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The interactive effects of age and sex on the neuro-cardiovascular responses during fatiguing rhythmic handgrip exercise

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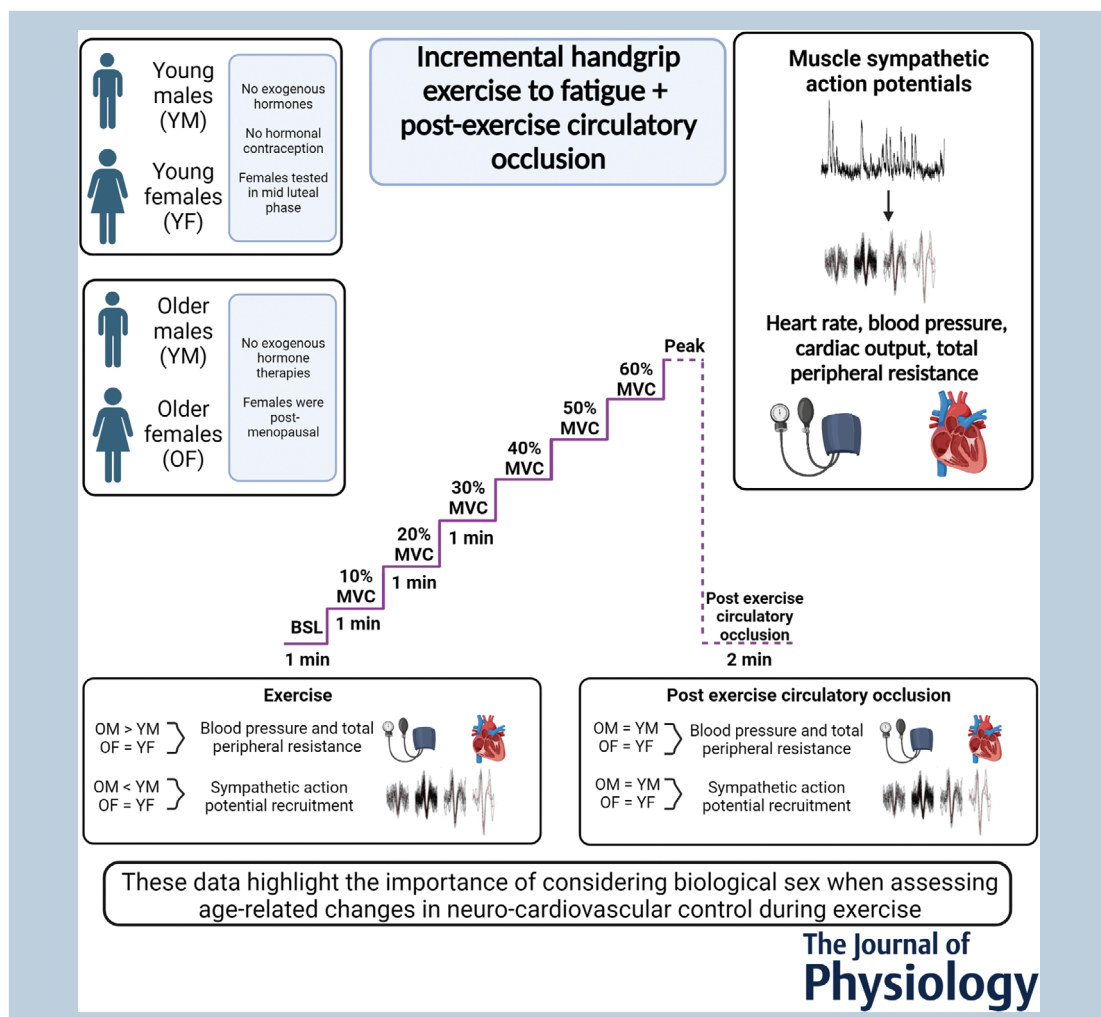
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Abstract The impact of age on exercise pressor responses is equivocal, likely because of sex-specific neuro-cardiovascular changes with age. However, assessments of the interactive effects of age and sex on muscle sympathetic nerve activity (MSNA) responses to exercise are lacking. We tested the hypothesis that older females would exhibit exaggerated increases in blood pressure (BP) and MSNA discharge patterns during handgrip exercise compared with similarly aged males and young adults. Twenty-five young (25 (2) years; mean (SD)) males (YM; $n = 12$) and females (YF; $n = 13$) and 23 older (71 (5) years) males (OM; $n = 11$) and females (OF; $n = 12$) underwent assessments of BP, total peripheral resistance (TPR; Modelflow) and MSNA action potential (AP) discharge patterns (microneurography) during incremental rhythmic handgrip exercise and post-exercise circulatory occlusion (PECO). OM demonstrated larger Δ BP and Δ TPR from baseline than YM (both $P < 0.001$) despite smaller increases in Δ APs/burst (OM: 0.4 (3) vs. YM: 5 (3) spikes/burst, $P < 0.001$) and Δ AP clusters/burst (OM: 0.1 (1) vs. YM: 1.8 (1) clusters/burst, $P < 0.001$) during exercise. Testosterone was lower in OM than YM ($P < 0.001$) and was inversely related to Δ BP but positively related to Δ AP clusters/burst in males (both $P = 0.03$). Conversely, YF and OF demonstrated similar Δ BP and Δ AP discharge during exercise (range: $P = 0.75$ – 0.96). Age and sex did not impact haemodynamics or AP discharge during PECO (range: $P = 0.08$ – 0.94). Altogether, age-related changes in neuro-cardiovascular reactivity exist in males but not females during fatiguing exercise and seem to be related to testosterone. This sex-specific impact of age underscores the importance of considering biological sex when assessing age-related changes in neuro-cardiovascular control during exercise.

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Abstract figure legend The effect of age on exercise pressor reflex activation is unclear, likely because of sex-specific neuro-cardiovascular changes with age. While there is some evidence supporting heightened sympathetic activity in older females during exercise, assessments of the interactive effects of age and sex on muscle sympathetic nerve activity (i.e. sympathetic outflow directed toward the skeletal muscle vasculature; MSNA) during exercise are lacking. We tested the hypothesis that older females would exhibit exaggerated increases in blood pressure and MSNA discharge patterns during handgrip exercise compared with similarly aged males and young adults. Using incremental handgrip exercise (1 min stages at a 50% duty cycle, increasing by 10% maximal voluntary contraction increments) and post-exercise circulatory occlusion, we were able to parse out the central and peripheral determinants of sympathetic discharge. We found age-related reductions in the AP discharge responses, and age-related increases in exercise mean arterial pressure and total peripheral resistance observed in males, but not females. Conversely, sympathetic action potential discharge patterns were unaffected during post-exercise circulatory occlusion. Altogether, these data highlight the critical importance of considering biological sex in the interpretation of age-related changes in sympathetic neuro-cardiovascular responses to exercise.

Key points

- Older males have the largest increase in blood pressure despite having the smallest increases in sympathetic vasomotor outflow during rhythmic handgrip exercise.
- Young males demonstrate greater increases in sympathetic action potential (AP) discharge compared with young females during rhythmic handgrip exercise.
- Older adults (regardless of sex) demonstrate smaller increases in muscle sympathetic nerve activity (MSNA) burst amplitude and total AP clusters compared with young adults during exercise, as well as smaller increases in integrated MSNA burst frequency, incidence and total MSNA activity during post-exercise circulatory occlusion (i.e. independent effect of age).
- Males, but not females (regardless of age), reflexively modify AP conduction velocity during exercise.
- Our results indicate that age and sex independently and interactively impact the neural and cardiovascular homeostatic adjustments to fatiguing small muscle mass exercise.

Introduction

The sympathetic nervous system plays a pivotal role in the homeostatic responses to exercise via increases in efferent sympathetic nerve discharge that serve to regulate blood pressure (BP) while redistributing blood flow to active muscle to meet metabolic demand (Delius et al., 1972; Mitchell & Schmidt, 1983). These neurocirculatory adjustments are mediated by central feed-forward signals originating from higher brain centres (i.e. central command) (Goodwin et al., 1972; Krogh & Lindhard, 1913), as well as peripheral feedback from group III and group IV skeletal muscle afferents (i.e. the exercise pressor reflex) (Alam & Smirk, 1937; Kaufman et al., 1983; Mark et al., 1985). Increases in BP and sympathetic nerve traffic during moderate-intensity exercise are primarily driven by the exercise pressor reflex, whereas during high-intensity exercise, central command also contributes to increases in sympathetic outflow directed toward inactive skeletal muscle vasculature (Victor et al., 1995). To partition the central *versus* peripheral determinants of sympathetic discharge during fatiguing exercise, many studies have used a post-exercise circulatory occlusion (PECO) as it represents a period where muscle sympathetic nerve activity (MSNA; a direct measure of sympathetic neural discharge directed toward skeletal muscle vasculature) remains elevated due to stimulation of chemically sensitive muscle afferents, independent of central processes related to effort or muscle mechanoreceptor stimulation (Alam & Smirk, 1937; Mark et al., 1985).

Among young adults, males demonstrate greater increases in MSNA and BP than females during both exercise and PECO (Ettinger et al., 1996; Jarvis et al., 2011), possibly due to a greater proportion of type I muscle fibres (and oxidative metabolism) in females (Simoneau & Bouchard, 1989), central sympatho-inhibitory effects of oestradiol (Ciriello & Roder, 2013; Xue et al., 2013), or oestradiol-mediated offsetting of sympathetic α -adrenergic vasoconstriction (Kneale et al., 2000). Conversely, the independent impact of age on the sympathetic neuro-cardiovascular responses to exercise and isolated muscle metaboreflex activation remains equivocal. Specifically, in primarily male samples, either no impact of age (Greaney et al., 2013) or attenuated

(Houssiere et al., 2006; Markel et al., 2003; Ng et al., 1994) sympathetic and pressor responses to exercise and PECO are reported in older adults. Conversely, post-menopausal females demonstrate exaggerated increases in MSNA and BP during isometric exercise and PECO compared with young, pre-menopausal females, likely due to the loss of oestradiol following menopause (Wenner et al., 2022). Taken together, these data suggest that there may be a sex-specific impact of age on the sympathetic neurocirculatory responses to exercise and PECO.

Thus far, only one study has assessed the impact of age and sex on the cardiovascular responses to rhythmic small muscle mass exercise, finding a greater rise in BP in older females compared with young females and similarly aged males that was driven primarily by increases in total peripheral resistance (TPR) (Trinity et al., 2018). Given that BP and TPR are positively associated with MSNA in older females (Hart et al., 2011), and that females exhibit greater age-related increases in MSNA than males (Keir et al., 2020; Matsukawa et al., 1998; Narkiewicz et al., 1999), it was thought that the exaggerated exercise pressor responses in older females was attributed to heightened sympathetic neural reactivity. However, direct assessments of sympathetic outflow were not conducted. Thus, the interactive effects of age and sex on the sympathetic neural responses to exercise remain unknown. Furthermore, it is unclear whether these exaggerated pressor responses in older females were primarily driven by central or peripherally mediated increases in sympathetic neural discharge.

To date, assessments of both age- and sex-related changes in sympathetic outflow during exercise have been completed using traditional, integrated MSNA analysis. Yet, bursts of MSNA represent periods of synchronously firing efferent action potentials (APs) that, in response to acute physiological stress, exhibit unique recruitment patterns that cannot be detected in the integrated MSNA neurogram (Salmanpour et al., 2010). We have previously found reduced AP discharge and recruitment, but not integrated MSNA responses, during apnoeic stress in older compared with young adults (Badrov, Lalande et al., 2016), indicating that the central features governing sympathetic neural emissions directed toward the skeletal muscle vasculature are attenuated with age. However, whether this is the case during exercise, and whether sex impacts

Andrew D'Souza is a doctoral candidate at Western University in London, Canada, under the supervision of Dr Kevin Shoemaker. His doctoral research is focused on the impact of age, biological sex and sex hormones on the sympathetic neural control of the circulation. Andrew has been funded by an Alexander Graham Bell Canada Graduate Scholarship through the Natural Sciences and Engineering Research Council of Canada and an Ontario Graduate Scholarship doctoral award.



age-related changes in axonal discharge and recruitment patterns remains unknown.

Therefore, the objective of the current study was to determine the impact of age and sex on the sympathetic and cardiovascular responses during incremental rhythmic handgrip exercise to fatigue and PECO. Incremental rhythmic handgrip exercise was used because MSNA increases during fatiguing, high-intensity – but not moderate-intensity – rhythmic exercise, primarily due to central command activation (Victor & Seals, 1989; Victor et al., 1995). Thus, unlike isometric exercise, where the progressive increases in MSNA are largely attributed to muscle mechano- and metaboreflex, with less contribution of central command (Mark et al., 1985; Victor et al., 1988, 1989), incremental rhythmic handgrip exercise provides a unique opportunity to assess the role of central command on sympathetic neural discharge patterns during exercise. Our hypotheses are that (1) older females will have the greatest increases in BP, integrated MSNA and AP recruitment during exercise and PECO compared with all other groups (i.e. interactive effect of age and sex), (2) young adults will demonstrate greater AP recruitment than older adults during exercise and PECO (i.e. independent effect of age), and (3) young males will have larger increases in BP, sympathetic discharge during exercise and PECO compared with young females (i.e. independent effect of sex).

Methods

Ethical approval

Written and informed consent was obtained from all participants prior to testing. Approval of experimental protocols was granted by the institutional review boards of the University of Texas Southwestern Medical Center, Texas Health Presbyterian Hospital Dallas (File no. STU-2022-0433), and The Western University Health Sciences Research Ethics Board (File no. 119 380). The study was conducted in accordance with the *Declaration of Helsinki*.

Participants

Fifty individuals (young males (YM): $n = 12$, young females (YF): $n = 13$, older males (OM): $n = 12$, older females (OF): $n = 13$) participated in the current study. Studies were conducted in a research laboratory at Western University ($n = 13$; 7 YM and 6 YF) and a clinical research laboratory at The Institute for Exercise and Environmental Medicine ($n = 37$; 5 YM, 7 YF, 12 OM, 13 OF). All participants were non-smokers, free of overt cardiovascular disease, metabolic syndrome, hepatic and renal disease, neurological disease and pulmonary disease, and were not pregnant. To minimize the impact of exogenous sex hormones on neural and cardio-

vascular outcomes, no participants were using hormonal replacement therapy or contraception (e.g. intrauterine devices, oral contraception). Young females were tested in the mid-luteal phase of the menstrual cycle (as determined by self-report ($n = 13$), blood draw ($n = 12$) and ovulation kits ($n = 9$)). Microneurographic recordings were unsuccessful in one older male and one older female. Thus, data are reported for 11 older males and 12 older females.

Experimental procedures

Participants reported to the laboratory in a fasted state (≥ 8 h), and having refrained from caffeine, alcohol and vigorous exercise for a minimum of 12 h. Prior to beginning the study, participants emptied their bladder to avoid any effects of bladder distention on sympathetic activity and BP (Fagius & Karhuvaara, 1989). Thereafter, participants laid in the supine position and blood samples were collected from the antecubital vein (oestradiol, progesterone, testosterone, sex hormone-binding globulin, and albumin). Participants were then instrumented for the study with an electrocardiogram and a finger BP cuff. Prior to microneurography, participants performed three brief (~ 3 s) maximal contractions using a handgrip dynamometer with the dominant hand to determine their maximal voluntary contraction (MVC) force. Data collection began after at least 10 min of quiet rest, and at least 10 min after an acceptable microneurographic nerve recording was obtained. Baseline data were collected for 1 min, and participants began exercise thereafter. The exercise protocol consisted of rhythmic handgrip exercise using a 50% duty cycle (2 s contraction, 2 s relaxation) at a rate of 15 contractions per minute. Participants began contractions at 10% MVC for 1 min. Each stage thereafter, the target intensity was increased by 10% MVC every minute until task failure. Task failure was defined as the inability to maintain the target force output for two consecutive contraction:relaxation cycles, or volitional fatigue. Once the participant was unable to meet the target force output, a BP cuff was inflated rapidly around the exercising forearm (distal to the elbow) to suprasystolic pressure (~ 250 mmHg) 4 s before the cessation of handgrip exercise to induce PECO for 2 min. Ratings of perceived exertion (RPE) were noted at the end of exercise using the 6–20 Borg scale (Borg, 1982). All experimental sessions were conducted in a thermoneutral environment ($\sim 24^\circ\text{C}$).

Experimental measures

Oestradiol, progesterone, testosterone sex hormone-binding globulin, and albumin were analysed via chemiluminescence (ARUP laboratories, Salt Lake City, UT, USA, and LifeLabs, London, Ontario, Canada).

Bioavailable testosterone was calculated from total testosterone, sex hormone-binding globulin and albumin using a previously validated equation (Vermeulen et al., 1999). Heart rate (HR) was recorded using a standard lead II electrocardiogram (BioAmplifier, ADInstruments, Dunedin, New Zealand). Brachial BP was measured by electrophygmomanometry (model 4240; SunTech Medical Instruments, Raleigh, NC) with a microphone placed over the brachial artery to detect Korotkoff sounds. Beat-by-beat BP was determined via finger photoplethysmography (Finapres Medical Systems, Amsterdam, the Netherlands, and Human NIBP Nano system, ADInstruments). Multi-unit MSNA was obtained via microneurography of the peroneal nerve, as previously described (Jarvis et al., 2011). Briefly, a 200 μm diameter tungsten microelectrode, tapering to an uninsulated 1–5 μm tip, was inserted percutaneously into the common peroneal nerve at the popliteal fossa, and a reference electrode was positioned subcutaneously ~1–3 cm from the recording site. A suitable MSNA site was obtained by manual manipulation of the microelectrode until a pulse-synchronous burst pattern was observed. An MSNA recording site was confirmed by the absence of skin paraesthesia, and an increase in sympathetic discharge during a maximal apnoea but not in response to a startling loud noise (Vallbo et al., 1979). The MSNA neurogram was recorded with a nerve traffic analyser (662C-3; Bioengineering Dept., University of Iowa, Iowa City, IA). The neural signal was amplified (gain: 70,000–160,000-fold), and band-pass filtered (bandwidth: 700–2000 Hz) before being rectified and integrated (leaky integrator; 0.1 s time constant). The raw, filtered, and integrated MSNA neurograms were sampled at 10,000 Hz, and stored for offline analysis using Powerlab (Labchart 8; ADInstruments, Colorado Springs, CO).

Data analysis

Finger photoplethysmography-derived BP waveforms were calibrated to the average of three brachial BP measures. Calibrated BP waveforms were then extracted on a beat-by-beat basis to determine systolic BP (SBP), diastolic BP (DBP) and mean arterial pressure (MAP), as defined as the maximum, minimum and mean BP of each waveform. Stroke volume and cardiac output were estimated from the BP waveform using the Modelflow method (Labchart, ADInstruments), which incorporates age and biological sex, and were presented as stroke volume index and cardiac index to account for differences in body size between individuals. TPR was calculated as the quotient of MAP and cardiac output and multiplied by 80.

Integrated bursts of MSNA were identified in accordance with recently published guidelines (Hart et al., 2017), and quantified as burst frequency (bursts/min) and incidence (bursts/100 heart beats). Burst amplitude was measured in volts and normalized to the largest burst at baseline, which was given a value of 100. Total activity of MSNA was calculated as the product of burst amplitude and burst frequency.

Postganglionic sympathetic APs were detected and extracted from the raw filtered neurogram using a wavelet-based methodology, as described previously (Salmanpour et al., 2010). Briefly, APs were binned on peak-to-peak amplitude and histogram analysis was performed to group APs into amplitude-based clusters using Scott's rule (Scott, 1979). As such, the number of total clusters varied between participants. Within-participant cluster characteristics were normalized to ensure that bin width, maximum bin centre and the total number of AP clusters would be identical across conditions (i.e. baseline to 10%, 20%, 30%, etc.). This normalization process assures that corresponding clusters across conditions contain APs with similar peak-to-peak amplitudes. This process was done separately for exercise and PECO, guaranteeing that an increase in the number of active clusters at peak exercise or during PECO represents recruitment of subpopulations of previously silent, larger-sized AP clusters.

AP discharge was quantified as follows: (1) AP frequency (spikes/min) and AP incidence (spikes/100 heart beats) as well as the number of active APs firing within an MSNA burst (spikes/burst), reflecting total sympathetic discharge; (2) the number of active AP clusters per integrated burst (clusters/burst) and the number of total AP clusters detected, reflecting the recruitment of larger axonal subpopulations; and (3) the conduction latency of individual APs, established as the time delay between the R-wave of the preceding cardiac cycle and the negative deflection of the AP waveform, reflecting modifications in synaptic delays and overall conduction velocity. AP cluster latency was determined for each cluster. Because the number of AP clusters recruited varied between individuals, the number of total clusters was normalized to 10 bins, each containing 10% ranges (i.e. 10–20%, 20–30%, etc.) of the largest detected cluster, which was given a value of 100% (Badrov et al., 2015).

Statistical analysis

All data are presented as means (SD). Data are expressed as a 1 min average of peak exercise (defined as the final completed 1 min stage of exercise) or an average of each minute of PECO (i.e. PECO1, PECO2). Analysis for outliers was conducted using the ROUT method

Table 1. Participant characteristics, resting blood pressure and sex hormones

	Young		Older		ANOVA P-values		
	Male	Female	Male	Female	Age	Sex	Age × Sex
Age (yrs)*	26 (5)	25 (4)	71 (5)	70 (4)	<0.001	0.346	0.890
Height (cm) †	178 (8)	164 (6)	172 (8)	166 (7)	0.384	<0.001	0.067
Weight (kg) †	79 (11)	67 (12)	78 (7)	71 (15)	0.587	0.009	0.489
BSA (m ²) †	2.0 (0.2)	1.7 (0.2)	1.9 (0.1)	1.8 (0.2)	0.744	<0.001	0.174
BMI (kg/m ²)	24.9 (2.9)	25.0 (4)	26.6 (2.1)	25.8 (4.3)	0.247	0.730	0.676
Heart rate (beats/min)*	67 (11)	68 (7)	57 (4)	65 (7)	0.011	0.067	0.090
Brachial SBP (mmHg)*	116 (7)	114 (12)	133 (11)	131 (9)	<0.001	0.557	0.861
Brachial DBP (mmHg)*	70 (6)	71 (8)	79 (2)	75 (4)	<0.001	0.448	0.110
Brachial MAP (mmHg)*	85 (6)	86 (7)	97 (4)	94 (5)	<0.001	0.408	0.255
Cardiac index (L/min/m ²)*	2.9 (0.7)	3.1 (0.6)	2.6 (0.3)	2.8 (0.3)	0.032	0.116	0.728
Stroke volume index (mL/m ²)	44 (9)	47 (9)	45 (6)	44 (7)	0.790	0.530	0.346
Total peripheral resistance (dynes/s/cm ⁵)*	1247 (389)	1403 (275)	1592 (243)	1481 (177)	0.014	0.788	0.113
MVC (kg)*†	42 (5)	32 (8)	39 (6)	25 (5)	0.009	<0.001	0.274
Oestradiol (pmol/L)	93 (25) ^α	687 (386) [‡]	86 (21)	15 (11)	<0.001	<0.001	<0.001
Progesterone (nmol/L)	0.8 (0.5) ^α	38 (18) [‡]	<0.3 ^β	<0.3 ^{‡β}	<0.001	<0.001	<0.001
Total testosterone (nmol/L) †	21 (7)	1 (0.5)	18 (7)	0.5 (0.4)	0.193	<0.001	0.376
Albumin (g/L)*	46 (3)	44 (5)	41 (3)	39 (2)	<0.001	0.071	0.985
Sex hormone-binding globulin (nmol/L)	29 (13) ^α	78 (51)	49 (16)	59 (26)	0.909	0.002	0.032
Bioavailable testosterone (nmol/L)	11 (2) ^{α‡}	0.3 (0.2)	7 (2) ^α	0.2 (0.2)	<0.001	<0.001	<0.001

Data are means (standard deviation). BMI, body mass index. BSA, body surface area. DBP, diastolic blood pressure. MAP, mean arterial pressure. MVC, maximum voluntary contraction force. SBP, systolic blood pressure. Statistical comparisons were carried out using linear mixed model analyses. Significance was set to $P < 0.05$.

*Significant difference between young and older adults, independent of sex (main effect of age).

†Significant difference between males and females, independent of age (main effect of sex).

‡Significant difference between young males and older males or between young and older females (within-sex *post hoc* comparison for the interaction term).

^αSignificant difference between young males and females or older males and females (within-age *post hoc* comparison for the interaction term).

^βProgesterone values for all older males and females were below the lower limit of detection of our assay (0.3 nmol/L). For statistical comparisons, a value of 0.3 nmol/L was assigned to each older adult. Due to participant comfort ($n = 1$) and technical difficulties ($n = 1$), blood was drawn from 11 young males and 12 young females.

(GraphPad Prism version 9.3, San Diego, CA). Two-factor linear mixed model analyses of variance (ANOVA) were used to evaluate the effects of age and sex on participant characteristics, resting haemodynamics, sex hormones and peak exercise neuro-cardiovascular outcomes. The time course effects of age, sex and stage (%MVC or PECO1 and 2) on all cardiovascular and neural outcomes were assessed using a three-factor linear mixed model analysis with a compound symmetry structure. Bonferroni-corrected *post hoc* comparisons were performed to evaluate specific differences between means when applicable. Pairwise comparisons were restricted to within age and sex. Thus, young males were not compared with older females and young females were not compared with older males. For the AP analysis, one young female was excluded due to a poor signal-to-noise ratio (<3.7) in the MSNA neurogram. Additionally, blood samples were obtained in 11 of 12 young males and 12 of 13 young females due to

participant discomfort ($n = 1$) and technical difficulties ($n = 1$). Partial regression analyses were conducted to assess the relationship between bioavailable testosterone and MAP, TPR and AP recruitment while adjusting for age. Statistical significance was accepted at $P \leq 0.05$. Statistical analyses were performed using SPSS statistics (Version 28.0; IBM, Armonk, NY).

Results

Participant characteristics, resting haemodynamics and sex hormones

Participant characteristics, resting haemodynamics and sex hormone data are presented in Table 1. Regardless of age, females were shorter ($P < 0.001$), and had a lower body mass ($P = 0.009$) and smaller body surface area ($P < 0.001$) than males, whereas body mass index was not impacted by age ($P = 0.247$) or sex ($P = 0.730$). Older

Table 2. Absolute haemodynamics, integrated MSNA and AP discharge/recruitment patterns in young and older males and females at peak exercise (i.e. final completed stage of exercise)

	Young		Older		ANOVA <i>P</i> -values		
	Male	Female	Male	Female	Age	Sex	Age × Sex
Haemodynamics							
Heart rate (beats/min)	88 (14)	86 (8)	72 (10)*	78 (9)*	<0.001	0.664	0.182
Mean arterial pressure (mmHg)	98 (6)	105 (11)	123 (16) † ^α	107 (9)	<0.001	0.155	<0.001
Cardiac index (L/min/m ²)	4.3 (1.1)	3.8 (0.8)	2.8 (0.3)*	3.1 (0.4)*	<0.001	0.604	0.079
Stroke volume index (mL/m ²)	49 (10)	45 (10)	41 (6)*	40 (8)*	0.016	0.479	0.549
Total peripheral resistance (dynes/s/cm ⁵)	526 (159) ^α	795 (213)	961 (360) †	927 (360)	<0.001	0.555	0.011
Integrated MSNA							
MSNA burst frequency (bursts/min)	20 (8)	21 (8)	37 (9)*	30 (10)*	<0.001	0.280	0.157
MSNA burst incidence (bursts/100 heart beats)	23 (8)	25 (10)	52 (13) † ^α	39 (11) †	<0.001	0.110	0.016
MSNA burst amplitude (AU)	92 (24)	93 (32)	76 (17)	83 (17)	0.072	0.601	0.659
Total MSNA activity (AU/min)	1819 (906)	1934 (876)	2748 (700)*	2523 (975)*	0.005	0.830	0.509
AP discharge and recruitment							
AP frequency (spikes/min)	302 (306)	223 (166)	475 (341)	289 (138)	0.110	0.078	0.465
AP incidence (spikes/100 heart beats)	332 (304)	275 (222)	646 (394)*	372 (177)*	0.017	0.052	0.195
APs/burst	14 (10)	10 (5)	12 (6)	10 (4)	0.642	0.107	0.673
AP clusters/burst	6 (2)	4 (1)	6 (3)	5 (1)	0.569	0.053	0.770
Total AP clusters	14 (5)	12 (2)	17 (7)	14 (5)	0.090	0.113	0.684

Data are means (standard deviation). AP, action potential. MSNA, muscle sympathetic nerve activity. Statistical comparisons were carried out using linear mixed model analyses. Significance was set to $P < 0.05$.

* Significant difference between young and older adults, independent of sex (main effect of age).

† Significant difference between males and females, independent of age (main effect of sex).

‡ Significant difference between young males and older males or between young and older females (within-sex *post hoc* comparison for the interaction term).

^α Significant difference between young males and females or older males and females (within-age *post hoc* comparison for the interaction term). $n = 12$ for the AP indices in young females as one participant was excluded due to a poor signal-to-noise ratio.

adults had higher SBP, DBP and MAP compared with young adults (all $P < 0.001$); however, sex did not impact resting BP (range: $P = 0.408$ – 0.557). MVC was higher in males ($P < 0.001$) and young adults ($P = 0.009$) compared with females and older adults, respectively. Oestradiol and progesterone concentrations were greater in young females relative to older females (both $P < 0.001$) and young males (both $P < 0.001$). Bioavailable testosterone was greater in young compared with older males ($P < 0.001$), but not different between young and older females ($P = 0.785$). Sex-based comparisons within each age group revealed that both older and young males had greater levels of bioavailable testosterone than similarly aged females (both $P < 0.001$).

Incremental exercise to fatigue

The peak haemodynamic responses to incremental handgrip exercise to fatigue are presented in Fig. 1, and absolute haemodynamic data at peak exercise are presented in Table 2. The increase in HR at peak exercise was greater in young, relative to older adults (Fig. 1F; $P = 0.012$). Significant age-by-sex-by-stage interactions

were observed for the peak increase in MAP from baseline (Fig. 1B; $P < 0.001$), with *post hoc* analyses revealing a larger rise in MAP in older males than similarly aged females and young males (both $P < 0.001$). At peak exercise, young males demonstrated larger increases in cardiac index (Fig. 1C) and stroke volume index (Fig. 1D), and larger reductions in TPR (Fig. 1E) than older males (all $P < 0.001$) and young females (range: $P < 0.001$ – 0.014). Furthermore, older females demonstrated smaller increases in cardiac index than young females ($P = 0.007$), and smaller changes in TPR than older males ($P = 0.009$). Young adults (regardless of sex) had a higher group mean absolute HR ($P < 0.001$), cardiac index ($P < 0.001$) and stroke volume index ($P = 0.016$) at peak exercise. Furthermore, a significant age-by-sex interaction was observed for MAP ($P < 0.001$), with *post hoc* analyses revealing greater MAP in older males compared with young males ($P < 0.001$) and older females ($P < 0.001$). Similarly, a significant age-by-sex interaction was found for peak exercise TPR ($P = 0.011$) and *post hoc* analyses indicated that young males had lower TPR than young females ($P = 0.024$) and older males ($P < 0.001$). RPE at peak exercise was similar in all participants (YM: 18(1),

YF: 17(2), OM: 17(2) and OF: 17(3); $P_{interaction} = 0.669$). Furthermore, a significant positive relationship was observed between MVC and the peak change in MAP across all participants (Fig. 2; $P = 0.005$).

Peak integrated MSNA responses to incremental handgrip exercise are presented in Fig. 3. The peak change in MSNA burst frequency (Fig. 3A; $P = 0.024$), incidence

(Fig. 3B; $P = 0.003$) and amplitude (Fig. 3C; $P = 0.048$) were smaller in older compared with young adults. Furthermore, females demonstrated greater increases in MSNA burst incidence than males, regardless of age ($P = 0.015$). Neither age ($P = 0.116$) nor sex ($P = 0.318$) impacted the peak change in total MSNA activity (Fig. 3H). However, absolute MSNA burst frequency

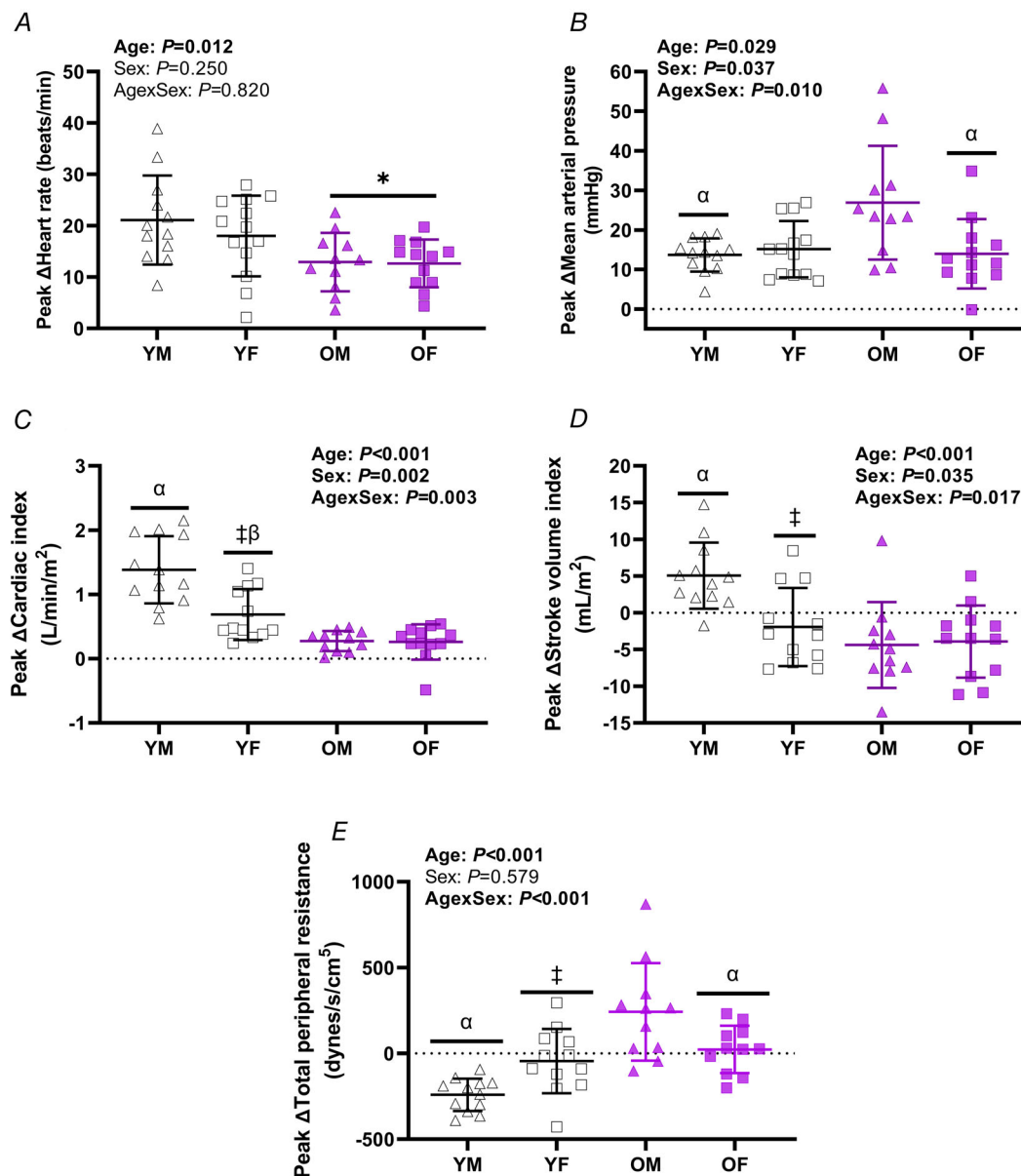


Figure 1. Peak changes in haemodynamics during exercise

Peak changes (Δ) in heart rate (A), mean arterial pressure (B), cardiac index (C), stroke volume index (D) and total peripheral resistance (E) in young males (YM, $n = 12$; open triangles and solid black lines), young females (YF, $n = 13$; open squares and solid black lines), older males (OM, $n = 11$; purple triangles and dashed lines) and older females (OF, $n = 12$; purple squares and dashed lines). Data were analysed using two-factor (age and sex) analyses of variance (ANOVA). *Post hoc* comparisons were carried out using Bonferroni multiple comparisons tests in the event of a significant interaction effect. *Significantly different between young and older adults, independent of sex. # Significant difference between older males and females. †Significantly different versus young males. α Significantly different versus older males. β Significantly different versus older females. Significance was set to $P \leq 0.05$. [Colour figure can be viewed at wileyonlinelibrary.com]

($P < 0.001$) and total MSNA activity ($P = 0.005$) were higher in older compared with young adults at peak exercise (Table 2). Furthermore, *post hoc* analyses on a significant interaction for MSNA burst incidence ($P = 0.016$) revealed that older males ($P < 0.001$) and older females ($P = 0.002$) had higher absolute MSNA burst incidence compared with young males and young females, respectively. Additionally, older males had higher MSNA burst incidence relative to older females at peak exercise ($P = 0.006$).

The peak AP discharge responses to incremental handgrip exercise are presented in Fig. 4. The peak changes in AP frequency (Fig. 4A) and incidence (Fig. 4B) from baseline were smaller in older males than older females and young males (range: $P = 0.001$ – 0.052). Additionally, young males demonstrated greater increases in APs/burst (Fig. 4C) and AP clusters/burst (Fig. 4D) than older males (both $P < 0.001$) and young females (range: $P = 0.001$ – 0.010), whereas the number of total AP clusters recruited was lower in older than young adults, regardless of sex (Fig. 4E; $P = 0.045$). When assessing the peak absolute AP discharge and recruitment during exercise (Table 2), AP incidence was higher in older than young adults ($P = 0.017$). Age and sex did not impact any other indices of absolute AP discharge or recruitment (range: $P = 0.053$ – 0.770).

In all groups, at baseline (YM: $r^2 = 0.69$, YF: $r^2 = 0.89$, OM: $r^2 = 0.83$ and OF: $r^2 = 0.90$; range: $P = 0.003$ – 0.033) and during peak exercise (YM: $r^2 = 0.91$, YF: $r^2 = 0.81$, OM: $r^2 = 0.92$ and OF: $r^2 = 0.82$; range: $P = 0.001$ – 0.020), a pattern emerged whereby AP cluster latency decreased as normalized AP cluster size increased (Fig. 5). The AP cluster size–latency relationship profile was shifted downward at peak exercise in young (range: -15 to -44 ms,

mean: -27 (9)) and older males (range: -5 to -39 ms, mean: -26 (13)) (both $P < 0.001$) whereby APs of all sizes expressed faster conduction velocities. Conversely, females did not demonstrate a shift in the mean AP cluster size–latency relationship during exercise (YF: range: 11 to -21 ms, mean: -13 (20), $P = 0.116$; OF range: 20 to -48 ms, mean: 0.6 (27), $P = 0.955$).

Post-exercise circulatory occlusion

The HR, BP, integrated MSNA and AP recruitment responses to PECO are presented in Fig. 6. No age-by-sex-by-stage interactions were observed for any cardiovascular or sympathetic outcome during PECO (range: $P = 0.462$ – 0.883). The changes in HR (Fig. 6A; range: $P = 0.342$ – 0.845) and MAP (Fig. 6B; range: $P = 0.056$ – 0.942) during PECO were not impacted by age or sex. The changes in MSNA burst frequency (Fig. 6C) and burst incidence (Fig. 6D) were smaller in older males and females compared with their younger counterparts throughout PECO (both $P < 0.001$). Changes in MSNA burst amplitude during PECO were not impacted by age or sex (range: $P = 0.109$ – 0.880), whereas the change in total MSNA activity was smaller in older compared with young adults throughout PECO ($P = 0.001$), but unaffected by sex ($P = 0.876$). Older adults had higher absolute MSNA burst frequency, incidence and total MSNA activity than young adults throughout PECO (Table 3; all $P \leq 0.05$). AP discharge and recruitment patterns during PECO are presented in Fig. 7. Neither age nor sex impacted the changes in any index of AP discharge or recruitment (range: $P = 0.299$ – 0.923). However, absolute AP incidence was higher in older males compared with young males throughout PECO (Table 3; $P = 0.003$). No other indices of absolute AP discharge or recruitment were impacted by age or sex (range: $P = 0.059$ – 0.928). In all groups, at baseline (YM: $r^2 = 0.79$, YF: $r^2 = 0.67$, OM: $r^2 = 0.71$ and OF: $r^2 = 0.83$; range: $P = 0.001$ – 0.039) and during PECO2 (YM: $r^2 = 0.87$, YF: $r^2 = 0.93$, OM: $r^2 = 0.86$ and OF: $r^2 = 0.78$; range: $P < 0.001$ – 0.001), a pattern emerged whereby AP cluster latency decreased as normalized AP cluster size increased. Contrary to exercise, the AP cluster size–latency relationship profile was not altered during PECO in any group (range: $P = 0.547$ – 0.740).

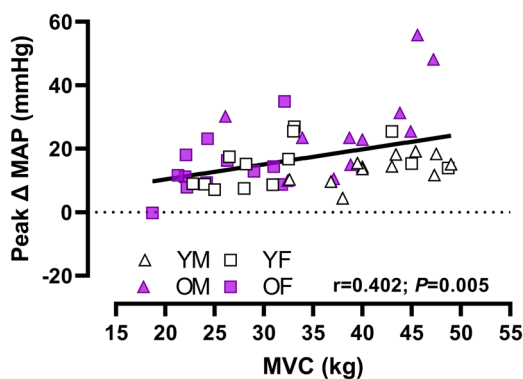


Figure 2. Relationship between maximal voluntary contraction force (MVC) and peak exercise mean arterial pressure (MAP)

Young males (YM; open triangles), young females (YF; open squares), older males (OM; purple triangles) and older females (OF; purple squares). A positive linear relationship was observed between MVC and the peak change in MAP during exercise across all participants. [Colour figure can be viewed at wileyonlinelibrary.com]

Relationships between endogenous sex hormones and haemodynamics, and AP recruitment

After accounting for age, partial regression analyses revealed that bioavailable testosterone was inversely related to Δ MAP ($r_{\text{adj}} = -0.463$; $P = 0.035$) and trended toward a significant negative relationship with Δ TPR ($r_{\text{adj}} = -0.414$; $P = 0.062$) in males (Fig. 8A, B). Additionally, bioavailable testosterone was positively

Table 3. Absolute integrated MSNA and AP discharge patterns in young and older males and females during post-exercise circulatory occlusion

	Stage			P-values			
	BSL	PECO1	PECO2	Age × Stage	Sex × Stage	Age × Sex	Age × Sex × stage
MSNA burst frequency (bursts/min)							
Young males	10 (7)	20 (9)	21 (12)	<0.001	0.363	0.062	0.873
Young females	10 (6)	20 (8)	19 (8)				
Older males	33 (12)*	32 (11)*	35 (11)*				
Older females	22 (11)*	24 (11)*	23 (11)*				
MSNA burst incidence (bursts/100 heart beats)							
Young males	15 (9)	29 (12)	32 (17)	<0.001	0.400	0.010	0.754
Young females	15 (10)	30 (10)	29 (12)				
Older males [†]	57 (20) *	52 (17) *	59 (18) *				
Older females	34 (15) *	35 (16) *	36 (16) *				
MSNA burst amplitude (AU)							
Young males	64 (15)	75 (25)	74 (22)	0.119	0.840	0.859	0.626
Young females	67 (12)	81 (26)	82 (23)				
Older males	61 (9)	63 (10)	68 (15)				
Older females	66 (7)	70 (10)	70 (10)				
Total MSNA activity (AU/min)							
Young males	598 (307)	1407 (686)	1522 (999)	<0.001	0.404	0.066	0.883
Young females	620 (312)	1609 (609)	1558 (753)				
Older males	2005 (793)*	1940 (663)	2398 (1062)*				
Older females	1396 (606)*	1625 (737)	1640 (759)*				
AP frequency (spikes/min)							
Young males	138 (216)	270 (356)	304 (358)	0.107	0.372	0.146	0.820
Young females	87 (72)	185 (164)	181 (160)				
Older males	432 (307)	393 (233)	505 (419)				
Older females	148 (89)	197 (105)	190 (90)				
AP incidence (spikes/100 heart beats)							
Young males	190 (258)	388 (454)	447 (477)	0.069	0.379	0.059	0.659
Young females	130 (108)	265 (227)	267 (227)				
Older males	755 (538)	647 (377)	846 (679)				
Older females	234 (155)	296 (142)	291 (131)				
APs/burst							
Young males	10 (8)	12 (9)	12 (9)	0.980	0.928	0.525	0.462
Young females	8 (4)	8 (5)	9 (7)				
Older males	12 (6)	12 (4)	13 (7)				
Older females	7 (3)	8 (3)	8 (3)				
AP clusters/burst							
Young males	4 (2)	5 (2)	5 (2)	0.442	0.650	0.262	0.860
Young females	4 (1)	4 (1)	4 (2)				
Older males	5 (2)	6 (2)	6 (2)				
Older females	4 (1)	4 (1)	4 (1)				
Total AP clusters							
Young males	9 (4)	11 (4)	12 (4)	0.094	0.622	0.225	0.719
Young females	8 (3)	10 (4)	11 (5)				
Older males	14 (5)	14 (3)	16 (5)				
Older females	10 (3)	12 (3)	11 (3)				

Data are means (standard deviation). AP, action potential. BSL, baseline. MSNA, muscle sympathetic nerve activity. PECO, post-exercise circulatory occlusion. Data are presented as a function of exercise intensity until the last common time point (i.e. 50%) and the final exercise stage (i.e. peak) in all participants. Statistical comparisons were carried out using three-factor (age, sex and stage) linear mixed model analyses. *Post hoc* comparisons were performed in the event of a significant interaction effect. Significance was set to $P < 0.05$.

*Significant difference between young and older adults, independent of sex (i.e. *post hoc* analyses for the age-by-stage interaction term). $n = 12$ for the AP indices in young females as one participant was excluded due to a poor signal-to-noise ratio.

related to Δ AP clusters/burst ($r_{\text{adj}} = 0.469$; $P = 0.032$) but not Δ APs/burst ($r_{\text{adj}} = 0.045$; $P = 0.848$) in males (Fig. 8C, D). Conversely, bioavailable testosterone was not related to Δ MAP ($r_{\text{adj}} = -0.212$; $P = 0.332$; Fig. 8E), Δ TPR ($r_{\text{adj}} = 0.064$; $P = 0.776$; Fig. 8F), Δ AP clusters/burst ($r_{\text{adj}} = -0.259$; $P = 0.245$; Fig. 8G), or Δ APs/burst ($r_{\text{adj}} = -0.306$; $P = 0.166$; Fig. 8H) in females. Oestradiol was not related to Δ APs/burst (YF: $r = 0.05$; $P = 0.882$; Fig. 9A, OF: $r = -0.36$; $P = 0.25$, Fig. 9B) or Δ AP clusters/burst (YF: $r = 0.13$; $P = 0.715$; Fig. 9C, OF: $r = -0.22$; $P = 0.492$, Fig. 9D) in young or older females.

Discussion

The present study examined the independent and interactive effects of age and biological sex on the sympathetic neural and haemodynamic responses during

fatiguing rhythmic handgrip exercise and isolated skeletal muscle metaboreflex activation in humans. Overall, this study indicates that age and sex interactively impact neuro-cardiovascular responses to exercise. The major findings of the current study were: (1) older males, not females, demonstrated larger increases in BP despite smaller increases in AP discharge (frequency, incidence and APs/burst) and recruitment (AP clusters/burst) compared with young males. Furthermore, compared with young females, young males demonstrated greater increases in APs/burst and AP clusters/burst during exercise, whereas sex differences in AP recruitment were not observed in older adults; (2) regardless of sex, older adults demonstrated smaller increases in integrated MSNA burst amplitude and total AP clusters compared with young adults during exercise, and smaller increases in MSNA burst frequency and incidence compared with young adults during exercise

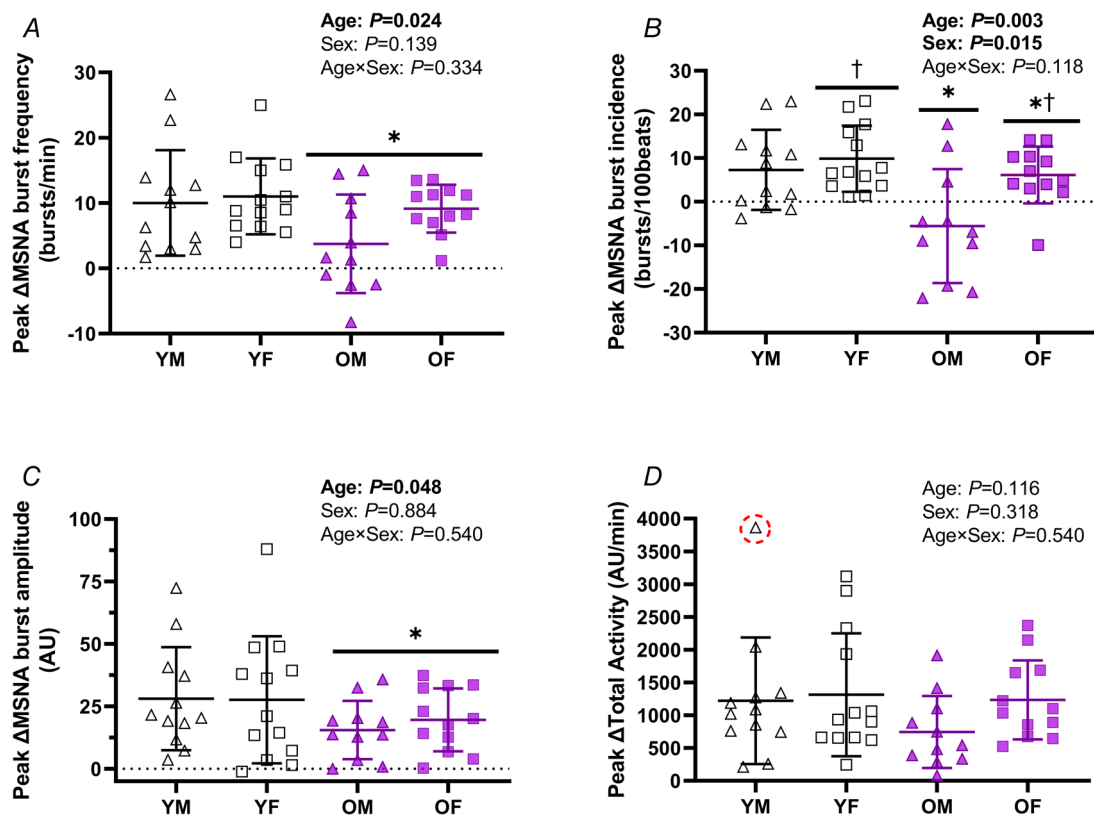


Figure 3. Peak changes in integrated muscle sympathetic nerve activity (MSNA) during exercise

Peak changes (Δ) in MSNA burst frequency (A), burst incidence (B), burst amplitude (C) and total MSNA activity (D) in young males (YM, $n = 12$; open triangles and solid black lines), young females (YF, $n = 13$; open squares and solid black lines), older males (OM, $n = 11$; purple triangles and dashed lines) and older females (OF, $n = 12$; purple squares and dashed lines). Data were analysed using two-factor (age and sex) analyses of variance (ANOVA). *Post hoc* comparisons were carried out using Bonferroni multiple comparisons tests in the event of a significant interaction effect. *Significantly different between young and older adults, independent of age. †Significant difference between males and females, independent of age. Significance was set to $P \leq 0.05$. One outlier was identified (dashed red circle) in the peak change in total MSNA activity; however, these data were incorporated in the statistical model as exclusion of their data did not change the statistical outcomes. [Colour figure can be viewed at wileyonlinelibrary.com]

and PECO; and (3) regardless of age, males, but not females, demonstrated reflex changes in AP conduction velocity during exercise. Collectively, these data provide novel evidence for sex-specific impacts of ageing on the

sympathetic and cardiovascular responses to exercise; however, contrary to our hypothesis, older females did not exhibit exaggerated neuro-cardiovascular reactivity to exercise.

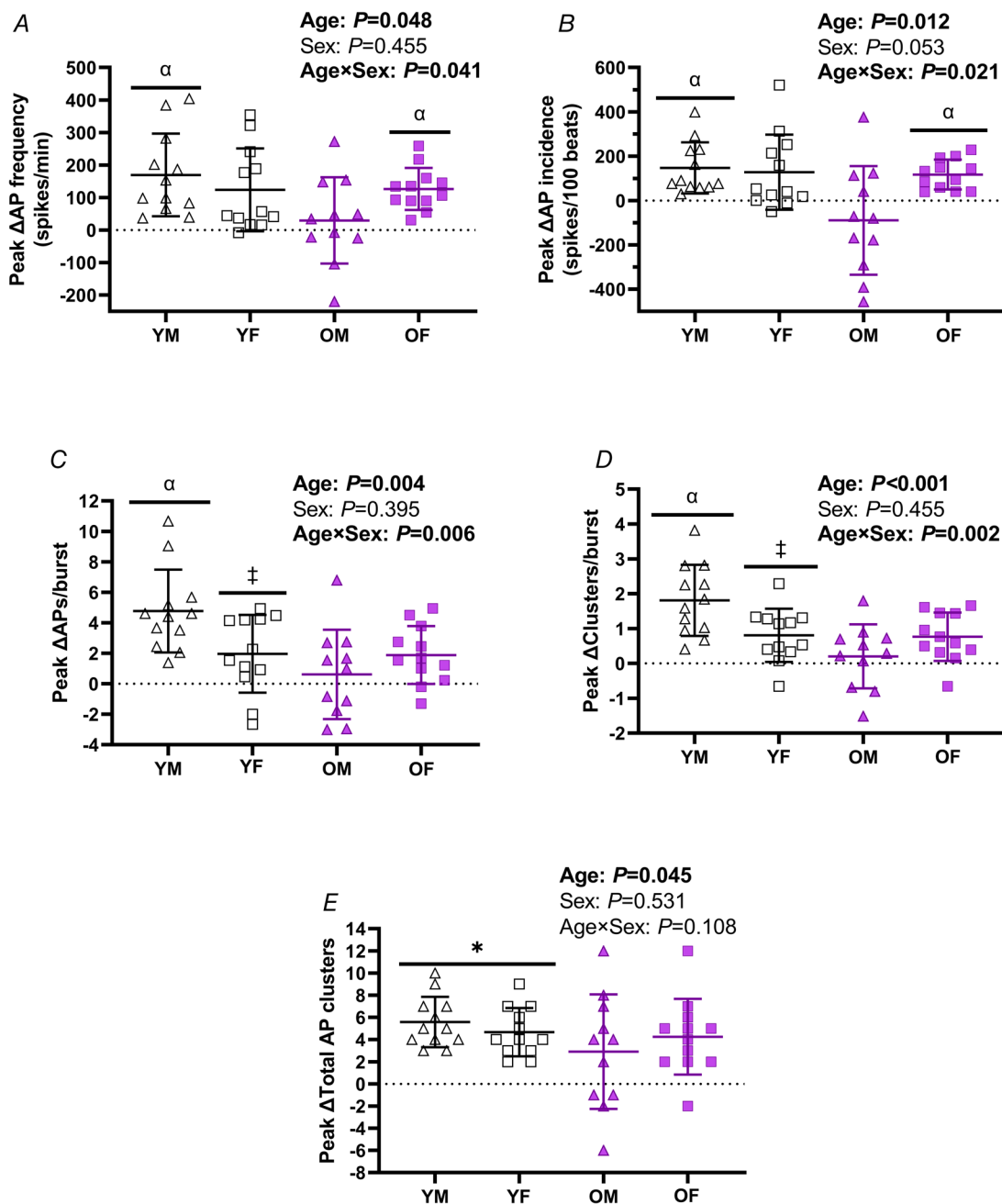


Figure 4. Peak changes in action potential (AP) recruitment during exercise

Peak changes (Δ) in AP frequency (A), AP incidence (B), APs/burst (C), AP clusters/burst (D) and total AP clusters (E) in young males (YM, $n = 12$; open triangles), young females (YF, $n = 12$; open squares), older males (OM, $n = 11$; purple triangles) and older females (OF, $n = 12$; purple squares). Data were analysed using two-factor (age and sex) analyses of variance (ANOVA). *Post hoc* comparisons were carried out using Bonferroni multiple comparisons tests in the event of a significant interaction effect. *Significantly different between young and older adults, independent of age. †Significantly different versus young males. ‡Significantly different versus older males. Significance was set to $P \leq 0.05$. [Colour figure can be viewed at wileyonlinelibrary.com]

Interactive effects of sex and age on the neuro-cardiovascular responses to incremental handgrip exercise and PECO

Reports regarding the impact of age on the pressor and sympathetic neural responses to exercise and PECO are inconsistent, likely due to either single-sex studies or a lack of consideration for the interactive effects of age and sex. To date, only one study has examined the effects of age and sex on the cardiovascular responses during exercise, finding larger increases in BP and TPR in older females compared with similarly aged males and young females during rhythmic plantar flexion exercise, but no age-related differences in males (Trinity et al., 2018). Although the authors attributed the exaggerated TPR and BP responses in older females to heightened sympathetic vasoconstrictor drive, MSNA was not measured. In contrast, in the current study, we found no age-related differences in BP, TPR or sympathetic outflow in females during exercise, whereas older males exhibited greater pressor and TPR responses to rhythmic handgrip exercise compared with young males and similarly aged females

despite smaller increases in integrated MSNA and AP recruitment.

The reasons for the discrepancy between our work and that of Trinity and colleagues (Trinity et al., 2018) are unclear but warrant discussion. BP is determined by cardiac output and TPR (i.e. Ohm's law). Compared with young adults, cardiac output responses to exercise decline with age due to smaller increases in HR and stroke volume (Ogawa et al., 1992). Thus, to raise BP during exercise, there is a shift from a cardiac output-driven increase in BP to greater TPR-mediated pressor responses in older adults. Indeed, like Trinity et al. (2018) this was observed in the current study; however, contrary to their work, we found that older males exhibited the largest increases in BP and TPR. This discrepancy may be explained by group differences in MVC. Specifically, in the current study, MVC was not different between young and older males (42 (5) vs. 38 (6) kg; $P = 0.231$) but was lower in older compared with young females (25 (5) vs. 32 (8) kg; $P = 0.018$), whereas older males in the study by Trinity et al. had a lower plantar flexion MVC than young males, and no difference in MVC

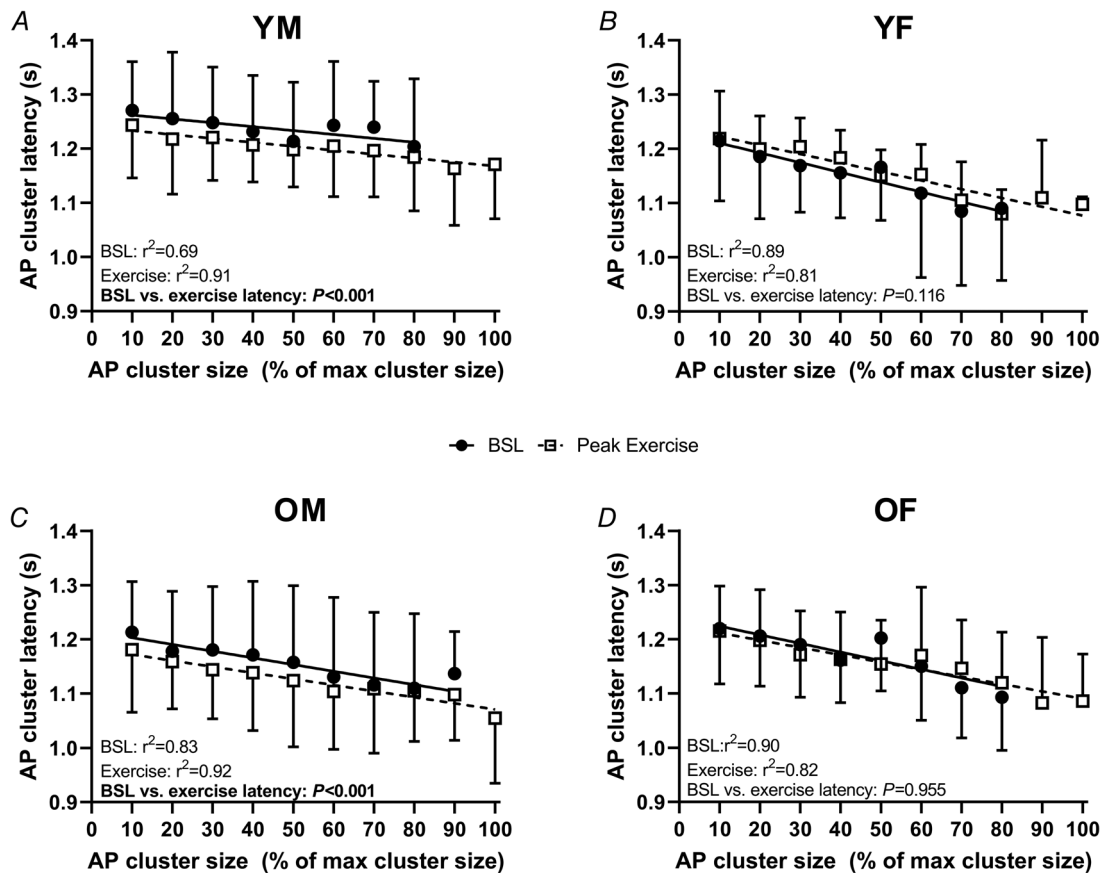


Figure 5. Action potential (AP) latency during exercise
 AP cluster latency as a function of AP cluster size at baseline (BSL; filled circles) and peak exercise (open squares) in young males (YM, $n = 12$; panel A), young females (YF, $n = 12$; panel B), older males (OM, $n = 11$; panel C), older females (OF, $n = 12$; panel D). The AP cluster size–latency relationship was fit with an exponential decay function.

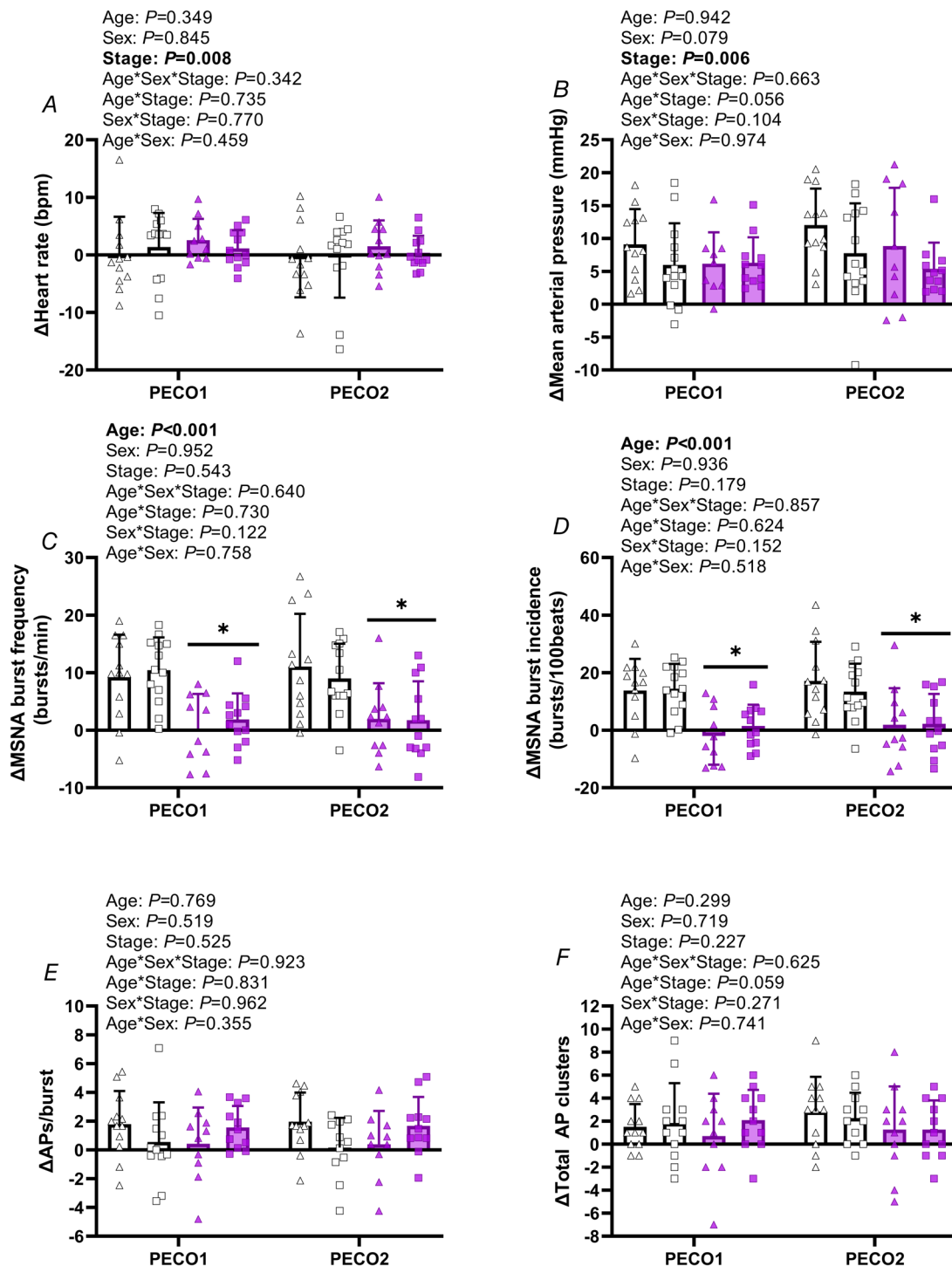


Figure 6. Changes in haemodynamics, integrated muscle sympathetic nerve activity (MSNA) and action potential (AP) recruitment during post-exercise circulatory occlusion (PECO)

Changes (Δ) in heart rate (A), mean arterial pressure (B), MSNA burst frequency (C), MSNA burst incidence (D), APs/burst (E) and total AP clusters (F) in young males (YM, $n = 12$; open triangles and bars), young females (YF, $n = 12$; open squares and bars), older males (OM, $n = 11$; purple triangles and bars) and older females (OF, $n = 12$; purple squares and bars). Data were analysed using three-factor (Age, Sex, Stage) linear mixed model analyses with a compound symmetry structure. *Post hoc* comparisons were carried out using Bonferroni multiple comparisons tests in the event of a significant main effect or interaction. *Main effect of age. Significance was set to $P \leq 0.05$. [Colour figure can be viewed at wileyonlinelibrary.com]

was observed in females (Trinity et al., 2018). In the current study, RPE at peak exercise were similar in all participants, indicating that differences in effort are unlikely to contribute to the discrepancy between studies. Rather, less active muscle mass in older compared with young females would have required a smaller proportion of cardiac output, thus minimizing the need for larger TPR increases to redistribute blood to the active muscle. Indeed, when assessing a subset of young ($n = 7$) and older females ($n = 7$) with similar MVC (YF: 26.8 (3) vs. OF: 26.6 (4) kg; $P = 0.907$, $d = 0.057$), young females demonstrated slightly smaller increases in MAP (YF: 11 (4) vs. OF: 17 (9) mmHg; $P = 0.097$, $d = 0.862$), and larger reductions in TPR (YF: -60 (88) vs. OF: 70 (141) mmHg; $P = 0.068$, $d = 1.11$) compared with older females. However, these comparisons did not reach statistical significance, likely because of the small sample size of this sub-analysis. Furthermore, in line with previous work in young males and females (Lee, Lutz et al., 2021; Lee, Notay et al., 2021), a positive relationship was observed between MVC and BP at peak exercise across all

groups (Fig. 2), indicating that individuals with a higher MVC had a greater pressor response. Thus, the lower MVC in our older females compared with young females may have masked age-related increases in exercise pressor reflex activation in females.

The larger BP and TPR responses observed in older males suggest that more sympathetically mediated vasoconstriction was required to redistribute the limited cardiac output towards active muscle. Thus, it was expected that older males would have greater overall MSNA responses that include larger levels of AP recruitment, because larger bursts of MSNA elicit greater neurogenic vasoconstriction (Fairfax et al., 2013) and comprise more and larger APs than smaller bursts (Ninomiya et al., 1993; Steinback et al., 2010). However, AP discharge was lower in older compared with young males during exercise. This discrepancy between sympathetic neural recruitment and haemodynamics may be explained by numerous factors. First, sympathetic neural discharge between organs is non-uniform (Esler, Jennings, Korner et al., 1984; Esler, Jennings, Leonard

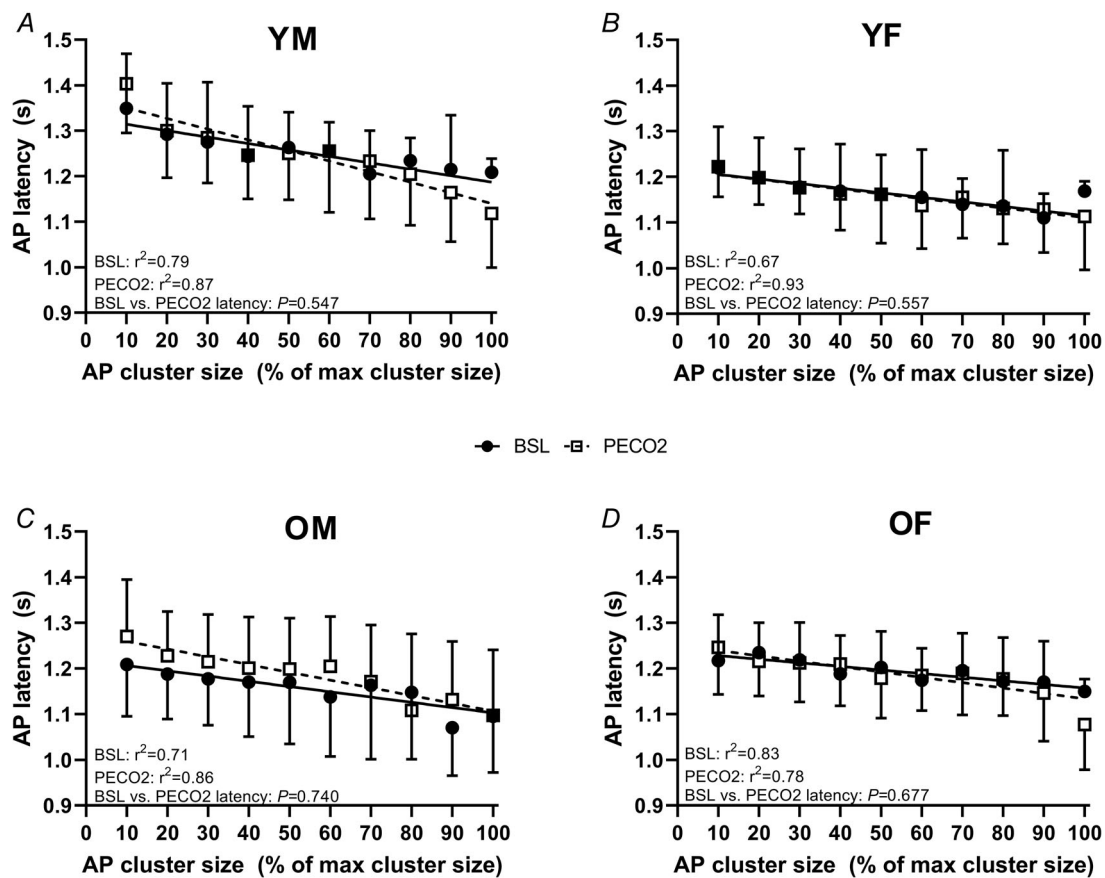


Figure 7. Action potential (AP) latency during post-exercise circulatory occlusion (PECO)
 AP cluster latency as a function of AP cluster size at baseline (BSL; filled circles) and the second minute of PECO (PECO2; open squares) in young males (YM, $n = 12$; panel A), young females (YF, $n = 12$; panel B), older males (OM, $n = 11$; panel C), older females (OF, $n = 12$; panel D). The AP cluster size–latency relationship was fit with an exponential decay function.

et al., 1984), and older males may have experienced larger increases in sympathetically mediated vasoconstriction of other vascular beds during exercise, such as the kidney (Momen et al., 2004). Second, age- and sex-specific changes in muscle metabolic responses to incremental fatiguing exercise may contribute to the smaller sympathetic AP discharge in older males. Older adults demonstrate smaller reductions in muscle cell pH and produce fewer metabolites (e.g. H_2PO_4) during fatiguing rhythmic exercise compared with young adults (effect of age), and females demonstrate smaller skeletal muscle metabolic perturbations during exercise compared with males (effect of sex) (Kent-Braun et al., 2002). However, females (young and older) exhibit greater sensitivity to metabolic perturbations than males, with older females possibly demonstrating the greatest sensitivity to changes in the muscle metabolic milieu compared with young males and females as well as older males (Kent-Braun et al., 2002). Therefore, smaller reductions in muscle cell pH without compensatory enhancement of metabolic sensitivity in older males may result in less group III/IV muscle afferent stimulation and subsequently less efferent sympathetic neural discharge. Third, compared with static contractions, rhythmic handgrip exercise induces a considerably smaller reduction in muscle pH, despite

similar increases in MSNA, indicating that the skeletal muscle metaboreflex likely contributes less to the MSNA responses during rhythmic exercise (Batman et al., 1994). Thus, other mechanisms, such as central command or the muscle mechanoreflex, may mediate sympathetic AP discharge and recruitment during rhythmic handgrip exercise. Indeed, central command plays a prominent role in the regulation of sympathetic neural discharge during high-intensity intermittent handgrip exercise in young adults (Victor et al., 1995). However, the impact of age and sex on central command during exercise remains unknown. Further, the similar RPE between groups suggests that central command activation was unaffected by age or sex in the current study. Nonetheless, the current data extend our previous observations of blunted AP recruitment in a mixed sex sample of older adults during an apnoeic stress (Badrov, Lalande et al., 2016), as we demonstrate that males may experience larger age-related changes in the central features governing sympathetic neural discharge during fatiguing rhythmic exercise than females. Specifically, during PECO, a period of peripherally mediated elevations in BP and sympathoexcitation (Mark et al., 1985), AP discharge and recruitment, as well as BP were not affected by age or sex. These data provide further

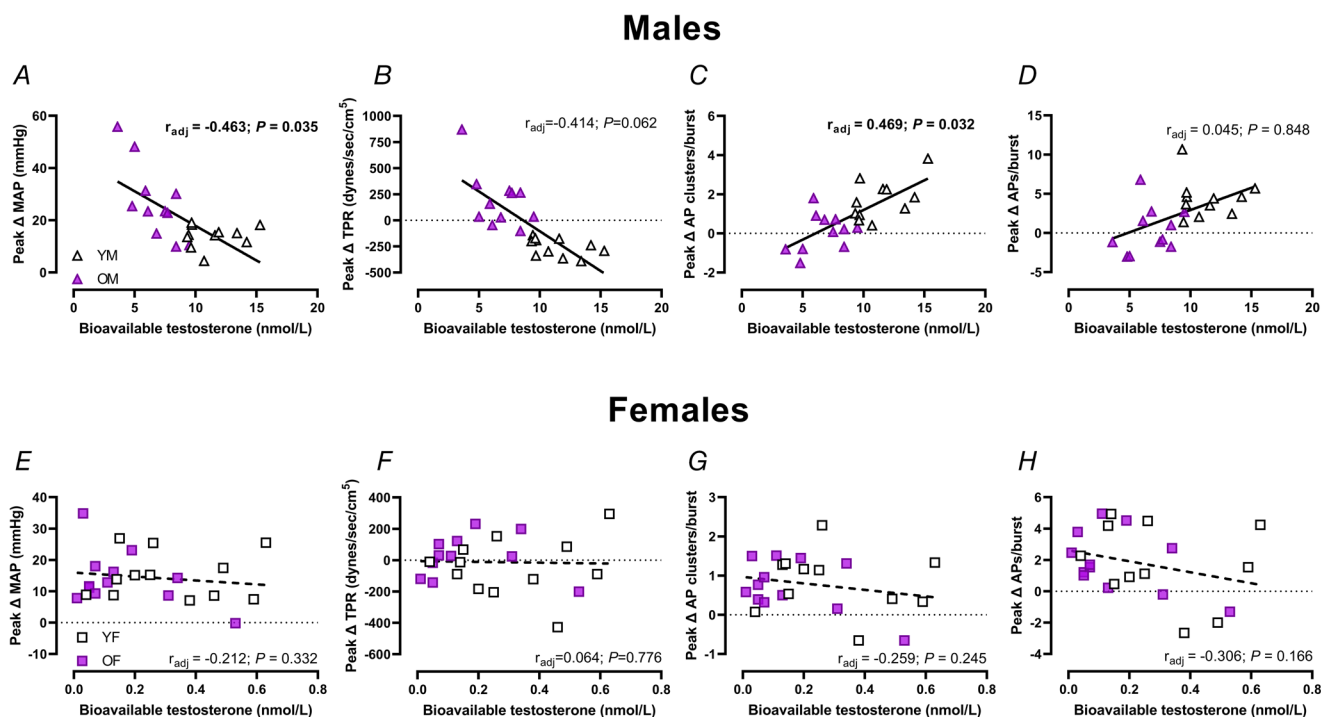


Figure 8. Relationship between bioavailable testosterone, blood pressure, total peripheral resistance and action potential (AP) discharge patterns during exercise

Linear relationships between bioavailable testosterone and mean arterial pressure (MAP), total peripheral resistance (TPR), as well as APs/burst and AP clusters/burst in males (A, B, C and D; $n = 23$) and females (E, F, G and H; $n = 24$). Partial regression analyses were used to determine the linear relationships between bioavailable testosterone and sympathetic/haemodynamic variables of interest, while accounting for the impact of age. [Colour figure can be viewed at wileyonlinelibrary.com]

support that the exaggerated pressor and attenuated axonal discharge responses in older males during exercise are likely driven by central, not peripheral, mechanisms.

Alternatively, the smaller increases in AP discharge, as well as the greater increases in BP and TPR in older males compared with young males during exercise may have been driven by age-related reductions in testosterone. Previous work found a positive relationship between testosterone and sympathetic outflow (Carter et al., 2012) and a negative relationship between testosterone and resting BP in males (Khaw & Barrett-Connor, 1988). We extend these observations by demonstrating a positive relationship between bioavailable testosterone and sympathetic neural recruitment, but not total AP discharge (Fig. 8). Furthermore, we found a negative relationship and a trend toward a significant negative relationship between bioavailable testosterone and exercise MAP and TPR, respectively, in males, but not females (Fig. 8). It is currently not known how testosterone impacts efferent sympathetic nerve traffic in males. The significant relationship between testosterone and the number of active AP clusters per burst, but not mean AP content per burst, in males suggests that testosterone may

impact the ability to recruit larger axons more than the ability to reflexively increase total axonal discharge during stress. This may also partly explain the greater increases in AP clusters per integrated burst in young males compared with young females during exercise. Conversely, low testosterone is associated with greater arterial stiffness (Corrigan et al., 2015) and reduced endothelial function (Babcock et al., 2022; Empen et al., 2012), which may contribute to the larger increases in exercise BP and TPR observed in older males. Future studies with larger sample sizes are warranted to determine the impact of testosterone on exercise BP and its determinants (e.g. preload, afterload, contractility).

In females, oestradiol exhibits central sympatho-inhibitory effects (Saleh & Connell, 2003; Xue et al., 2009) and is inversely related to resting sympathetic outflow in young females (Carter et al., 2013). Furthermore, exogenous oestradiol therapy dampens the sympathetic neural responses to isometric handgrip exercise in post-menopausal females (Wenner et al., 2022). Thus, the reduction in oestradiol following menopause is thought to contribute to exaggerated sympathetic neural discharge in older females during exercise. However, in the current study, oestradiol was not related to AP recruitment

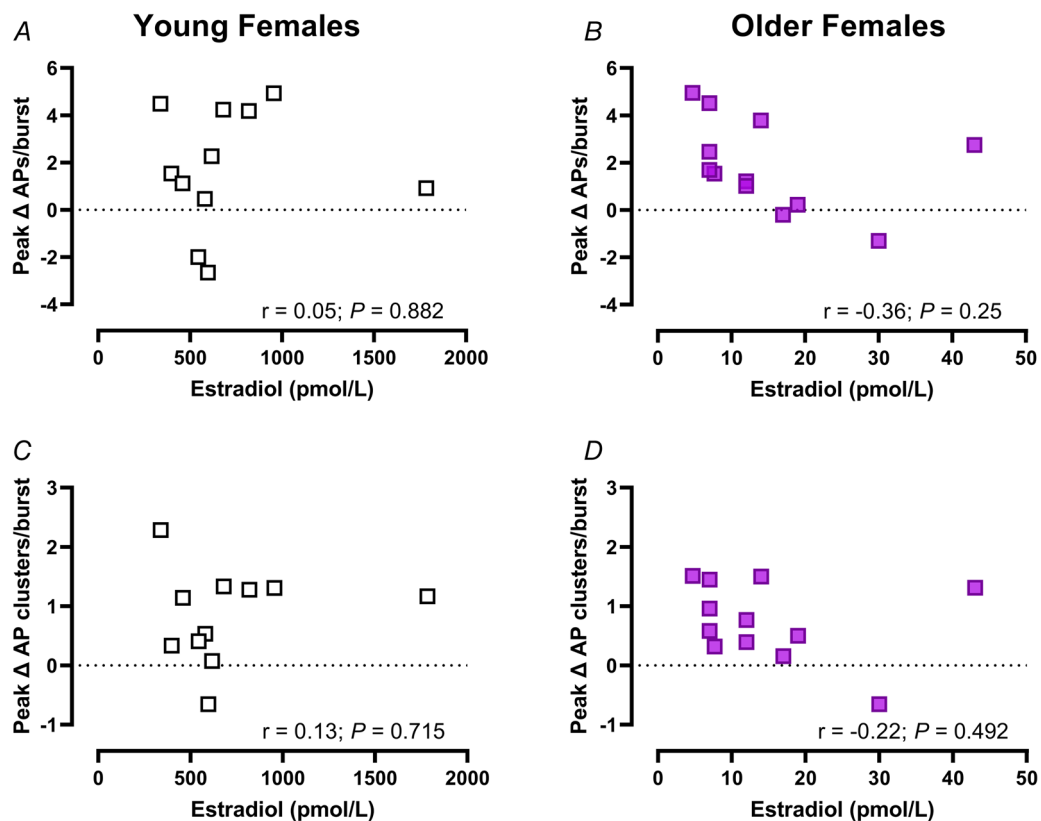


Figure 9. Relationship between oestradiol and action potential (AP) discharge patterns during exercise Linear relationships between oestradiol and APs/burst, as well as AP clusters/burst in young (A and C; $n = 11$) and older females (B and D; $n = 12$). Linear regression analyses were used to determine the relationships between oestradiol and sympathetic AP discharge patterns. [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/terms-and-conditions)]

during exercise in older or young females (Fig. 9). It is unclear why no relationship between oestradiol and sympathetic neural discharge was observed in the current study. However, contrary to isometric exercise (Wenner et al., 2022), oestradiol treatment does not impact the MSNA responses to rhythmic handgrip exercise (Fadel et al., 2004) suggesting that exercise mode may impact the relationship between oestradiol and MSNA. Nonetheless, the impact of sex hormones on sympathetic neural reactivity to exercise remains an emerging area of research, and our data highlight the important need for further assessments in this domain.

Independent effect of age on integrated MSNA and AP discharge during incremental handgrip exercise

Older adults demonstrated smaller increases in MSNA burst occurrence (frequency and incidence), burst strength (amplitude), and the number of AP clusters recruited compared with young adults during exercise. The smaller rise in MSNA burst occurrence among older adults may reflect an age-related increase in the ability of the baroreflex to defend against elevations in BP during exercise (Fisher et al., 2010). Contrary to MSNA burst occurrence, the arterial baroreflex exerts weak regulation of integrated MSNA burst strength (Kienbaum et al., 2001) and large AP clusters (D'Souza, Klassen et al., 2022; Salmanpour et al., 2011). Thus, the smaller increases in MSNA burst amplitude and less recruitment of larger AP clusters in older adults is likely not explained via a baroreflex-mediated mechanism. However, our data align with previous work which demonstrated smaller increases in integrated MSNA burst amplitude during a cold pressor test (Keller-Ross et al., 2020), and smaller increases in AP recruitment during an apnoeic stress (Badrov, Lalande et al., 2016), in older compared with young adults. Taken together, the reduced ability to increase integrated MSNA burst amplitude and recruit larger axons in older adults may be attributed to age-related changes in the central features governing sympathetic neural recruitment.

Independent impact of sex on sympathetic neural discharge responses to incremental handgrip exercise

Regardless of age, we found that AP latency was reduced in males during exercise but not females. AP latency reflects the time required for the brainstem-generated neural signal to reach the postganglionic recording site. The ability to alter AP latency represents a fundamental recruitment pattern employed by the sympathetic nervous system (Shoemaker et al., 2018). The current results are consistent with previous findings that physiological challenges with an intense volitional effort component

(e.g. exercise, apnoea) cause a downward shift in the AP cluster size–latency relationship (Badrov, Olver et al., 2016; Badrov, Lalande et al., 2016). Of note, a downward shift in AP latency during exercise was previously observed in a male-only sample (Badrov, Olver et al., 2016) whereas we recently demonstrated that females with and without post-traumatic stress disorder did not modify AP conduction velocity during handgrip exercise (D'Souza, Yoo et al., 2022). Although these prior findings suggest that sex differences in AP latency shifts exist, the current data provide the first direct evidence of a sex difference in the ability to modify AP conduction velocity.

Central command is speculated to contribute to AP latency modifications as a downward shift in the AP cluster size–latency relationship is observed during effortful tasks but not during passive sympathetic stressors (e.g. cold pressor test) (Badrov et al., 2015; Yoo et al., 2020). Although oestradiol reduces the autonomic responses to central command activation (i.e. mesencephalic locomotor region stimulation) in cats (Hayes et al., 2002), it is unlikely that sex hormones primarily mediate the sex differences in central processing times during rhythmic handgrip exercise because the similar resting and exercise AP latencies were also observed in older post-menopausal females who have low oestradiol levels. Furthermore, central command-mediated neuro-cardiovascular responses to exercise are dictated more by effort perception than force production (Williamson, 2010). Thus, despite smaller MVCs in females compared with males, RPE was similar between all groups, suggesting similar levels of central command activation during exercise. As such, we do not believe that sex differences in central command explain the lack of shift in AP latency in females during exercise. While this feature of modifiable AP latency is consistent across many of our studies, the mechanisms governing this neural communication strategy, and its functional relevance in the context of BP regulation, remain to be determined.

Limitations

We acknowledge certain limitations in the current study. First, strength training status was not strictly controlled for, which may have contributed to the variability in the sympathetic neural responses to handgrip exercise (Mostoufi-Moab et al., 1998; Somers et al., 1992). Second, Modelflow-derived cardiac index and stroke volume index may have underestimated the change in haemodynamics during exercise (Dyson et al., 2010). Third, measures of skeletal muscle metabolite production were not made. Additional research is required to quantify concomitant measures of sympathetic AP discharge and local metabolite production and examine the degree of

metaboreflex activation in young and older males and females.

Conclusions

The present study demonstrated that sex and age interactively impact muscle sympathetic AP recruitment patterns and systemic haemodynamics during rhythmic exercise, with age-related reductions in the AP discharge responses, and age-related increases in exercise MAP and TPR observed in males, but not females. Furthermore, sex differences in AP recruitment were observed in young (young males > young females) but not older adults. Additionally, regardless of sex, older adults demonstrated smaller increases in integrated MSNA burst amplitude and total AP clusters compared with young adults during exercise, as well as smaller increases in integrated MSNA burst frequency, incidence and total MSNA activity during PECO (i.e. independent effect of age). Thus, older adults (especially older males) exhibit a reduced ability to reflexively alter sympathetic neural recruitment strategies during rhythmic handgrip exercise, indicating that there may be age-related changes in the central features governing sympathetic vasoconstrictor discharge. Lastly, we found that males, but not females (i.e. independent effect of sex), reflexively modify AP conduction velocity during exercise. Altogether the interactive effects of age and sex on the neural and cardiovascular homeostatic adjustments to fatiguing small muscle mass exercise highlight the critical importance of considering biological sex in the interpretation of age-related changes in sympathetic neuro-cardiovascular responses to exercise.

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Additional information

Data availability statement

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

Competing interests

None declared.

Author contributions

A.W.D. and J.K.S. conceived and designed the study; A.W.D., R.T., K.M., S.L.H., T.W., G.B.C., B.S. and Q.F. performed the experiments; A.W.D. analysed the data; A.W.D., Q.F. and J.K.S. interpreted the results; A.W.D. drafted the manuscript; all authors edited, revised and approved the final version of the manuscript.

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Keywords

action potential, exercise pressor reflex, metaboreflex, micro-neurography, MSNA, sex differences

Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

Statistical Summary Document

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