The influence of habitual endurance exercise on carotid artery strain and strain-rate in young and middle-aged men.

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New Findings:

What is the central question of this study?

- Carotid artery peak circumferential strain (PCS) and strain-rate attenuate with age, but appear to be modulated by cardiorespiratory fitness status in young males. However, the relationship between habitual endurance exercise (running) and these parameters has not been studied in young and middle-aged men.

What is the main finding and its importance?

- Young and middle-aged runners exhibited elevated PCS and systolic strain-rate (S-SR) compared to nonrunners, but habitual running did not influence diastolic strain-rate (D-SR).
- Habitual exercise is associated with comparable improvements in carotid strain parameters in young and middle-aged men, but the age-related decline in PCS and S-SR may be more amenable to habitual endurance exercise than D-SR.

ABSTRACT

Central arterial stiffness is an independent predictor of cardiovascular risk that can be modified by exercise training. However, conventional local measures of carotid artery stiffness display conflicting responses to habitual endurance exercise in young and older adults. 2D-Strain imaging of the common carotid artery (CCA) quantifies circumferential deformation (strain) of the arterial wall across the cardiac cycle, which is more sensitive at detecting age-related alterations in CCA stiffness than conventional methods. Therefore, the study was designed to examine the relationship between habitual endurance exercise (running) and CCA 2D-Strain parameters in young and middle-aged men. Short-axis ultrasound images of the CCA were obtained from 13 young nonrunners (23 years [95% CI: 21-26]), 19 young runners (24 [22-26]), 13 middle-aged nonrunners (54 [52-56]) and 19 middle-aged runners (56 [54-58]). Images were analysed for peak circumferential strain (PCS; magnitude of deformation) as well as systolic and diastolic strain-rate (S-SR and D-SR; deformation velocity) and group differences were examined via two-way ANOVA. PCS, S-SR and D-SR were attenuated in middle-aged males when compared to young men (all $P \leq 0.001$). PCS and S-SR were elevated in young and middle-aged runners when compared to nonrunners ($P = 0.002$ and $P = 0.009$ respectively), but no age*training status interaction was observed. In contrast, there was no influence of habitual running on D-SR. Habitual exercise is associated with comparable improvements in CCA 2D-Strain parameters in young and middle-aged men, but the age-related decline in PCS and S-SR may be more amenable to habitual endurance exercise than D-SR.

Introduction

Arteriosclerosis, a hallmark of advanced ageing, is characterised by alterations to the elastic composition of the arterial wall matrix of large central arteries, which promotes arterial stiffening (Lakatta & Levy, 2003) and impairs a vessel's ability to buffer pulsatile blood flow during systole (Liao et al., 1999). Consequently, increased aortic and common carotid artery (CCA) stiffness are both associated with elevated blood pressure (Kaess et al., 2012; Liao et al., 1999), impaired cardiac function (Bruno et al., 2017; Kawaguchi, Hay, Fetics, & Kass, 2003) and target organ microvessel damage (O'Rourke & Safar, 2005; van Sloten & Stehouwer, 2016) and are independent predictors of cardiovascular disease and all-cause mortality (Laurent et al., 2006; van Sloten et al., 2014). Therefore, management strategies capable of attenuating the age-related progression of aortic and CCA stiffening have considerable clinical implications.

Regular endurance exercise can attenuate both aortic and CCA stiffening in middle-aged and older healthy adults (Tanaka et al., 2000; Tanaka & Safar, 2005; Vaitkevicius et al., 1993), while cardiorespiratory fitness (CRF) is inversely associated with aortic and CCA stiffness in ageing populations (Tanaka, DeSouza, & Seals, 1998; Vaitkevicius et al., 1993). However, the influence of CRF (Ferreira et al., 2005; Ferreira, Twisk, Stehouwer, van Mechelen, & Kemper, 2003; Tanaka et al., 2000) and exercise training (Heydari, Boutcher, & Boutcher, 2013; Kakiyama et al., 2005; Montero, Breenfeldt-Andersen, Oberholzer, & Haider, 2017; Tanaka et al., 2000) on aortic and CCA stiffness appears to be less consistent in young asymptomatic individuals, leading some to propose that only aged arteries can be modulated by exercise (Montero et al., 2017).

The majority of these studies utilised aortic pulse wave velocity (aPWV) as the 'gold-standard' non-invasive measurement of 'central' arterial stiffness. Whilst aPWV is a valuable marker of central artery stiffness and an independent predictor of cardiovascular risk (Laurent et al., 2006), it is limited to the aorta and assumes vascular homogeneity. This is problematic, as adults with similar aPWV can demonstrate differences in local indices of CCA stiffness (Priest, Shenouda, & MacDonald, 2018), inferring that arteriosclerosis has a heterogeneous effect at distinct regions of the central arterial tree. Since the CCA is a frequent site of atheroma formation (Laurent et al., 2006), local measures of CCA stiffness provide important prognostic information beyond aPWV and are independently associated with incident stroke (van Sloten et al., 2015). Accordingly, sensitive measures of local CCA stiffness are required alongside

aPWV to comprehensively characterise artery stiffness across the arterial tree. Although conventional CCA stiffness indices, such as β-stiffness index (β-stiffness), Peterson's Elastic Modulus (E_p) and distensibility coefficient have valuable reference standards (Engelen et al., 2015; Uejima et al., 2020), these parameters also assume vascular homogeneity, are limited to one-dimensional measurement of lumen distension and display low intra- and inter-observer reliability (Bjallmark et al., 2010). This emphasizes that a sensitive 'gold-standard' measure of CCA stiffness does not yet exist, and may explain why the influence of exercise training on conventional CCA stiffness parameters is varied in young individuals (Montero et al., 2017; Tanaka et al., 2000) and not always consistent with aPWV responses in older populations (Shibata et al., 2018).

Recently, two-dimensional strain imaging (2D-Strain) has emerged as a valuable technique to detect heterogeneous wall motion of the CCA, which provides localised deformation characteristics of the entire arterial wall (Bjallmark et al., 2010; Pugh et al., 2018; Rosenberg et al., 2018; W. I. Yang et al., 2018). This technique quantifies the magnitude and rate of circumferential deformation (strain) of the CCA wall across the cardiac cycle (Bjallmark et al., 2010); which has been reported to offer a more sensitive characterisation of age-related increases in CCA stiffness than conventional measures, as well as superior intra- and inter-observer reliability (Bjallmark et al., 2010; Rosenberg et al., 2018). Specifically, peak circumferential strain (PCS) as well as systolic and diastolic strain rate (S-SR and D-SR) are attenuated in healthy middle-aged adults compared to younger counterparts (Bjallmark et al., 2010), and are further diminished in stroke patients (W. I. Yang et al., 2018). Furthermore, we have recently demonstrated that CCA PCS and S-SR are elevated in young males with high CRF compared to those with lower CRF, despite no differences in conventional indices of CCA stiffness or blood pressure (Pugh et al., 2018). As PCS likely reflects overall loading capacity of the CCA, whereas S-SR and D-SR relate more to the intrinsic elastic properties of the vessel (Teixeira et al., 2015), it is plausible that the influence of habitual endurance exercise on these distinct CCA stiffness parameters may differ between young and middle-aged individuals. Therefore, 2D-Strain imaging may provide novel insight into the interaction between habitual endurance exercise and healthy ageing of large elastic central arteries. Accordingly, the primary aim of this study was to examine the relationship between habitual endurance exercise and CCA 2D-Strain parameters in young and middle-aged men. We hypothesised that male habitual runners would exhibit elevated 2D-Strain parameters compared to age-matched male nonrunners, while ageing would attenuate 2D-Strain parameters irrespective of training status.

Methods

Participants

Sixty-four male participants were recruited, including 13 young nonrunners (23yrs [95% CI; 21 - 26]) 19 young runners (24yrs [22, 26]), 13 middle-aged nonrunners (54yrs [52, 56]) and 19 middle-aged runners (56yrs [54, 58]). All participants were normotensive non-smokers with no history of cardiovascular, metabolic or renal disease or any contraindications to exercise. None of the participants reported taking prescribed medication and all middle-aged participants were screened for ECG abnormalities at rest and during exercise (SECA CT8000P, Vogel & Halke, Hamburg, Germany) (Drezner et al., 2013). Middle-aged runners had performed ≥ 15 miles per week of moderate to intense training for ≥ 10 years, whereas young runners had performed ≥ 30 miles per week of moderate to intense training for ≥ 2 years. Middle-aged and young male nonrunners were recreationally active i.e. ≤ 3 hours of structured physical activity per week for ≥ 10 years and ≥ 2 years respectively. We specifically chose to recruit middle-aged runners (i.e. 50-60 years), rather than older runners onto the study, as this decade appears to represent a focal point during which central arterial stiffness (as determined by aPWV) exponentially increases as part of healthy vascular ageing (AlGhatrif et al., 2013). In addition, due to the recognised differences in haemodynamic regulation between men and women (Hart & Charkoudian, 2014), temporal changes in arterial function across the menstrual cycle (Green et al., 2016) and well-established fluctuations in haemodynamic and vascular control during menopause (Low, Hubing, Del Coso, & Crandall, 2011), only male participants were recruited onto the study. Ethical approval was provided by Cardiff Metropolitan University's School of Sport and Health Sciences Research Ethics Committee (16/7/02R) and the study conformed to the Declaration of Helsinki (2008) except for registration in a database. All participants were informed of the methods and study design verbally and in writing before providing written informed consent.

Experimental Procedures

The study implemented a cross-sectional design and was conducted in a quiet, temperature controlled room. Participants reported to the laboratory on two separate occasions having refrained from strenuous exercise, alcohol, and caffeine intake for 24 hours prior to each visit. During visit one, preliminary screening was conducted including anthropometric measurements, brachial blood pressure via manual sphygmomanometry and a maximal exercise test on an upright cycle ergometer (Corival, Lode B.V., Gronigen, Netherlands) to

obtain $\dot{V}O_{2peak}$ as previously described (Wakeham et al., 2019). For visit two, participants attended the laboratory having fasted for a minimum of 6 hours. Participants rested supine for 10 minutes before aPWV and central blood pressure were assessed. Additionally, short-axis and longitudinal ultrasound images of the right CCA were recorded for post-hoc analysis of 2D-Strain parameters and conventional measures of CCA stiffness.

Aortic Pulse Wave Velocity (aPWV)

A high-fidelity micromanometer tipped probe was used to obtain sequential ECG-gated pressure waveforms at the site of maximal arterial pulsation of the carotid and femoral arteries to calculate aPWV in accordance to applanation tonometry guidelines (Van Bortel et al., 2012). Central blood pressure was estimated by applying a validated transfer function (Van Bortel et al., 2012) to radial artery waveforms collected via the same probe (SphygmoCor, AtCor Medical, Sydney, AUS).

Carotid Artery Ultrasonography and Two-Dimensional Strain Imaging

Short-axis grey-scale cine loops of the right CCA were recorded 1-2cm proximal to the carotid bulb using a commercially available ultrasound system (Vivid Q, GE Medical, Norway) and a 12-MHz linear array transducer at a consistent frame rate of 92.3 frames per second. Longitudinal B-mode images were then obtained at an insonation angle <60° to simultaneously record CCA diameter and mean blood flow velocity. All images were acquired by a single trained sonographer at a constant depth.

Ultrasound Image Analysis

2D-Strain. Speckle-tracking software quantifies the motion of the arterial wall by automatically identifying speckles in the short-axis ultrasound image, which are subsequently tracked across the cardiac cycle (Catalano, Lamberti-Castronuovo, Catalano, Filocamo, & Zimbalatti, 2011). For quantification of strain and strain rates, a region of interest (ROI) was manually placed over the entire CCA wall circumference, ensuring accurate alignment with the lumen-wall interface. Within this ROI, movement of speckles were tracked frame by frame throughout systole and diastole within six evenly distributed segments using a speckle-tracking algorithm inherent to the software (EchoPac Version 112, GE Vingmed Ultrasound, Horten Norway), which generated strain and strain rate curves. PCS, S-SR and D-SR were measured as ‘global’ values reflecting the averaged values obtained from each ROI segment over three consecutive cardiac cycles and therefore represent the circumferential motion of the entire CCA wall. PCS

was identified as the greatest peak in the interpolated circumferential strain curve and represents the magnitude of CCA circumferential deformation from diastole to peak systole. S-SR was identified as the largest positive peak in the strain rate curve that occurred after the QRS complex, while D-SR was determined as the largest negative peak in the strain rate curve after the T-wave of the ECG.

CCA Blood Flow. Analysis of CCA diameter and flow were performed using semi-automated custom-designed edge-detection and wall-tracking software, which is independent of investigator bias and has been comprehensively described elsewhere (Townsend et al., 2015; Woodman et al., 2001). From synchronized diameter and velocity data, blood flow (the product of lumen cross-sectional area and Doppler velocity) was calculated at 30 Hz. Longitudinal B-mode ultrasound images were analysed continuously over 1 minute of recording to acquire average systolic (maximum) and diastolic (minimum) CCA diameter, as well as mean CCA blood flow. CCA systolic and diastolic diameters were then used to calculate CCA E_p and β-stiffness (Bjallmark et al., 2010; Pugh et al., 2018).

Statistical Analysis

Power analyses of CCA 2D-Strain parameters were conducted *a priori* by sampling data from two separate studies; one investigating the effect of ageing on 2D-Strain parameters in untrained individuals (Bjallmark et al., 2010) and the other investigating the effect of high CRF on 2D-Strain parameters in young males (Pugh et al., 2018). A mean difference in PCS of 3.8% (d = 4.19) was detected between young and old individuals, whereas a mean difference of 2.3% (d = 0.7) was detected between high and moderately fit young males. Accordingly, we estimated that a sample of 60 participants within a four-group cross-sectional study design would detect a 2.3% (d=0.7) difference in PCS with 90% power at a two-sided 0.05 significance level. Statistical analysis was conducted on SPSS statistical software package (Version 23.0, Chicago, USA). All data are presented as mean differences and confidence intervals (95% CI) with statistical significance set to $P \leq 0.05$. A two-factor analysis of variance (ANOVA) was used to determine the main effects of age, training status and whether there was an interaction between these factors (age*training status). When a significant interaction was observed, post-hoc comparisons with Bonferroni corrections were conducted to identify significant differences among group mean values. Additionally, analysis of covariance (ANCOVA) was conducted with central mean arterial pressure and CCA diastolic diameter individually set as covariates to control for their potential cofounding influence on 2D-Strain

parameters. Finally, linear regression analysis was also conducted to quantify the relationship between CRF ($\dot{V}O_{2peak}$), 2D-Strain parameters and aortic stiffness (aPWV) in young and middle-aged males by grouping all of the young and middle-aged participants into two separate groups.

Results

Participant Characteristics

$\dot{V}O_{2peak}$ was higher in runners compared to nonrunners ($P \leq 0.001$) and lower with age ($P \leq 0.001$; Table 1), but no age*training status interaction was observed. Nonrunners had a greater BMI than runners ($P \leq 0.001$), but there were no age-related differences. Runners demonstrated lower resting heart rates than nonrunners ($P = 0.001$), as well as lower brachial systolic and diastolic blood pressure ($P = 0.032$ and $P = 0.003$ respectively), whereas diastolic pressure was elevated with age irrespective of training status ($P \leq 0.001$). No age*training status interaction was observed for any of these parameters (Table 1).

(TABLE 1)

Central Haemodynamics and Aortic Pulse Wave Velocity

Central systolic blood pressure and pulse pressure were higher in middle-aged men compared to young men (both $P \leq 0.001$), whereas both central diastolic and mean arterial pressure were lower in runners compared to nonrunners ($P = 0.018$ and $P = 0.008$ respectively; Table 2). No other main effects of age or training status were observed for central pressures, nor were any age*training status interactions. aPWV was elevated in middle-aged men compared to young men ($P \leq 0.001$) and lower in runners than nonrunners ($P \leq 0.001$). However, no age*training status interaction was observed (Table 2).

Common Carotid Artery Morphology & Conventional Stiffness Indices

Systolic, diastolic and mean CCA diameters were elevated in middle-aged men compared to young men ($P = 0.015$, $P \leq 0.001$ and $P = 0.005$ respectively). However, there was no main effect of training status or an age*training status interaction for any of these parameters (Table 2). There were no main effects of age or training status on mean blood flow. CCA distension, EM_p and β-stiffness were all lower with age (all $P \leq 0.001$) and elevated in runners compared

to nonrunners ($P \leq 0.001$, $P = 0.016$ and $P = 0.039$ respectively), but no age*training status interaction was observed for any of these parameters (Table 2).

(TABLE 2)

Carotid Artery 2D-Strain Parameters

PCS, S-SR and D-SR were attenuated in middle-aged males when compared to young men (all $P \leq 0.001$). PCS and S-SR were elevated in runners when compared to nonrunners ($P = 0.002$ and $P = 0.009$ respectively), but no age*training status interaction was observed for either parameter (Figure 1). In contrast, there was no main effect of training status on D-SR, however a significant age*training status interaction was observed ($P = 0.024$; Figure 1C). Subsequent pairwise analysis revealed that D-SR was significantly reduced in middle-aged runners when compared to young runners (-0.16 $l \cdot s^{-1}$ [-0.10, -0.22]; $P \leq 0.001$), but no other differences were observed between the groups. All significant main effects of age and training status for PCS and S-SR remained following covariate adjustment for both CCA diastolic diameter and central mean arterial pressure, as did the main effect of age and age*training status interaction for D-SR.

(FIGURE 1)

Relationship between Cardiorespiratory Fitness and Carotid Artery 2D-Strain Parameters

CRF was associated with PCS ($r^2 = 0.26$, $P \leq 0.001$) and S-SR ($r^2 = 0.25$, $P \leq 0.001$) when data from all groups were pooled, but there was no association between CRF and D-SR. When grouped by age (young vs. middle-aged), the relationship between CRF and PCS remained in both groups (young: $r^2 = 0.18$. middle-aged: $r^2 = 0.18$; both $P = 0.015$; Figure 2A). Conversely, the relationship between CRF and S-SR remained in young males ($r^2 = 0.31$; $P \leq 0.001$) but abolished in middle-aged males ($r^2 = 0.07$; $P = 0.133$; Figure 2B).

Relationship between Aortic Pulse Wave Velocity and Carotid Artery 2D-Strain Parameters

aPWV was associated with PCS ($r^2 = 0.32$, $P \leq 0.001$), S-SR ($r^2 = 0.32$, $P \leq 0.001$) and D-SR ($r^2 = 0.15$, $P = 0.002$) when data from all groups were pooled. When grouped by age (young vs. middle-aged), the relationship between aPWV and PCS remained in the young and disappeared in the middle-aged group ($r^2 = 0.18$; $P = 0.016$ and $r^2 = 0.03$; $P = 0.381$

respectively; Figure 2C). Grouping by age had a similar effect on the relationship between aPWV and S-SR (young: $r^2 = 0.19$; $P \leq 0.013$; middle-aged: $r^2 = 0.0$; $P = 0.975$; Figure 2D). There was no relationship between aPWV and D-SR when grouped by age (young: $P = 0.389$; middle-age: $P = 0.600$).

(FIGURE 2)

Discussion

The primary aim of this study was to examine the relationship between habitual endurance exercise and CCA 2D-Strain parameters in young and middle-aged men. Our main findings were as follows: (1) PCS, S-SR and D-SR were attenuated in middle-aged males compared to young males, and (2) young and middle-aged runners exhibited elevated PCS and S-SR compared to nonrunners. To our knowledge, this is the first study to demonstrate that habitual endurance exercise is associated with superior CCA 2D-Strain parameters in both young and middle-aged males, which provides novel insight into the benefits of regular exercise on healthy arterial ageing.

In agreement with the literature, PCS, S-SR and D-SR were attenuated in healthy middle-aged males compared to young counterparts (Bjallmark et al., 2010; Rosenberg et al., 2018) and this age-related decline occurred irrespective of training status. However, we also observed that PCS and S-SR were elevated in runners compared to nonrunners and the lack of an age*training status interaction would indicate that the influence of habitual running on these parameters is comparable in young and middle-aged men. In contrast, we did not observe any differences in D-SR between runners and non-runners, suggesting that D-SR may be less amenable to habitual endurance exercise than PCS and S-SR in young and middle-aged men. A superior magnitude (PCS) and rate (S-SR) of deformation during cardiac systole likely reflects a training-induced improvement in the capacity of the CCA to buffer pulsatile ejection and thus ensure smooth consistent blood flow to the cerebral circulation (Tomoto, Sugawara, Nogami, Aonuma, & Maeda, 2015). The precise reason why we did not also observe a main effect of training status on D-SR is unclear, but may relate to the fact that during the diastolic phase of the cardiac cycle, there is less of a requirement to buffer pulsatile pressure and therefore less demand for an increased rate of arterial recoil (D-SR). Furthermore, D-SR is likely to be more *passive* in nature and may be influenced by a greater reliance on stiffer collagen fibres within

the arterial wall in order to maintain structural integrity during arterial recoil (Rossow et al., 2010).

Healthy arterial ageing is characterized by alterations to the composition of the extracellular matrix within the arterial wall, including the degeneration of elastin fibres, as well as the accumulation of collagen, connective tissue and advanced glycation end-products, all of which promote arterial stiffening (Lakatta & Levy, 2003). In rat models, exercise training has been shown to improve the elastin-to-collagen ratio within the extracellular matrix of central arteries (Matsuda, Nosaka, Sato, & Ohshima, 1993), supporting that our observation of modulated 2D-Strain parameters in endurance trained males may reflect favourable alterations to the elastic properties of the CCA. It should be noted that we did not observe an age*training status interaction for PCS or S-SR. However, our regression analysis revealed a similar linear relationship between CRF and PCS in both young and middle-aged men, whereas CRF was only associated with S-SR in young males and not in middle-aged males (Figure 2). Although more research across a wider age range is required, this distinction may indicate that the relative influence of habitual endurance training (high CRF levels) on PCS and S-SR differs with advancing age.

Indeed, it is plausible that the constraining effect of age-related accumulation of irreversible cross-linked collagen and advanced glycation-end products may limit the extent to which habitual endurance exercise can improve *'global'* CCA elasticity in ageing males (Fujimoto et al., 2010; Lakatta & Levy, 2003). Exercise-mediated improvements in the relative contribution of elastin within the CCA extracellular matrix may be sufficient to increase the magnitude of circumferential deformation (PCS), but irreversible cross-linked collagen accumulation may hinder any concomitant improvement in the rate of deformation (S-SR). This supposition is supported by a recent animal study that reported exercise training improved arterial elasticity solely via increasing elastin content despite negligible changes to collagen accumulation (Hanna et al., 2014). This constraining remodelling may also explain our surprising observation that the age-related decline in D-SR appears to be exacerbated in middle-aged male runners; where the inherent accumulation of cross-linked collagen coupled with a greater magnitude of deformation during systole gives rise to a blunted elastic transfer of potential energy and a slower rate of diastolic recoil. Clearly, without being able to obtain in-vitro CCA samples in humans, attributing age- and training-induced changes in 2D-Strain derived parameters to alterations in the composition of the extracellular matrix is speculative. Nevertheless, our data

demonstrate that 2D-Strain parameters provide novel insight into the varied and intricate effects of habitual endurance exercise on CCA elasticity in young and middle-aged men.

Importantly, PCS and S-SR were elevated in young and middle-aged runners, despite displaying similar CCA diameter and blood flow to their untrained counterparts. Although not measured in the present study, it is well established that total blood- and stroke volume increase with long-term endurance exercise training (Carrick-Ranson et al., 2014). However, it would seem that this increase in volume is not proportionately distributed to the carotid region at rest, presumably due to an unaltered demand in basal cerebral perfusion. Rather, we speculate that the increase in PCS represents a chronic training adaptation required to buffer repeated bouts of high CCA blood flow and normalise wall stress during intensive exercise. This proposition is consistent with the Law of Laplace and would explain the lack of association between PCS and CCA flow or diameter at rest. Furthermore, our ANCOVA confirmed that neither the age nor habitual exercise related differences in 2D-Strain parameters were influenced by CCA diastolic diameter. Similarly, all age-related differences in 2D-Strain parameters remained following covariate adjustment for central mean arterial pressure, as did all differences between runners and nonrunners. Taken together, these observations indicate that both age- and exercise-related alterations in 2D-Strain parameters are largely independent of CCA diameter, blood flow and central arterial pressure at rest.

Similar to our PCS and S-SR observations, significant main effects of age and training status were observed for conventional measures of CCA (EMp and β) and aortic (aPWV) stiffness, but no age*training status interaction was seen in any of these parameters. Although several previous studies have demonstrated that regular exercise attenuates the age-related increase in β-stiffness and aPWV (Tanaka et al., 2000; Vaitkevicius et al., 1993), those investigating the effect of exercise in young healthy individuals report far less consistent results (Heydari et al., 2013; Kakiyama et al., 2005; Montero et al., 2017; Pugh et al., 2018; Tanaka et al., 2000). However, the findings of the present study infer that, like PCS and S-SR, the positive influence of habitual exercise on EMp, β-stiffness and aPWV is similar in young and middle-aged males. This finding is arguably in tension with our previous observation that PCS and S-SR are elevated in young males with high CRF compared to less fit counterparts, despite no differences in EMp, β-stiffness and aPWV (Pugh et al., 2018). However, this apparent discrepancy is likely explained by the considerable differences in both training history and volume between the two respective cohorts of young males. Interestingly, our regression analysis (Figure 2) reveals that whilst PCS and SSR are closely related to aPWV in young males, this relationship disappears

during middle-age. This observation suggests that the mechanisms that influence aortic and CCA stiffness are similar during early adulthood, but become distinct during middle-age, which may relate to the reported exponential increase in aortic stiffness beyond 50 years of age (AlGhatrif et al., 2013; McEniery et al., 2005). Accordingly, future longitudinal training studies should include 2D-Strain parameters alongside aPWV in order to gain greater insight into any age-related differences in the therapeutic effects of endurance exercise at distinct regions of the central arterial tree.

Our findings have a number of important implications that relate to the cardio-protective role of habitual exercise throughout healthy ageing. Increased CCA stiffness is associated with a variety of age-related cerebrovascular diseases (van Sloten & Stehouwer, 2016) and is an independent predictor of cardiovascular events and all-cause mortality (van Sloten et al., 2014). Specifically, as a primary role of the CCA is to aid the regulation of cerebral blood flow (Hirata, Yaginuma, O'Rourke, & Kawakami, 2006), the observation of enhanced PCS and reduced EMp and β-stiffness in young and middle-aged runners may offer cerebral protection, including a reduced risk of stroke (Mattace-Raso et al., 2006; E. Y. Yang et al., 2012). Furthermore, effective buffering of dynamic elevations in blood flow throughout the central arterial tree may also reduce the age-related increase in peripheral artery dysfunction and microvessel damage (O'Rourke & Safar, 2005), which are hallmark morbidities of sedentary cardiovascular ageing.

Study Limitations

Although this study benefitted from a number of methodological strengths, including the comprehensive range of arterial stiffness measures and high training status of both the young and middle-aged runners, there are some noteworthy limitations. Firstly, our findings are restricted to the CCA and therefore cannot be applied systemically. Peripheral muscular arteries may be more susceptible to endurance-training than elastic central arteries (Dawson et al., 2008), therefore, 2D-Strain parameters should also be studied in peripheral vascular beds in the future. Secondly, data reported are only relevant for healthy young and middle-aged males. Due to recognised sex differences in haemodynamic regulation (Hart & Charkoudian, 2014) and vascular function (Green, Hopman, Padilla, Laughlin, & Thijssen, 2017), we acknowledge that females might display different age- and exercise-related changes in 2D-Strain parameters. Therefore, specific studies that are adequately powered to explore sex differences and examine whether sex hormones and/or the menopause influence the effect of habitual endurance

exercise on healthy arterial aging are warranted. Third, although the reported training volumes were sufficiently high and ecologically realistic for the respective age-groups, the middle-aged male runners demonstrated a lower training volume than the younger runners.

Conclusions

In conclusion, this is the first study to demonstrate that habitual endurance exercise is associated with enhanced PCS and S-SR in young and middle-aged males, but does not influence D-SR in either age group. This novel insight indicates that regular exercise is associated with comparable improvements in CCA 2D-Strain parameters in young and middle-aged men, but the age-related decline in PCS and S-SR may be more amenable to habitual endurance exercise than D-SR.

Competing Interests

The authors have no competing interests to report.

Author Contributions

The study was performed in the physiology laboratories in the Cardiff School of Sport and Health Sciences at Cardiff Metropolitan University **JT:** Conception of study design, data acquisition, analysis, interpretation and drafting of final manuscript. **RNL:** Data acquisition and critical revision of final manuscript. **DJW:** Data acquisition and critical revision of final manuscript. **TGD:** Data acquisition and critical revision of final manuscript. **BAC:** Data acquisition and critical revision of final manuscript. **MB:** Data acquisition and critical revision of final manuscript. **FML:** Data acquisition and critical revision of final manuscript. **CJP:** Conception of study design and critical revision of final manuscript. All persons designated as authors qualify authorship, approved of the final version to be published and agree to be accountable for all aspects of the work.

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Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Table 1. Descriptive characteristics of young male nonrunners, young male runners, middle-aged male nonrunners and middle-aged male runners. Data reported as group mean (95% CI). Abbreviations: BMI, body mass index; PB, personal best; $\dot{V}O_{2peak}$, maximal aerobic capacity; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure.

	Young male nonrunners	Young male runners	Middle-aged male nonrunners	Middle-aged male runners	Age Effect (*P*-value)	Training Status Effect (*P*-value)	Age*Training Status Interaction (*P*-value)
Age (y)	23 (21, 26)	24 (22, 26)	54 (52, 56)	56 (54, 58)	≤0.001	0.220	0.577
Resting Heart Rate (bpm)	63 (58, 67)	45 (41, 49)	59 (54, 63)	45 (41, 48)	0.259	≤0.001	0.393
Anthropometrics							
Body Mass (kg)	82.0 (76.6, 87.5)	69.4 (64.9, 73.9)	80.4 (74.9, 85.8)	67.7 (63.2, 72.2)	0.502	≤0.001	0.999
Height (cm)	177.8 (174.4, 181.1)	180.6 (177.8, 183.3)	175.3 (171.9, 178.6)	177.2 (174.4, 179.9)	0.058	0.127	0.770
BMI ($kg \cdot m^2$)	25.9 (24.4, 27.4)	21.3 (20.0, 22.5)	26.2 (24.7, 27.7)	21.5 (20.3, 22.8)	0.719	≤0.001	0.992
Training Status							
Training History (y)	-	7 (2, 11)	-	27 (22, 31)	≤0.001	-	-
Weekly Running (miles)	-	57 (51, 64)	-	33 (26, 40)	≤0.001	-	-
Recent 5km PB (mm:ss)	-	16:12 (15:33, 16:50)	-	19:54 (19:11, 20:28)	≤0.001	-	-
$\dot{V}O_{2peak}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	37.7 (33.7, 41.7)	61.0 (57.7, 64.3)	31.8 (27.8, 35.8)	51.3 (48.0, 54.6)	≤0.001	≤0.001	0.294
Brachial Blood Pressure							
SBP (mmHg)	122 (117, 127)	113 (109, 117)	119 (114, 124)	118 (114, 122)	0.642	0.032	0.101
DBP (mmHg)	72 (68, 75)	65 (62, 68)	77 (73, 80)	73 (70, 76)	≤0.001	0.003	0.458
MAP (mmHg)	86 (82, 89)	78 (75, 81)	91 (87, 95)	87 (84, 94)	≤0.001	≤0.001	0.217
PP (mmHg)	50 (46, 54)	48 (44, 51)	42 (38, 47)	45 (41, 48)	0.110	0.955	0.199

Table 2. Central haemodynamics, carotid artery morphology and conventional indices of arterial stiffness for young male nonrunners, young male runners, middle-aged male nonrunners and middle-aged male runners. Data reported as group mean (95% CI). Abbreviations: cSBP, central systolic blood pressure; cDBP, central diastolic blood pressure; cMAP, central mean arterial pressure; cPP, central pulse pressure; aPWV, aortic pulse wave velocity; EMP, Peterson's elastic modulus.

	Young male nonrunners	Young male runners	Middle-aged male nonrunners	Middle-aged male runners	Age Effect (*P*-value)	Training Status Effect (*P*-value)	Age*Training Status Interaction (*P*-value)
Central Blood Pressure							
cSBP (mmHg)	102 (97, 107)	95 (91, 99)	106 (101, 111)	106 (102, 110)	≤0.001	0.106	0.126
cDBP (mmHg)	74 (70, 77)	68 (65, 71)	75 (71, 79)	71 (68, 75)	0.223	0.018	0.535
cMAP (mmHg)	86 (81, 90)	77 (73, 81)	87 (82, 92)	84 (80, 88)	0.062	0.008	0.192
cPP (mmHg)	28 (26, 31)	27 (25, 30)	31 (28, 34)	34 (32, 37)	≤0.001	0.451	0.092
CCA Diameters & Blood Flow							
Systolic Diameter (mm)	6.57 (6.26, 6.89)	6.45 (6.19, 6.71)	6.83 (6.52, 7.15)	6.90 (6.65, 7.16)	0.015	0.843	0.500
Diastole Diastolic (mm)	5.88 (5.57, 6.18)	5.66 (5.40, 5.91)	6.34 (6.03, 6.65)	6.26 (6.01, 6.52)	≤0.001	0.289	0.605
Mean Diameter (mm)	6.22 (5.94, 6.51)	6.12 (5.88, 6.36)	6.64 (6.36, 6.93)	6.47 (6.23, 6.70)	0.005	0.289	0.763
Distension (mm)	0.69 (0.61, 0.78)	0.79 (0.72, 0.86)	0.49 (0.41, 0.58)	0.64 (0.57, 0.71)	≤0.001	0.004	0.559
Blood Flow ($ml \cdot min^{-1}$)	570 (500, 640)	602 (549, 655)	660 (586, 732)	615 (558, 673)	0.362	0.861	0.238
Conventional Arterial Stiffness Indices							
aPWV ($m \cdot s^{-1}$)	5.8 (5.4, 6.3)	5.1 (4.7, 5.4)	7.4 (7.0, 7.9)	6.7 (6.3, 7.1)	≤0.001	≤0.001	0.986
EM_p (kPa)	33.1 (26.3, 39.9)	26.1 (20.4, 31.7)	57.5(50.7, 64.3)	48.1 (42.5, 53.7)	≤0.001	0.016	0.706
B-stiffness ($cm^2 \cdot kPa$)	2.87 (2.3, 3.5)	2.43 (1.9, 2.9)	4.85 (4.3, 5.4)	4.16 (3.7, 4.6)	≤0.001	0.039	0.634

FIGURE LEGENDS

Figure 1. CCA 2D-Strain parameters in the young male nonrunners (YNR), young male runners (YR), middle-aged male nonrunners (MNR) and middle-aged male runners (MR) (Panel A – PCS [%], Panel B – S-SR [$l \cdot s^{-1}$] and Panel C – D-SR [$l \cdot s^{-1}$]). Data are reported as individual participant data points with corresponding group means (95% CI). * Indicates a significant post-hoc difference between groups ($P \leq 0.05$).

Figure 2. Panel A (PCS [%], Young: 18%. Middle-aged: 18%; both $P = 0.015$) and Panel B (S-SR [$l \cdot s^{-1}$], Young: 31%; $P \leq 0.001$. Middle-aged: 7%; $P = 0.133$) illustrate regression analyses examining the relationship of CCA 2D-Strain parameters and $\dot{V}O_{2peak}$ in Young (○) and Middle-aged (▪) males. Panel C (PCS, Young: $r^2 = 0.18$; $P = 0.016$. Middle-aged: $r^2 = 0.30$; $P = 0.381$) and Panel D (S-SR, Young: $r^2 = 0.19$; $P = 0.013$. Middle-aged: $r^2 = 0.0$; $P = 0.975$) illustrate regression analyses examining the relationship of CCA 2D-Strain parameters and aPWV.