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<https://orcid.org/0000-0001-6572-7874>, Stöhr, Eric J.,  
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Experimental Physiology

**EP-RP-2018-087067R1**

**Title:** Carotid artery wall mechanics in young males with high cardiorespiratory fitness

**Authors:** Christopher J. APugh  
Keeron J Stone  
Eric J Stöhr  
Barry J McDonnell  
Jane E. S Thompson  
Jack S Talbot  
Denis J Wakeham  
John Cockcroft  
Robert Shave

**Author Conflict:** No competing interests declared

**Running Title:** Cardiorespiratory fitness and carotid artery stiffness

**Abstract:** The influence of cardiorespiratory fitness (CRF) on arterial stiffness in young adults remains equivocal. Beyond conventional measures of arterial stiffness, 2D strain imaging of the common carotid artery (CCA) provides novel information related to the intrinsic properties of the arterial wall. Therefore, this study aimed to assess the effect of CRF on both conventional indices of CCA stiffness and 2D strain parameters, at rest and following about of aerobic exercise in young healthy males. Short-axis ultrasound images of the CCA were recorded in 34 healthy men [22 years (95% CI, 19-22)] before, and immediately after 5-minutes of aerobic exercise (40% VO<sub>2</sub>max). Images were analysed for arterial diameter, peak circumferential strain

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(PCS), and peak systolic and diastolic strain rates (S-SR, D-SR). Heart rate (HR), systolic and diastolic blood pressure (SBP, DBP) were simultaneously assessed and Petersons' elastic modulus ( $E_p$ ) and Beta stiffness ( $\beta_1$ ) were calculated. Participants were separated post hoc into moderate and high fitness groups [ $VO_{2max}$ : 48.9 ml.kg<sup>-1</sup>min<sup>-1</sup> (95%CI, 44.7-53.2) vs 65.6 ml.kg<sup>-1</sup>min<sup>-1</sup> (95%CI, 63.1-68.1);  $P < 0.001$ ].  $E_p$  and  $\beta_1$  were similar between groups at baseline ( $P > 0.13$ ) but were elevated in the moderate-fitness group post-exercise ( $P < 0.04$ ). PCS and S-SR were elevated in the high-fitness group at both time-points [3.0% (95%CI=1.2, 4.9);  $P = 0.002$ ; 0.401/s (95%CI =0.085, 0.72);  $P = 0.02$ , respectively]. No group differences were observed in diameter, HR, SBP, DBP or D-SR throughout the protocol ( $P > 0.05$ ). High-fit individuals exhibit elevated CCA PCS and S-SR, which may reflect training-induced adaptations that help to buffer the rise in pulse-pressure and stroke volume during exercise.

**New Findings:** Common carotid artery (CCA) 2D strain imaging detects intrinsic arterial wall properties beyond conventional measures of arterial stiffness, however the effect of cardiorespiratory fitness (CRF) on 2D strain derived indices of CCA stiffness is unknown. 2D strain imaging of the CCA revealed greater peak circumferential strain (PCS) and systolic strain rate (S-SR) in high fit males compared to their less fit counterparts. Altered CCA wall mechanics may reflect intrinsic training-induced adaptations that help to buffer the rise in pulse-pressure and stroke volume during exercise.

# **Carotid artery wall mechanics in young males with high cardiorespiratory fitness**

Christopher J.A Pugh<sup>1</sup>, Keeron J Stone<sup>1,2</sup>, Eric J Stöhr<sup>1,4</sup>, Barry J McDonnell<sup>1</sup>, Jane E.S Thompson<sup>1</sup>, Jack. S. Talbot<sup>1</sup>, Denis. J. Wakeham<sup>1</sup>, John Cockcroft<sup>1</sup>, Robert Shave<sup>1,3</sup>

<sup>1</sup>Cardiff School of Sport & Health Science, Cardiff Metropolitan University, Cardiff, UK.

<sup>2</sup>School of Sport & Exercise, University of Gloucester, Gloucester, UK.

<sup>3</sup>Centre for Heart, Lung & Vascular Health, University of British Columbia, Kelowna, Canada.

<sup>4</sup>Columbia University Irving Medical Center, Columbia University in the City of New York, New York City, NY, USA

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**Corresponding author:**

Dr Christopher Pugh,  
Cardiff School of Sport,  
Cardiff Metropolitan University,  
Cyncoed Campus,  
Cyncoed Road,  
Cardiff, CF23 6XD, UK

### **What is the central question of this study?**

Common carotid artery (CCA) 2D strain imaging detects intrinsic arterial wall properties beyond conventional measures of arterial stiffness, however the effect of cardiorespiratory fitness (CRF) on 2D strain derived indices of CCA stiffness is unknown.

### **What is the main finding and its importance?**

2D strain imaging of the CCA revealed greater peak circumferential strain (PCS) and systolic strain rate (S-SR) in high fit males compared to their less fit counterparts. Altered CCA wall mechanics may reflect intrinsic training-induced adaptations that help to buffer the rise in pulse-pressure and stroke volume during exercise.

### **Abstract**

The influence of cardiorespiratory fitness (CRF) on arterial stiffness in young adults remains equivocal. Beyond conventional measures of arterial stiffness, 2D strain imaging of the common carotid artery (CCA) provides novel information related to the intrinsic properties of the arterial wall. Therefore, this study aimed to assess the effect of CRF on both conventional indices of CCA stiffness and 2D strain parameters, at rest and following a bout of aerobic exercise in young healthy males. Short-axis ultrasound images of the CCA were recorded in 34 healthy men [22years (95%CI, 19-22)] before, and immediately after 5-minutes of aerobic exercise (40% VO<sub>2</sub>max). Images were analysed for arterial diameter, peak circumferential strain (PCS), and peak systolic and diastolic strain rates (S-SR, D-SR). Heart rate (HR), systolic and diastolic blood pressure (SBP, DBP) were simultaneously assessed and Petersons' elastic modulus (Ep) and Beta stiffness ( $\beta$ 1) were calculated. Participants were separated post hoc into moderate and high fitness groups [VO<sub>2</sub>max: 48.9ml.kg<sup>-1</sup>min<sup>-1</sup> (95%CI, 44.7-53.2) vs 65.6ml.kg<sup>-1</sup>min<sup>-1</sup> (95%CI, 63.1-68.1); P<0.001]. Ep and  $\beta$ 1 were similar between groups at baseline (P>0.13) but were elevated in the moderate-fitness group post-exercise (P<0.04). PCS and S-SR were elevated in the high-fitness group at both time-points [3.0% (95%CI =1.2, 4.9); P=0.002; 0.401/s (95%CI =0.085, 0.72); P=0.02, respectively]. No group differences were observed in diameter, HR, SBP, DBP or D-SR throughout the protocol (P>0.05). High-fit individuals exhibit elevated CCA PCS and S-SR, which may reflect training-induced adaptations that help to buffer the rise in pulse-pressure and stroke volume during exercise.

## Introduction

Large central arteries such as the common carotid artery (CCA) act as low resistance conduits and buffer the rise in blood pressure during cardiac systole. The ability of these elastic arteries to distend and recoil in response to the pulsatile ejection is essential in order to ensure myocardial efficiency and smooth consistent blood flow to the periphery (Greenwald, 2007; Nichols, 2011). However, advanced ageing and/or the presence of cardiovascular disease can alter the elastic composition of the arterial wall matrix, which causes large central arteries to stiffen. As a consequence, increased arterial stiffness can elevate systolic blood pressure and cardiac afterload as well as reduce coronary perfusion (Greenwald, 2007; Nichols, 2011) and is associated with microvessel and target organ damage (O'Rourke & Safar, 2005). Accordingly, arterial stiffness is an important independent predictor of CVD risk and all-cause mortality (Laurent *et al.*, 2006).

Due to its clinical significance, several non-invasive indices of arterial stiffness have emerged, and interventions capable of preventing or reversing arterial stiffness have become highly desirable. Regular exercise training has been shown to reduce arterial stiffness in both healthy and diseased populations (Ashor *et al.*, 2014). Indeed, several studies have reported an inverse relationship between cardiorespiratory fitness (CRF) and conventional measures of arterial stiffness; including aortic pulse wave velocity (aPWV) (Vaitkevicius *et al.*, 1993; Tanaka *et al.*, 1998), augmentation index (AIx) (Binder *et al.*, 2006), beta stiffness index ( $\beta_1$ ) (Tanaka *et al.*, 2000) and Peterson's elastic modulus ( $E_p$ ) of the CCA (Ferreira *et al.*, 2005). However, despite it being well accepted that regular exercise training can attenuate the age-related increase in arterial stiffness (Seals *et al.*, 2009), the influence of CRF in young individuals is less clear. Some studies report CRF to be positively associated with CCA distensibility and compliance (Ferreira *et al.*, 2002; Ferreira *et al.*, 2005), and inversely associated with aPWV (Eugene *et al.*, 1986; Boreham *et al.*, 2004), whereas, others report that CCA compliance (Tanaka *et al.*, 2000) and AIx (Gando *et al.*, 2010) are not influenced by CRF in young adults. Interestingly, these studies have principally assessed arterial stiffness at rest, however, little is known about the influence of CRF on arterial stiffness in response to physiological stress.

Exercise may be a valuable tool to examine the influence of CRF on central arterial stiffness. Compared to resting conditions, arterial stiffness plays a greater role in determining cardiac

afterload, and thus myocardial performance, during physiological stress (Kingwell, 2002; Otsuki *et al.*, 2006). While CRF may only have a modest influence on central arterial stiffness at rest in young adults (Tanaka *et al.*, 2000; Rakobowchuk *et al.*, 2008; Gando *et al.*, 2010; Montero *et al.*, 2017), it is possible that in response to an exercise challenge, high fit individuals may display differential arterial characteristics in comparison with their low-fit counterparts. Reduced central artery stiffness during physiological stress may help buffer the dynamic rise in blood flow and pressure required to meet increased oxygen demand, whilst protecting the smaller down-stream vessels from the significant rise in pulsatile flow and pressure (Kingwell, 2002).

Conventional measures of arterial stiffness, including aPWV, AIx,  $\beta_1$  and  $E_p$  assume vascular homogeneity and tell us very little about the localised deformation characteristics of the arterial wall. Nevertheless, these measures have frequently been used when attempting to reveal the influence of CRF on arterial stiffness in the young (Tanaka *et al.*, 2000; Ferreira *et al.*, 2002; Ferreira *et al.*, 2003; Ferreira *et al.*, 2005; Rakobowchuk *et al.*, 2008; Montero *et al.*, 2017). In contrast, two-dimensional speckle-tracking strain (2D strain) imaging detects heterogeneous motion pattern and local variations in arterial wall compliance, which likely provide a superior index of whole artery wall stress (Bjallmark *et al.*, 2010). Indeed, this technique allows for the assessment of intrinsic arterial wall characteristics, including circumferential strain (extent of arterial wall deformation) and strain rate (rate of arterial wall deformation), which are more sensitive at detecting age-related alterations in the elastic properties of the CCA than conventional measures (Bjallmark *et al.*, 2010). Accordingly, 2D strain imaging may be a valuable tool when attempting to unmask the influence of CRF on central arterial stiffness in the young. Therefore, we aimed to recruit participants across a wide range of aerobic fitness in order to examine the effect of CRF on CCA stiffness at rest and immediately following a brief bout of aerobic exercise in young healthy males using both conventional and 2D strain imaging derived parameters. It was hypothesised that (i) 2D strain imaging would be more sensitive at detecting fitness-induced differences in CCA stiffness than conventional methods at rest; and (ii) a brief bout of aerobic exercise would augment resting differences in 2D strain parameters and cause differences in conventional measures of CCA stiffness to emerge.

## **Methods**

### ***Ethical Approval***

The study conformed to the Declaration of Helsinki, except for registration in a database, and was approved by the Cardiff Metropolitan University School of Sport Research Ethics Committee (15-7-02S). Participants were informed of the methods and study design verbally and in writing before providing written informed consent.

### ***Participants***

Thirty-four male participants were recruited to the study (age;  $22 \pm 3$  yr, body mass index;  $23.6 \pm 2.0$  kg/m<sup>2</sup>). All participants were normotensive, non-smokers with no history of cardiovascular, musculoskeletal, or metabolic disease or any contraindications to exercise. None of the participants reported taking any prescribed medication. Participants were recruited across a wide range of aerobic fitness with the aim of determining whether aerobic capacity influences carotid artery stiffness in a general young population. The thirty-four participants were split *post hoc* by the median [58.4 (IQR: 17.5) ml kg<sup>-1</sup>min<sup>-1</sup>] into a moderate and high VO<sub>2max</sub> group [48.9 ml kg<sup>-1</sup>min<sup>-1</sup> (95% CI, 44.7–53.2) vs 65.6 ml kg<sup>-1</sup>min<sup>-1</sup> (95% CI, 63.1–68.1);  $P < 0.001$ ; Table 2].

### ***Experimental Procedures***

Participants reported to the laboratory on two separate occasions separated by 7 days, and were asked to abstain from alcohol, caffeine and strenuous exercise for 24 hours prior to each visit. During visit one, maximal oxygen consumption (VO<sub>2max</sub>) and peak power output (PPO) were assessed using a standardised incremental ramp exercise test on an upright cycle ergometer (Lode Excalibur, Groningen, Netherlands). Workload was initially set at 120W and continuously increased at a rate of 20W per minute. VO<sub>2max</sub> was measured using a breath-by-breath analyser (Oxycon Pro, Jaeger, Hoechberg, Germany) and calculated as the highest 30 second average of oxygen uptake prior to volitional exhaustion. Criteria for the attainment of  $\dot{V} O_{2max}$  included two of the following: a respiratory exchange ratio (RER)  $\geq 1.15$ , maximal heart rate within 10 beats/minute of age-predicted maximum, or a  $\dot{V} O_2$  plateau with an increase in power output.

During visit two, following ten minutes of rest on a supine cycle ergometer, brachial blood pressure (BP) and heart rate (HR) were assessed and ultrasound images of the right common carotid artery (CCA) were recorded on a commercially available ultrasound system (Vivid Q,

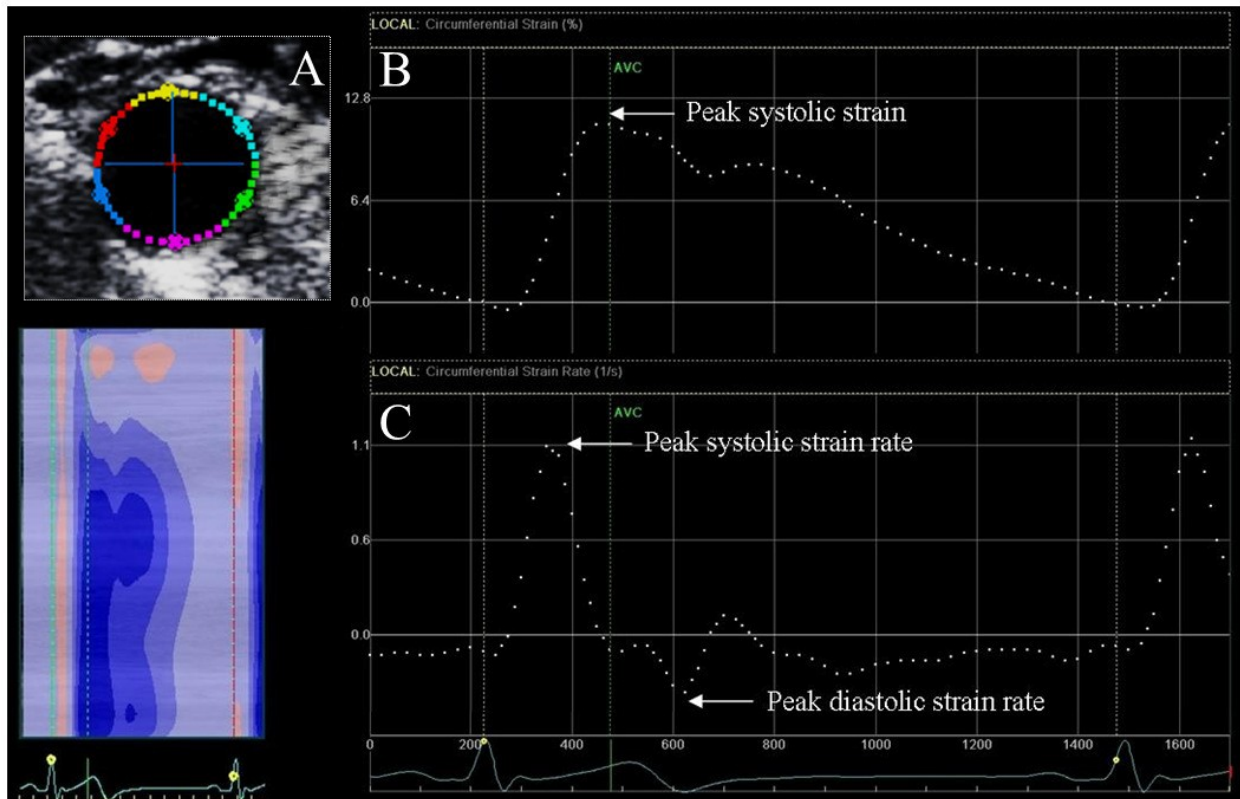


GE Healthcare, Amersham, UK). In addition, conventional measures of CCA stiffness and wave reflection (aPWV and AIx), were also assessed (SphygmoCor, AtCor Medical, Sydney, Australia). BP was obtained with standard auscultation and HR was recorded continuously from a 3-lead ECG inherent to the ultrasound system. Following resting measurements, participants completed a 5 minute bout of supine cycling exercise at an intensity of 40% of the peak power achieved during the  $VO_{2max}$  test, at a fixed cadence of 60 rpm. The brief low intensity exercise stimulus was chosen to minimise the influence of changes in systemic factors upon arterial stiffness (Sugawara *et al.*, 2003). Following the completion of exercise, conventional indices of CCA stiffness and 2D strain parameters were repeated within 2 minutes of exercise cessation.

### ***Vascular Ultrasonography and 2D-Strain Imaging***

Two-dimensional short-axis gray-scale cine loops of the right CCA were recorded 1–2 cm below the carotid bulb over a minimum of three consecutive cardiac cycles using a commercially available ultrasound system with a 12-MHz linear array transducer (Vivid Q, GE Medical Systems Israel Ltd., Tirat Carmel, Israel). Image acquisition was performed by a trained sonographer; frame rate, imaging depth and probe position were kept constant within subjects throughout the protocol to ensure the same section of the CCA was imaged at both time points. Images were stored for subsequent offline analysis using dedicated speckle-tracking 2D-strain software (EchoPac Version 112, GE Vingmed Ultrasound, Horten Norway). Two-dimensional strain software quantifies vascular tissue motion by automatically identifying speckles in the ultrasound image, which are subsequently tracked across the cardiac cycle (Bjallmark *et al.*, 2010). For quantification of strain and strain rates, a region of interest (ROI) was manually placed over the cross-sectional area of the CCA ensuring accurate alignment with the posterior wall (Figure 1A). Within this ROI, movement of speckles were tracked frame by frame throughout systole and diastole using a speckle-tracking algorithm inherent to the software which generated strain and strain rate curves (Figure 1A). Appropriate tracking of the vessel wall was verified automatically by the software and visually confirmed by the operator who manually adjusted the ROI if necessary. Peak circumferential strain (%), systolic strain rate (1/s) and diastolic strain rate (1/s) were measured ‘globally’, reflecting the averaged values obtained from the entire circumference of the arterial wall. Systolic strain rate was defined as the first positive peak in the strain rate curve that occurred after the QRS complex, whilst diastolic strain rate was defined as the first negative peak in the strain rate curve after the T-wave of the ECG (Bjallmark *et al.*, 2010).

Vessel diameters were measured by obtaining an M-mode trace through the centre of the short-axis image. Systolic and diastolic diameters were defined as the maximum and minimum diameters during the cardiac cycle, respectively, and were measured from the leading edge of the intima-lumen interface of the anterior wall to the leading edge of the lumen-intima interface of the posterior wall (Oishi *et al.*, 2008).



**Figure 1.** The region of interest identifying the cross-sectional area of the common carotid artery on a short-axis image (A) and typical global peak circumferential strain (B) and strain rate (C) curves generated using two-dimensional strain imaging.

To characterise local CCA stiffness, Peterson's elastic modulus ( $E_p$ ),  $\beta_1$  stiffness index,  $\beta_2$  stiffness index and distensibility (the inverse of  $E_p$ ) were calculated.  $E_p$ ,  $\beta_1$  and distensibility are conventional measures of arterial stiffness and adjust changes in arterial diameter during the cardiac cycle for changes in pulse pressure (Laurent *et al.*, 2006).  $\beta_2$  relates peak circumferential strain to distending pulse pressure (Oishi *et al.*, 2008). An increase in  $E_p$ ,  $\beta_1$  and  $\beta_2$  stiffness indices indicate an increase in arterial stiffness, whereas, an increase in distensibility indicates a greater magnitude of arterial distension per unit of pressure (Laurent *et al.*, 2006). Stiffness indices were calculated as follows:

$$\text{Distensibility} = [(D_s - D_d) / (SBP - DBP)] / D_d \text{ in } mmHg \times 10^{-3}$$

$$E_p = (SBP - DBP) / ((D_s - D_d) / D_d) \quad \text{in } kPa$$

$$\beta_1 = \ln (SBP / DBP) / ((D_s - D_d) / D_d) \quad \text{in } mm^2/kPa$$

$$\beta_2 = \ln (SBP / DBP / PCS) \quad \text{in } AU$$

Where SBP and DBP indicate brachial systolic and diastolic pressures, respectively,  $D_s$  and  $D_d$  indicate maximal systolic and minimum diastolic CCA diameters, respectively and PCS indicates peak circumferential strain. The reproducibility of the 2D strain imaging and conventional arterial stiffness variables was determined in 10 participants and intra-observer variability was assessed by calculating coefficients of variation (CV) (Table 1). Intra-observer reliability was assessed by performing two ultrasound assessments one hour apart, following a 20 minute period of quiet supine rest. The variability of the 2D strain variables agreed well with previously reported data from our lab (Black *et al.*, 2016) and was considerably lower than the variability reported elsewhere (Bjallmark *et al.*, 2010; Yuda *et al.*, 2011; Charwat-Resl *et al.*, 2016).

**Table 1. The intra-observer variability of 2D strain and conventional local arterial stiffness variables.**

Measured Variable	Mean	SD	Intra-observer CV (%)
<b>Global circumferential variables</b>			
Peak strain (%)	11	2.3	4.9
Peak systolic strain rate (1/s)	1.1	0.2	3.4
Peak diastolic strain rate (1/s)	-0.3	0.1	9.7
$\beta_2$ stiffness index	16.1	3	4.9
<b>CCA diameters</b>			
Systolic (mm)	6.7	0.6	1.0
Diastolic (mm)	5.6	0.5	1.3
<b>Conventional variables</b>			
$E_p$ (kPa)	34.7	4.5	5.7
$\beta_1$ stiffness index	2.8	0.4	5.7

CV: coefficient of variation; CCA: common carotid artery.  $E_p$ : Peterson's elastic modulus.

### ***Aortic Pulse Wave Velocity (aPWV) and Augmentation Index (AIx)***

aPWV and AIx were assessed by an experienced operator using a high fidelity micromanometer tipped probe (SphygmoCor, AtCor Medical, Sydney, AUS) in accordance to applanation tonometry guidelines (Townsend *et al.*, 2015). For the assessment of aPWV, the probe was used to obtain sequential ECG-gated pressure waveforms of the right carotid and femoral artery, at the site of maximal arterial pulsation. Using the R-wave of the ECG as a reference frame, pulse-wave transit time was determined automatically by the SphygmoCor system as the time delay between the carotid and femoral “foot” waveforms. Pulse wave path length was measured as the distance from the femoral sampling site to the sternal notch minus the distance from the carotid sampling site to the sternal notch. aPWV was thereafter calculated as the distance to transit time ratio, expressed in metres per second and normalised to mean arterial pressure (Townsend *et al.*, 2015).

Central AIx was determined by pulse wave analysis by placing the micromanometer tipped probe on the radial artery, just proximal of the radial-ulnar joint. From the radial pressure waveforms obtained, a corresponding central pressure waveform and thus AIx were calculated using a previously validated generalised transfer function inherent to the SphygmoCor system (Chen *et al.*, 1996; Pauca *et al.*, 2001; Sharman *et al.*, 2006). AIx was defined as the difference between the first and second peaks of the central arterial waveform, expressed as a percentage of pulse pressure (Townsend *et al.*, 2015). Measurements of aPWV and AIx were obtained in duplicates with eight to ten cardiac cycles being recorded for each assessment.

### ***Statistical Analysis***

Differences in participant characteristics between moderate and high fit groups at rest were assessed using independent samples t-tests. A two-factor ANOVA (group vs time) was used to identify group differences in arterial stiffness at rest and immediately following exercise. If group differences were observed at rest, additional analysis of post-exercise data was performed, whereby delta ( $\Delta$ ) change from rest was calculated and analysed using analysis of covariance (ANCOVA) with resting data as a covariate. Analyses were performed using the Statistics Package for Social Sciences for Windows, version 21.0 (SPSS Chicago, IL). Data are presented as means (95% confidence intervals), unless otherwise stated. All data were analysed for distribution and logarithmically transformed where appropriate. Logarithmically transformed data were back-transformed to the original units for presentation in the text, and

statistical significance was set *a priori* to  $P < 0.05$  ( $P$  values of “0.000” provided by the statistics package are reported as “ $<0.001$ ”).

## Results

### *Participant characteristics*

All participant characteristics are listed in Table 2. There were no significant differences between the two groups for age, height, body mass or body mass index ( $P > 0.05$ ). By study design, the high fitness group displayed a significantly higher  $VO_{2max}$  than the moderate fitness group [ $16.6 \text{ ml kg}^{-1} \text{ min}^{-1}$  (95% CI = 11.9, 21.4);  $P < 0.001$ ] and subsequently achieved a higher PPO [65W (95% CI = 18, 112);  $P = 0.008$ ]. aPWV was not different between groups ( $P > 0.05$ ), however, AIx was significantly lower in the high fitness group [ $-13.8\%$  (95% CI = -4.8, -22.8);  $P = 0.004$ ].

**Table 2. Baseline characteristics of study participants.**

Characteristics	Moderate Fitness (n=17)	High Fitness (n=17)
Age (y)	21 (20, 22)	21 (19, 22)
Height (cm)	181.1 (176.7, 185.4)	178.0 (174.2, 181.8)
Body mass (kg)	76.6 (71.8, 84.4)	72.6 (69.2, 76.1)
BMI ( $\text{kg/m}^2$ )	23.3(22.6, 24.0)	22.9 (21.8, 24.1)
$VO_{2max}$ ( $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ )	49.2 (43.8, 54.5)	66.7 (63.3, 70.1)*
40% PPO (W)	138 (122, 153)	164 (152, 176)*
aPWV ( $\text{m}\cdot\text{s}^{-1}$ )	5.4 (4.9, 6.0)	5.1 (4.7, 5.4)
Central AIx (%)	8.5 (-0.65, 17.6)	-5.0 (-0.23, -9.7)*

$VO_{2max}$ : Maximal oxygen consumption; PPO: Peak power output; aPWV: aortic pulse wave velocity adjusted for mean arterial pressure; AIx: central augmentation index; \*:  $P < 0.05$  vs. moderate fitness; Data are presented as means (95% CI).

### ***Resting Comparisons***

There were no differences in HR, SBP, DBP, PP or MAP between the moderate fitness and high fitness groups, nor were there any group differences in systolic, diastolic or mean CCA diameter ( $P > 0.05$ ; Table 3). Similarly, conventional parameters of CCA stiffness;  $E_p$ ,  $\beta_1$  and distensibility did not differ between groups ( $P > 0.05$ ; Table 3).

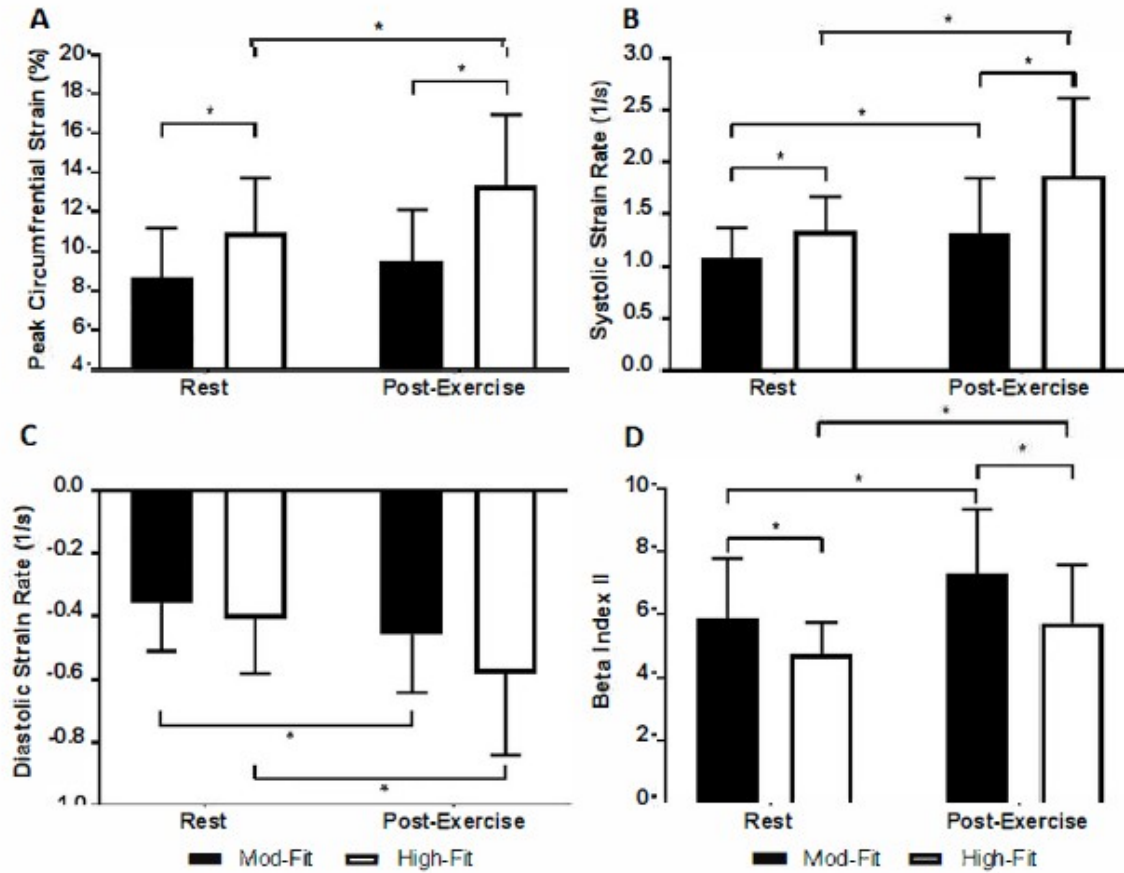
PCS [2.3% (95% CI = 0.43, 4.2);  $P = 0.02$ ; Figure 2A] and S-SR [0.25<sub>1</sub>/s (95% CI = 0.038, 0.46);  $P = 0.02$ ; Figure 2B] were significantly higher in the high fitness group compared to the moderate fitness group, whereas,  $\beta_2$  was significantly lower in the high fitness group [-1.1 (95% CI = -0.02, -2.2);  $P = 0.05$ ; Figure 2D]. There was no difference in D-SR between the high fitness and moderate fitness groups (Figure 2C).

### ***Post-Exercise Comparisons***

There were no group differences in systolic, diastolic or mean CCA diameter or any haemodynamic parameter post-exercise ( $P > 0.05$ ; Table 3).  $E_p$  and  $\beta_1$  were significantly higher in the moderate fitness group post-exercise when compared to the high fitness group [18.3 (95% CI = 1.0, 40.0);  $P = 0.04$ ; 1.2mm<sup>2</sup>/kPa (95% CI = 0.6, 2.4);  $P = 0.04$  respectively]. In addition, distensibility tended towards being greater ( $P = 0.07$ ; Table 3) in the high fitness group following exercise.

PCS was elevated in the high fitness group post-exercise when compared with the moderate fitness group [3.7% (95% CI = 1.6, 5.9);  $P = 0.001$ ; Figure 2A]. Similarly, S-SR was significantly greater [0.55<sub>1</sub>/s (95% CI = 0.10, 1.01);  $P = 0.02$ ; Figure 2B] and  $\beta_2$  significantly lower [-1.6 (95% CI = -0.21, -2.9);  $P = 0.03$ ; Figure 2D] in the high fitness group following exercise. No group differences in D-SR were observed following exercise (Figure 2C).

**Figure 2.** Peak circumferential strain (A; group effect:  $P = 0.002$ ), systolic strain rate (B; group effect:  $P = 0.02$ ), diastolic strain rate (C; group effect:  $P = 0.18$ ) and Beta stiffness index II (D; group effect:  $P = 0.02$ ) of the common carotid artery (CCA) at rest and immediately following 5-min of moderate intensity cycling in moderate and high fitness groups. \*:  $P < .05$  after ANOVA post-hoc analysis; Values are means  $\pm$  SD.



**Table 3. Haemodynamic variables and common carotid artery (CCA) diameters and conventional stiffness indices at rest and following 5-min of moderate intensity cycling.**

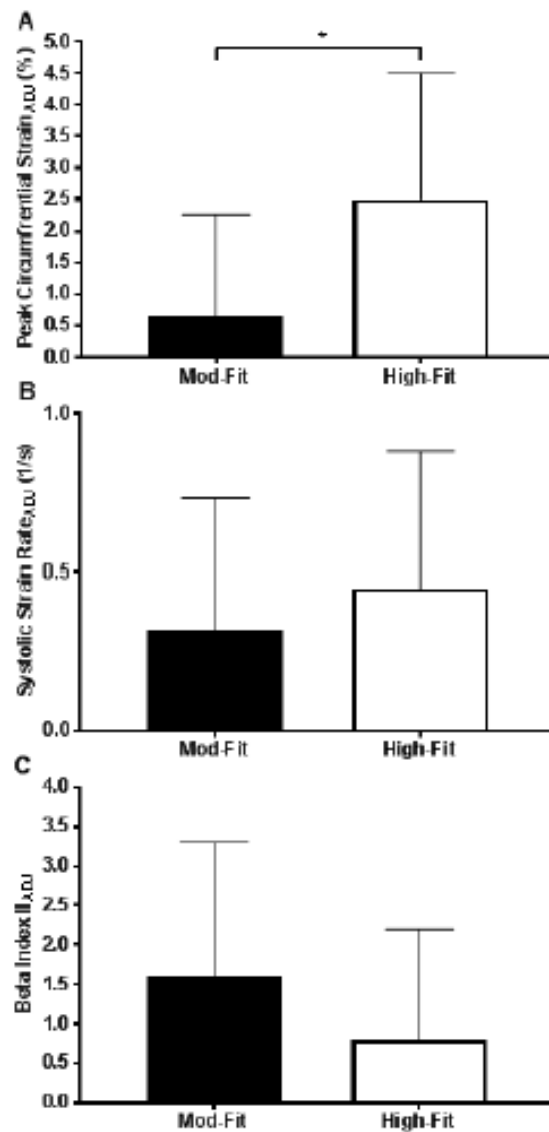
Measured variable (n = 17)	Moderate fitness (n = 17)		High fitness	
	Rest	Post Exercise	Rest	Post Exercise
<b>Haemodynamics</b>				
HR (bpm)	59 (55,62)	76 (70, 82) <sup>†</sup>	54 (48,57)	68 (62,74) <sup>†</sup>
SBP (mmHg)	123 (120,129)	141 (133,149) <sup>†</sup>	120 (116,125)	144 (136,151) <sup>†</sup>
DBP (mmHg)	78 (73,83)	75 (69, 80)	73 (67,79)	70 (65,77)
PP (mmHg)	45 (42,51)	68 (60,76) <sup>†</sup>	47 (43,52)	73 (65,81) <sup>†</sup>
MAP (mmHg)	92 (86,96)	96 (90,101) <sup>†</sup>	89 (84,93)	94 (90,101) <sup>†</sup>
<b>CCA diameters</b>				
Systolic (mm)	6.7 (6.15, 6.74)	6.56 (6.26, 6.86)	6.4 (6.1, 6.69)	6.55 (6.27, 6.82)
Diastolic (mm)	5.63 (5.36, 5.9)	5.64 (5.38, 5.9)	5.45 (5.18, 5.72)	5.38 (5.13, 5.64)
Mean (mm)	6.04 (5.76,6.33)	6.1 (5.81, 6.4)	5.92 (5.65, 6.2)	6.0 (5.68, 6.22)
<b>Stiffness variables</b>				
Ep (kPa)	40 (37, 50)	64 (45, 83) <sup>†</sup>	38 (34, 40)	46 (41, 50) <sup>*</sup>
$\beta_1$ (mm <sup>2</sup> /kPa)	3.1 (2.9, 3.8)	4.6 (3.4, 5.7) <sup>†</sup>	3.0 (2.7, 3.3)	3.4 (3.0, 3.7) <sup>*</sup>
Distensibility (mmHg x10 <sup>-3</sup> )	3.3 (2.9, 3.8)	2.5 (2.0, 3.0) <sup>†</sup>	3.7 (3.4, 4.0)	3.1 (2.7, 3.4) <sup>†</sup>

HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; PP: pulse pressure; MAP: mean arterial pressure; Ep: Peterson's elastic modulus;  $\beta_1$  Beta stiffness index. †: Significantly different to resting value (P <0.05); \* Significant difference between moderate and high fitness groups (P <0.05). Data are presented as means (95% CI).

### *Analysis of Covariance*

Post-exercise group differences in S-SR and  $\beta_2$  disappeared following covariate adjustment for resting data (P > 0.19; Figure 3), however, PCS remained elevated following covariate adjustment in the high fitness group when compared with the moderate fitness group [1.8 (95% CI = 0.25, 3.4); P = 0.03; Figure 3].





**Figure 3.** Delta ( $\Delta$ ) change in peak circumferential strain (A; group effect:  $P = 0.03$ ), systolic strain rate (B; group effect:  $P = 0.39$ ) and Beta stiffness index II (C; group effect:  $P = 0.19$ ) of the common carotid artery (CCA) from rest to post-exercise in moderate and high fitness groups. Data presented following covariate-adjustment (ANCOVA) for resting data. \*:  $P < .05$  after post-hoc analysis; Values are means  $\pm$  SD.

## Discussion

The aim of this study was to assess the effect of high CRF on conventional and 2D strain derived indices of CCA stiffness at rest and immediately following a brief bout of aerobic exercise. In line with our hypothesis, no differences in conventional measures of CCA stiffness were observed between high and moderately fit males at rest, however, 2D strain imaging of

the CCA revealed greater resting PCS and S-SR in high fit males when compared with their less fit counterparts. Immediately following exercise, the magnitude of difference in PCS between groups increased and differences in conventional measures of CCA stiffness emerged, with moderately fit males displaying an elevated  $E_p$  and  $\beta_1$  stiffness compared to high fit males. Taken together, our findings suggest that high fit individuals exhibit elevated PCS and S-SR, which may reflect intrinsic adaptations to the composition of the CCA.

### ***The influence of cardiorespiratory fitness on conventional measures of arterial stiffness at rest***

It is well established that normal healthy ageing is associated with stiffening of large elastic arteries (Lakatta & Levy, 2003; Greenwald, 2007). An abundance of data indicates that regular exercise training can attenuate the age-related increase in arterial stiffness (Seals *et al.*, 2009), however, the influence of CRF on arterial stiffness in young individuals is less clear. In the present study, there was no influence of CRF on conventional measures of local CCA stiffness in young males at rest. These findings are consistent with those from Tanaka *et al.* (Tanaka *et al.*, 2000) who also report no difference in resting CCA stiffness between sedentary, recreationally active and endurance trained young men, despite significant differences in  $VO_{2max}$ . However, our data conflict with the findings of the Amsterdam Growth and Health Longitudinal Study, which reported CRF to be positively associated with both the distensibility and compliance of the CCA in young individuals (Ferreira *et al.*, 2002; Ferreira *et al.*, 2005). Similarly, a recent meta-analysis has demonstrated that aerobic exercise improves regional central arterial stiffness (aPWV and AIx) in young and old individuals, but is most effective in those with greater arterial stiffness at baseline (aPWV  $>8.0 \text{ m}\cdot\text{s}^{-1}$ ) (Ashor *et al.*, 2014). In the present study, our pooled cohort of healthy young males exhibited relatively low arterial stiffness (aPWV  $5.2\pm 0.7 \text{ m}\cdot\text{s}^{-1}$ ), therefore it is perhaps unsurprising that no group differences in aPWV were observed between high- and moderately-fit individuals. Nevertheless, similar to previous research (Edwards & Lang, 2005), the high fit males in the present study did display a significantly lower central AIx than the lower fitness group. AIx has been shown to be a more sensitive measure of arterial stiffness in younger individuals than aPWV (McEniery *et al.*, 2005), which may account for the disparity between these measures in the present study. However, AIx is a derived measure which is reliant on a transfer function to predict the central waveform from a peripheral waveform and is independently influenced by gender, age, height, heart rate and diastolic blood pressure (Hope *et al.*, 2003; Williams, 2004). Nevertheless, as the present participants were well

matched, we suggest that the difference in AIx between the high and moderately fit groups is likely related to the difference in CRF.

***The influence of cardiorespiratory fitness on 2D strain measures of arterial stiffness at rest***

Although numerous studies have reported that exercise training can attenuate the age-related increase in local and regional arterial stiffness (Seals *et al.*, 2009), the limited number of studies

investigating the effect of exercise in young healthy individuals suggest that conventional measures of CCA stiffness remain unaltered following training (Tanaka *et al.*, 2000; Rakobowchuk *et al.*, 2008; Montero *et al.*, 2017). These findings have lead some authors to propose the notion of a *ceiling effect*, which implies that further improvement of young healthy elastic arteries is not achievable (Montero *et al.*, 2017). However, as the arterial wall is not homogeneous, conventional stiffness measures such as  $E_p$ ,  $\beta_1$  and distensibility that assume homogeneity and are limited to 1D measurement of lumen distension may be inaccurate, as they cannot reflect whole arterial wall stress. Furthermore, conventional measures only tell us about the magnitude of change in arterial wall diameter in relation to distension pressure, and nothing about the rate of change. In contrast, the speckle tracking method allows for 2D detection of heterogeneous motion pattern and local variations in arterial wall mechanics, which likely provide a superior index of whole artery wall stress (Bjallmark *et al.*, 2010). In support of this, it has recently been reported that 2D strain imaging is more sensitive at detecting age-related alterations in CCA elastic properties than  $E_p$  and  $\beta_1$  (Bjallmark *et al.*, 2010).

In the present study, resting differences in conventional measures of arterial stiffness were not observed between groups, whereas, PCS and S-SR were elevated and  $\beta_2$  lower in high fit males compared to their less fit counterparts. To our knowledge, this is the first study to investigate the effect of CRF status on 2D circumferential strain and strain rate of the CCA. However, previous research has shown that healthy ageing is associated with reductions in PCS, S-SR and D-SR of the CCA (Kawasaki *et al.*, 2009; Bjallmark *et al.*, 2010), which may reflect age-related degeneration of elastin fibres and compensatory increases in collagen within the extracellular matrix of the arterial wall (Lakatta & Levy, 2003; Greenwald, 2007). Moreover, in the presence of coronary artery disease, PCS and S-SR are further reduced compared to age-matched healthy controls (Kawasaki *et al.*, 2009) and a strong inverse correlation between PCS and Framingham Risk Scores has been observed in asymptomatic individuals (Park *et al.*, 2012). Whilst pathological alterations to intrinsic arterial wall properties may, in part, explain the reduction in CCA PCS and S-SR in older and diseased

populations, it is possible that exercise-induced improvements in the relative proportion of elastin and collagen explain the differences in PCS and S-SR between the high- and moderately-fit young males in the present study. Indeed, animal studies have reported that exercise training increases elastin content within central arterial walls and reduces the percentage of collagen, frayed elastin fibers and the calcium content of elastin within the extracellular matrix tissue (Matsuda *et al.*, 1993; Koutsis *et al.*, 1995). Alternatively, resting PCS and S-SR may be elevated in high fit individuals due to training-induced alterations in systemic vascular tone. A combination of enhanced endothelial function, increased basal levels of nitric oxide, reduced oxidative stress and alterations in sympathetic tone are frequently observed following exercise training (Green *et al.*, 2011; Green *et al.*, 2017), which may also contribute to reductions in arterial stiffness.

### ***The value of exercise in the assessment of local arterial stiffness***

PCS and S-SR remained elevated in the high fit group immediately following the acute bout of moderate intensity exercise. In addition, PCS increased in response to exercise in the high fit group but remained unaltered in the moderate-fitness group. Importantly, this was observed following covariate adjustment for group differences in resting PCS and despite comparable changes in heart rate, blood pressure, MAP and arterial diameter between the groups. In contrast, post-exercise group differences in S-SR and  $\beta_2$  disappeared following covariate adjustment for resting data. It is likely that a superior magnitude and rate of artery deformation during cardiac systole will facilitate an enhanced ability to buffer the exercise-induced elevation in blood pressure and blood flow in the high fit individuals and may represent a training-induced adaptation. An enhanced ability to buffer this dynamic pulsation is likely to provide a smooth consistent blood flow to the periphery and improve myocardial efficiency (Kingwell, 2002), ultimately facilitating an enhanced fitness level. Furthermore, the efficient buffering of the dynamic elevation in blood pressure and flow may also prevent microvessel and target organ damage further down the arterial tree (O'Rourke & Safar, 2005). Given that a primary role of the CCA is to aid the regulation of cerebral blood flow (Hirata *et al.*, 2006), a reduced ability to buffer blood pressure and flow elevations may have significant pathological consequences, including increased risk of stroke (Mattace-Raso *et al.*, 2006; Yang *et al.*, 2012). Consequently, the association between CRF and carotid artery characteristics may have greater importance with advancing age, especially as circumferential strain and strain rate have been shown to reduce with healthy aging (Bjallmark *et al.*, 2010).

Central arterial stiffness has previously been shown to not change (Munir *et al.*, 2008) or to be reduced (Kingwell *et al.*, 1997; Sugawara *et al.*, 2003) during recovery from brief, low/moderate-intensity cycling. In the present study, we did not observe any group differences in  $E_p$  and  $\beta_1$  at rest, however, both parameters increased in response to exercise in the moderate fitness group but remained unaltered in the high fitness group. These observations may reflect an enhanced capacity to modulate acute exercise-induced alterations in sympathetic adrenergic vasoconstrictor tone, endothelial function, humeral vasoconstrictor release and oxidative stress in high fit individuals (Green *et al.*, 2011; Green *et al.*, 2017). Importantly, these findings indicate that exercise is a valuable stimulus capable of revealing fitness-induced differences in conventional measures of arterial stiffness that were unidentified under resting conditions. Additionally, this finding also supports the observation of superior PCS and S-SR following exercise in high fit individuals, which together may reflect a greater ability to buffer exercise-induced increases in pulse-pressure than their less fit counterparts.

### ***Limitations and Future Research***

We acknowledge that the present findings were obtained in healthy young males and that female, elderly and diseased populations may demonstrate a different interaction between aerobic fitness and 2D strain derived parameters of CCA stiffness. We also recognise that not collecting data during exercise is a limitation of present study. It was felt that the movement associated with exercise would have compromised the ability to collect acceptable 2D ultrasound images. In future studies, with practice and appropriate participant familiarisation, it may be possible to collect these data during exercise. Comparisons between fitness groups at higher absolute and relative exercise intensities may also help to further unmask the influence of CRF on CCA properties in the young. Applanation tonometry of the CCA would have provided a more accurate representation of central arterial pressure and we also acknowledge that our findings are restricted to the CCA and therefore cannot be applied systemically. Future studies should also measure 2D strain indices within peripheral arteries to compare the impact of CRF on the intrinsic arterial wall mechanics of both elastic and muscular arteries. Finally, it is important to acknowledge that whilst we assessed CRF, we did not record training history of the participants nor did we recruit a sedentary control group. As such, we are not able to delineate between the influence of intrinsic CRF and the influence of exercise training-induced adaptation or the independent deleterious effect of sedentary behaviour on arterial stiffness. Future studies should investigate the independent impact of sedentary behaviour on CCA stiffness and examine the possible interaction between sedentary behaviour and CRF on arterial health.

## *Conclusion*

This is the first study to demonstrate that high fit individuals exhibit distinct CCA wall mechanics to their less fit counterparts. Elevated PCS and S-SR may reflect training-induced adaptations that help to buffer the significant rise in pulse-pressure and stroke volume that occur during exercise. Longitudinal studies that adopt 2D strain imaging techniques are required to further investigate the influence of exercise training on intrinsic arterial wall mechanics.

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**Competing Interests**

None declared

**Author contributions**

CJAP, K.S and R.S. contributed to the conception and design of the experiment, data collection, analysis, interpretation of the data and the drafting of the manuscript. E.J.S., B.J.M., J.T., J.S.T., D.J.W and J.C. contributed to data collection and analysis and the critical revision of the manuscript for its intellectual content. All authors have approved the final version of the manuscript.

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