Effect of breaking up sedentary time with calisthenics on endothelial function

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Abstract

Background: Periods of prolonged sitting impairs endothelial function in lower limb conduit arteries, which is attenuated with physical activity breaks. The effect of activity breaks on upper limb arteries has not been examined. This study assessed changes in brachial artery endothelial function following either a prolonged sitting period or breaking up this sedentary time by performing sets of calisthenics exercises. **Methods:** Ten healthy participants (6 men) completed two conditions in a counterbalanced order: a) 1-hr 26-min sitting, or b) breaking up this period every 20-min by performing a set of five calisthenics exercises. Brachial artery endothelial function was assessed via ultrasound using the flow-mediated dilation (FMD) technique prior to and following each condition, while brachial shear rate (SR) was acquired after each set of calisthenics. **Results:** There was no significant change in FMD over time ($p=0.09$) or between conditions ($p=0.12$). Compared to sitting, brachial SR increased following each set of calisthenics, with a significant difference after the third break (Sit: 33.94±12.79 s^{-1}; Calisthenics: 57.16±30.48 s^{-1}, $p=0.02$). **Conclusion:** Alterations in SR in the upper limbs suggest calisthenics may be an effective intervention to break up sedentary time and attenuate the potentially deleterious effects of prolonged sitting on cardiovascular health.

Introduction

Sitting is the most prevalent sedentary behaviour (Dunstan et al., 2012a; Owen et al., 2010), particularly in workplaces (Parry et al., 2013), and is emerging as an independent health risk factor (Dunstan et al., 2012a; Healy et al., 2008). Periods of prolonged sitting impairs cardiometabolic health, however this is attenuated by frequently breaking up this time (Dunstan et al., 2012b; Henson et al., 2015; Peddie et al., 2013). Research is emerging to suggest that vascular health is also negatively influenced by prolonged sedentary periods. Sitting for three (Thosar et al., 2014; 2015; McManus et al., 2015) and six (Restaino et al., 2015) hours caused superficial femoral and popliteal artery endothelial function to decrease respectively. Whether upper limb vascular function is also negatively influenced by sitting is less clear, as over these time periods no decline in brachial artery endothelial function was observed (Thosar et al., 2014; Restaino et al., 2015).

Reductions in shear stress appear to strongly mediate sitting-induced impairments in vascular function. Shear stress is a key physiological mechanism in the regulation of endothelial function (Carter et al., 2013; Padilla et al., 2011; Tinken et al., 2009), chronic reductions of which augments the atherosclerotic process (Malek et al., 1999). Prolonged sitting causes shear rate (SR) to decline in the superficial femoral (Thosar et al., 2014; 2015), popliteal (Restaino et al., 2015, 2016) and brachial (Thosar et al., 2014; Restaino et al., 2015) arteries. As brachial artery endothelial function is maintained despite reductions in SR it suggests this vessel may be more resistant to this negative effect of sitting (Restaino et al., 2015).

Using activity breaks to disrupt prolonged sitting periods appears to contribute to the preservation of endothelial function by increasing shear stress. Frequently breaking up prolonged sitting with walking bouts prevents the decline in femoral artery endothelial function that is otherwise observed (Thosar et al., 2015). Physical activity enhances endothelial function (Di Francescomarino et al., 2009) via increased blood flow and shear stress (Tinken et al., 2009; 2010), but this improvement is abolished if shear stress is attenuated using cuff inflation methods (Birk et al., 2013; Tinken et al., 2009; 2010). Moreover, ~~E~~exercise training increases endothelial function due to exposing the vasculature to repeated episodic elevations in shear stress (Green et al., 2011; Tinken et al., 2009). Consequently, this pattern of repeated increases in shear stress is replicated using activity breaks, which may explain their protective role. However, in their study Thosar et al., (2015) assessed SR 25 minutes after the activity breaks therefore any immediate changes were not recorded, which may explain the lack of difference observed between the activity and sitting conditions. Moreover, changes to upper limb endothelial function were not considered.

Upper limb vascular function was assessed when a single ten minute walking bout was completed following an extended sitting period (Restaino et al., 2015). This intervention restored the decrease in popliteal but not brachial microvascular function, possibly due to the lack of increased blood flow to the upper limbs (Restaino et al., 2015). Consequently, a whole body exercise modality such as calisthenics exercises (using body weight as a resistance) may be more effective in enhancing vascular function as this activates both upper and lower limb musculature. Additionally, calisthenics make an ideal workplace intervention as individuals are

not required to leave their working environment, purchase equipment or make workplace adaptations (Carr et al., 2012; Carter et al., 2015).

The purpose of this study was to determine changes in brachial artery endothelial function following either a prolonged sitting period or breaking up this sedentary time performing sets of calisthenics exercises. We also aimed to assess the changes in SR over this time period, to provide a greater mechanistic understanding of the effects of sitting with or without activity breaks on upper limb vascular function.

Methods

Study population

Ten healthy participants (6 men) were screened prior to testing (PAR-Q) and exclusion criteria included: smoker, current medication and presence of apparent cardio-metabolic disease. The experimental procedures and potential risks were explained to participants prior to testing and written informed consent obtained. The University of Essex ethics committee approved the experimental protocol, which conformed to the Declaration of Helsinki.

Study design and procedures

Participants attended the temperature controlled (20-22°C) laboratory at the same time of day (between 9.00-9.30 am) on two separate occasions. Prior to testing participants avoided strenuous exercise for 48-hr and any exercise for 12-hr prior, and completed an overnight fast and abstinence from caffeine. Women were assessed in days 1–7 of the menstrual cycle. Participants randomly completed either 1-hr 26-min of: a) uninterrupted sitting (Sit) or b) disrupting this sitting every 20-min performing a set of calisthenics (Calisthenics). This time period was chosen as previous work has shown changes in SR occur within the first two hours of sitting (Restaino et al., 2015; Thosar et al., 2015). Moreover, due to the novelty of the calisthenics, it was important to assess the feasibility of participants completing this intervention without too much participant burden.

After arrival, participants were fitted with a 3-lead electrocardiogram and separate heart rate (HR) monitor, to assess HR during endothelial function measures and during the physical activity breaks respectively. Participants rested in a supine

position for 20-min followed by baseline assessment of right brachial artery endothelial function (Pre Condition) using the noninvasive flow mediated-dilation (FMD) technique. Participants then moved to a seated position and baseline right brachial artery mean SR was acquired, with SR used as an estimation of shear stress (Johnson et al., 2011). Following this, participants completed one of the experimental conditions. In the Calisthenics condition, mean brachial artery SR was acquired following each activity break (Post Break). Time points were matched in the Sit condition. After the condition baseline FMD and SR measures were repeated (Post Condition) (Figure 1). The same researcher completed all measures.

Condition 1: Sit

Participants remained seated in a chair at a desk for 1-hr 26-min. During this time, non-vigorous arm and leg movements were permitted, enabling participants to complete desk-based activities such as reading and working on a computer (Figure 1a).

Condition 2: Calisthenics

The sedentary period was broken up every 20-min with a 2-min set of calisthenics. This was repeated three times (Break 1, Break 2 and Beak 3), followed by a final 20-min sitting period, therefore totalling 1-hr 26-min (Figure 1b).

Five different exercises were performed: squats, arm circles, calf raises, knees to elbows and forward lunges. Exercises alternatively activated upper and lower body muscles groups to minimise fatigue. Participants performed 8 repetitions of each exercise across a 3 second cycle (24 seconds per exercise) in time to an audible and

visual metronome. Prior to testing written and verbal instructions and demonstrations for each exercise were given, and participants were provided the opportunity to practise any unfamiliar exercises. Participants were instructed to keep exercise technique consistent for each set of exercises.

To ensure consistent duration of break, a standardised transition time between sitting to starting each activity bout and then returning to sitting was included of 25 and 15 seconds respectively.

Vascular endothelial function testing

Assessment of brachial artery FMD was performed according to published guidelines (Harris et al., 2010; Stoner et al., 2012; Thijssen et al., 2011) using M-mode imaging. Arterial blood flow and SRs were assessed using a linear array 10 Mhz Doppler ultrasound in triplex mode (Prosound Alpha 6, Hitachi Aloka Medical, Tokyo, Japan), with an insonation angle of 60°. The ultrasound had built in software enabling automated edge detection for diameter analysis in real time and fast Fourier transform of raw audio data to determine mean blood velocity. Testing procedures were completed as previously described (Carter et al., 2014).

For data analysis, basal arterial diameter was determined as the average of 30 heart cycles prior to cuff inflation; while peak vessel dilation was calculated from the highest average diameter of three consecutive heart cycles after cuff deflation. FMD was calculated as: ((Peak post-occlusion arterial diameter – Baseline mean arterial diameter)/Baseline mean arterial diameter) x 100 %). SR area under the curve

(AUC) was calculated from post cuff deflation until the point of peak vessel diameter and formulated using the trapezoid rule (Harris et al., 2010).

Shear rate measurements

Brachial artery mean SR measurements were acquired at the same location as FMD measures, as indicated by an indelible marking at the site. SRs were measured at Pre Condition, Post Condition and Post each Break (at 22, 44 and 66-min during each condition). Measures were collected at 1.5 minutes following each set of calisthenics, providing time for probe positioning and imaging. Time points were replicated in the Sit condition (Time 1, Time 2 and Time 3; Figure 1).

For each SR measurement 20 heart cycles of blood velocity and arterial diameter data were collected. From this mean SR (4 x [mean blood velocity/arterial diameter at the measurement time]) could be calculated in accordance to published guidelines (Harris et al., 2010).

Heart rate and blood pressure

HR was continually monitored (S610i, Polar Electro Oy, Kempele, Finland) with measures taken at 5 second intervals. Blood pressure (BP) was measured at left brachial artery (MX3 plus, Omron, USA) at the same time points as SR measures and mean arterial pressure (MAP) subsequently calculated.

Physical activity and sedentary behaviour assessment

Participants completed the International Physical Activity Questionnaire (Long form, IPAQ) (Booth, 2000), a validated and reliable measure of adult physical activity (Craig et al., 2003) and sedentary behaviour (Rosenberg et al., 2008).

Statistical analyses

~~All data are presented as mean ± standard deviation (SD).~~ Data were normally distributed, assessed using Shapiro-Wilk test. Technical reasons during data collection meant ten data sets for endothelial function and eight sets for brachial SR were analysed.

Two-way repeated measures ANOVA were used to compare the effect of condition on relative FMD, basal arterial diameters, mean SR and MAP. All post hoc analyses were performed via paired samples t-test with Bonferroni adjustment. Data are presented as means ± standard deviation (SD) from which effects sizes were calculated (partial eta squared η^2). These were interpreted as: η^2=0.01 considered small, η^2=0.06 considered medium and η^2=0.14 considered large (Cohen, 1988). Data was analysed using statistical software (SPSS Version 18.0, IBM Corporation, Somers, NY, USA), with significance accepted as $p \leq 0.05$.

Results

Descriptive statistics

Descriptive statistics are shown in Table 1. Participants were classified as highly active but spent over 6.5 hours per day seated (Table 1).

Endothelial function

For relative FMD, no significant interaction ($F(1,9)= 0.9$, $p=0.37$, $\eta^2=0.09$), condition ($F(1,9)= 3.6$, $p=0.12$, $\eta^2=0.29$) or time ($F(1,9)= 2.9$, $p=0.09$, $\eta^2=0.25$) effects were observed (Pre Sit: 4.58 ±5.20 %; Post Sit: 5.33 ±5.81 %; Pre Calisthenics: 5.36 ±4.07 %; Post Calisthenics: 8.32 ±6.10 %; Figure 2). For SR AUC there were no significant effects for interaction ($F(1,9)= 0.9$, $p=0.36$, $\eta^2=0.14$), condition ($F(1,9)= 0.5$, $p=0.48$, $\eta^2=0.10$) or time ($F(1,9)= 0.8$, $p=0.40$, $\eta^2=0.12$) (Pre Sit: 3261.6 ±2887.8 s^{-1}; Post Sit: 3191.3 ±2619.4 s^{-1}; Pre Calisthenics: 3903.9 ±1948.5 s^{-1}; Post Calisthenics: 3249.8 ±1893.3 s^{-1}). Normalising FMD for SR AUC (dividing the percentage of FMD by SR AUC) resulted in no significant interaction ($F(1,9)= 1.2$, $p=0.31$, $\eta^2=0.16$), condition ($F(1,9)= 0.7$, $p=0.44$, $\eta^2=0.11$) or time ($F(1,9)= 2.9$, $p=0.12$, $\eta^2=0.33$) effects (Pre Sit: 0.001 ±0.004 %/s^{-1}; Post Sit: 0.001 ±0.002 %/s^{-1}; Pre Calisthenics: 0.001 ±0.001 %/s^{-1}; Post Calisthenics: 0.003 ±0.002 %/s^{-1}). There was no main effect for time ($F(1,9)= 2.5$, $p=0.15$, $\eta^2=0.21$) or condition ($F(1,9)= 3.9$, $p=0.52$, $\eta^2=0.05$) for basal arterial diameters (Pre Sit: 3.72 ±0.69 mm; Post Sit: 3.55 ±0.76 mm; Pre Calisthenics: 3.60 ±0.66 mm; Post Calisthenics: 3.57 ±0.79 mm).

Shear rate

A significant interaction effect was observed for brachial SR ($F(4,32)=2.0$ $p=0.02$, $\eta^2=0.12$). Post Break SRs were increased in the Calisthenics condition compared to the same time points in the Sit condition, however post-hoc analysis revealed this difference was only statistically significant following Break 3 with Calisthenics elevating SR by 23.22 s^{-1} more compared to Sit at Time 3 ($p=0.02$, $\eta^2=0.51$; Table 2). Post Condition, Sit SR remained depressed compared to Calisthenics ($p=0.07$, $\eta^2=0.36$). Finally, Pre Condition SRs were not significantly different between conditions ($p=0.88$, $\eta^2=0.01$).

Cardiovascular response

For MAP no significant interaction ($F(5,45)= 0.8$, $p=0.56$, $\eta^2=0.08$), time ($F(5,45)= 1.2$, $p=0.33$, $\eta^2=0.12$) or condition ($F(1,9)= 0.8$, $p=0.41$, $\eta^2=0.08$) effects were observed (Sit Pre: 91.1 ±10.2 mmHg, Post: 88.1 ±8.2 mmHg; Calisthenics Pre: 90.0 ±9.9 mmHg, Post: 90.9 ±7.4 mmHg). HR in the Calisthenics condition showed a significant main effect for time ($F(11,88)= 23.3$, $p=0.001$, $\eta^2=1.0$). Post hoc analysis revealed calisthenics significantly increased HR (Break 1: 90.05 ±8.26 $b.min^{-1}$; Break 2: 92.24 ±6.16 $b.min^{-1}$; Break 3: 92.51 ±7.24 $b.min^{-1}$; Figure 3) compared to ~~Baseline~~ Pre Condition (68.90 ±9.10 $b.min^{-1}$; $p=0.001$) and HR remained elevated above ~~Baseline~~ Pre Condition at 30 seconds following each break ($p=0.001$). In the Sit condition, no main effect for time was observed ($F(4,36)= 1.2$, $p=0.34$, $\eta^2=0.33$) with HR only slightly reduced over time (Pre: 70.25 ±8.92 $b.min^{-1}$; Post: 66.21 ±8.72 $b.min^{-1}$).

Discussion

This study investigated changes in brachial artery endothelial function and SR in response to a prolonged sitting period or breaking up this sedentary time. Sitting did not attenuate brachial SR or FMD. Using calisthenics to disrupt this sitting time caused significant transient increases in HR and brachial mean SR, however this did not significantly increase brachial FMD. Results indicate that brachial artery endothelial function is resistant to the negative effects of one hour of sitting. Data also suggests that over a longer time period using calisthenics to break up sedentary time may improve vascular function.

Calisthenics activity breaks and endothelial function

Using calisthenics to break up sedentary time did not significantly increase brachial endothelial function. Despite what is known about activity-related improvements in endothelial function (Di Francescomarino et al., 2009), this current result is in support of existing research. Although in previous work, short walking bouts to break up sitting attenuated the decline in FMD that otherwise occurred, FMD in the activity condition was also not significantly increased compared to baseline (Thosar et al., 2015). Whilst the FMD results from this current study were not significantly different between conditions, unlike in Thosar et al. (2015) study, FMD was not attenuated in the sitting condition. There are currently no guidelines as to clinically meaningful changes in FMD and consequently, the 2.99 % greater increase in the calisthenics condition compared to the sit condition ~~difference between the conditions~~ may have important vascular heath implications; especially considering the relative acute nature of this study.

For the first time, changes in SR immediately after activity breaks to sitting have been characterised. Brachial SR was elevated following each set of calisthenics and, of particular interest, the increase in SR was greater after break three than break one, suggesting a possible accumulative effect of the calisthenics interventions. Moreover, SR measured 20 minutes after the final activity break had returned to a value similar to baseline; highlighting the need to frequently disrupt sedentary periods in order to maintain elevated SR.

Repeated episodic increases in SR are a suggested mechanism underlying exercise related improvements in endothelial function (Padilla et al., 2008; Tinken et al., 2010). In the current study, although over a short time period, each break replicated this repetitive stimulus, suggesting that SR alterations could be a mechanism to explain the slight increase brachial FMD observed. Indeed, this has previously been proposed as an explanation for the maintenance of femoral artery endothelial function when sedentary time was broken up with walking bouts (Thosar et al., 2015). However, in the study by Thosar et al. (2015) SR measures were taken over 20 minutes after each walk, consequently assessing SR immediately post-activity allows a clearer mechanistic explanation to describe the effects activity breaks during sitting have on the vascular system. Future work should seek to characterise the SR response following an activity break at more regular intervals to determine the length of time over which elevations in SR persist.

Despite calisthenics not significantly changing brachial FMD, this does not imply it is not an effective intervention choice to break up sedentary time. Results show that

mean brachial SR can be acutely increased using this intervention and, as the calisthenics routine employed was a whole body exercise, such elevations may also have been present in the lower limbs. Previous work (Thosar et al., 2015; Restaino et al., 2015; 2016) has shown this vasculature is more susceptible to decreases in endothelial function following prolonged sitting periods. Consequently, future research should use calisthenics and investigate changes in lower limb vascular function.

Sitting did not attenuate endothelial function

In agreement with existing literature (Thosar et al., 2014; Restaino et al., 2015) continuous sitting did not decrease brachial FMD compared to baseline values. Additionally, no significant reductions in brachial SR were observed. This may provide an explanation for this outcome, as decreased SR is a proposed mechanism for the reduction in lower limb endothelial function following sitting (Thosar et al., 2014; 2015; Restaino et al., 2016). Importantly during these studies participants' lower limbs remained motionless (Restaino et al., 2015; Thosar et al., 2015) whereas in this current study participants were permitted to move their upper limbs to complete desk-based tasks. This may have resulted in low level muscular contractions, maintaining blood flow and SR. In support of this, upper limb movement during bed rest enhanced brachial FMD (Hamburg et al., 2007), whilst reducing daily step count attenuated femoral but not brachial FMD, related to a larger reduction in lower limb perfusion compared to the upper limbs (Boyle et al., 2013).

The absence of a decline in mean brachial SR after one hour and six minutes of sitting is in line with existing research, as SR was not significantly reduced after the first hour of a three hour sedentary period (Thosar et al., 2014). However, mean brachial SR was significantly attenuated after two hours of sitting and remained depressed for the further five hours of assessment (Restaino et al., 2015). This indicates that the brachial artery is susceptible to SR reductions, however the results from this current study and Thosar et al. (2014) suggest within an hour of sitting this is not apparent. In the current study, the measure taken after one hour and six minutes resulted in the largest reduction in SR which then remained slightly depressed for the post-condition measure, indicating that reductions may have continued if the time period was extended, in line with existing research (Restaino et al., 2015). Taken together these results support the idea that the brachial artery is more ~~resistance~~ resistive to the negative effects of sitting (Restaino et al., 2015), as popliteal and femoral mean SR are reduced within the first hour of sitting (Thosar et al., 2015; Restaino et al., 2015). Moreover, even when reductions in brachial SR have been observed the magnitude of decline was less than that in the lower limbs and only microvascular function was attenuated (Restaino et al., 2015).

Limitations and future research

As aforementioned, it is likely that the continuous sitting condition duration was not long enough for significant changes to endothelial function and SR to occur. As there is no definition as to what is considered prolonged sitting (Dunstan et al., 2012a) the current design was chosen to provide initial data on calisthenics and endothelial function without excessive participant demands. Previous research considering metabolic health and changes to endothelial function has utilised longer assessment

periods of up to nine hours (Dunstan et al., 2012b; Peddie et al., 2013; Thosar et al., 2014; 2015; Restaino et al., 2015). Additionally, the population assessed were young, healthy and had high levels of physical activity. Larger changes to endothelial function may have occurred with an older or less active population. Indeed, exercise status and age are known to influence endothelial responses (Black et al., 2009). Furthermore, participants' transport to the laboratory was not standardised, therefore pre-testing physical activity may have varied between participants. However, based on the location of the university campus, it is assumed participants would have used car or bus transportation; moreover participants were asked to use the same form of transport for each test visit. Finally, dDue to the pilot nature of this research, it is possible that the study was underpowered to detect changes in FMD., Although as previous work has shown significant differences with a sample size of 12 (Thosar et al., 2015) a power calculation using our data suggests our study may be underpowered and that 30 participants might be needed to detect a significant change in relative -FMD. This As this study shows that calisthenics are feasible for participants to complete therefore future work should therefore use a larger sample size.

This study presents results following a single sitting period, however of greater ecological interest is the influence of repeatedly performing either the calisthenics intervention or prolonged sitting over several days or weeks. This would determine if, as in exercise training studies, the repeated exposure of the vasculature to increases in SR can lead to longer term improvements in endothelial function. Furthermore, if repeated prolonged sitting periods does decrease endothelial function it would further support the need for a reduction of this behaviour in the population.

In practical terms, longer term research within a workplace environment would determine the feasibility of individuals carrying out multiple breaks to their daily routine, alongside assessing behavioural change and adherence.

Conclusions

For the first time, this study demonstrates breaking up sedentary time with calisthenics can lead to transient increases in HR and brachial artery mean SR. This did not significantly increase brachial artery endothelial function, however a longer assessment period more representative of a working day may be needed to result in a significant improvement. Additionally results show that brachial artery endothelial function is not negatively affected by an acute siting period, when upper limb movement typical of daily living is permitted. Overall, the SR results from this study present calisthenics as a potential intervention to break up sedentary time and enhance or maintain cardiovascular health. Longer term research, assessing upper and lower limb endothelial function is required to assess whether repeatedly exposing the vascular system to increases in SR can lead to larger improvements in endothelial function.

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Additional Information

Competing interests:

None declared.

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None declared.

Tables

Table 1: Descriptive characteristics, self-reported physical activity scores and total sitting time of participants (n= 10)

Variable	Mean	SD
Age (years)	27.3	8.1
Body Mass (kg)	82.6	19.7
Height (cm)	172.3	10.4
Physical Activity Score (MET-minutes/week)	3844.0	3271.8
Sitting Time Per Day (Hours)	6.6	2.3
Sitting Time Per Week (Hours)	46.2	16.3

Table 2: Brachial artery shear rate (SR) at baseline (Pre) and after (Post) two experimental conditions: a) uninterrupted sitting for 1-hr 26-min (Sit) or b) disrupting this sitting every 20-min performing a set of calisthenics (Calisthenics). In Calisthenics SR was measured after each break (Break 1, Break 2 and Break 3) and these time points were matched in Sit condition (Time 1, Time 2 and Time 3).

	Sit					Calisthenics				
Measurement	**Pre**	**Time 1**	**Time 2**	**Time 3**	**Post**	**Pre**	**Break 1**	**Break 2**	**Break 3**	**Post**
Brachial SR (s^{-1})	40.95 ±14.67	42.17 ±17.31	46.22 ±14.20	33.94 ±12.79	36.32 ±10.73	40.13 ±16.19	47.26 ±19.37	65.86 ±36.43	57.16 ±30.48*	46.41 ±20.22

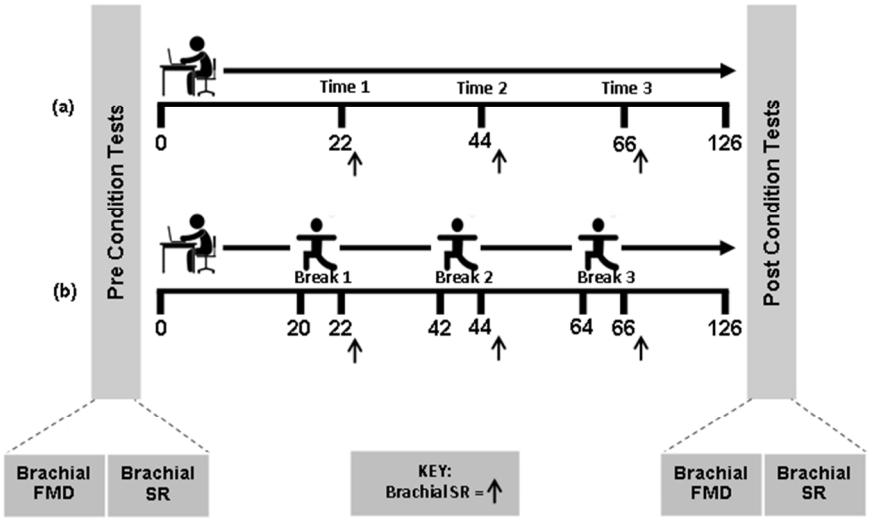
* Indicates Calisthenics was significantly greater than Sit at Break 3 ($p<0.05$).

Figure Legends

Figure 1: The experimental design and measurement time points for the two conditions, completed in a randomised order on separate days. (a) Sitting for 1-hr 26-min, or (b) breaking up this period every 20-min with a 2-min set of calisthenics exercises. FMD- flow mediated dilation; SR- shear rate.

Figure 2: Brachial artery flow mediated dilation (FMD) before (Pre) and following (Post) either 1-hr 26-min uninterrupted sitting (Sit) or, breaking up this period every 20-min with 2-min of performing calisthenics exercises (Calisthenics). (Error bars= ±SD)

Figure 3: Mean heart rate (HR) prior to each condition (Pre), during each calisthenics activity break (Break 1, Break 2 and Break 3), and during the 5-min of recovery following this intervention. * indicates a significantly greater HR than Pre (p=0.001). (Error bars= ±SD)



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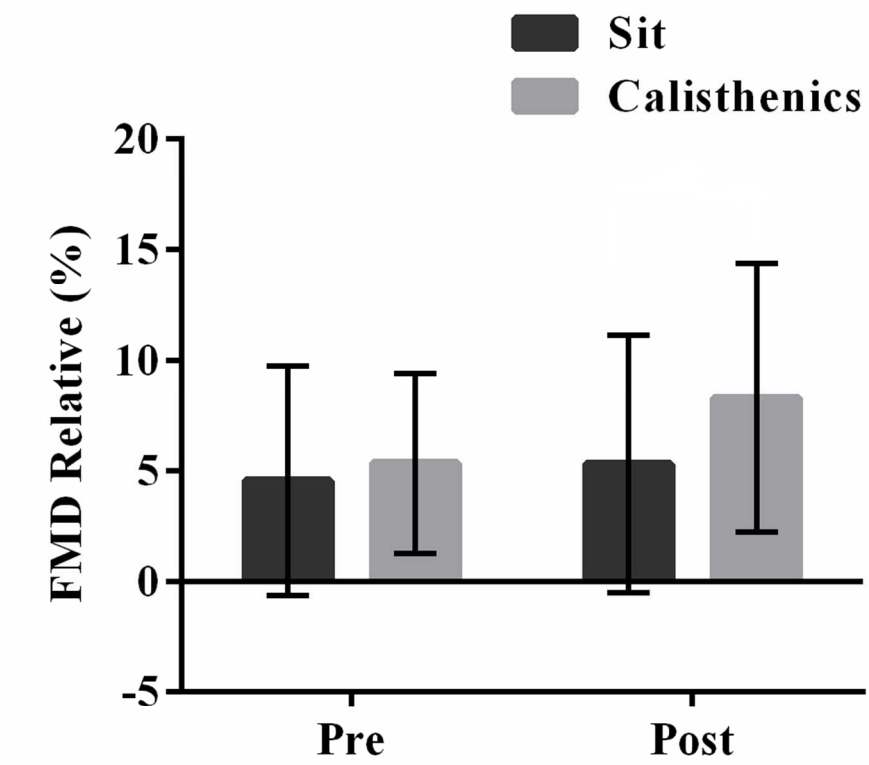

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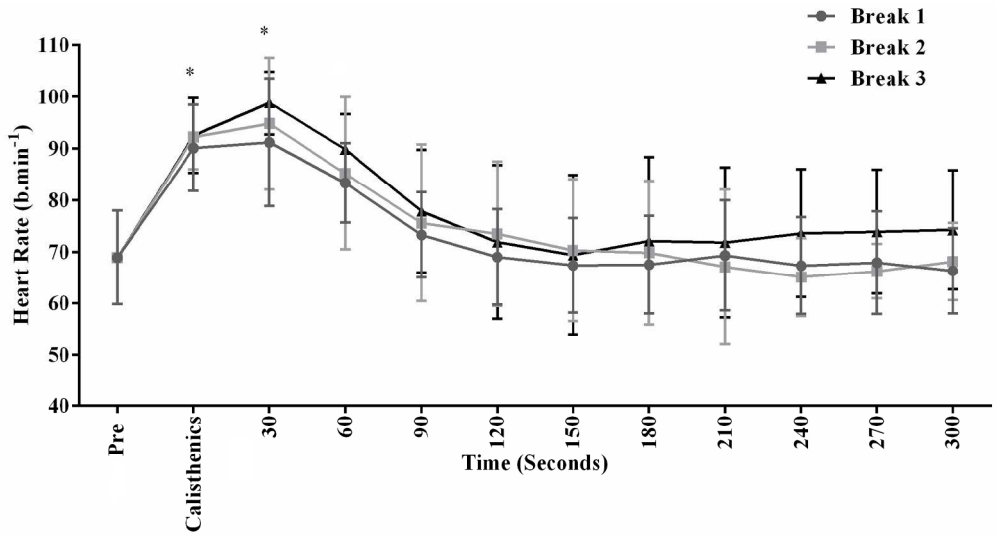

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472x255mm (120 x 120 DPI)