Upward resetting of the vascular sympathetic baroreflex in middle-aged male runners

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ABSTRACT

This study focussed on the influence of habitual endurance exercise training (i.e. committed runner or non-runner) on the regulation of muscle sympathetic nerve activity (MSNA) and arterial pressure in middle-aged (50 to 63 years, n= 23) and younger (19 to 30 years; n=23) normotensive men. Haemodynamic and neurophysiological assessments were performed at rest. Indices of vascular sympathetic baroreflex function were determined from the relationship between spontaneous changes in diastolic blood pressure (DBP) and MSNA. Large vessel arterial stiffness and left ventricular stroke volume also were measured. Paired comparisons were performed within each age-category. Mean arterial pressure and basal MSNA bursts·min^{-1} were not different between age-matched runners and non-runners. However, MSNA bursts·100 heartbeats^{-1}, an index of baroreflex regulation of MSNA (vascular sympathetic baroreflex operating point) was higher for middle-aged runners (P=0.006), whereas this was not different between young runners and non-runners. The slope of the DBP-MSNA relationship (vascular sympathetic baroreflex gain) was not different between groups in either age-category. Aortic pulse wave velocity was lower for runners of both age-categories (P<0.03), although carotid β stiffness was lower only for middle-aged runners (P=0.04). For runners of both age-categories, stroke volume was larger, while heart rate was lower (both P<0.01). In conclusion, we suggest that neural remodelling and upward setting of the vascular sympathetic baroreflex compensates for cardiovascular adaptations after many years committed to endurance exercise training, presumably to maintain arterial blood pressure stability.

NEW AND NOTEWORTHY

Exercise training reduces muscle sympathetic burst activity in disease; this is often extrapolated to infer a similar effect in health. We demonstrate that burst frequency of middle-aged and younger men committed to endurance training is not different compared with age-matched casual exercisers. Notably, well-trained middle-aged runners display similar arterial pressure but higher sympathetic burst occurrence than untrained peers. We suggest homeostatic plasticity and upward setting of the vascular sympathetic baroreflex maintains arterial pressure stability following years of training.

INTRODUCTION

Human ageing exerts a marked influence on blood pressure, which is the primary regulated variable of the cardiovascular system. Two hallmarks of cardiovascular aging are large-vessel arterial stiffening (30), and chronic elevation of muscle sympathetic nerve activity, (MSNA) (27). The conventional wisdom is that these factors, amongst others, contribute to the age-related increase in arterial blood pressure observed in western society beyond 50 years of age (13).

Arterial baroreflex control of MSNA (i.e. vascular sympathetic baroreflex) is the primary mechanism through which the autonomic nervous system regulates vasomotor tone, and thus plays a pivotal role in blood pressure homeostasis. The age-related increase in MSNA is underpinned by resetting of the vascular sympathetic baroreflex (31), whereby the 'operating point' (i.e. mean resting diastolic blood pressure [DBP] and corresponding MSNA bursts per 100 heartbeats, a measure of the probability of a burst occurrence) resets upward and rightward. Vascular sympathetic baroreflex 'resetting' with age occurs in the absence of a change of reflex 'gain' (i.e. responsiveness to acute changes in blood pressure) (11, 25, 26). Notably, however, it appears that a rise in arterial pressure does not necessarily follow progressive elevation in resting vascular sympathetic activity with advancing age (49). In contrast, baroreflex-mediated cardiac parasympathetic control (i.e. cardiovagal baroreflex gain) is progressively impaired with advancing age (11, 32). Alterations to mechanosensory transduction and neural control (44) may explain these changes to the vascular sympathetic and cardiovagal limbs of the arterial baroreflex with human ageing.

Long-term aerobic exercise training mitigates against some of the hallmarks of cardiovascular aging. For example, lifelong endurance exercise training offsets age-related stiffening of the aorta (51) and carotid artery (47). However, the interaction of committed exercise training and age-related changes to vascular sympathetic activity is unclear. To date, relatively little consensus exists among previous microneurographic studies, which have found basal MSNA burst frequency for middle-aged and older endurance-trained men

77 is either higher (37), not different (38) or lower (44), compared to untrained peers.
78 Furthermore, quantification of the number of burst occurrences relative to the number of
79 opportunities for a burst (i.e. burst incidence) does not provide clarity. However, the method
80 of burst quantification provides different neurophysiological insight into regulation of vascular
81 sympathetic activity (5). Burst frequency is reflective of the amount of sympathetic activity
82 (or neurotransmitter release) that the vasculature is exposed to in a given time period (53).
83 In contrast, burst incidence indicates the probability of a sympathetic burst occurring at a
84 given arterial pressure (29). Furthermore, baroreceptor signals over a wide pressure range
85 influence both the timing and the probability of sympathetic bursts. We contend, therefore,
86 that burst incidence is an index of the baroreflex 'gating' sympathetic bursts (20, 21), rather
87 than sympathetic outflow *per se*. In the only study to consider the influence of aging and
88 chronic exercise training on vascular sympathetic baroreflex control, the training status of
89 healthy older males had no effect on MSNA burst incidence (vascular sympathetic baroreflex
90 operating point), or the MSNA responsiveness (gain) to a modified Oxford baroreceptor test
91 (44). In contrast, cross-sectional evidence from middle-aged and older men indicate that
92 vigorous long-term endurance training attenuates the ageing-related decline in cardiovagal
93 baroreflex responsiveness (34).

94 Taking the various aforementioned uncertainties into account, the primary aim of this
95 cross-sectional study was to investigate the effect that habitual endurance exercise training
96 has on regulation of vascular sympathetic burst activity and resting blood pressure in healthy
97 middle age. Because of marked sex differences in sympathetic regulation (18) and
98 autonomic support of blood pressure (6), men only were studied to experimentally isolate the
99 influence of long-term endurance training as much as possible. Furthermore, in order to
100 examine the effect of exercise training independently of ageing, a secondary aim was to
101 compare the sympathetic control of blood pressure between young runners and young non-
102 runners. To address these aims, we performed comprehensive haemodynamic and
103 neurophysiological assessment, and measured central artery stiffness and left ventricular

stroke volume, in four groups of healthy normotensive men: middle-aged committed runners, middle-aged non-runners, younger runners and younger non-runners. Based upon limited data, we hypothesised that the vascular sympathetic baroreflex control would not be different between well-trained runners and non-runners.

METHODS

Ethical Approval

This study conformed to the most recent Declaration of Helsinki, except for registration in a database. The Research Ethics Committee at the Cardiff School of Sport and Health approved all study procedures (16/7/02R) and participants provided written informed consent prior to entering the study.

Participants

Between August 2016 and August 2017, the eligibility to participate was assessed for seventy men. Forty-six participants completed the study. Each participant was categorised according to his age (i.e. middle-aged or young) and training status (i.e. committed runner or non-runner) (Table 1). Among middle-aged men, runners performed ≥ 25 miles of moderate to intense training per week for ≥ 10 years (n=13), whereas non-runners were casually recreationally active i.e. ≤ 3 hours of structured physical activity per week for ≥ 10 years (n=10). In the case of the young men, runners performed ≥ 50 miles of training per week (n=13) and non-runners performed ≤ 3 hours of structured physical activity per week, for ≥ 2 years (n=10). All participants were free of known cardiovascular, metabolic or other chronic diseases, normotensive (<140/90 mmHg when supine), non-smokers, and non-obese (BMI < 30 $kg \cdot m^2$) as assessed by a medical history, manual sphygmomanometry (Welch Allyn, UK) and measurement of height and body mass. Middle-aged men were further evaluated by resting and maximal exercise electrocardiogram.

Experimental overview

Participants completed one screening visit and 2 days of physiological testing, with a minimum of one week between the tests. All screening and physiological tests were performed at the Cardiff School of Sport and Health Sciences in a quiet, temperature controlled (22-24°C) environment. We requested that participants abstain from caffeine, alcohol and strenuous exercise for twenty-four hours prior to arrival at the laboratory on each visit; none took medication at the time of testing. On one testing day, assessment of body composition (Bioelectrical impedance analysis; Bodystat 1500, Bodystat Ltd, Douglas, Isle of Man) and measurement of arterial stiffness were followed by a maximal incremental exercise test. On the other testing day, having fasted for six hours, participants underwent cardiovascular and sympathetic neural assessments.

Assessment of arterial stiffness

Sequential ECG-gated arterial pressure waveforms were recorded in accordance with current guidelines (50) from the carotid and femoral arteries, at the site of maximal arterial pulsation, enabling the calculation of aortic pulse wave velocity (aPWV; SphygmoCor, Cardie X Ltd, Australia). Furthermore, the β stiffness index of the right common carotid artery was determined via high-resolution ultrasonography, using a 12-MHz linear array transducer (Vivid Q, GE Medical, Norway), as previously described (19). Central blood pressure was estimated to calculate β stiffness index, by applying a generalized transfer function (41) to radial arterial waveforms, collected via a high fidelity micromanometer tipped probe (SphygmoCor, Cardie X Ltd, Australia). Carotid artery β stiffness index is reported in 44 individuals (9 young non-runners, 12 young runners, 10 middle-aged non-runners, 13 middle-aged runners).

Cardiopulmonary exercise test

All participants completed an incremental exercise test to exhaustion on a cycle ergometer (Lode Corival, Groningen, The Netherlands) to assess $\dot{V}O_2$ peak. Cycling was chosen for

reasons of safety and assessment of the exercise electrocardiogram. Each increment corresponded to an increase 20 watts per minute (Middle-aged runners started at 90W and young runners started at 120W; middle-aged and young non-runners started at 30W and 50W, respectively). During the maximal exercise test oxygen consumption was measured continuously via a breath-by-breath analyser (Oxycon Pro, Jaeger, Hoechberg, Germany). Heart rate was measured throughout the exercise test via either a chest strap in the young groups (Polar Electro, RS400, Finland) or 12-lead electrocardiography in middle-aged men (Oxycon Pro, Jaeger, Hoechberg, Germany).

Hemodynamics and sympathetic neural activity

Heart rate and blood pressure were monitored continuously via three-lead electrocardiography and finger photoplethysmography (FinometerPro, FMS, Groningen, Netherlands), with participants supine. The arterial pressure waveform was calibrated at regular intervals to the average resting systolic and diastolic pressures measured via manual sphygmomanometry. Echocardiograms were acquired using a commercially available ultrasound system (Vivid E9, GE Medical, Norway) with a 1.5 to 4 MHz array probe. Images were obtained from apical 4 and 2 chamber views by a single experienced sonographer (RNL) and saved for offline analysis with commercially available software (EchoPAC, BT12, GE Medical, Norway).

Multiunit muscle sympathetic nerve activity was obtained by microneurography using a recording system (Nerve Traffic Analyser, Model 663 C, University of Iowa, Iowa City, IA) and following a recognized technique (45). In brief, a unipolar tungsten microelectrode (FHC, Bowdoin, ME), with shaft diameter of 0.1 mm (impedance 1-5 MW), was placed across the skin at the popliteal fossa and inserted into the peroneal nerve by an experienced microneurographer (JPM). A reference electrode was placed subcutaneously approximately 2-3 cm above from the site of the recording electrode. The recorded neurogram was amplified (70, 000 to 160, 000 fold), band-pass filtered (700 to 2000 Hz), full-wave rectified and integrated with a resistance-capacitance circuit (time constant 0.1 sec). Satisfactory

recordings of MSNA were identified, dependent on the following criteria (54), (i) pulse-synchronous “bursts” of activity, (ii) increased “burst” occurrence in response to voluntary apnoea, (iii) unaffected “burst” pattern during stroking of the skin, and (iv) 3:1 signal to noise ratio. At least 10 minutes after an acceptable MSNA recording site was found, echocardiograms and other baseline data were acquired. Hemodynamic and neural data were sampled at 1000Hz using a commercial data acquisition system and stored for offline data analysis (Chart Version 8, Lab Chart Pro, AD Instruments, UK).

Assessment of arterial baroreflex function

Hemodynamic and neural recordings were acquired for six minutes in order to characterize the arterial baroreflex regulation of MSNA and interbeat RR interval. Respiratory rate was monitored via a nasal cannula (Capnocheck® Sleep Capnograph, Smiths Medical, UK), to ensure that the participants had a regular breathing pattern, due to the influence of breath-hold on MSNA (9). Examples of the dynamic relationship between beat-by beat arterial pressure and bursts of MSNA are shown in Figure 1.

Data Analyses

Stroke volume was estimated using the Simpson’s-biplane method (24), thus permitting determination of cardiac output ($\dot{Q}$; heart rate x stroke volume) and the total peripheral resistance (TPR; $\dot{Q}$/mean arterial pressure). Satisfactory images for the quantification of stroke volume were not recorded in one individual (one middle-aged runner); accordingly, stroke volume, $\dot{Q}$ and TPR data are reported for forty-five individuals.

Multi-unit bursts of MSNA were verified by two investigators (DJW/JPM) via visual inspection following adjustment for baroreflex latency (54) (time between R wave and peak burst height), which aligned each burst with the appropriate R wave of the ECG. MSNA was quantified as burst frequency (bursts per minute [bursts·min^{-1}]) and burst incidence (bursts per 100 heartbeats [bursts·100hb^{-1}]).

206 The slope of the stimulus-response relationship between DBP and MSNA burst
207 probability was calculated to represent vascular sympathetic baroreflex gain (21, 45). Briefly,
208 DBP was averaged into two mmHg bins, to minimize the influence of respiration on MSNA
209 and to maximize the number of data points for inclusion in the linear regression model. The
210 percentage of cardiac cycles associated with a burst of MSNA (ranging from zero to 100%),
211 per bin of DBP, was used to calculate burst probability. Data were included for further
212 analysis if, (i) at least five data points for each linear regression were available and (ii) a
213 correlation coefficient of ≥ - 0.5 was present (14). Mean values and tests of statistical
214 significance are presented for 20 middle-aged (11 runners) and 20 younger men (11
215 runners). Statistical weighting was adopted for this analyses to minimize the influence of
216 differences in the number of cardiac cycles within each DBP bin (21). The operating point of
217 the vascular sympathetic baroreflex was determined from mean diastolic pressure and
218 corresponding average burst incidence.

219 Cardiovagal baroreflex gain was assessed by the sequence method using
220 customized computer software (Cardioseries version 2.4, Ribeirao Preto, São Paulo, Brazil).
221 If R-R interval was ≥800 milliseconds a delay of 1 beat was applied so that the SBP was
222 regressed against the following R-R interval (12). Data were included for further analysis
223 upon condition of (i) a minimum of three data points for a linear regression were available
224 and (ii) a correlation coefficient of ≥ 0.8 was present (40). The operating point of the
225 cardiovagal baroreflex was determined from mean prevailing SBP and corresponding
226 average RR interval. Data, including positive and negative ramp gains, and the number of
227 sequences, are presented for 20 middle-aged (11 runners) and 21 younger men (11
228 runners).

229 **Statistical Analyses**

230 In line with our primary (i.e. middle-aged runner *versus* age matched non-runner) and
231 secondary (i.e. younger runner *versus* age matched non-runner) aims, and after checking
232 compliance with basic parametric assumptions, we assessed between-group differences for

middle-aged runners and non-runners, and for young runners and non-runners, via independent t-tests. Alpha was set a-priori as $P<0.05$. All statistical analyses were completed using Statistics Package for Social Sciences for Windows, (Version 23, Chicago, IL) and data are reported as mean (95% Confidence Intervals).

RESULTS

Participant demographics

By design, training and cardiorespiratory fitness ($\dot{V}O_{2peak}$) were greater for runners compared to age-matched non-runners (middle-aged and young, $P<0.001$; Table 1). Runners had lower body mass (middle-aged, $P=0.001$; young, $P=0.003$) and body mass index (middle-aged and young, $P<0.001$), and less body fat percentage (middle-aged and young, $P<0.001$), than age-matched non-runners. Systolic BP ($P=0.041$) and Diastolic BP ($P=0.027$) were lower for young runners compared to age-matched non-runners. Screening blood pressures were not different among middle-aged runners and untrained peers.

Resting hemodynamics and vascular sympathetic neural activity

Stroke volume was higher (middle-aged, $P=0.03$; young, $P<0.01$) and heart rate was lower (middle-aged, $P<0.001$; young, $P<0.001$) between both groups of runners compared to age-matched non-runners (Table 2). There were no other differences in resting haemodynamic parameters between runners and non-runners for either age-category. Resting MSNA burst frequencies were not different among middle-aged runners and non-runners, or among young runners and age-matched non-runners. Burst incidence data is considered in the following section.

Arterial baroreflex function

Among middle-aged men, there was no difference between runners and non-runners for the diastolic operating pressure of the vascular sympathetic baroreflex ($P=0.57$); however, the corresponding operating MSNA (i.e. bursts·100hb^{-1}) was higher in the runners ($P<0.01$;

Figure 2A). Among young men, there was no significant difference in vascular sympathetic operating point between runners and non-runners (DBP, P=0.23; corresponding MSNA bursts·100hb^{-1}, P=0.24). The vascular sympathetic baroreflex gain (i.e. slope of the DBP-MSNA relationship) was not influenced by the training status of either middle-aged (-6.07 [-8.80 to -3.55] vs -7.30 [-10.49 to -4.12] %·mHg^{-1}, P=0.55) or younger men (-6.68 [-13.1 to -2.33] vs. -5.82 [-7.15 to -4.49] %·mHg^{-1}, P=0.58).

Among middle-aged runners and non-runners, there was no difference in the prevailing systolic pressure for the cardiovagal baroreflex (P=0.58), but the corresponding RR interval was higher for runners (P<0.01; Figure 2B). Among young men, the prevailing systolic pressure was lower (P=0.02) and the corresponding RR interval was higher for runners (P<0.01). The cardiovagal baroreflex gain was not different between runners and non-runners of both age groups; data for positive and negative pressure ramps and the number of sequences *per* ramp are presented in Table 3.

Arterial stiffness

Runners had lower aPWV (middle-aged, P=0.026; young, P=0.027) compared to age-matched non-runners (Table 2). In contrast, the β stiffness index of the carotid artery was lower only for the middle-aged runners compared to age-matched non-runners (P=0.041).

DISCUSSION

The principal findings are as follows: 1) for middle-aged men, many years of moderate to vigorous endurance exercise training sets the operating point of the vascular sympathetic baroreflex at a burst occurrence that is higher than for peers that have not trained; 2) higher burst occurrence does not influence overall reflex gain, basal burst frequency, or resting arterial pressure; 3) for younger men, endurance training has a limited effect on the operating point and there are no differences in vascular sympathetic baroreflex reflex gain or basal burst frequency compared with untrained peers. Taken together, these findings indicate that some form of remodelling in middle-aged men following many years of

committed endurance exercise training plays a critical role in the baroreflex control of vascular sympathetic bursts and resting blood pressure.

The effect of training on vascular sympathetic baroreflex control

Regardless of the training status, we observed similar frequencies of sympathetic bursts in microneurographic recordings taken from middle-aged males during supine rest. An intriguing finding, however, is that the well-trained men exhibit a greater MSNA burst occurrence, and by some margin (40 to 50 % approximately); this occurs without any obvious difference in the corresponding diastolic pressure stimulus. Together, we interpret these data for MSNA burst frequency and occurrence as evidence that many years of training alters the gating of sympathetic bursts (i.e. baroreflex control) without influencing the frequency of sympathetic bursts *per* minute (i.e. rate of neurotransmitter release). Although this might seem contradictory, burst frequency and occurrence provide slightly different neurophysiological information (5, 29, 53). Furthermore, reciprocal interplay between exercise bradycardia (i.e. fewer opportunities for a burst) and the higher MSNA operating point (i.e. greater burst occurrence) explain why the burst frequency for trained runners and non-runners is similar.

Our data indicate that an exercise training-induced upward setting for the MSNA operating point in middle age occurs without any change in the ability to increase or decrease vasoconstrictor outflow during fluctuations of resting arterial pressure. In other words, vascular sympathetic baroreflex overall gain is unaffected by training. Stüdinger and colleagues (44), using the modified Oxford baroreceptor test, also observed that overall gain was similar among older trained and untrained men. Unlike the present study, however, no difference was observed for sympathetic burst occurrence, and resting burst frequency was marginally lower for endurance-trained versus untrained middle-aged males.

Other studies of trained and untrained middle-aged and older people have recorded resting MSNA without specifically addressing vascular sympathetic baroreflex function.

Notarius and colleagues (38) observed that burst occurrence was higher, while basal sympathetic burst frequency was similar, for endurance trained middle-aged men compared with sedentary peers. In contrast, Ng and co-workers reported higher sympathetic burst occurrence and burst frequency for older-endurance trained athletes; however, these findings may reflect an older cohort, or inclusion of endurance trained females, for whom burst frequency was markedly higher compared with untrained peers (37). Whilst we cannot explain this lack of consensus, it may reflect the differences in the endurance phenotype across the studies. Factors that influence basal vascular sympathetic outflow with human ageing, such as abdominal adiposity (15), distensibility of the barosensory vessel walls (48), and blood volume (2), all are influenced by the dose of endurance exercise training.

To isolate the effect of endurance exercise training from human ageing, we also studied younger males. As with older men, we found no difference for basal burst frequency between well-trained runners and non-runners. The burst occurrence was marginally higher for the runners, but this difference was modest in comparison to that between the older groups. These findings in young men are similar to previous cross-sectional studies (7, 43, 46). Furthermore, vascular sympathetic baroreflex gain is similar for trained runners and non-runners. Thus, our data suggest that the endurance phenotype traits of young men does not include a higher operating point for the vascular sympathetic baroreflex.

Differences for aortic compliance and resting heart rate between young runners and non-runners are comparable with those for the middle-aged men. However, one noteworthy distinction relates to the difference in resting stroke volume. For young, well-trained men, stroke volume during supine rest was 50% greater than that of age-matched non-runners. For older men, resting stroke volume was only 12% greater for runners compared with age-matched non-runners. This lesser difference in stroke volume may explain why endurance training effects the operating point for the vascular sympathetic baroreflex only for committed middle-aged runners. That is, older runners rely more on vascular sympathetic neural activity than cardiac output to support arterial pressure. However, further investigation of potential

interaction of left ventricular stroke volume and the vascular sympathetic baroreflex is required.

Our interpretation for young men in this study is consistent with a previous report that endurance training does not influence autonomic support of blood pressure in the young (17). However, our findings do contrast with those of a study by Alvarez and colleagues (1). As in the present study, burst occurrence was marginally higher in trained men, while basal MSNA burst frequency was similar. However, when adiposity is taken into account, burst occurrence and burst frequency both were greater for endurance trained versus untrained men (1). Furthermore, in contrast to the present study, sympathetic baroreflex gain was lower for endurance-trained compared with untrained young men, an effect regardless of percentage body fat. This suggests that body composition may be important, at least in younger men.

The effect of training on cardiovagal baroreflex control

It is well known that endurance athletes display exercise-induced bradycardia, although considerable debate exists surrounding the mechanism(s) involved (3, 4). Furthermore, arterial baroreceptor control of blood pressure is mediated predominantly via sympathetic vascular regulation, rather than by reflex changes in heart period (8). Nonetheless, we determined how habitual endurance exercise influenced the responsiveness of the cardiovagal baroreflex in middle age. For well-trained middle-aged men, as expected, the cardiovagal baroreflex operated around a considerably longer RR interval at rest; however, the baroreflex gain was similar among runners and non-runners. Previous work has shown that middle-aged endurance trained men display greater cardiovagal baroreflex gain than sedentary controls, but not moderately-active, age-matched peers (34). In the case of the younger trained men in this study, the cardiovagal baroreflex also operated around a longer heart period, without any difference in baroreflex gain compared with age-matched non-runners; this finding for gain is in agreement with previous studies in younger men (1, 7, 34).

364 **Remodelling of the vascular sympathetic baroreflex**

365 Mechanosensory transduction, central mediation, and efferent neurotransmission are
366 integrated into the baroreflex regulation of vasomotor tone and arterial pressure.
367 Furthermore, it is proposed that human aging may have opposing influences on mechanical
368 and neural events (44). However, we can only speculate upon potential sites where
369 additional remodelling might have occurred in committed middle-aged runners to explain our
370 findings. Many years of training may influence the strength and/or timing of mechanosensory
371 signals controlling efferent sympathetic burst occurrence; this could arise from altered
372 vascular mechanics and/or a change to the threshold for baroreceptor activation.
373 Specifically, well-trained middle-aged men have less stiff barosensory regions; furthermore,
374 more complete elastic recoil during a longer diastolic period could lead to a longer interval of
375 'silence' in the afferent baroreceptor signal (21). However, the apparent lack of a similar
376 upward setting of the MSNA operating point for younger trained men, who also possess
377 lesser vascular stiffness and display bradycardia, argues against this. However, endurance-
378 training induced cardiovascular remodelling may only lead to upward vascular sympathetic
379 baroreflex resetting in middle-aged men due to increased autonomic support of blood
380 pressure with age (16).

381 Animal studies indicate that chronic exercise training potentially influences
382 baroreceptor control of sympathetic bursts at brain structures including, the nucleus tractus
383 solitairius, the paraventricular nucleus of the hypothalamus, and the rostral ventrolateral
384 medulla (36). Brain imaging studies have identified some of the same sites as regions of
385 baroreflex control in humans (22) (23). It is possible, therefore, that neural plasticity and
386 exercise-induced central remodelling previously observed in animals underpins the higher
387 sympathetic burst occurrence in middle-aged trained males.

388 Changes to efferent neurotransmission may also mediate upward vascular
389 sympathetic baroreflex setting. Short-term exercise training reduces alpha-adrenergic
390 vasoconstrictor responsiveness in (35), and a reduction of sympathetic vascular transduction

has been proposed to contribute to orthostatic intolerance observed in some highly-trained individuals (52). Vasoconstrictor responsiveness to noradrenaline declines with advancing age (10), which may counteract the effects of elevated MSNA burst frequency (16). Furthermore, Notarius and colleagues (38) observed that sympathetic vascular transduction during baroreflex-mediated sympathoexcitation may be altered further in trained middle-aged men. Another possibility is that vascular sympathetic baroreflex resetting may be a compensatory mechanism to offset training-induced vascular changes (42) (33). All of these aforementioned possibilities require investigation. Notably, irrespective of the location(s), exercise-induced remodelling does not alter vascular sympathetic baroreflex gain, at least not the integrated gain.

Experimental Considerations

Vascular sympathetic baroreflex gain was calculated by associating spontaneous fluctuations in DBP to the occurrence of bursts of MSNA. We did not take strength (amplitude) of sympathetic bursts into account, because baroreceptor signals modulate burst occurrence, whereas less is known of the mechanisms that govern amplitude (21, 29). Furthermore, we did not assess vascular sympathetic baroreflex gain to rising and falling pressures independently and we acknowledge that this does not take baroreflex hysteresis into account (14).

It is reported that dietary salt and nitrate can influence sympathetic burst activity (28, 39). However, we did not control for diet in our study, therefore we cannot exclude some influence on our data. Every effort was made to accurately record the number of years over which an individual had exercised at their current level. In addition, we recorded lifetime physical activity and exercise and observed a clear difference in maximal aerobic capacity between the trained and untrained groups. However, group allocation, determined by habitual endurance training, may limit the conclusions based on other components of exercise training. These components include mode, intensity, duration, all of which may have an impact on cardiac, vascular and neural remodelling. Because sex of the participants

418 was controlled for in this study, future studies are required to properly address potential sex
419 differences. Although our participants were non-obese, we did not specifically control for
420 adiposity, which is known to influence sympathetic burst activity. However, post hoc analysis
421 suggest that percentage body fat was not a significant covariate for any indices of
422 sympathetic activity in this study. Finally, the *a priori* intention of our study was to investigate
423 the effect that committed endurance exercise training has on elevated sympathetic neural
424 activity and vascular sympathetic baroreflex control of resting blood pressure in healthy
425 middle-aged men. However, we also studied young men in order to investigate the effect of
426 endurance training independently of cardiovascular ageing. The use of independent samples
427 t-tests reflects these *a priori* questions. To limit the chance of a type 1 error, we did not
428 perform statistical comparisons between middle-aged runners and young runners, or middle-
429 aged non-runners and young non-runners.

430 **CONCLUSION**

431 This study demonstrates upward setting of arterial baroreflex regulation of vascular
432 sympathetic bursts following committed endurance training in middle-aged men. Importantly,
433 vascular sympathetic baroreflex resetting coupled to exercise-induced bradycardia, results in
434 a similar basal burst frequency compared with untrained peers. Furthermore, the study
435 demonstrates that training status does not influence the MSNA operating point for younger
436 well–trained men, who also display similar sympathetic burst frequency compared with
437 untrained peers. In our view, remodelling within the vascular sympathetic baroreflex arc,
438 culminating in a higher MSNA operating point, is another example of phenotypic adaptation
439 to lifelong (> 25 years) training. This occurs, presumably, to maintain resting vasomotor tone
440 and blood pressure stability and to complement cardiac and vascular adaptations to many
441 years of endurance exercise training.

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446 **CONFLICT OF INTEREST**

447 None of the authors has any conflicts of interest, financial or other.

448 **AUTHOR CONTRIBUTIONS**

449 DJW, RS, CJP and JPM conception and design; DJW, RNL, JST, FML, BAC, TGD, LLS,
450 CJAP, and JPM performed experiments. DJW, JST, RNL, and JPM data analysis. DJW and
451 JPM data interpretation. DJW and JPM drafted manuscript. DJW, RS, CJP and JPM revised
452 manuscript. All authors approved the manuscript.

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Table 1 - Participant Characteristics

	Young non-runners (n=10)	Young runners (n=13)	Middle-aged non-runners (n=10)	Middle-aged runners (n=13)
Demographics				
Age, years	23 (21-25)	22 (21-24)	53 (52-55)	57 (54-59)
Stature, cm	178.1 (174.0-182.3)	179.9 (176.9-183.0)	175.6 (170.5-180.6)	174.7 (170.9-178.5)
Body Mass, kg	80.4 (68.8-92.0)	67.0 (63.9-70.0) *	80.9 (73.8-88.0)	66.1 (61.3-70.9) †
BMI, $kg \cdot m^2$	25.4 (22.1-28.8)	20.8 (19.9-21.6) *	26.2 (24.0-28.5)	21.6 (20.7-22.6) †
Body fat (%)	19.7 (15.2-24.1)	10.7 (7.8-13.6) *	26.8 (20.4-33.3)	17.5 (15.6-19.3) †
Blood Pressure				
SBP, mmHg	119 (109-128)	111 (108-114) *	119 (113-124)	118 (113-123)
DBP, mmHg	71 (67-76)	66 (62-69) *	76 (73-80)	74 (70-78)
Cardiorespiratory Fitness				
$\dot{V}O_2$ Peak, $mL \cdot kg^{-1} \cdot min^{-1}$	36.5 (31.9-41.0)	60.6 (55.0-66.2) *	32.6 (26.6-38.6)	50.7 (47.0-54.4) †
$\dot{V}O_2$ Peak, % Predicted	86 (82-103)	116 (116-141) *	106 (87-129)	143 (129-155) †
Training History				
Exercise per week, miles		65 (56-73)		34 (28-39)
Training history, years		8 (5-11)		29 (28-40)

Data are presented as mean (95% Confidence Intervals). Symbols represent significant between-group differences ($P<0.05$), * = Young runner vs. Young non-runner; † = Middle-aged runner vs. middle-aged non-runner.

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Table 2 - Resting Haemodynamics and Basal Sympathetic Nervous System Activity

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	Young		Middle-aged	
	non-runners (n = 10)	**runners (n = 13)**	**non-runners (n = 10)**	**runners (n = 13)**
Central Artery Stiffness				
aPWV, $m \cdot s^{-1}$	5.8 (5.2-6.3)	5.1 (4.8-5.3) *	7.5 (6.9-8.1)	6.8 (6.2-7.3) †
β stiffness index	2.96 (2.52-3.40)	2.38 (2.03-2.74)	5.06 (3.94-6.19)	4.06 (3.25-4.88) †
Haemodynamics				
Heart rate, $beats \cdot min^{-1}$	64 (57-70)	45 (41-48) *	56 (49-62)	43 (38-47) †
Stroke volume, ml	61 (57-64)	92 (87-97) *	62 (56-68)	70 (63-77) †
Cardiac output, $L \cdot min^{-1}$	3.8 (3.5-4.2)	4.1 (3.8-4.4)	3.4 (3.0-3.8)	3.0 (2.6-3.3)
TPR, $mmHg \cdot L \cdot min^{-1}$	24.3 (21.2-27.4)	21.3 (19.2-23.3)	29.1 (26.8-31.3)	31.6 (28.4-34.7)
MAP, mmHg	90 (83-97)	84 (81-88)	95 (89-101)	93 (90-96)
Respiration rate, $breaths \cdot min^{-1}$	13 (10-15)	15 (14-16)	11 (9-13)	12 (10-14)
Muscle Sympathetic Nerve Activity				
Burst Frequency, $bursts \cdot min^{-1}$	18 (12-23)	16 (10-21)	28 (19-38)	31 (27-34)
Burst Incidence, $bursts \cdot 100hb^{-1}$	27 (19-36)	36 (23-50)	50 (33-66)	72 (63-81) †

Data are presented as mean (95% Confidence Intervals). Symbols represent significant between-group differences ($P<0.05$), * = Young runner vs. Young non-runner; † = Middle-aged runner vs. middle-aged non-runner.

Note: We were unable to quantify β stiffness index in one young non-runner and one young runner; accordingly, data are reported for forty-four individuals. Furthermore, stroke volume was unobtainable for one middle-aged runner. Accordingly, stroke volume, $\dot{Q}$ and TPR data are reported in forty-five individuals.

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Table 3 – Cardiovagal baroreflex gain and the number of sequences for positive and negative pressure ramps

	Young		Middle-aged	
	non-runners (n = 10)	**runners (n = 11)**	**non-runners (n = 9)**	**runners (n = 13)**
'Up' Gain (ms·mmHg^{-1})	31 (23-39)	41 (27-55)	28 (18-37)	34 (25-44)
# sequences	20 (12-29)	8 (5-11)	17 (10-24)	11 (8-16)
'Down' Gain (ms·mmHg^{-1})	24 (17-32)	33 (22-45)	23 (14-32)	33 (22-45)
# sequences	30 (20-39)	9 (6-12)	20 (13-26)	13 (8-19)

Data are presented as mean (95% Confidence Intervals).

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Figure Legends

Figure 1 Example recordings of muscle sympathetic nerve activity and blood pressure during supine rest. 20 seconds of resting muscle sympathetic nerve activity (MSNA) and blood pressure (BP) data are shown from one representative participant per group: (A) Middle-aged runner; (B) Middle-aged non-runner; (C) Young runner; (D) Young non-runner.

Figure 2 Sympathetic and cardiac baroreflex function. (A) Group mean regressions between diastolic blood pressure (DBP) and muscle sympathetic nerve activity (MSNA) are presented with the sympathetic operating points superimposed on the regression lines. Middle-age runners had similar operating DBP compared to middle-aged non-runners but the corresponding level of MSNA was higher (by 22 bursts·100hb^{-1}; red arrow), despite similar sympathetic baroreflex gain. However, in young men training status had no influence on the operating DBP, corresponding level of MSNA or sympathetic baroreflex gain. **(B)** Group mean regressions between systolic blood pressure (SBP) and R-R interval (sequence method) are shown with the operating points of the cardiac baroreflex overlaid on the regression lines. Middle-aged runners had similar operating SBP and cardiovagal baroreflex gain (33.6 [24.5-42.8] vs 25.5 [16.2-34.7], *P*=0.16) compared to middle-aged non-runners, but the corresponding R-R interval was longer (by 352 msec; red arrow). In contrast, when compared to young non-runners, the operating SBP was set leftward (by 9 mmHg; green dashed arrow) in young runners with a longer corresponding R-R interval (by 418 msec; green solid arrow), despite similar cardiac baroreflex gain (37.2 [28.1-46.3] vs 26.4 [19.1-33.8], *P*=0.06). Abbreviations: M, Middle-aged non-runners; MR, Middle-aged runner; Y, Young non-runner; YR, Young runner. NB: Baroreflex responsiveness data are presented from: 10 young non-runners, 11 young runners, 9 middle-aged non-runners, 11 middle-aged runners.