting that prolonged sitting increased central arterial stiffness, and that breaking-up sitting with regular standing does not effect this increase in arterial stiffness.(Barone Gibbs et al., 2017) Collectively, findings from these previous studies and the current study indicate that simple exercises to break-up sitting may be effective at maintaining leg vascular health, but may not be effective in terms of central or cerebrovascular health.

Future studies are warranted which simultaneously measure cerebral artery blood flow, perfusion and autoregulation, including in those using older populations and those with chronic disease. It is also recommended that future studies utilize executive function tests which are similarly short and unlikely to induce prolonged mental stimulation, but which are more challenging. Such executive function tests may include part B of the Trail making test. Lastly, it is also recommended that the executive function test(s) are only administered pre and post prolonged sitting to avoid mental stimulation during the sitting protocol.

4.4 CONCLUSIONS

The purpose of this study was to determine, in young healthy adults, (i) the effects of prolonged (3 h) sitting on prefrontal cortex perfusion and executive function, and (ii) the effects of breaking-up prolonged sitting, using intermittent calf raise exercises, on cerebral perfusion and executive function. Contrary to expected, we found cerebral perfusion and executive function did not change during prolonged sitting, but that calf raise exercise decreased both cerebral perfusion and executive function. While simple exercises, such as fidgeting or calf raises, may preserve vascular function in the legs, such exercise may not be of benefit to the heart or the brain. Future studies are warranted which simultaneously measure cerebral artery blood flow, perfusion and autoregulation during prolonged sitting, including in older populations and those with chronic diseases.

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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