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Aortic stiffness contributes to greater pressor responses during static hand grip exercise in healthy young and middle-aged normotensive men

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Abstract

Central arterial stiffness can influence exercise blood pressure (BP) by increasing the rise in arterial pressure per unit increase in aortic inflow. Whether central arterial stiffness influences the pressor response to isometric handgrip exercise (HG) and post-exercise muscle ischemia (PEMI), two common laboratory tests to study sympathetic control of BP, is unknown. We studied 46 healthy non-hypertensive males (23 young and 23 middle-aged) during HG (which increases in cardiac output [$\dot{Q}c$]) and isolated metaboreflex activation PEMI (no change or decreases in $\dot{Q}c$). Aortic stiffness (aortic pulse wave velocity [aPWV]; applanation tonometry via SphygmoCor) was measured during supine rest and was correlated to the pressor responses to HG and PEMI. BP (photoplethysmography) and muscle sympathetic nerve activity (MSNA) were continuously recorded at rest, during HG to fatigue (35% maximal voluntary contraction) and 2-minutes of PEMI. aPWV was higher in middle-aged compared to young males (7.1±0.9 vs 5.4±0.7 m/s, *P*<0.001). Middle-aged males also exhibited greater increases in systolic pressure (Δ30±11 vs 10±8 mmHg) and MSNA (Δ2313±2006 vs 1387±1482 %/min) compared to young males during HG (both, *P*<0.03); with no difference in the $\dot{Q}c$ response (*P*=0.090). Responses to PEMI were not different between groups. Sympathetic transduction during these stressors (MSNA-diastolic pressure slope) was not different between groups (*P*>0.341). Middle-aged males displayed a greater increase in SBP per unit change of $\dot{Q}c$ during HG (ΔSBP/$\Delta\dot{Q}c$; 21±18 vs 6±10 mmHg/L/min, *P*=0.004), with a strong and moderate relationship between the change in systolic (r=0.53, *P*<0.001) and diastolic pressure (r=0.34, *P*=0.023) and resting aPWV, respectively; with no correlation during PEMI. Central arterial stiffness can modulate pressor responses during stimuli associated with increases in cardiac output and sympathoexcitation in healthy males.

Introduction

Static handgrip exercise (HG) is often employed in the laboratory or clinic to study autonomic adjustments that regulate arterial blood pressure (BP). Notably, the basis of this approach is that the arterial BP response to HG is underpinned primarily by reflex increases in sympathetic vasomotor outflow (i.e. muscle sympathetic nerve activity [MSNA] (Fisher et al., 2015). Hence, the magnitude of the exercise pressor reflex response provides an index of activation of MSNA. Furthermore, an exaggerated increase in arterial blood pressure with exercise is predictive of the future diagnosis of hypertension (Kayrak et al., 2010; Matthews et al., 1993; Schultz et al., 2015) and cardiovascular events (Lewis et al., 2008), as reviewed previously (Schultz et al., 2017). The cold pressor test is another classical laboratory/clinical test used to elicit increases in MSNA and arterial BP (Victor et al., 1987). Recently, Borner and colleagues showed that resting aortic stiffness (aortic pulse wave velocity [aPWV]) positively correlates with the change in BP induced by a cold pressor test in a cohort of young and older individuals (Borner et al., 2017). This finding raises the intriguing possibility that, in addition to reflex sympathoexcitation, the arterial BP response to CPT is influenced by central arterial stiffness.

Stiffer central arteries contribute to a greater pressor response during increases in cardiac output ($\dot{Q}c$) due to a reduced ability of the central arteries to distend and accommodate aortic inflow. This is demonstrated as a greater rise in arterial pressure per unit increase in flow during whole body dynamic exercise (Miyai et al., 2021; Sarma et al., 2020; Thanassoulis et al., 2012). Therefore, age-related central artery stiffening (McEniery et al., 2005) could contribute to greater pressor responses during HG in older, compared to younger, individuals as suggested previously (Lalande et al., 2014). However, it is unknown whether arterial stiffness correlates with the pressor responses to HG; if the correlation exists, previously reported differences in pressor responses to small muscle mass exercise (i.e. HG) may be confounded by differences in arterial stiffness rather than reflecting a difference in the effectiveness of sympathetic nerve activity in eliciting vasoconstriction. Thus, the knowledge of whether arterial stiffness contributes to pressor responses to HG is highly-relevant for the field and interpretation of these tests.

Herein, we assessed whether arterial stiffness correlates with pressor responses in healthy non-hypertensive young and middle-aged men during HG and post-exercise muscle ischaemia (PEMI). HG is associated with an increase in $\dot{Q}c$ and sympathoexcitation while PEMI is associated with sympathoexcitation and no change, or even a decrease, in $\dot{Q}c$ (Kiviniemi et al., 2012). The use of these two stimuli in younger and middle-aged individuals, known to have differences in aortic stiffness (Talbot et al., 2020; Wakeham et al., 2022), facilitates investigation as to whether arterial stiffness influences the pressor responses to changes in $\dot{Q}c$.

Methodology

All data presented were collected during 2015-2017 as part of a cross-sectional study in 23 young (23 ± 3 [18 - 30] years) and 23 middle-aged (55 ± 4 [50 - 63] years) endurance-trained and recreationally-active healthy men designed to address several *a-priori* research aims (Lord et al., 2020; Talbot et al., 2020; Wakeham et al., 2022; Wakeham et al., 2019). These prior aims were to assess the independent effects of age and habitual exercise on integrative cardiovascular control by studying men only due to the well-known sex differences in autonomic support of blood pressure, MSNA, BP, sympathetic transduction at rest or during HG and PEMI in both younger and older age (Best et al., 2014; Christou et al., 2005; Hart et al., 2011; Jarvis et al., 2011; Vianna et al., 2012), as reviewed previously (Joyner et al., 2015).

We recruited forty-six males, all were non-smokers, normotensive and reported no chronic diseases. Recruitment criteria were either young (18-30 years) or middle-aged (50-65 years) men who were recreationally-active (≤3hours of structured physical activity for ≥2 or ≥10years for the young and middle-aged men, respectively) or endurance-trained (Young: ≥50 miles of moderate to intensity training for ≥2 years; Middle-aged: ≥25 miles of moderate to intensity training for ≥10 years). Participants were requested to abstain from caffeine, alcohol, nutritional supplements, and heavy exercise for 24 hours prior to testing; furthermore, participants arrived having fasted for the previous 6 hours. All participants provided written informed consent. The study conformed to the *Declaration of Helsinki*, except for registration as a clinical trial and was approved by the Cardiff Metropolitan University School of Sport and Health Sciences Research Ethics Committee (16/7/02R).

Participants attended the laboratory on two occasions, first for a screening visit and second for the experimental visit. Study visits were separated by a minimum of 24 hours. During the screening visit, aPWV and cardiorespiratory fitness ($\dot{V}O_2$ Peak) were assessed, as described previously (Wakeham et al., 2022; Wakeham et al., 2019). aPWV was assessed via applanation tonometry (SphygmoCor) measuring the transit time between the foot of the carotid and femoral arterial waveforms divided by the path length (measuring tape). $\dot{V}O_2$ Peak was assessed with an incremental (ramp, 20 Watts/min) test to exhaustion on a cycle ergometer. During the experimental visit, heart rate (electrocardiography), beat-by-beat blood pressure (finger photoplethysmography; FinometerPro, FMS, Groningen, Netherlands), multiunit MSNA (microneurography; Nerve Traffic Analyzer, Model 663 C, University of Iowa, Iowa City, IA) and left ventricular volumes (2-D echocardiography; Vivid E9, GE Medical, Norway) were recorded at rest (Wakeham et al., 2019) and during HG and PEMI. Briefly, following a 1-minute baseline, participants performed HG at 35% of their maximal voluntary contraction with their left hand (MLT004/D, ADInstruments, Oxford, UK; 5 participants were left hand dominant). When a participant was unable to maintain the required force for >3 seconds, a cuff positioned around the left forearm (to enable ultrasonography at the level of the brachial artery; data not shown) was rapidly inflated (E20 Rapid Cuff Inflation System, D.E. Hokanson, Bellevue, USA) to suprasystolic pressure (220mmHg), to elicit a period of PEMI for two minutes. To account for inter-individual variability in time to task failure, HG duration was divided into five equal quintiles (20%, 40%, 60%, 80%, 100% HG duration); whereas PEMI was assessed in quartiles (30 second bins).

Data Acquisition and Analyses

Echocardiographic images were acquired with a 4-MHz array probe (Vivid E9, GE Medical, Norway) over five cardiac cycles by an experienced cardiac sonographer (RNL) and stored for analysis off-line. Left ventricular stroke volume was derived in the single plane from apical 4-chamber views in 2D echocardiograms as the as the absolute difference between end diastolic and systolic volumes using commercially available software (EchoPAC, BT12 GE Medical, Norway).

The beat-by-beat arterial pressure waveform was calibrated against the average of three systolic (SBP) and diastolic (DBP) blood pressures, measured at rest, using a manual sphygmomanometer. We calculated pulse pressure (PP [SBP-DBP]). Heart rate was determined from the R-R interval recorded in the electrocardiogram (Lead II). The assessment of stroke volume and arterial pressure permitted the calculation of $\dot{Q}c$ (heart rate x stroke volume), total vascular conductance (TVC, $\dot{Q}c$/mean arterial pressure [MAP]) and total peripheral resistance (TPR, MAP/ $\dot{Q}c$). Stroke volume could not be obtained in 4 middle-aged males, therefore stroke volume and associated hemodynamics are reported for 42 individuals during all stimuli (23 young and 19 middle-aged males).

MSNA (raw and integrated) signals, arterial BP, electrocardiogram, and grip force data were sampled at 1 kHz using analog-to-digital data acquisition hardware (Powerlab 8/35, ADInstruments). Multiunit bursts of integrated MSNA were inspected independently by two researchers (DJW/JPM) and verified. To account for variation in the microelectrode position, and the effect this has on MSNA burst amplitude, the height of the largest burst appearing under resting conditions was assigned a value of 100 units. All other bursts were expressed relative to this value.

MSNA was quantified as burst frequency (bursts/min), burst incidence (bursts/100hb; hb, heartbeats), mean burst amplitude (%) and total activity (burst amplitude x burst frequency, %/min). We were unable to record MSNA from one middle-aged participant. MSNA total activity was used as the primary index of sympathetic responses, as it accounts for changes in both burst occurrence and burst size. Thirty seconds of representative MSNA and blood pressure data are presented from one young (Figure 1, A-C) and one middle-aged male (Figure 1, D-F) during baseline, at the end of SHG and at the end of PEMI.

Sympathetic transduction to pressure was assessed via the calculation of slopes from linear regression analyses (Halliwill et al., 1996) between MSNA burst frequency and DBP and MSNA total activity and DBP, respectively (see representative data in Figure 2). DBP was used as it is reproducible, and a target variable of sympathetic outflow; furthermore, DBP indicates systemic vascular responses (Briant et al., 2016). To generate sympathetic vascular transduction slopes, the relationship between MSNA burst frequency and total activity was plotted against DBP, for each quintile (HG) or quartile (PEMI). The baseline value for each variable was included in the regression analyses. Due to similar between-group differences when sympathetic vascular transduction was determined using MSNA burst frequency and total activity, only data using total activity are reported.

Statistical Analysis

All analyses were completed using IBM SPSS (version 26, IBM statistics, Armonk, NY). Data were tested for normality (Shapiro-Wilks), sphericity (Mauchly's test) and the presence of significant outliers (≥ 3 standard deviations). Participant characteristics, HG duration and sympathetic transduction were compared via independent samples t-tests. Neural and haemodynamic data were analysed using a linear mixed effects model including subject as a random factor, to determine main effects of group (young *versus* middle-aged) and condition (pre-test baseline *versus* final quintile of HG or final quartile of PEMI). In the event of a significant interaction, SIDAK post hoc-multiple comparisons were performed. The relationship between variables was assessed via Pearson product-moment correlation coefficients. Statistical significance was defined at a level of $P < 0.05$. Values are reported in text as mean ± standard deviation (SD).

Results

Middle-aged males were older (55 ± 4 vs 23 ± 3 years, $P < 0.001$), of greater stature (179.1 ± 5.4 vs 175.1 ± 6.7 cm, $P < 0.05$), had a higher body fat percentage (22.0 ± 7.8 vs 14.8 ± 7.0 %, $P < 0.05$; via bioelectrical impedance) and aPWV (7.1 ± 0.9 vs 5.4 ± 0.7 m/s, $P < 0.001$) compared to young males. Body mass (72.3 ± 11.6 vs 72.8 ± 12.9 kg, $P = 0.896$), $\dot{V}O_2$ Peak (43.8 ± 10.9 vs 50.1 ± 14.6 mL/kg/min, $P = 0.108$) and HG duration (250 ± 108 vs 190 ± 136 seconds, $P = 0.119$) were not different between groups.

Baseline cardiovascular haemodynamics and neural activity

Heart rate, stroke volume, cardiac output and TVC were lower in middle-aged males; whereas, TPR, blood pressure (all) and MSNA burst frequency, burst incidence and total activity were higher (main effects of group, $P < 0.05$; Figure 2 and Table 1).

Handgrip exercise

In response to HG, in both groups, heart rate, $\dot{Q}c$, total vascular conductance (TVC), and all indices of blood pressure and MSNA increased, whereas stroke volume decreased (main effects of condition, $P < 0.05$) and TPR did not change. However, compared to their younger counterparts, there were greater increases in MSNA total activity, systolic blood pressure (SBP) and MAP in middle-aged males (group*condition interactions, $P < 0.05$; Table 1 and Figure 2). Sympathetic transduction to pressure was not different ($P = 0.341$) between young (0.004 ± 0.008 mmHg·%·min^{-1}) and middle-aged groups (0.006 ± 0.004 mmHg·%·min^{-1}).

Post-exercise muscle ischaemia

Heart rate, stroke volume, cardiac output and TVC were lower and TPR, systolic blood pressure (SBP), and MSNA (except burst amplitude) were higher in middle-aged males during PEMI (main effects of group, $P < 0.05$). Stroke volume, $\dot{Q}c$ and total vascular conductance (TVC) decreased during PEMI; whereas, TPR, blood pressure (all) and MSNA (all) increased (main effects of condition, $P < 0.05$), with no significant effect of age on the neural or haemodynamic responses to PEMI (Table 1 and Figure 3). Sympathetic transduction to pressure was not different ($P = 0.807$) between young (0.003 ± 0.004 mmHg·%·min^{-1}) and middle-aged groups (0.002 ± 0.007 mmHg·%·min^{-1}).

Correlation analysis

With all data pooled, aPWV exhibited a significant positive linear correlation with the systolic, diastolic and mean pressor responses during HG, but not PEMI (Figure 4). Furthermore, there were significant correlations between aPWV and the change in pulse pressure for both HG (r = 0.54, $P < 0.001$) and PEMI (r = 0.55, $P < 0.001$); there was no significant correlation between aPWV and baseline pulse pressure (r = 0.03, $P = 0.849$). In line with this, middle-aged men displayed a greater increase in SBP per unit change of $\dot{Q}c$ during HG than young men (ΔSBP/$\Delta\dot{Q}c$; 21 ± 18 vs 6 ± 10 mmHg/L/min, $P = 0.004$), likely due to the higher aPWV.

When age was included as a covariate for partial correlational analyses, there were no longer any significant relationships between aPWV and the pressor responses during HG (SBP: $r = -0.03$, $P = 0.865$; MAP: $r = -0.04$, $P = 0.781$; DBP: $r = -0.03$, $P = 0.845$; PP: $r = -0.01$, $P = 0.942$). However, there were no significant relationships between aPWV and pressor responses to HG in young (SBP: $r = 0.19$, $P = 0.396$; MAP: $r = -0.001$, $P = 0.995$; DBP: $r = -0.11$, $P = 0.628$; PP: $r = 0.36$, $P = 0.092$) or middle-aged (SBP: $r = -0.12$, $P = 0.614$; MAP: $r =$

-0.04, *P* = 0.881; DBP: *r* = 0.07, *P* = 0.765; PP: *r* = -0.25, *P* = 0.280) men when assessed separately. Furthermore, all correlations remained (either significant or non-significant) when adjusted for either $\dot{V}O_2$ Peak or baseline MAP (Table 2).

Discussion

Herein, middle-aged men displayed a greater increase in SBP, MAP and MSNA total activity, during HG; whereas, there was no effect of age on the arterial pressure and sympathetic neural responses to PEMI. Since $\dot{Q}c$ increases during HG and decreases during PEMI, we speculate that arterial stiffening, such as that induced by ageing, exaggerates the pressor response during HG. Moreover, when data from young and middle-aged men were combined, a positive relationship exists between baseline aPWV and the absolute change in SBP, MAP and DBP during HG. Notably, there is no relationship between baseline aPWV and the pressor responses to PEMI. Considering these findings together, we suggest that the arterial BP response to HG is influenced by central arterial stiffness. Hence, there is potential for vascular stiffness to be a confounding factor in studies utilizing HG exercise to study neural control and autonomic regulation of blood pressure.

The magnitude of the systolic and mean pressor responses during HG, but not PEMI, were greater for middle-aged males. Several possible mechanisms may contribute to the exaggerated pressor response to HG in middle-aged males. First of which may be higher central arterial stiffening, which is commonly observed in western society (McEniery et al., 2005). Central arterial stiffening would exaggerate the pressor response during increases in $\dot{Q}c$, due to a reduced ability of the central arteries to distend and accommodate aortic inflow, thereby increasing arterial pressure per unit increase in flow. Indeed, the middle-aged males, who exhibited a greater aPWV (i.e., index of arterial stiffness), presented with a greater change in systolic for a given change in $\dot{Q}c$, compared to the younger males. Furthermore, in response to HG, during which cardiac output increased, we identified a significant positive relationship between aPWV and the absolute change in SBP, DBP and MAP (Figure 3A, 3C and 3E). This suggests that those with greater arterial stiffness exhibit more exaggerated pressor responses to increases in $\dot{Q}c$, further highlighting the important role of arterial stiffness. However, during PEMI, where cardiac output fell compared to baseline, there was no significant relationship between baseline aPWV and the pressor responses (Figure 3B, 3D and 3F). Importantly, to highlight the effect of age, and associated arterial stiffening, when including age as a covariate in partial correlational analyses, there were no longer any significant relationships between aPWV and the pressor responses during HG (SBP: r = -0.027, P = 0.865; MAP: r = -0.044, P = 0.781; DBP: r = -0.031, P = 0.845), suggesting that this association is a function of differences in age. As aortic stiffening appears to correlate with the pressor response during sympathoexcitation associated with increases in cardiac output (i.e. exercise), studies comparing groups with known differences in aPWV (i.e. age) should consider including measurement of arterial stiffness to include in covariate analysis.

An alternative mechanism mediating greater pressor responses during HG in middle-aged men could be the greater increase in MSNA total activity eliciting greater decreases in vascular conductance (or increases in resistance) and ultimately DBP. Although there was no significant effect of age on the response of total vascular conductance (or resistance) during HG, there was a trend for a greater DBP response with age (group*condition interaction, P = 0.050; Table 1). This is of relevance as DBP is a target variable of vascular sympathetic activity (Briant et al., 2016).Indeed, sympathetic transduction to pressure was similar between young and middle-aged men, suggesting the greater increase in total MSNA activity would result in a greater increase in BP, compared to young individuals. However, there was no correlation between the change in MSNA and the change in arterial pressure (SBP: r = 0.278, P = 0.06; MAP: r = 0.024, P = 0.114; DBP: r = 0.017, P = 0.262). Thus, it appears unlikely that the greater increase in MSNA total activity contributed to the larger increase in arterial pressure with age during HG.

Our findings contrast previous studies that report no effect of age on the MSNA or pressor responses to HG (Greaney et al., 2013; Houssiere et al., 2006; Krzeminski et al., 2012; Markel et al., 2003; Momen et al., 2004; Ng et al., 1994; Tan et al., 2013). Nevertheless, only the change in MSNA total activity was greater with age and there were no age-related effects on the response of MSNA burst frequency, amplitude or incidence. Although we cannot determine the reasons for the disparity between our findings and those of other studies, it likely reflects between study sample differences in either hemodynamic (Watanabe et al., 2014) or genetic (Notay et al., 2018) factors. Furthermore, the influence of comparing absolute or relative responses in previous data also may partly contribute to differences in study findings. However, the greater response of MSNA and blood pressure here occurred despite higher MSNA in middle-aged men with no difference in blood pressure between groups at baseline. Thus, it is unlikely that baseline differences influenced the age-related difference in the responses observed here. Together, it appears that the interaction between increases in cardiac output, higher aortic stiffness and greater elevations in sympathetic vasomotor outflow contribute to greater increases in arterial pressure during HG in middle-aged compared to young males.

Notably, we observed no significant effect of age on changes in MSNA burst frequency or amplitude during HG, suggesting it is the interaction of burst rate and size which culminates in a greater increase in MSNA total activity in middle-aged males. The contributing mechanism(s) to the larger increase in MSNA total activity during HG with age are unclear but appear to be independent of afferent feedback from skeletal muscle metaboreceptors, as the augmented MSNA response was not maintained during PEMI. A greater feedforward (central command) or feedback (muscle mechanoreflex) signal could contribute to the greater level of sympathoexcitation with age during HG. In addition, venous compliance decreases (Monahan et al., 2001) and pulmonary vascular stiffness increases (Dawes et al., 2016) with age; accordingly, during increases in $\dot{Q}c$, there could be a greater activation of the sympathoexcitatory venous distention and pulmonary baroreceptor reflexes (Moore et al., 2022). Despite these effects being difficult to isolate in humans, future studies should attempt to investigate the mechanisms contributing to this greater level of sympathoexcitation during HG with age.

Methodological Considerations

There are several strengths of our study, including the comprehensive assessment of cardiovascular responses to exercise using echocardiography and microneurography, as well as characterizing arterial stiffness in a large sample size. However, our inclusion of males only represents a major limitation of our study, and this limits the generalisability of our findings. Future studies employing a balanced sex ratio are required to assess whether these same correlations exist in both sexes. Also, resting aortic stiffness was measured on a separate day to the sympathetic and hemodynamic responses to HG and PEMI to address a separate *a-priori* study aim (Wakeham et al., 2022). However, the pressor responses to HG and PEMI and resting aPWV have been reported to have good reproducibility (Dillon et al., 2020; Yasmin et al., 1999). Accordingly, the difference in days of assessment of aortic stiffness and HG and PEMI responses is unlikely to affect the conclusions of this study.

Conclusion

This study provides new information regarding the influence of aortic stiffness on pressor responses during HG in men. The greater pressor response to HG in middle-aged men likely occurs due to central artery stiffening, which increases arterial pressure per unit increase in flow (i.e. $\dot{Q}c$). The systolic pressor difference between groups suggests central artery stiffening plays a larger role than does the greater increase in MSNA in middle-aged men, as there was no difference in the DBP or TPR response. These findings suggest that pressor responses to stimuli associated with increases in $\dot{Q}c$ and sympathoexcitation (e.g. exercise) are likely to be exaggerated in healthy middle-aged men, who exhibit higher arterial stiffness.

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Declarations

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Conflicts of interest/Competing interests

Not Applicable.

Availability of data and material

Data are available from the corresponding author upon reasonable request.

Code availability

Not Applicable.

Author Contributions

All testing was completed at the Cardiff School of Sport and Health Sciences, Cardiff Metropolitan University, Cardiff, Wales, UK. D.J.W., C.J.P., R.S., and J.P.M contributed to conception and design of the work and acquisition, analysis and interpretation of the data and writing of the manuscript. R.N.L., J.S.T., F.M.L., B.A.C., T.G.D. and L.L.S., contributed to acquisition, analysis and interpretation of the data and critically revised the manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons included as an author qualify for authorship, and all those who qualify for authorship are listed.

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Figures and Tables

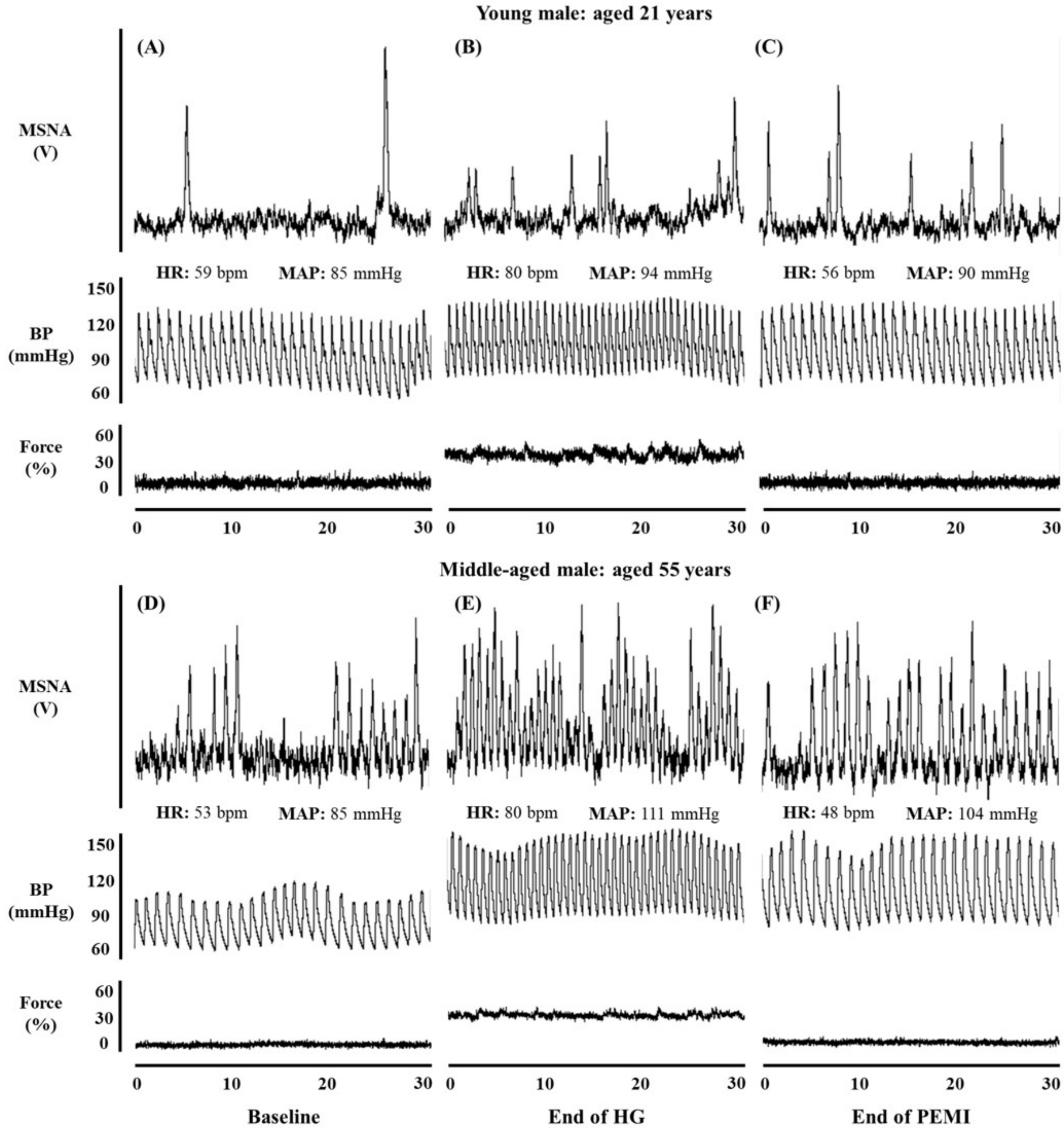

Figure 1 - Representative raw data from one young and one middle-aged man. Thirty seconds of raw muscle sympathetic nerve activity (MSNA) and blood pressure (BP) data are shown from one young (panels A, B and C) and one middle-aged male participant (panels D, E and F) at baseline, the end of static hand grip (HG) and the end of post-exercise muscle ischemia (PEMI).

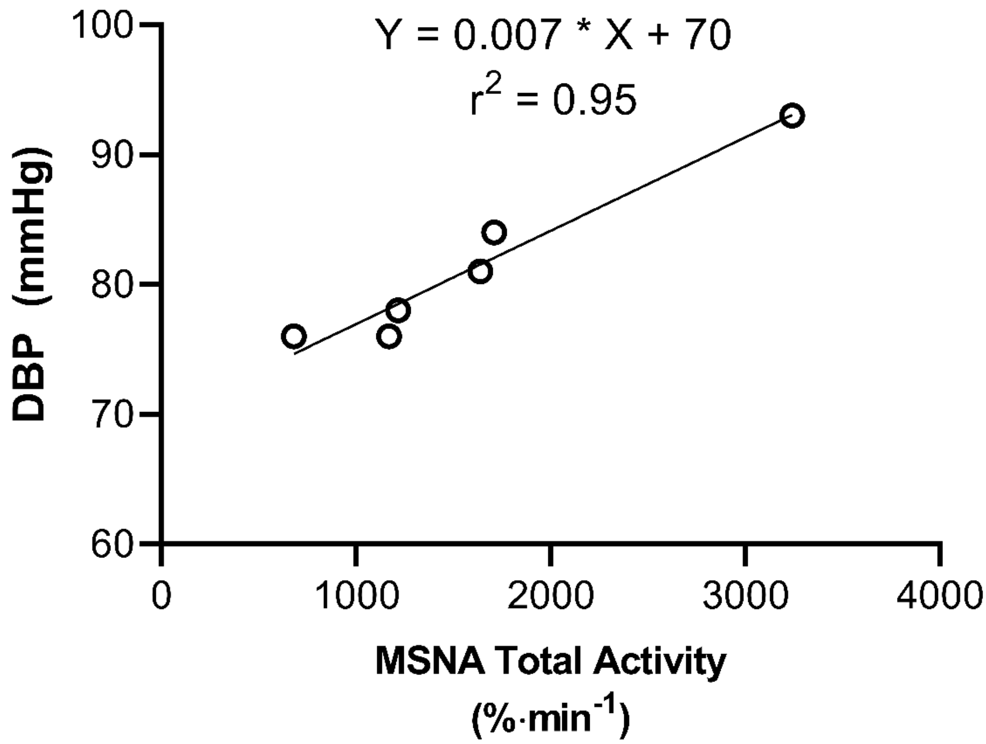

Figure 2 - A representative sympathetic vascular transduction slope from one young participant. Sympathetic vascular transduction was assessed as the slope of the linear relationship between MSNA total activity and diastolic blood pressure during hand grip exercise.

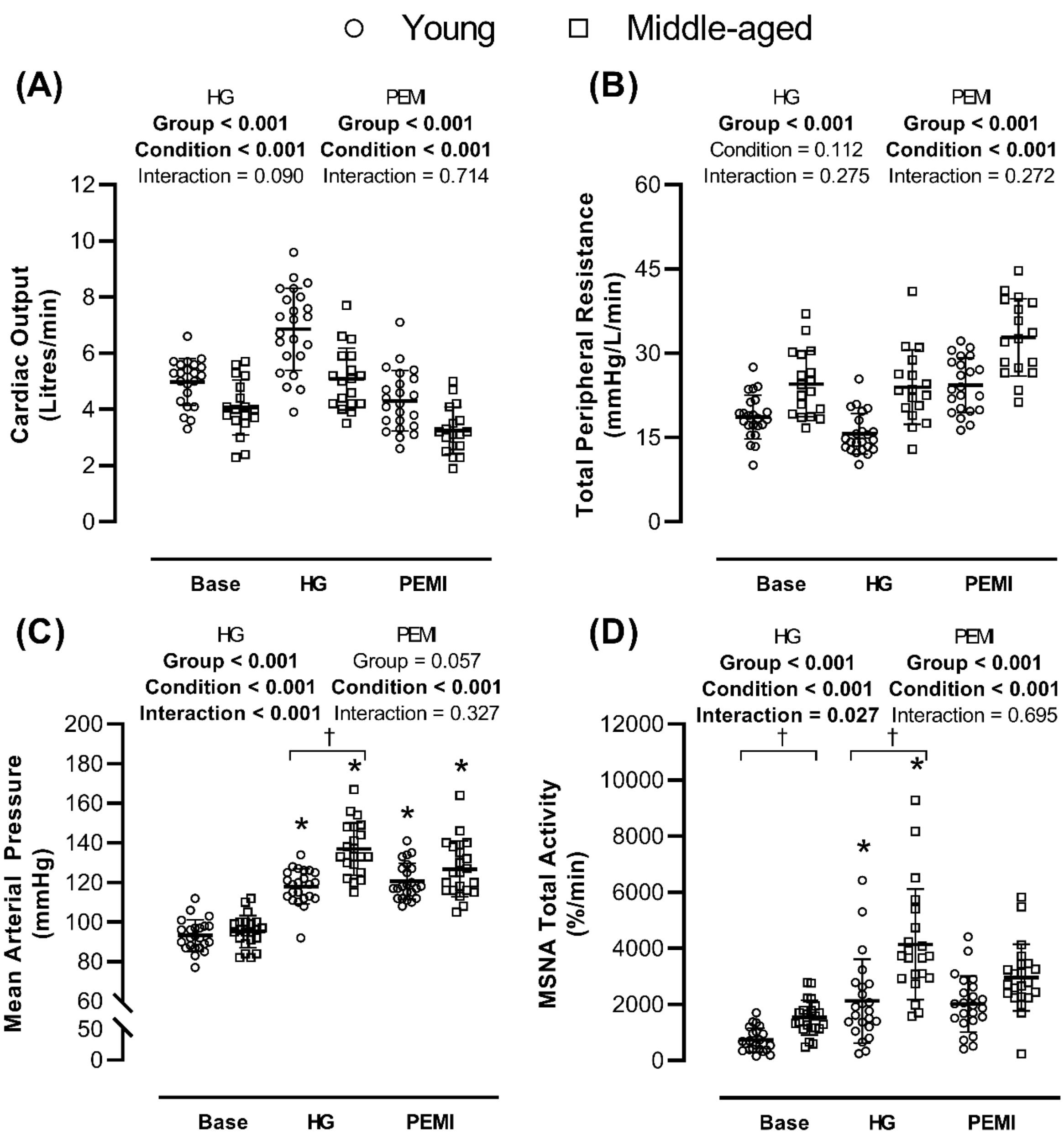

Figure 3 - The sympathetic and haemodynamic responses to HG and PEMI in young and middle-aged men. The changes in cardiac output (Panel A) and total peripheral resistance (Panel B) were not different between groups. The increases in mean arterial pressure (Panel C) and MSNA Total Activity (Panel D) during HG were greater in middle-aged men when compared to younger counterparts. There were no significant group differences in the sympathetic or haemodynamic responses to PEMI. *Notes: Significant main effects or interactions are shown in bold above each respective panel, as determined via separate linear mixed models for HG and PEMI. Symbols display results from SIDAK post hoc analyses; * and † represent within-group and between-group differences (P < 0.05), respectively. Abbreviations: Base, baseline; HG, static hand grip exercise; MSNA, muscle sympathetic nerve activity; PEMI, post-exercise muscle ischaemia.*

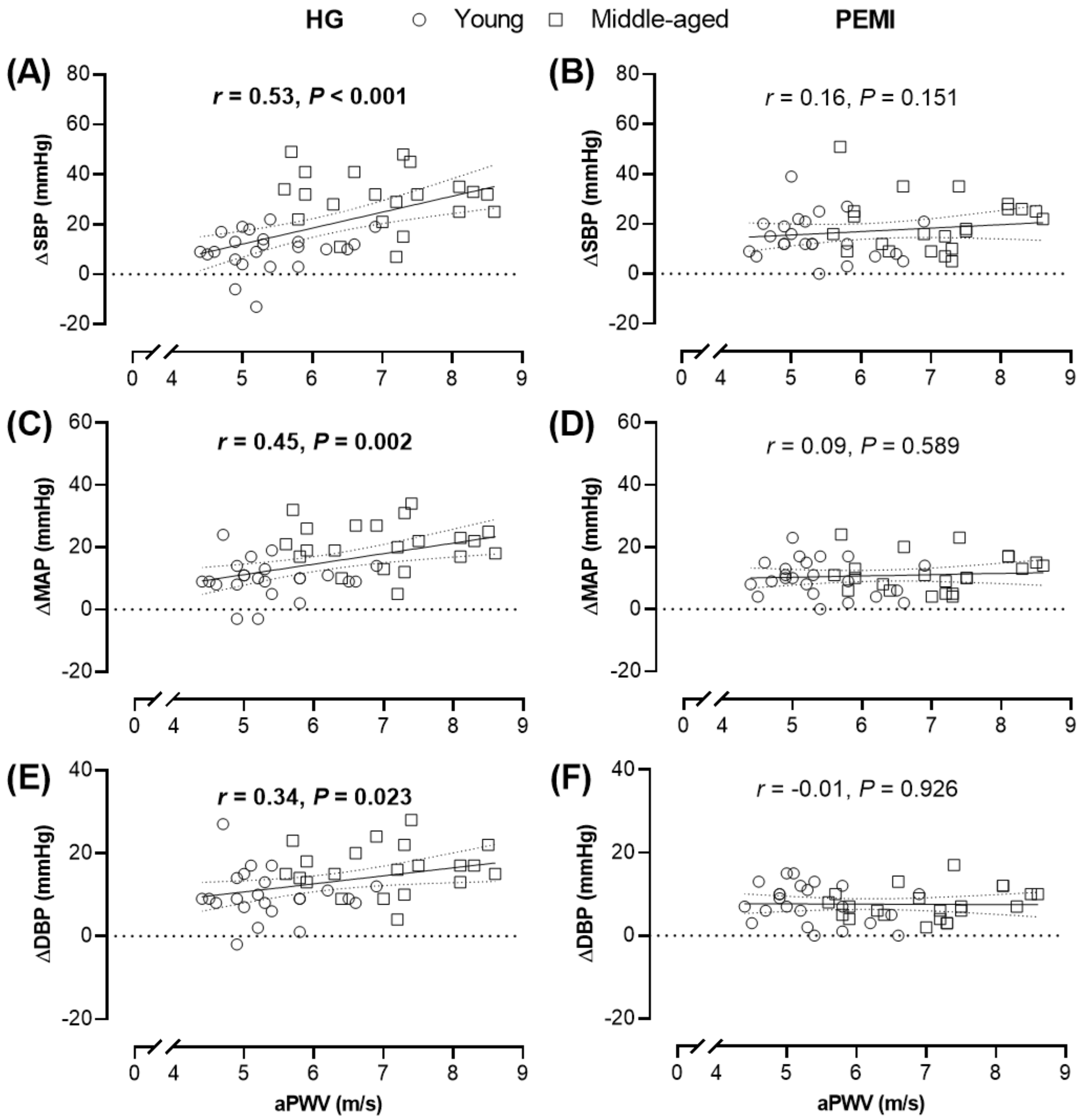

Figure 4 - Correlations between baseline aortic stiffness and the delta pressor responses to HG and PEMI. aPWV significantly correlated with the change in systolic, mean and diastolic pressure from rest to the final quintile of HG (Panels A, C and E, respectively). However, there were no significant correlations between aPWV and pressor responses to the final 30-seconds of PEMI (Panels B, D and F). *Abbreviations: aPWV, aortic pulse wave velocity; DBP, diastolic blood pressure; HG, static handgrip exercise; MAP, mean arterial pressure; PEMI, post-exercise muscle ischaemia; SBP, systolic blood pressure.*

Table 1 – Sympathetic and haemodynamic responses to HG and PEMI in young and middle-aged men

				HG				PEMI			
Variable	**Group**	**n**	**Baseline**	**End HG**	**Group *P***	**Condition *P***	**Interaction *P***	**End PEMI**	**Group *P***	**Condition *P***	**Interaction *P***
Haemodynamic											
Heart Rate	Y	23	57 ± 15	86 ± 18	**0.007**	**< 0.001**	0.431	55 ± 13	**0.044**	0.528	0.830
(bpm)	MA	22	52 ± 10	76 ± 17				47 ± 7			
Stroke Volume	Y	23	92 ± 16	80 ± 12	**< 0.001**	**< 0.001**	0.974	79 ± 14	**< 0.001**	**< 0.001**	0.945
(ml)	MA	18	79 ± 10	67 ± 10				66 ± 9			
TVC	Y	23	0.056 ± 0.014	0.067 ± 0.014	**< 0.001**	**0.028**	0.108	0.042 ± 0.010	**< 0.001**	**< 0.001**	0.689
(l/min/mmHg)	MA	18	0.043 ± 0.010	0.045 ± 0.013				0.031 ± 0.008			
SBP	Y	23	124 ± 9	133 ± 10 *	**< 0.001**	**< 0.001**	**< 0.001**	138 ± 11	**0.038**	**< 0.001**	0.325
(mmHg)	MA	22	127 ± 11	158 ± 17 *†				147 ± 18			
DBP	Y	23	78 ± 10	87 ± 9	**0.006**	**< 0.001**	0.050	85 ± 8	0.315	**< 0.001**	0.894
(mmHg)	MA	22	80 ± 7	96 ± 8				87 ± 7			
Sympathetic activity											
MSNA BF	Y	23	13 ± 8	26 ± 15	**< 0.001**	**< 0.001**	0.147	26 ± 10	**< 0.001**	**< 0.001**	0.449
(bursts/min)	MA	22	27 ± 11	47 ± 14				37 ± 11			
MSNA BI	Y	23	26 ± 18	35 ± 22	**< 0.001**	**0.007**	0.623	50 ± 23	**< 0.001**	**< 0.001**	0.504
(bursts/100hb)	MA	22	54 ± 24	68 ± 17				72 ± 21			
MSNA BA	Y	23	58 ± 11	81 ± 27	0.207	**< 0.001**	0.302	77 ± 21	0.359	**< 0.001**	0.525
(%)	MA	22	60 ± 11	94 ± 46				84 ± 38			

Data are presented as mean ± standard deviation. Significant main effects or interactions are highlighted in bold, determined via separate linear mixed models for HG and PEMI. Symbols display results from SIDAK post hoc analyses; * and † represent within-group and between-groups differences, respectively. *Abbreviations: BA, burst amplitude; BF, burst frequency; BI, burst incidence; DBP, diastolic blood pressure; HG, hand grip exercise; MA, middle-aged; MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity; n, sample size; PEMI, post-exercise muscle ischaemia; SBP, systolic blood pressure; TVC, total vascular conductance; Y, young.*

Table 2 - Correlation Matrix

	∆SBP (mmHg)	**∆DBP (mmHg)**	**∆PP (mmHg)**	**∆MAP (mmHg)**
HG				
aPWV, m/s	**r = 0.53**	**r = 0.34**	**r = 0.54**	**r = 0.45**
(unadjusted)	**P < 0.001**	**P = 0.023**	**P < 0.001**	**P = 0.002**
aPWV, m/s	**r = 0.53**	**r = 0.34**	**r = 0.53**	**r = 0.45**
(adj V̇O2peak)	**P < 0.001**	**P = 0.027**	**P < 0.001**	**P = 0.002**
aPWV, m/s	**r = 0.52**	**r = 0.38**	**r = 0.53**	**r = 0.47**
(adj BL MAP)	**P < 0.001**	**P = 0.012**	**P < 0.001**	**P = 0.002**
PEMI				
aPWV, m/s	r = 0.16	r = -0.01	**r = 0.55**	r = 0.09
(unadjusted)	P = 0.151	P = 0.926	**P < 0.001**	P = 0.589
aPWV, m/s	r = 0.20	r = 0.05	**r = 0.57**	r = 0.139
(adj V̇O2peak)	P = 0.191	P = 0.728	**P < 0.001**	P = 0.374
aPWV, m/s	r = 0.14	r = 0.05	**r = 0.52**	r = 0.101
(adj BL MAP)	P = 0.365	P = 0.770	**P < 0.001**	P = 0.521

Unadjusted data are presented as Pearson's product-moment correlation coefficients, with associated significance values. Adjusted correlations for V̇O2peak (ml/kg/min) and baseline MAP were performed using Partial correlation analysis within SPSS v20 (IBM Corp). Significant correlations are presented in bold. *Abbreviations: aPWV, aortic pulse wave velocity; BL, baseline; HG, handgrip exercise; MAP, mean arterial pressure; PEMI, post-exercise muscle ischemia; V̇O2peak, peak oxygen uptake.*