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## Title Page

**Title:** Central and peripheral arterial stiffness responses to uninterrupted prolonged sitting combined with a high-fat meal: a randomized, controlled cross-over trial

**Running title:** Effect of sitting and a high-fat meal on vascular function

\* Simon Fryer<sup>a</sup>, Keeron Stone<sup>a</sup>, Craig Paterson<sup>a</sup>, Meghan Brown<sup>a,b</sup>, James Faulkner<sup>c</sup>, Danielle Lambrick<sup>d</sup>, Daniel Credeur<sup>e</sup>, Gabriel Zieff<sup>f</sup>, Aitor Martínez Aguirre-Betolaza<sup>g</sup>, Lee Stoner<sup>f</sup>

<sup>a</sup> School of Sport and Exercise, University of Gloucestershire, Gloucestershire, UK

<sup>b</sup> School of Health Sciences, Birmingham City University, Birmingham, UK

<sup>c</sup> Department of Sport and Exercise, University of Winchester, Hampshire, UK

<sup>d</sup> School of Health Sciences, University of Southampton, Hampshire, UK

<sup>e</sup> Department of Biology, Ave Maria University, Ave Maria, Florida, USA

<sup>f</sup> Department of Sport and Exercise, University of North Carolina, Chapel Hill, USA

<sup>g</sup> Department of Physical Education and Sport, Faculty of Education and Sport, University of the Basque Country (UPV/EHU), Vitoria-Gasteiz, Araba/Álava, Basque Country, Spain

### \* Corresponding author:

Dr Simon Fryer

University of Gloucestershire

Oxstalls Campus

Longlevens

Gloucester

GL29HW

E: [dr.s.fryer@gmail.com](mailto:dr.s.fryer@gmail.com)

T: +44 (0) 1242 715221

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### Previous presentations

This work has not been presented and is not under review elsewhere.

## Abstract

Independently, prolonged uninterrupted sitting and the consumption of a meal high in saturated fats acutely disrupt normal cardiovascular function. Currently the acute effects of these behaviours performed in combination on arterial stiffness, a marker of cardiovascular health, is unknown. This study sought to determine the effect of consuming a high-fat meal ( $\Delta= 51$  g fat) in conjunction with prolonged uninterrupted sitting (180 min) on measures of central and peripheral arterial stiffness. Using a randomized crossover design, thirteen young healthy males consumed a high-fat (61 g) or low-fat (10 g) meal before 180 min of uninterrupted sitting. Carotid-femoral (cf-) and femoral-ankle (fa-) pulse wave velocity (PWV), aortic-femoral stiffness gradient (af-SG), superficial femoral PWV beta ( $\beta$ ), and oscillometric pulse wave analysis outcomes were assessed pre and post sitting. cfPWV increased significantly more following the high-fat (mean difference [MD]=  $0.59 \text{ m}\cdot\text{s}^{-1}$ ) when compared to the low-fat (MD=  $0.2 \text{ m}\cdot\text{s}^{-1}$ ) meal, with no change in faPWV in either condition. The af-SG significantly decreased (worsened) ( $\eta_p^2= 0.569$ ) overtime in high and low-fat conditions (ratio= 0.1 and 0.1 respectively). Superficial femoral PWV $\beta$  significantly increased over time in high- and low-fat conditions ( $\eta_p^2= 0.321$ ;  $0.8$  and  $0.4 \text{ m}\cdot\text{s}^{-1}$  respectively). A significant interaction found that triglycerides increased over time in the high fat trial only ( $\eta_p^2= 0.761$ ). There were no significant changes in blood pressures. Consuming a high-fat meal prior to 180 min of uninterrupted sitting augments markers of cardiovascular disease risk more than sitting following a low-fat meal.

**Key words:** vascular function; sedentary behaviour; dietary behaviour; hypertension

## Introduction

Epidemiological data suggests that prolonged sitting time <sup>1</sup>, and the consumption of meals high in saturated fats <sup>2</sup> are behaviours which independently increase cardiovascular disease (CVD) risk <sup>3</sup>. There also appears to be a tendency for individuals to perform these two behaviours in combination <sup>4</sup>, potentially further increasing CVD risk <sup>5</sup>. Fortunately these risk factors are suggested to be two of the most modifiable behaviours for CVD risk reduction <sup>3</sup>. However, there is a paucity of research targeted at understanding the physiological mechanisms associated with prolonged sitting in combination with the consumption of meals of different nutritional compositions. In the only study to date, Cho, Bunsawat, Kim, Yoon, Jae <sup>6</sup> reported that following 240 min of prolonged sitting with a high-fat meal, popliteal blood flow and shear rate were reduced, and this was subsequently improved with physical activity interruptions in the form of stair climbing <sup>6</sup>. Whilst these findings support the implementation of PA interventions to attenuate the deleterious effects of sitting, the study had no low-fat 'control group'. Consequently, it is unclear whether the impaired arterial health, was predominantly from the high-fat meal, the prolonged sitting period, or a consequence of these behaviours performed in combination. Currently, it is not known what the effects of prolonged uninterrupted sitting with and without a high-fat meal are on cardiovascular function.

Independently, the cardiovascular consequences of prolonged uninterrupted sitting and a meal high in saturated fat have been well characterised <sup>7,8</sup>. Several studies provide evidence to support the association between post-prandially elevated triglyceride concentrations and endothelial dysfunction. For example, Vogel, Corretti, Plotnick <sup>9</sup> reported that consumption of a one-off meal which is high in triglyceride rich lipoproteins (50 g) reduces endothelial function by 11 % between two- and four-hours post meal, compared to a low-fat meal (0 g). This triglyceride-induced endothelial dysfunction is likely due to a combination of both acute morphological and cellular changes. Independently, endothelial function, assessed using flow mediated dilation, has also been shown to be reduced (2.12 %) by periods of prolonged uninterrupted sitting <sup>7</sup>. Interestingly, prolonged sitting has also been shown to

negatively impact systemic arterial health with changes in both central and peripheral PWV<sup>10</sup>. PWV is considered to be the gold standard assessment for arterial stiffness<sup>11,12</sup>, is a proxy for endothelial function<sup>13</sup>, and can be used to reliably and accurately assess both central and peripheral vascular function. In addition to regional PWV measures, the interaction between central and peripheral vasculature, termed the 'stiffness gradient' has been employed as a novel way to investigate vascular function<sup>14</sup>. Indeed, a reduction in the stiffness gradient has been previously linked to target end organ damage<sup>15</sup> and likely decreases with prolonged uninterrupted sitting or a single high-fat meal.

As such, this study sought to determine the effect of consuming a high-fat meal ( $\Delta$  50 g) in conjunction with a period of prolonged uninterrupted sitting (180 min) on central and peripheral arterial stiffness. Specifically, the aims were to determine the effects of a high-fat meal vs. a low-fat meal in conjunction with prolonged sitting on: 1) central and peripheral PWV, and 2) the novel af-SG and 3) local femoral artery blood flow and stiffness measures.

## **Methodology**

This study is reported in accordance with CONSORT (Consolidated Standards of Reporting Trials) guidelines<sup>16</sup>.

### *Participants*

Thirteen healthy non-smoking male participants were recruited. Participant characteristics were: age  $22.3 \pm 2.2$  yrs.; height  $178 \pm 6$  cm; mass  $76.7 \pm 9.2$  kg. Self-reported data suggested all participants were physically active, exercising  $3.8 \pm 2.1$  times per week, for  $2.2 \pm 1.8$  hours per session. Written informed consent and institutional ethical approval was obtained.

### *Experimental protocol*

Participants visited the laboratory on three separate occasions. During visit one participants were familiarised with all equipment and experimental procedures. Height, body mass, and physical activity status and food allergies or intolerances were determined. The following two experimental visits were randomized (using <https://www.randomizer.org/>) and separated by at least 48 hours but no more than 10 days. Participants were blinded to the meal type until the start of their first trial. Each visit began at 08:30 following an overnight fast. Alcohol and strenuous exercise were abstained for a 24-hour period prior to each visit. Prior to the first visit, the evening meal was recorded and participants were asked to repeat this before their subsequent assessment. This study utilized 180 min of sitting as it has been shown to impair PWV<sup>10</sup>, and for comparative purposes it is currently the most frequently reported time period used for assessing vascular function<sup>7</sup>. During the 180 min sitting period, participants were asked not to partake in boisterous activities, but were allowed to watch a non-stimulatory television documentary.

For each experimental visit, participants quietly lay supine on a test bed for twenty-minutes whilst being fitted with an oscillometric blood pressure cuff (SphygmoCor Xcel, Atcor Medical, Sydney, Australia) over the left upper arm, and thigh and ankle cuffs on the left side of the body to determine central and peripheral blood pressures and cfPWV and faPWV respectively. A continuous wave near infrared spectroscopy (NIRS) (Artinis Medical Systems, BV Zetten, Netherlands) device was placed over the muscle belly of the dominant gastrocnemius to determine changes in blood volume (blood pooling). To ensure the NIRS unit was on the same portion of the gastrocnemius for each trial, the skin was marked with indelible pen, distances from anatomical locations were recorded and photos were taken.

Following the twenty-minute supine rest, a Doppler ultrasound (Terason T3300, Burlington, MA; USA) with a linear array probe (15-4 MHz) collected 3 x 10 s videos of the superficial femoral artery (SFA) on the left side of the body 2 cm distal to the bifurcation. Oscillometric pressure waveforms were assessed using brachial cuff inflation. Following this the SphygmoCor Xcel was used to determine

cfPWV followed by faPWV; the af-SG was subsequently calculated off-line. The participant was then manually moved into a seated position using an electronic three-way tilt table (Plinth 2000, Plinth Medical, Suffolk, UK), and baseline triglyceride concentration was taken. Calf circumference was determined on the dominant gastrocnemius by measuring the area of greatest girth, which was subsequently marked and distance from anatomical landmarks were recorded for following trials. The participant was then given 10 minutes to eat their breakfast. All participants finished their allocated meals. Participants were asked not to move their legs during the 180 min of sitting as leg fidgeting has previously been shown to improve lower limb vascular function<sup>17</sup>, and our study aimed to minimize potential confounding variables between trials as best as possible. Participants were able to urinate in situ if required. No participants needed to empty their bowels during any visits. Blood samples for determination of triglycerides were taken at 30, 60, 120, and 170 min of sitting. Following 180 minutes of sitting, all assessments were repeated.

## Experimental Procedures

### *Meal type*

In accordance with previous research<sup>9</sup>, we used a McDonald's Corporation breakfast meal which included a double sausage and egg McMuffin, two hash browns and a hot chocolate with added double cream (1066 kcal, 4.5 MJ, 61 g fat [of which 20 g was saturated fat], 86 g carbohydrates, 40 g protein and 5 g salt). The low-fat meal consisted of two large English crumpets (Kingsmill Inc.) each with 10 g of low-fat spread (Tesco PLC.), 5 g of Marmite, and 200 ml skimmed milk beverage with 22 g of unflavoured whey protein powder (MyProtein) (601 kcal, 2.5 MJ, 10 g fat [of which 3 g was saturated fat], 86 g carbohydrate, 40 g protein, 5 g salt). A difference of 51 g in fat was used as 50 g has previously been shown to independently cause endothelial function in healthy individuals<sup>9</sup>.

### *Regional pulse wave velocity*

The SpygmoCor XCEL device was used to assess cfPWV and faPWV. PWV is calculated by dividing pulse transit time (PTT) by arterial path length ( $D$ ). For cfPWV, the tonometer was placed on the left carotid artery and the oscillometric cuff was placed on the upper left thigh, following recommended manufacturer guidelines<sup>18</sup>. The carotid-femoral  $D$  was estimated by measuring the linear distance from the suprasternal notch to the top of the leg cuff and subtracting the distance from the suprasternal notch to the carotid artery. For faPWV, the tonometer was placed on the SFA, whilst the ankle cuff (SC10, Hokanson) was positioned at the malleolus. Femoral-ankle  $D$  was estimated by measuring the linear distance between these two points. Femoral-ankle PTT was corrected prior to the calculation of PWV as previously described<sup>19</sup>. A PWV stiffness gradient was calculated as cfPWV / faPWV<sup>15</sup>.

### *Local pulse wave velocity and blood flow*

Local measures of femoral artery  $PWV_{\beta}$  and blood flow provide additional mechanistic information which complement regional arterial stiffness measures when determining the effects of prolonged sitting on arterial function<sup>20</sup>. Three 10s videos of the ultrasound readings were recorded using external video capturing software (LiteCam HD, Englewood Cliffs, NJ; USA). During each 10 s video capture, participants were instructed to hold their breath (without having a large inhalation) throughout the measurement. The video clips were analyzed offline using automated edge-detecting software (FMD Studio, Quipu, Italy) by a trained operator blinded to the condition. Custom written Excel Visual Basic code was used to fit peaks and troughs to the diameter waveforms in order to calculate diastolic, systolic, and mean diameters. Blood flow was calculated from continuous diameter and mean blood velocity recordings using the equation:  $3.14 \times (\text{diameter}/2)^2 \times \text{mean blood velocity} \times 60$ . A local, single-point measure of PWV was calculated using the  $PWV_{\beta}$  equation, previously described in detail by Fryer et al.,<sup>20</sup>

### *Pulse Wave Analysis*

The SphygmoCor Xcel was used to conduct pulse wave analysis (PWA). Oscillometric pressure waveforms are assessed during a brachial cuff inflation, of which a corresponding aortic waveform is generated using a validated transfer function <sup>21</sup>. From sub-diastolic recordings, central: systolic blood pressure (cSBP), diastolic blood pressure (cDBP), pulse pressure (cPP), augmentation pressure (cAP), augmentation index (AIx), augmentation index normalized to a heart rate of 75 bpm (AIx@75), forward aortic pressure (Pf), backward aortic pressure (Pb) and subendocardial viability ratio (SEVR) were derived.

### *Blood sampling*

Using a 1.6 mm lancet, all capillary blood samples were collected using a 32 µL lithium heparin capilliette (Sarstedt Aktiengesellschaft & Co, Germany). Samples were extracted onto a Reflotron test strip (Hoffmann-La Roche LTD) for determination of triglyceride concentration (mg·dL) using a Reflotron Plus (Hoffmann-La Roche LTD).

### *Near infrared spectroscopy (NIRS)*

A Portalite continuous-wave NIRS device, was used to determine blood volume in the gastrocnemius as a measure of blood pooling, pre- and post-180 min of uninterrupted sitting. The Portalite permits determination of oxy-haemoglobin (O<sub>2</sub>Hb) and deoxy-haemoglobin (HHb), the sum of which is total haemoglobin (tHb). Changes in tHb using NIRS have previously been shown to be both valid and reliable for the assessment of lower-limb blood pooling during an orthostatic challenge <sup>22</sup>.

### *Sample size*

Using the effect size of 0.36 derived from the main effect of time i.e. change in cfPWV between pre- and post-180 min of sitting<sup>10</sup>, and the maximum chances of type 1 error set at 5 % (i.e. very unlikely) and power set at 0.8, the approximate number of participants required using G\*Power, was 12. However, 13 were recruited to account for any data errors or participant attrition throughout the study.

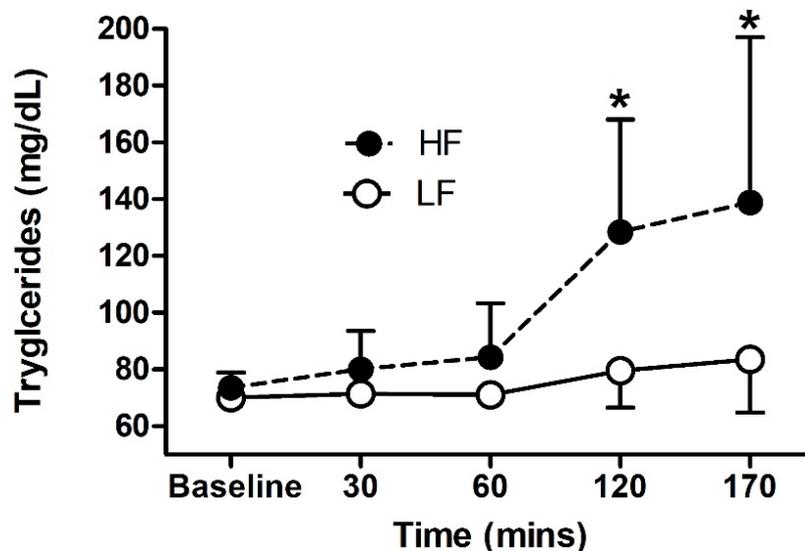
### *Statistical analysis*

Statistical analyses were performed using Statistical Package for Social Sciences version 25 (IBM, Chicago, Illinois). Using the Shapiro-Wilk and Mauchly's test of sphericity, all dependent variables were found to be normally distributed and spherical. In accordance with Fryer, Stone, Zieff, Faulkner, Credeur, Stoner<sup>20</sup> all PWV, PWA and local blood flow measures were collected three times and the average of the closest two were reported. All data are reported as mean, standard deviation (SD), mean difference (MD) and 95 % confidence intervals (CI) unless otherwise stated. For all two-way repeated measures ANOVAs where a significant interaction of time x condition was found, separate one-way ANOVAs with post-hoc Bonferroni were conducted on each condition. When a main effect was reported, paired samples *t*-tests with MD and 95 % CI were reported. As measures of effect size for ANOVA,  $\eta_p^2$  was used where 0.01, 0.06, and 0.14 represent a small, medium, and large effect, respectively<sup>23</sup>. As measures of effect size for *t*-tests, Cohen's *d* was used where 0.2, 0.5 and 0.8 represent a small, medium, and large effect, respectively<sup>23</sup>.  $\alpha$  was set at  $p < 0.05$  (two tailed).

## Results

### Triglyceride concentration

As shown in Figure 1, there was a significant interaction for triglyceride concentration ( $\eta_p^2 = 0.615$ ,  $p = 0.0001$ ). Follow up analyses revealed that triglyceride concentration did not change over time in the low-fat condition. However, there was a significant main effect of time for the high fat condition ( $\eta_p^2 = 0.761$ ,  $p = 0.001$ ), with triglyceride concentration significantly increasing from pre to post 120 min (MD= 55, 95% CI= 18-91 mg/dL, Cohens' $d = 1.85$ ) and 170 min (MD= 65, 95% CI= 11-120 mg/dL, Cohens' $d = 1.52$ ).



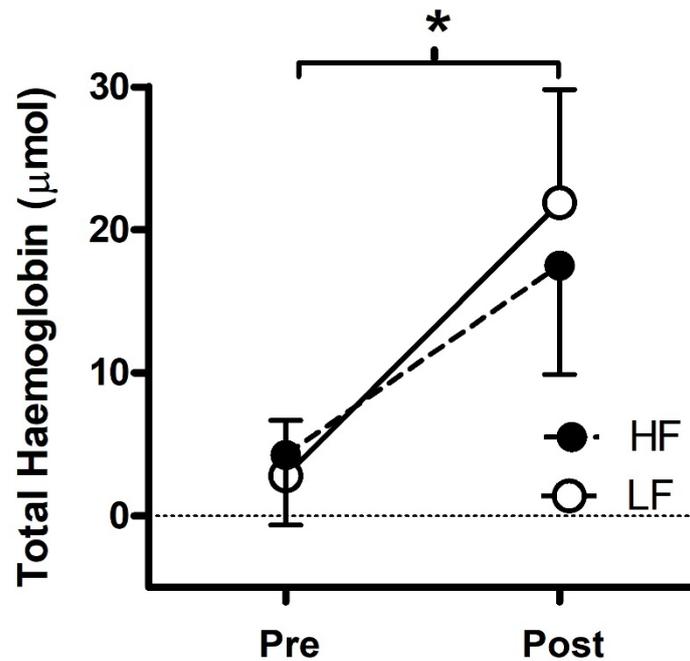
NB: mg·dL= milligram per decilitre; HF= high-fat; LF= low-fat; \*= significantly different from baseline

Figure 1 Mean  $\pm$  SD triglyceride concentrations (mg·dL) during prolonged sitting following high and low-fat meal consumption.

### Calf circumference and blood pooling (tHb)

There was no significant interaction ( $p = 0.089$ ,  $\eta_p^2 = 0.378$ ) for tHb. However, as seen in Figure 2 there was a significant main effect of time ( $p < 0.001$ ,  $\eta_p^2 = 0.877$ ) but not condition ( $p = 0.492$ ,  $\eta_p^2 = 0.061$ ). tHb increased similarly in the high (MD= 14.3, 95% CI= 8.4-20.3  $\mu\text{mol}$ ) and low (MD= 19.1, 95% CI= 14.5-23.7  $\mu\text{mol}$ ) fat conditions. For calf circumference, there was no significant interaction and no

main effect of group, however there was a significant main effect of time ( $p < 0.001$ ,  $\eta_p^2 = 0.928$ ). Calf circumference increased similarly over time in both low-fat (MD= 1.42, 95% CI= 0.94-1.89 cm) and high-fat (MD= 1.66, 95% CI= 0.18-2.06 cm) conditions, indicating similar effects on blood pooling.



NB: \*= significant ( $p < 0.05$ ) main effect of time;  $\mu\text{mol}$ = micromoles

Figure 2 Mean  $\pm$  SD total haemoglobin ( $\mu\text{mol}$ ) in the gastrocnemius at 0 and 180 min of sitting following a low and high-fat meal.

#### Pulse wave velocity

As reported in Table 1, a significant interaction for cfPWV ( $F_{(1,12)} = 7.221$ ,  $p = 0.02$ ;  $\eta_p^2 = 0.376$ ) was found. Follow up analyses revealed no significant change in cfPWV over the 180 min sitting for the low-fat condition (post hoc MD= 0.14, 95% CI 0.05 – 0.34  $\text{m}\cdot\text{s}^{-1}$ , Cohens'  $d = 0.44$ ), but a significant increase following the high-fat condition (post hoc MD= 0.59, 95% CI 0.29 – 0.89  $\text{m}\cdot\text{s}^{-1}$ , Cohens'  $d = 1.12$ ). For faPWV there were no significant interaction or main effects. For the af-SG there was no significant interaction effect or group effect, but there was a significant main effect of time.

Table 1 Mean ( $\pm$  SD), interaction, and main effects for central and peripheral PWV pre (0 min) and post (180 min) prolonged sitting following a low and high-fat meal.

		cfPWV (m·s <sup>-1</sup> )	faPWV (m·s <sup>-1</sup> )	af-SG
<b>Mean Average (SD)</b>				
<b>Low Fat</b>	0 min	5.8 (0.7)	9.3 (1.1)	1.6 (0.2)
	180 min	6.0 (0.5) <sup>+</sup>	9.2 (1.1)	1.5 (0.2) <sup>+</sup>
<b>High Fat</b>	0 min	5.5 (0.5)	9.1 (1.1)	1.6 (0.1)
	180 min	6.1 (0.6) <sup>*+</sup>	9.1 (1.1)	1.5 (0.2) <sup>+</sup>
<b>Interaction Effect</b>				
	<i>p</i>	0.020	0.689	0.154
	$\eta_p^2$	0.376	0.014	0.162
<b>Time Effect</b>				
	<i>p</i>	0.001	0.538	0.002
	$\eta_p^2$	0.640	0.032	0.569
<b>Condition Effect</b>				
	<i>p</i>	0.671	0.418	0.751
	$\eta_p^2$	0.016	0.055	0.009

SD = standard deviation;  $\eta_p^2$  = partial Eta<sup>2</sup>; *p* = significance; \* = significant interaction effect; <sup>+</sup> = significant time effect; cfPWV = carotid to femoral pulse wave velocity; faPWV = femoral to ankle pulse wave velocity; m·s<sup>-1</sup> = meters per second; min = minute; af-SG = aortic-femoral stiffness gradient.

### Local femoral artery measures

As shown in Table 2, there were no significant interactions or main effects for blood flow, diameter, or shear rate at the femoral artery. However, there was a significant main effect of time for femoral artery diameter and PWV<sub>f</sub>.

### Pulse wave analysis

As presented in Table 3, there was a significant interaction for Alx, and Alx@75. Follow up analyses found a non-significant change in Alx over the 180min sitting period for the low-fat condition, but a significant decrease during the high-fat condition (MD= 6.8, 95% CI 4 – 9.6, Cohens' *d*= 0.84). For HR there was a significant main effect of time with HR increasing over the 180min of sitting in both the

low-fat (MD= 3, 95% CI 5 – 0.3 bts·min<sup>-1</sup>, Cohens'd= 0.45) and high-fat (MD= 2, 95% CI 5 – 0.6 bts·min<sup>-1</sup>, Cohens'd= 0.24) conditions. For all other PWA variables in Table 3, there were no significant interaction or main effects.

Table 2 Mean ( $\pm$  SD), interaction, and main effects for local superficial femoral artery measures pre (0 min) and post (180 min) prolonged sitting following a low and high-fat meal.

		Superficial Femoral Artery			
		Blood flow (ml·min)	Shear rate (S <sup>-1</sup> )	Avg. Diameter (mm)	PWV- $\beta$ (m·s <sup>-1</sup> )
<b>Mean Average (SD)</b>					
<b>Low Fat</b>	0 min	290 (162)	87 (24)	6.6 (0.9)	5.9 (1.6)
	180 min	281 (158)	89 (15)	6.4 (0.9)	6.3 (1.1)
<b>High Fat</b>	0 min	296 (117)	93 (32)	6.5 (0.9)	5.6 (0.7)
	180 min	264 (116)	100 (32)	6.3 (0.9) <sup>+</sup>	6.4 (0.9) <sup>+</sup>
<b>Interaction Effect</b>					
	<i>p</i>	0.271	0.493	0.637	0.409
	$\eta_p^2$	0.132	0.044	0.019	0.063
<b>Time Effect</b>					
	<i>p</i>	0.060	0.196	0.030	0.044
	$\eta_p^2$	0.340	0.147	0.336	0.321
<b>Condition Effect</b>					
	<i>p</i>	0.834	0.091	0.599	0.400
	$\eta_p^2$	0.005	0.238	0.024	0.065

SD = standard deviation;  $\eta_p^2$ = partial Eta<sup>2</sup>; *p* = significance; \* = significant interaction effect; <sup>+</sup> = significant time effect; m·s<sup>-1</sup> = meters per second; PWV- $\beta$ = pulse wave velocity beta; S<sup>-1</sup> = Shear; mm= millimetres; ml·min= millilitres per minute; min= minute.

## Discussion

The current study is the first to determine the effects of uninterrupted prolonged sitting in combination with a high-fat meal compared to uninterrupted sitting with a low-fat meal. The main findings were: 1) a high-fat meal immediately prior to 180 min of prolonged uninterrupted sitting caused a significant increase in cfPWV but not faPWV, 2) 180 min of prolonged uninterrupted sitting caused a significant decrease in the af-SG (which is detrimental), and an increase in local (femoral artery) single point PWV $\beta$ , irrespective of meal type.

Table 3 Mean ( $\pm$  SD), interaction, main effects for pulse wave analysis data pre (0 min) and post (180 min) prolonged sitting following a low and high-fat meal.

		Heart rate (bts·min)	SBP (mmHg)	MAP (mmHg)	cSBP (mmHg)	DBP (mmHg)	cPulse Pressure (mmHg)	Alx (%)	Alx@75 (%)	Pf (mmHg)	Pb (mmHg)	SEVR (%)
<b>Mean Average (SD)</b>												
<b>Low Fat</b>	0 min	57 (6)	114 (10)	78 (6)	99 (8)	66 (5)	33 (6)	3 (9)	-6 (8)	25 (5)	14 (9)	171 (24)
	180 min	60 (6)	116 (9)	76 (8)	101 (6)	65 (3)	36 (8)	3 (7)	-4 (7)	27 (3)	12 (1)	160 (18)
<b>High Fat</b>	0 min	59 (9)	118 (7)	78 (5)	102 (6)	65 (4)	37 (7)	7 (9)	-2 (9)	28 (4)	13 (2)	160 (23)
	180 min	61 (9)*	117 (10)	77 (6)	104 (7)	64 (7)	40 (8)	0.4 (7)*, +	-6 (6)*	28 (5)	11 (2)	155 (23)
<b>Interaction Effect</b>												
	<i>p</i>	0.704	0.145	0.690	0.060	0.626	0.065	0.045	0.047	0.192	0.974	0.551
	$\eta_p^2$	0.012	0.168	0.014	0.321	0.020	0.255	0.295	0.290	0.138	<0.001	0.030
<b>Time Effect</b>												
	<i>p</i>	0.029	0.687	0.446	0.626	0.567	0.803	0.010	0.319	0.153	0.416	0.126
	$\eta_p^2$	0.340	0.014	0.049	0.020	0.028	0.005	0.439	0.083	0.162	0.056	0.182
<b>Condition Effect</b>												
	<i>p</i>	0.324	0.091	0.676	0.452	0.429	0.078	0.485	0.537	0.456	0.457	0.195
	$\eta_p^2$	0.081	0.219	0.015	0.048	0.053	0.237	0.041	0.033	0.008	0.047	0.136

SD = standard deviation;  $\eta_p^2$ = partial Eta<sup>2</sup>; *p* = significance; \* = significant interaction effect; + = significant time effect; mmHg = pressure; bts·min<sup>-1</sup> = beats per minute; mmHg= millimetre of mercury; Alx= augmentation index, Pf= pressure forwards, Pb= pressure backwards; bts·min= beats per minute; SEVR subendocardial variability ratio; SBP= systolic blood pressure; MAP= mean arterial pressure; cSBP central systolic blood pressure; cPP= central pulse pressure

### *Limitations and Strengths*

Whilst this is the first study to assess the effects of prolonged sitting with and without a high-fat meal on vascular function, in order to appropriately contextualise the findings, it is important to highlight any limitations and strengths. Firstly, as meal fat content was not prescribed relative to body mass, some participants will have consumed *relatively* more than others. However, the consumption of 50 g of fat irrespective of participants body mass, has previously been shown to reduce endothelial function in healthy adults <sup>24</sup>, and given the SD for participants body mass in the current study is small, any impact on our primary findings is likely to be minimal. Second, all participants were asked to ensure their pre-trial (night before) meals were consistent between visits. Whilst it cannot be guaranteed that participants adhered to this instruction, they were reminded prior to the second session, and a food diary was completed to ensure compliance. Third, due to the sample population being habitually active males, the study's findings are not able to be generalized beyond this. Fourth, as extraneous variables such as leg fidgeting<sup>17</sup> and short walking breaks<sup>25</sup> for the toilet may improve vascular functions such as blood flow, these were not allowed in the current study and therefore the ecological validity is lower. Fifth, we did not assess habitual sitting time before either trial, and as a reduced step count prior to prolonged sitting can negatively impact vascular function<sup>26</sup>, unaccounted reductions in physical activity may have affected our results. Compared to prior literature, a significant strength of the study is the within-participant design, whereby participants are their own control. A further strength of our study was the use of the af-SG, as a novel marker of the integration of central and peripheral vasculature which may provide additional information beyond standard PWV assessments <sup>15</sup>.

### *Comparison to the literature*

cfPWV is considered the gold standard assessment of arterial stiffness <sup>27</sup>, and is an independent predictor of CVD risk <sup>28</sup>. As such, our key finding that a high-fat meal followed by 180 min of

uninterrupted sitting significantly increased cfPWV (MD= 0.6 m·s<sup>-1</sup>) more than a low-fat meal followed by sitting is an important one. Given that we found a significant interaction for triglyceride concentration (Figure 1), with the high-fat trial showing large increases between baseline and 120 (MD= 55 mg/dL) and 170 min (MD= 65 mg/dL) post meal, it seems likely that the increase in cfPWV is caused by the addition of the high-fat meal. Further, independent of sitting induced changes in cfPWV, postprandial elevations in circulating triglycerides after a single meal containing 50 g of triglyceride rich lipoproteins has been shown to impair endothelial function (flow mediated dilation) by 11 %<sup>9</sup>. It also seems likely that the high-fat meal contributed to the increased cfPWV as the response in our study is greater than that previously reported in other non-meal controlled studies<sup>10</sup>. Previously, Credeur, Miller, Jones, Stoner, Dolbow, Fryer, Stone, McCoy<sup>10</sup> reported that prolonged uninterrupted sitting alone (i.e. not dietary manipulation) produced a comparatively smaller increase in cfPWV (0.4 m·s<sup>-1</sup>) compared to the high-fat trial in the current study (0.6 m·s<sup>-1</sup>). As such, findings of the current study suggest that the combined effect of a high-fat meal prior to 180 min of prolonged uninterrupted sitting, augments cfPWV, likely as a result of the combined behaviours worsening endothelial function. However, as the baseline measures in the current study were lower for the high fat trial (0.27m·s<sup>-1</sup>), further research should look to understand whether a lower cfPWV is more sensitive to the deleterious effects caused by sitting and a high fat meal. It should also be noted that as we did not assess step count prior to each trial, and Boyle, Credeur, Jenkins, Padilla, Leidy, Thyfault, Fadel<sup>26</sup> found that reducing step count before sitting worsened popliteal endothelial function following prolonged sitting, we cannot rule out that this did not play a part in the lower baseline high-fat cfPWV seen in Table 1.

In addition to the cfPWV assessment, we also determined regional (faPWV) and local (PWB<sub>β</sub>) measures of arterial stiffness in the leg (Table 2). Interestingly, unlike cfPWV, there was no significant time effect for the regional stiffness measure faPWV but there was a time effect for PWB<sub>β</sub>. These differences in PWV measures may be caused by either the non-uniformity in stiffness across an artery<sup>29</sup>, as endothelial function changes due to the increase in muscularity towards the periphery<sup>30</sup>, or the local

measure (PWB<sub>R</sub>) may be more sensitive given that it is a direct measurement of endothelial function<sup>13</sup>. Previous research into the effects of prolonged uninterrupted sitting have found that 180 min caused a reduction in popliteal endothelial function<sup>17</sup>, which was largely thought to be caused by a reduction in blood flow. Whilst the current study found a reduction in SF artery blood flow over time, it was not significantly different ( $p= 0.06$ ) and the effect size was small ( $\eta_p^2= 0.340$ ). However, this reduction combined with the significant and large increase ( $\eta_p^2= 0.877$ ) in venous pooling, and calf circumference, may have in part caused the increase in local stiffness (PWB<sub>R</sub>) at the SF artery.

Recently, a stiffness gradient has been presented as a novel marker of hemodynamic integration and CVD risk<sup>14</sup>. A stiffness gradient may provide a unique insight into the acute consequences of prolonged sitting and high-fat meals on arterial health. Indeed, reductions in the central to peripheral arterial stiffness gradient can increase the transmission of pulsatile forces to the micro-circulation, potentially leading to target organ damage<sup>15,31</sup>. In the present study, the af-SG was significantly reduced (worsened) in response to 180 min of uninterrupted sitting; but this was not augmented by the high-fat meal (Table 1). The consequences of this reduced gradient are likely to be limited in the relatively young population employed in the present study, given that the stiffness gradient values were maintained above what is considered to be physiologically normal (i.e. values greater than 1.0). However, the central to peripheral arterial stiffness gradient reduces with age, and is accelerated by the presence of disease<sup>14</sup>. Accordingly, if the baseline af-SG is already below one, then any further reduction in the stiffness gradient caused by prolonged sitting would likely heighten the risks of target organ damage. Whilst there is currently no clinical af-SG threshold, future work should seek to identify whether the measure would be informative in elderly and at-risk populations.

Lastly, our study found that tHb significantly increased within the gastrocnemius following 180 min of sitting with no difference between meal types. This increase in lower limb pooling likely plays a large part in explaining the significant decrease in both Alx and Alx@75 following 180 min of uninterrupted sitting. This finding complements that of Credeur, Miller, Jones, Stoner, Dolbow, Fryer, Stone, McCoy

<sup>10</sup> who also reported a decrease in AIX over 180-min with an increase in calf circumference. Whilst not investigated with prolonged sitting, studies using orthostatic stressors (e.g. tilt test) have suggested that the increased pooling leads to a dampened pulse wave reflection <sup>32</sup>, thus reducing AIX. To account for this, the reflected wave (Pb) is believed to mirror peripheral resistance to pressure waves descending from the aorta <sup>21,33</sup>. However, as there were no changes in Pb or blood pressure (Table 3) in either the high-fat or low-fat trials, it is unlikely that peripheral resistance, or changes in blood pressure contributed to the decreased AIX. However, given the effects that lower limb pooling appears to have on AIX, its interpretation as a marker of systemic arterial stiffness during prolonged sitting trials, may be questionable. Further research should determine the validity of using AIX and AIX@75 to assess systemic aortic stiffness in studies which encounter significant venous pooling in the legs.

### *Implications*

Prolonged uninterrupted sitting time <sup>1</sup>, and the consumption of high saturated fats <sup>2</sup> are two of the most important, yet modifiable independent risk factors for cardiovascular disease risk <sup>3</sup>. Our data suggests that when 180 min of uninterrupted sitting is combined with a high-fat meal, cfPWV is augmented compared to uninterrupted sitting with a low-fat meal in a young healthy population. Given that cfPWV is an established predictor of coronary artery outcomes and morbidity <sup>34</sup>, this finding is an important one. Further, whilst there was no effect of meal type, this is the first study to show a significant decrease in the af-SG following 180 min of uninterrupted sitting. Collectively, these findings indicate that consuming a meal high in saturated fat prior to a period of uninterrupted prolonged sitting may cause additional stress on the cardiovascular system, beyond sitting alone. Future studies should seek to determine 1) whether these effects are present in the female population, as well as at-risk and elderly populations, and 2) whether the effects of novel interruption strategies such as sit-to-stand and leg fidgeting, which have been shown to offset sitting-induced dysfunction<sup>7</sup>, reduce this additional cardiovascular burden caused by a high-fat meal and uninterrupted prolonged sitting.

Consuming a meal high in saturated fat prior to prolonged uninterrupted sitting acutely augments known markers of cardiovascular disease risk more than sitting following a low-fat meal. Future studies are needed to 1) identify the long-term effects of combining these behaviours, and 2) determine appropriate interruption strategies to moderate the determinantal changes in central and peripheral cardiovascular function.

### *Conflicts of interest*

There are no conflicts of interests and or relationships between any of the authors and any external partners or companies

### *Author Contributions*

Research design was conducted by SF, KS, LS, DC, MB, JF, GZ & DL. Data collection was conducted by SF, KS, CP, AM, MB. Data analysis and interpretation was conducted by SF, CP, GZ, LS, DC, AM. Manuscript was written and approved by all authors.

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