SIMPLE INTERMITTENT RESISTANCE ACTIVITY MITIGATES THE DETRIMENTAL EFFECT OF PROLONGED UNBROKEN SITTING ON ARTERIAL FUNCTION IN OVERWEIGHT AND OBESE ADULTS

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Short title: Prolonged sitting and arterial function

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Abstract

Background. Prolonged sitting contributes to cardiovascular disease (CVD) risk. The underlying mechanisms are unknown, but may include changes in arterial function and vasoactive mediators. We examined the effects of prolonged unbroken sitting, relative to regular active interruptions to sitting time, on arterial function in adults at increased CVD risk.

Methods. In a randomized crossover trial, 19 sedentary overweight/obese adults (mean±SD 57±12 yrs), completed two laboratory-based conditions: five hours uninterrupted sitting (SIT) and; five hours sitting interrupted every 30 minutes by three minutes of simple resistance activities (SRA). Femoral artery function (flow mediated dilation; FMD), blood flow and shear rate were measured at zero hour, 30 minutes, one, two and five hours. Brachial FMD was assessed at zero and five hours. Plasma was collected hourly for measurement of endothelin-1 (ET-1), nitrates/nitrites, vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1).

Results. There was a significant decline in femoral artery FMD, averaged over five hours in the SIT condition, relative to SRA ($p<0.001$). Plasma ET-1 total AUC over five hours increased in the SIT condition compared to SRA ($p=0.006$). There was no significant difference between conditions in femoral or brachial shear rate, brachial FMD, nitrates/nitrites, VCAM-1 or ICAM-1 ($p>0.05$ for all).

Conclusions. Five hours of prolonged sitting, relative to regular interruptions to sitting time, impaired femoral artery vasodilator function and increased circulating ET-1 in overweight/obese adults. There is the need to build on this evidence beyond acute observations to better understand the potential longer-term vascular-related consequences of prolonged sitting.

Key words: Obesity, arteries, sedentary lifestyle, blood flow, blood pressure.

Introduction

Arterial dysfunction, particularly that related to the inner (endothelial) lining, represents one of the earliest detectable stages of atherosclerotic disease (1, 2). Atherosclerotic lesions are not uniformly distributed, developing primarily in the coronary and carotid arteries as well as in the lower limb (3), which suggests that local factors, such as abnormal arterial shear stress, may play a role. Shear stress is modulated by physical (in)activity and by sedentary behaviors (i.e. prolonged sitting (4-7)), making such activities key contributors to endothelial (dys)function and atherosclerosis. Excessive time spent sitting is now ubiquitous in modern-day society, with the average adult spending nine hours a day sitting (8). Moreover, prolonged sitting (defined as >30 minutes of uninterrupted sitting) accounts for four hours per day (9). Importantly, high volumes of sitting are associated with elevated risk of CVD (10) along with other adverse health consequences (11) and it is likely that sitting-induced decrements in arterial function contribute to increased prevalence of CVD.

Previous research has shown that prolonged sitting leads to impairment in lower limb arterial function and dilation, effects that may be negated, or reversed, by light intensity activity (4-6). These studies have been restricted to young, healthy populations and it is unknown whether prolonged sitting may affect arterial function in those already at a heightened risk of CVD (such as overweight/obese adults). Further, mecanisms relating to the effect of prolonged sitting on vascular function remain unexplored. Candidate meachnisms include nitric oxide (NO), a potent dilator released from endothelial cells in response to shear stress (12). Moreover, high insulin concentration (such as in insulin resistant, type 2 diabetes or overweight/obese populations), is associated with endothelin-1 (ET-1; a vasoconstrictor) upregulation (13-15) and expression of pro-atherogenic molecules (intracellular adhesion molecule [ICAM-1] and vascular cell adhesion molecule [VCAM-1]) (16). It is conceivable that the combination of CVD risk factors and reduced shear stress in the lower limb associated with prolonged sitting

promotes an exaggerated pro-atherogenic environment. However, this hypothesis has not been directly addressed in those at increased risk of CVD.

Most earlier work has examined the effect of interrupting prolonged sitting with intermittent walking activity, but it has been suggested that a more pragmatic option for working adults could be to interrupt sitting without having to move away from their workstation, for example by performing simple resistance activities (SRA) in a static position using their own body weight (17). Indeed, a recent study in patients with type 2 diabetes demonstrated that interrupting prolonged sitting with brief bouts of SRA was as effective as light walking for reducing the impact of a day of uninterrupted sitting on postprandial glucose and insulin (17). However, it is unclear whether benefits in arterial function could be gained via SRA during periods of prolonged sitting. The aim of this study was to examine the effects on arterial function of prolonged unbroken sitting, relative to regular active interruptions to sitting time, in adults at increased CVD risk.

Methods

Study participants. Sedentary overweight/obese (body mass index [BMI] ≥25-40kg/m^2) adults were recruited via local advertisements. The exclusion criteria included: pregnancy, pre-menopausal, self-reported sitting less than five hours per day, self-reported regular engagement in moderate-to-vigorous intensity physical activity (≥ 150 minutes per week), diagnosed diabetes, use of glucose/lipid lowering medications, current smoker, or having any major acute or chronic illness that may limit their ability to perform the SRA.

Study overview and randomization. This study was a randomized crossover trial (ACTRN12316000578404), undertaken at the Baker Heart and Diabetes Institute research clinic. Potential participants were initially screened via a telephone questionnaire to determine

their eligibility and were asked about their general health and medical history. Eligible participants were requested to undergo a fasted screening blood test at a local pathology clinic (Melbourne Pathology; Sonic Healthcare Ltd) for glycated haemoglobin (HbA1c), glucose and lipid profile. Participants attended the laboratory on three separate occasions: a familiarisation visit and two trial visits (Figure 1). Trial condition order was randomized by a third party (block-randomization and balanced block sizes) and stratified by sex. Study personnel were blinded to the condition order until familiarisation and participants were blinded to the condition order until their first condition visit. Sample analyses (including arterial function analysis) were performed by trained personnel who were blinded to the condition order. The trial was approved by the Alfred Human Ethics Committee and was performed in accordance with the Declaration of Helsinki.

Study protocol. Participants attended a familiarization session one week prior to their first condition visit, where they were shown the testing procedures and measurements. Height, weight, waist and hip circumference were obtained in duplicate via standard methods. To minimize any diet-induced variability, participants consumed a standardized meal the evening prior, and on the day of, the experimental condition. Each meal was standardized to meet 33% of each participant's daily energy requirements, estimated using the Schofield equation (18). Using FoodWorks Software (FoodWorks Xryris, 2012), all meals were matched for macronutrients of 12-15% energy from proteins, 53-55% energy from carbohydrate, and 30-33% energy from fat. Participants were instructed to consume their standardized meal the evening prior to each condition between 1900 and 2100h and to fast until the next morning. Participants were also instructed not to alter their habitual daily activities while involved in the study but to avoid moderate-to-vigorous exercise, caffeine and alcohol for 48 hours prior to

each condition (15). This was confirmed upon presentation to the respective experimental condition days.

Experimental conditions

On the trial days, participants arrived at the laboratory between 0730 and 0800h in a fasted state (>10 hours). Weight was re-measured and body mass index (BMI) was calculated. An indwelling venous catheter was inserted in the antecubital vein for blood sampling. Each condition began with a one hour 'steady state' seated period. During the steady state period blood samples were collected, blood pressure (BP) was measured, femoral and brachial artery flow mediated dilation (FMD) were recorded and participants were then given 15 minutes to consume a standardized breakfast meal prior to the zero hour time point. Postprandial blood samples were collected at 30 minutes, one hour and then hourly up to the end of the five hour condition. Options for the breakfast meal consisted of bran-based cereal, fruit salad, ham-and-cheese croissant and juice (200mL). Lunch options included a salad and meat bread roll and juice. A note was made regarding each individual's meal choice, and these were replicated on the repeat attendance. Timing of medication (if applicable) was standardized to occur with the breakfast meal during each condition.

Participants were instructed to sit upright in a comfortable lounge chair for the duration of the condition and were asked to minimize excessive movement. In the SIT condition, participants sat uninterrupted for five hours, only rising from the chair to void. The SRA condition was similar, but sitting was interrupted every 30 minutes for three minutes of SRA. The SRA were light-intensity, body weight-resisted exercises undertaken in a standing posture including half squats, calf raises and single knee raises with gluteal contractions. Each exercise was performed for 20 seconds, three times, for a total of three minutes. To ensure appropriate movement standardization, tempo, and correct form, participants mimicked a video recording. Half squats

and knee raises were tailored to the range of motion of each participant where knee/hip angle was between 45° to 90° for half squats/knee raises, assessed during the familiarization session. The alternate trial condition was completed after a minimum of six days washout period.

Measurements

Arterial function

Participants were seated for 20 minutes in a dimly lit, temperature-controlled room (22-24°C) prior to the steady state recording of FMD. Brachial and superficial femoral artery function (i.e. FMD) were assessed in the seated position using a high-resolution ultrasound machine (Terason t3200™, Teratech, Burlington, MA, USA) in conjunction with a 10 MHz multifrequency linear array probe and insonation angle of 60° according to current guidelines (19). Femoral artery FMD was measured in the right leg with the foot placed flat on the floor. A rapid inflatable cuff (SCI12D™, D.E. Hokanson Inc., Bellevue, Washington) was placed around the thigh at the distal end of the femur. Once an optimal image of the artery was obtained, a one minute recording of continuous resting vessel diameter and blood velocity was measured (live duplex mode). The cuff was then inflated for five minutes (>200 mmHg). After five minutes of inflation, the cuff was released to induce reactive hyperemia. A further three minutes of continuous duplex ultrasound recording was then undertaken to observe the post deflation diameter profile and peak response. The FMD response is presented as the percentage change from preceding resting diameter to peak dilation. Femoral artery FMD was measured at the start of the condition during the steady state period (zero hour), 30 minutes, one, two and five hours. Brachial FMD, resting blood flow and resting shear rate were measured prior to the femoral measurements at the start of each condition, and again at five hours. The arm was extended and supported by a pillow at the level of the heart. The inflatable cuff was placed around the forearm, distal to the cubital fossa and the artery was imaged following a similar protocol as

that described for the femoral FMD measurement. FMD measurements were taken in the same limbs for all trial conditions. Importantly, all FMD measures occurred prior to the SRA to avoid measuring any transient effects of the SRA that may influence the measurement.

Analyses of artery diameter and blood velocity were performed offline using automated edge detection and wall tracking software (20). Resting diameter and peak diameter post cuff release was used to calculate FMD percentage change. Shear rate (sec-1), calculated from blood velocity and diameter, was used as an estimate of shear stress on the artery wall. The shear stimulus was calculated as the shear rate area under the curve (AUC) from time of cuff release to peak dilation, using the sum of trapezoids method.

Resting blood pressure

Hourly resting brachial BP was measured in triplicate at one minute intervals, using an automated oscillometric BP monitor (Dinamap Vital Signs Monitor 184465X, Criticon, Florida USA) and an appropriately sized cuff, as per recommended guidelines (21). All measurements were taken in the same arm for both conditions by trained research staff. The first measurement was discarded and the average of the second two was used in the analysis.

Biochemical analysis

Whole blood was collected into EDTA tubes and centrifuged within 5-minutes of collection (2000 rpm for 15 minutes at 4°C) and the plasma fraction was separated and stored at -80°C. Samples for ET-1, VCAM-1, ICAM-1 were analyzed by sandwich immunoassay technique using kits from R&D systems (Minneapolis, MN, USA) according to the manufacturer's instructions. The final product of the ELISA was quantified using a Benchmark Plus Microplate spectrophotometer and standard curve (Bio-Rad Laboratories, Hercules, CA, USA) at 450nm

(22). Plasma nitrate and nitrite were measured as an indirect measurement of total NO using a commercial colorimetric kit from Cayman Chemical Company (Ann Arbor, MI, USA).

Statistical analysis. The total AUC across the five hour protocol on each day was calculated for ET-1, nitrates plus nitrites, ICAM-1 and VCAM-1 using the trapezoidal method. We examined within and between condition effects using generalized linear mixed models with random intercepts in Stata 14.2 (StataCorp LP). All models were adjusted for potential covariates including age, sex, BMI, values at zero hour and condition order. A condition by time interaction with posthoc comparsions was used to compare individual time points between conditions and within condition relative to zero hour. Post hoc comparsions between time points were adjusted for multiple comparisons using a Šidák correction. Descriptive data are presented as means ± standard deviation (SD) and output from mixed model analyses are presented as marginal means ± standard error of the mean (SEM) where $p< 0.05$ was considered statistically significant.

Results

Participant characteristics. Of the 21 participants randomized, 19 completed the study (Supplementary Figure 1). The mean ± SD age was 57±12 years, participants were all overweight or obese (30.6±3.4 kg/m^2) and six were taking medication for hypertension. The participant characteristics are presented in Table 1.

Flow mediated dilation and hemodynamics. The raw hemodynamic and FMD data are presented in supplementary Table 1. Table 2 displays corrected data with statistical comparisons. Femoral artery FMD was not significantly different at the zero hour time point between conditions, nor at the 30 minute time point, but was significantly lower at one and two

hours in the SIT condition compared to SRA (3.3±0.6% vs. 9.3±0.6%, p<0.001 and 5.4±0.8% vs. 8.9±0.8%, p=0.007 respectively; Table 2, Figure 2). Femoral artery FMD at five hours was not significantly different between conditions (p>0.05). However, femoral artery FMD averaged across the five hour day was lower in the SIT condition compared to the SRA condition (5.3±0.6% vs. 8.4±0.5% respectively, p<0.001; Figure 2B). No significant differences between conditions were observed for brachial artery FMD at either the zero or five hour time point (p for both>0.05). Additional adjustment for resting diameter or shear stimulus had no significant impact on the models for femoral or brachial FMD (p>0.05) and so were not included as covariates.

Mean resting femoral shear rate averaged across five hours was lower in the SIT condition relative to SRA, although the difference did not reach statistical significance (23.1±9.7s^{-1} vs. 45.7±9.6s^{-1}, p=0.052). Mean resting femoral blood flow averaged across five hours was lower in the SIT condition relative to SRA (1.6±0.4mL·s^{-1} vs. 2.3±0.4mL·s^{-1}, p=0.049). No differences in resting systolic or diastolic BP averaged across five hours were observed between the SIT condition and SRA conditions (117±2 vs. 115±2mmHg, p=0.618 and 69±1 vs. 71±1mmHg, p=0.094 respectively). Mean heart rate averaged over five hours was significantly lower in the SIT relative to SRA condition (70±2bpm vs. 72±2bpm, p=0.003).

Blood biomarkers. Plasma ET-1 total AUC was 14% higher in the SIT condition relative to SRA (8.1±0.3 pg·hr·mL^{-1} vs 7.0±0.3 pg·hr·mL^{-1}, p=0.006; Figure 3). In the SRA condition only, ET-1 concentrations at one, two and five hours, were all significantly lower within condition relative to zero hour (-0.5±0.1pg/mL, -0.6±0.1pg/mL and -0.4±0.1 respectively, p<0.01 for all). In addition, a significant but weak negative correlation was observed between resting blood flow and ET-1 (r^2=-0.25, p=0.001) and resting shear rate and ET-1 (r^2=-0.36,

p<0.001). The plasma nitrate plus nitrite, ICAM-1 and VCAM-1 total AUC over five hours were not significantly different in the SIT condition compared to SRA (281±9 $\mu M \cdot hr \cdot L^{-1}$ vs. 283±9 $\mu M \cdot hr \cdot L^{-1}$, p=0.842; 906 $ng \cdot hr \cdot mL^{-1}$ vs. 912±23 $ng \cdot hr \cdot mL^{-1}$, p=0.847 and 3016±97 vs. 2809±97 $ng \cdot hr \cdot mL^{-1}$, p=0.142 respectively).

Discussion

The principal novel finding of this study was that femoral artery function (measured via FMD) was lower in the SIT condition relative to SRA at the one and two hour time points, suggesting that introducing intermittent activity breaks during the first two hours of prolonged sitting appears to exert the greatest impact on lower limb arterial function. We also found that ET-1 was significantly elevated following five hours of prolonged sitting, compared to the SRA condition. These findings provide pathophysiological insights into the impact of prolonged uninterrupted sitting on endothelial dysfunction and increased CVD risk in overweight/obese adults.

We measured femoral artery FMD at multiple time points across five hours. The magnitude of the decline in femoral FMD in the SIT condition was greatest after one hour of uninterrupted sitting. In line with previous work (5, 7), this suggests that the first hour of prolonged sitting could elicit the greatest impact on femoral artery function. The reasons for a sitting-induced decline in FMD are not completely understood, but may be due to reductions in shear stress and blood flow (20, 23) as well as an increase in blood viscosity (23) in the SIT condition. Although we observed a marked decrease in femoral artery FMD in the SIT condition, we did not observe a concomitant decrease in resting shear rate or blood flow, as observed in some previous studies (4, 5, 7, 22). The participants in these earlier studies were, however, young healthy adults which is in contrast to the relatively older, overweight to obese and sedentary population included in

the current analysis. It is possible that shear and function relationships differ with age and long term exposure to CVD risk factors (24).

Femoral FMD was elevated at one hour and two hours in the SRA condition relative to the SIT condition. This is similar to previous work in healthy populations which has demonstrated that interrupting sitting with light-intensity walking (4, 5), moderate-intensity activity breaks (6) or 'fidgeting' (25) improves lower limb artery function, relative to prolonged uninterrupted sitting. The increase in femoral FMD observed in the current study occurred despite no within-condition increase in resting blood flow or shear rate, which has been reported in previous work (4, 5, 25). This suggests that the improvement in FMD seen with the introduction of intermittent SRA could be due to intrinsic improvement in vessel wall function, and not merely an increase in the FMD stimulus. It is pertinent to mention that the FMD measurement occurred prior to the SRA break in our study, as opposed to immediately following exercise, as in some previous experiments (4, 26). We, therefore, avoided any transient impact of activity related blood flow and shear rate changes on FMD. McManus et al. (6) also measured shear rate and FMD prior to activity breaks and observed no difference in shear rate compared to the sedentary condition. Despite no within-condition differences in resting blood flow, we did observe a between condition difference in the resting blood flow averaged over five hours. Therefore, we cannot completely rule out the possibility that differences in blood flow and arterial haemodynamics may, in part, explain the differences observed in FMD.

Relative to SRA, ET-1 AUC was elevated across the 5 hours of the SIT condition. ET-1 is a potent vasoconstrictor which plays a role in regulating vascular tone and blood flow, especially in older populations (27, 28). There was a weak, but significant, correlation between ET-1 and resting blood flow and similarly between ET-1 and resting shear rate. Indeed, it has been

demonstrated that low levels of shear stress stimulate ET-1 secretion from cultured cells, while higher levels of shear stress have an inhibitory effect (29). In addition, sustained increases in shear stress following hand heating have been shown to result in uptake and clearance of arterial ET-1 via endothelin type B (ET_B) receptors (30). The authors of this study noted that by blocking the ET_B during hand heating, the decline in arterial ET-1 was prevented and radial artery FMD was reduced, despite sustained increases in shear stress. It is possible that the weak correlation between shear rate and ET-1 and lack of correlation between ET-1 and FMD in the current study may be due to measuring venous rather than arterial concentrations of ET-1. That said, our observation of elevated ET-1 in the SIT conidition suggests that in older, sedentary and at-risk populations, interactions between blood flow, shear stress and ET-1 may contribute to sitting-induced impairment in arterial function. More work is required to confirm our findings.

In keeping with previous studies (4, 7), we did not observe a significant reduction in brachial FMD in the SRA condition compared to SIT. It should be noted, however, that brachial FMD was only measured at the start and end of each condition, and the possibility remains that transient differences may have occurred throughout the day. This is supported by the time course of effect in the femoral artery FMD, where differences were less apparent at five hours than they were throughout the intervention period. Further, it is also likely that the SRA had a varried effect on the upper and lower limbs. Alternatively, sitting may differentially impact upper and lower limb artery function, given that in the seated position, the lower limbs are subjected to unique structural and functional milieu (7). Future studies utilizing similar measurement time points for both the brachial and femoral arteries will be necessary to establish, or definitively rule out, a generalized arterial effect of prolonged sitting (31-33).

Although we did not observe differences in BP between conditions, an increase in average heart rate across five hours was evident in the SRA condition, relative to SIT. The absence of a BP lowering impact of activity breaks contrasts with previous evidence demonstrating that regular walking breaks or SRA can lower BP, relative to prolonged sitting (34, 35). It should be noted that the resting BP of our study population was relatively low, and possibly indicates limited potential for improvement.

Limitations. This study was performed in a laboratory setting and while this environment allows for rigorously controlled trials to be conducted, it does not reveal the impact of prolonged sitting on arterial function in a real-life setting, such as in the workplace or at home. We only measured brachial FMD at zero and five hours, limiting the possibility to examine any transient effects in upper limb FMD across the day. Finally, our results cannot be generalized beyond the current study population and future research is needed to compare the effect of prolonged sitting, compared to breaks in sitting, on arterial function in other high risk groups.

Conclusion and perspectives. This is the first study to show that prolonged, uninterrupted sitting has detrimental effects on arterial function in older, overweight/obese adults at heightened risk of CVD across a five-hour day. We also demonstrate that brief periods of simple resistance exercise effectively mitigates this impairment. Given the ubiquitous high volumes of prolonged sitting in contemporary work and recreational settings, and the associated increased risk of CVD and all-cause mortality (36), short, frequent bouts of light-intensity resistance activities may provide a practical and easily translated approach to maintaining healthy arterial function, particularly within the first two hours of prolonged sitting. Future work should aim to examine the longer term impacts of prolonged unbroken sitting, and the impacts of different

interventions that interrupt this ubiquitous behaviour, on arterial (dys)function in high risk populations.

Conflicts of interest. The authors have no conflicts of interest.

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Novelty and Signficance.

What is new?

- This is the first randomized, controlled laboratory trial to demonstrate that prolonged sitting, relative to regular interruptions to sitting time, impaired femoral artery vasodilator function in overweight/obese adults.
- This study also showed that increased circulating vasoactive mediators are elevated during prolonged sitting in overweight/obese adults.

What is relevant?

- Prolonged sitting contributes to cardiovascular disease (CVD) risk and elevates resting blood pressure.
- The underlying mechanisms linking prolonged sitting and increased CVD risk are unknown.
- This study is the first to examine the effects of prolonged sitting on artery function in adults at an increased CVD risk.

Summary

Prolonged, uninterrupted sitting has detrimental effects on arterial function in older, overweight/obese adults at heightened risk of CVD. Short, frequent bouts of light-intensity resistance activities may provide a practical and easily translated approach to maintaining healthy arterial function.

Figure legends.

Figure 1. Study design and protocol. Participants were initially screened over the phone, followed by a screening blood test if eligible. Eligible participants then attended the laboratory on three occasions; familiarization followed by two experimental conditions in a random order. SIT, uninterrupted sitting condition; SRA, sitting interrupted by simple resistance activities condition; FMD, flow mediated dilation.

Figure 2. (A) Time-course of femoral artery FMD in the two conditions. Data are mean ± SD. (B) Mean femoral artery flow mediated dilation (FMD) over five hours in the uninterrupted sitting (SIT) and sitting interrupted by simple resistance activities (SRA) conditions, adjusted for mean FMD at zero hour, age, sex, body mass index, treatment order. Data are marginal means ± SEM, ***$p<0.001$ versus SIT.

Figure 3. (A) Plasma endothelin-1 levels over time. Data are mean ± SD. (B) The effect of uninterrupted sitting (SIT) and sitting interrupted with 3-min simple resistance activities (SRA) on plasma endothelian-1 total area under the curve over five hours, adjusted for age, sex, body mass index, treatment order, values at zero hour and change in plasma volume. Data are marginal means ± SEM. **$p\leq0.01$ versus SIT.

Table 1. Participant characteristics.

N	19
Sex (male/female)	11 / 8
Age (years)	57±12
Body mass index (kg/m^2)	30.6±3.4
Waist circumference (cm)	104.3±10.3
Clinic systolic blood pressure (mmHg)	121±11
Clinic diastolic blood pressure (mmHg)	74±10
Glycated haemoglobin (%)	5.4±0.4
Glycated haemoglobin (mmol/mol)	35.5±4.4
Fasting glucose (mmol/L)	5.2±0.8
Fasting insulin (mmol/L)	110±57
HOMA2-IR	2.0±1.0
Fasting cholesterol (mmol/L)	5.4±1.2
Fasting triglycerides (mmol/L)	1.5±0.6
Fasting HDL cholesterol (mmol/L)	1.7±0.4
Fasting LDL cholesterol (mmol/L)	3.1±0.9
Angiotensin II receptor blockers, n (%)	3 (16%)
Angiotensin converting enzyme inhibitors, n(%)	1 (5%)
Calcium channel blockers, n (%)	2 (11%)
Diuretic, n (%)	1 (5%)
Serotonin reuptake inhibitors, n (%)	2 (11%)

Data are mean±SD; HOMA2-IR, homeostatic model assessment index of insulin resistance; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol.

Table 2. Hemodynamics and flow mediated dilation (FMD) during five hours of uninterrupted sitting (SIT) and sitting interrupted with simple resistance activities (SRA).

	Femoral					Brachial	
	Zero hour	30 minute	One hour	Two hour	Five Hour	Zero hour	Five hour
SIT resting blood flow ($mL \cdot min^{-1}$)	60±8	118±40	101±52	81±27	99±55	78±44	100±26
SRA resting blood flow ($mL \cdot min^{-1}$)	60±8	43±43	165±54	146±26	216±59	85±58	93±25
SIT resting shear rate (s^{-1})	15.3±2.6	29.7±11.8	20.8±12.3	21.1±9.1	29.8±35.9	108.0±84.9	106.6±23.1
SRA resting shear rate (s^{-1})	17.2±2.6	21.4±12.2	42.7±12.3	40.2±8.5	84.8±37.08	105.0 92.6	101.0±23.1
SIT FMD (%)	7.4±0.7	6.3±0.9	3.3±0.6**†	5.5±0.8*	6.2±0.9	10.6±4.5	9.0±0.9
SRA FMD (%)	6.2±0.7	6.4±0.9	9.3±0.6**†	8.9±0.8*†	8.3±1.0	9.9±6.2	9.0±0.9

Data are marginal mean ± SEM; all models adjusted for age, sex, body mass index, treatment order and multiple comparisons. Time points 30 minute, one hour, two hour and five hour additionally adjusted for value at zero hour. * $p \leq 0.01$ between conditions, ** $p \leq 0.001$ between conditions, † $p < 0.05$ within condition vs. zero hour.