MICRONEUROGRAPHIC CHARACTERIZATION OF SYMPATHETIC RESPONSES DURING 1-LEG EXERCISE IN YOUNG AND MIDDLE-AGED HUMANS

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ABSTRACT

Muscle sympathetic nerve activity (MSNA) at rest increases with age. However, the influence of age on MSNA recorded during dynamic leg exercise is unknown. We tested the hypothesis that aging attenuates the sympatho-inhibitory response observed in young subjects performing mild to moderate 1-leg cycling. After pre-determining peak oxygen uptake (VO2peak), we compared contra-lateral fibular nerve MSNA during 2 minutes each of mild (unloaded) and moderate (30-40% of the work rate at peak VO_2, halved for single leg) 1-leg cycling in 18 young (23±1 years [mean±SE]) and 18 middle-aged (57±2 years) sex-matched healthy subjects. Mean height, weight, resting heart rate (HR), systolic blood pressure (BP) and percent predicted VO_2peak were similar between groups. Middle-aged subjects had higher resting MSNA burst frequency and incidence ($P<0.001$) and diastolic BP ($P=0.04$). During moderate 1-leg cycling, older subjects' systolic BP increased more (+21±5 vs.+10±1 mmHg; $P=0.02$) and their fall in MSNA burst incidence was amplified (-19±2 vs. -11±2 bursts/100heartbeats; $P=0.01$) but because HR rose less (+15±3 vs.+19±2 bpm; $P=0.03$), exercise induced similar reductions in burst frequency ($P=0.25$). Contrary to our initial hypothesis, with advancing age, mild to moderate intensity dynamic leg exercise elicits a greater rise in systolic BP and a larger fall in MSNA.

Keywords: aging, microneurography, dynamic exercise

INTRODUCTION

Human aging is accompanied by an increase in central sympathetic outflow to skeletal muscle (muscle sympathetic nerve activity; MSNA) (Sundlof & Wallin, 1978) without any concurrent diminution of its arterial baroflex modulation (Ebert *et al.*, 1992;Rudas *et al.*, 1999;Matsukawa *et al.*, 1996). This increased neural vasoconstrictor drive is paralleled by reductions in resting muscle blood flow, vascular conductance (Dinenno *et al.*, 2001;Hart *et al.*, 2009), arterial compliance (Tanaka *et al.*, 2000), and higher systolic blood pressure (Franklin *et al.*, 1997).

Less is known about the effects of healthy aging on MSNA during exercise,which engages simultaneously autonomic reflexes elicited by mechanically- and metabolically-sensitive group III/IV skeletal muscle afferents (exercise pressor reflex), stretch sensitive baroreflexes, and feed-forward signals from higher brainstem regions (central command) (Crisafulli *et al.*, 2015). The firing of single sympathetic units engaged by these stimuli integrate to comprise the multi-fiber neurogram recorded by microneurography (Valbo *et al.*, 2004).

To date, most exercise studies involving MSNA have employed static handgrip (HG) protocols. These investigations have not yielded consistent results: age either attenuated (Houssiere *et al.*, 2006) or had no effect (Ng *et al.*, 1994;Markel *et al.*, 2003;Greaney *et al.*, 2013;Lalande *et al.*, 2014) on the reflex increase in MSNA elicited by this stimulus.
Leg exercise at mild to moderate intensities is more representative of daily human activity than static HG. Whereas moderate intensity static HG increases MSNA in young healthy subjects, principally by stimulating group III/IV skeletal muscle afferents sensitive to metabolic stimuli (muscle metaboreflex) (Mark *et al.*, 1985;Victor *et al.*, 1987;Saito & Mano, 1991), dynamic leg exercise of comparable intensity decreases MSNA (Saito *et al.*, 1993;Ichinose *et al.*, 2008;Callister *et al.*, 1994;Katayama *et al.*, 2011;Doherty *et al.*, 2018).

This latter observation has been attributed to concurrent engagement of the skeletal muscle pump to increase cardiac filling pressure; the consequent stimulation of cardiopulmonary baroreceptors will inhibit reflexively central sympathetic outflow (Ray *et al.*, 1993;Katayama *et al.*, 2014). Whether 1-leg exercise elicits similar sympatho-inhibition in middle-aged subjects has not been reported.

Prior studies of aging in which cardiopulmonary mechanoreceptors were unloaded by lowering cardiac filling pressures reported either an augmented (Davy *et al.*, 1998;Davy *et al.*, 1998) or unaltered (Tanaka *et al.*, 1999) increase in MSNA. Conversely, dynamic exercise should stimulate these baroreceptors, decreasing MSNA reflexively. However, several age-related factors could modify the influence of the skeletal muscle pump on the autonomic adjutments to exercise (Proctor D.N. & Parker, 2006), including a decrease in muscle mass (Frontera *et al.*, 2000), a change in total blood volume (Davy & Seals, 1994), and/or changes in venous compliance (Olsen & Lanne, 1998).

The principal aim of the present study was to compare, in young and healthy middle-aged subjects, the magnitude of sympatho-inhibition evoked by short duration low intensity dynamic 1-leg cycling. We hypothesized that healthy aging attenuates the fall in multi-unit MSNA observed in young subjects over the first 4 minutes of such exercise.

METHODS

Subjects

Thirty-six healthy, medication-free, volunteers were recruited through local advertisement, screened by medical history, and divided into 2 groups: 18 young (mean age 23±1 years; range 18-28; 6 women) and 18 middle-aged (mean age 57±2 years; range 48-72; 6 women). By definition, all were normotensive and in sinus rhythm; none was diabetic or obese. This protocol represents one element of a larger study approved by the Research Ethics

Boards at both University Health Network (Reference number 09-0988-AE), Mount Sinai Hospital (10-0013-E), and the University of Guelph (15JN004). Informed written consent was obtained from all subjects.

Procedures

Subjects were studied in a quiet temperature-controlled laboratory at the same time of day, two hours following the last food intake, on two separate days. All participants abstained from caffeine as well as vigorous exercise for 12 hours beforehand.

On the first day, oxygen uptake at peak exercise (VO2peak) was determined by open circuit spirometry (Quark CPET system, Cosmed USA Inc., Chicago, IL or Moxus Modular VO2 System, AEI Technologies, Pittsburgh PA). Subjects performed a graded ramped bicycle ergometer test (17-30 watts/min) with both legs until pedal speed could not be maintained above 50 rpm and the respiratory exchange ratio (VCO_2/VO_2) exceeded 1.1. Work increments were individualized, based on physical activity status, to ensure that exercise duration would not exceed 14 minutes. VO2peak was expressed both as $ml/kg \cdot min^{-1}$ and as percent of predicted VO2 peak, accounting for age, sex, body weight and height (Jones *et al.*, 1985). The work rate at 30-40 percent of that at VO2peak was based on 2-legged cycling. This value was halved in order to determine an equivalent moderate intensity work rate for the 1-leg cycling protocol. Therefore the moderate work rate for 1-leg was approximately 15-20% of the peak work rate at VO2peak.

On the second day subjects sat in a comfortable chair with the left leg supported on a stool and the right leg secured to the pedal of a floor-mounted cycle ergometer (Monark Rehab Ergometer Trainer 881, Sweden). Right arm blood pressure was recorded automatically each minute (Dinamap Pro 100, Critikon, Tampa, FL) at rest and during exercise. In 10 of the young subjects, blood pressure during exercise was assessed during exercise by calculating 30 second averages of beat-by-beat blood pressure (Finometer MIDI, Finapres Medical Systems, The Netherlands), calibrated at rest (BPtru Model BPM-200, BpTRU, Coquitlam, BC). Heart rate was derived from lead II of an electrocardiogram. A respiratory belt encircled the abdomen. Multiunit recordings of post-ganglionic MSNA were obtained with a unipolar tungsten electrode inserted selectively into a left peroneal (fibular) muscle-nerve fascicle, as previously described (Notarius *et al.*, 2015). To compare between-group differences in exercise-induced reflex and central effects on efferent sympathetic nerve discharge (the principal variable of interest), MSNA was expressed as firing incidence (bursts/100 heartbeats). MSNA also was expressed as firing frequency (bursts/min), a measure that provides insight into the potential functional importance of changes in central sympathetic outflow for neural norepinephrine release and vascular resistance (Valbo *et al.*, 2004). MSNA was analyzed using a custom semi-analytic program constructed on LabVIEW® (National Instruments, Austin, Texas) (Notarius *et al.*, 2015).

Protocol

After 10 minutes of quiet rest, baseline signals were acquired over 7 minutes of spontaneous breathing. MSNA was recorded from the left leg at rest and during early onset, non-steady state 1-leg cycling (right leg) for 4 minutes (2 minutes at zero load and 2 minutes at 15-20% of the work rate at peak VO_2). Although the target intensity was 20 percent of the work rate at peak VO2, a slight reduction was required in 8 participants (3 older and 5 younger) who could not keep the stationary leg still when the absolute work rate was highest. Subjects pedalled at 60-70 rpm, Zero load refers to short duration unloaded cycling, i.e. no added resistance. After 2 minutes of unloaded 1 leg cycling a resistance comparable to 15-20% of the measured work rate at peak VO2 was applied for a further 2 minutes. At both work rates, subjects rated their perceived exertion (RPE) according to the modified Borg scale (0-10) (Noble *et al.*, 1983).

Statistical Analysis

Data are presented as mean ± standard error. Depending on the distribution of data, we compared differences between group means by unpaired t-tests or Mann-Whitney Rank sum tests. We compared absolute changes from baseline in dependent variables during the second minute of unloaded and loaded dynamic 1-leg cycling between the middle-aged and younger groups, by applying a two-factor repeated measures analysis of variance (ANOVA)(SigmaStat™ for Windows, Ver. 3.5, Systat Software Inc., Chicago, IL), with group (middle-aged vs. younger) and exercise intensity (mild and moderate work rate) as the two factors. A post-hoc Student Newman-Keuls test assessed individual differences between means. Pearson correlation analysis was applied to test for an association between peak oxygen uptake and MSNA.

RESULTS

Physical characteristics and baseline measures

Summary data appear in Table 1. Of note, the groups exhibited similar mean height, weight, resting heart rate, and systolic blood pressure (all $P>0.05$). Mean resting diastolic blood pressure was higher in the older cohort ($P=0.04$), as were resting MSNA burst frequency and burst incidence (both $P<0.001$). If expressed in absolute terms ($P=0.004$) or relative to body mass ($P<0.001$), peak $VO_2$was significantly less in the middle-aged group. However, there was no between-group difference when calculated as a percent of predicted VO_2peak ($P=0.52$), indicating that, relative to age, the exercise capacity of both groups was matched. As expected, there was no relationship between resting MSNA and VO2peak in either age group nor in the group as a whole.

Cycling Exercise

Blood pressure was not available in 6 of 18 middle-aged subjects during moderate exercise because of cuff malfunction due to increased muscle tension in the arm. The mean change in systolic blood pressure during moderate cycling was significantly greater in the middle-aged subjects (+21±5 vs. +10±1 mmHg, P=0.02) whereas diastolic blood pressure did not differ (P=0.22) (Figure 1). At the moderate work rate, the rating of perceived exertion (RPE) tended to be higher in the middle-aged group (3.9±0.4 vs.2.8±0.3, P=0.05), although their absolute work rate was lower (23±2 in middle-aged vs 30±2 watts in the young, P=0.04). Both exercise intensities elicited a fall in MSNA burst incidence; this reduction was greater in the middle-aged compared with younger cohort in response to both mild (-14±2 vs. -9±2, P<0.05) and moderate (-19±2 vs. -11±2 bursts/100 heartbeats, P=0.01) 1-leg cycling (Figure 2A).

In both groups, heart rate increased from baseline during both levels of exercise (main effect of time P<0.001) but less in middle-aged subjects (interaction P=0.03). Consequently, at each work rate there were similar reductions in the average MSNA burst frequency of young and middle-aged subjects (P=0.25) (Figure 2B).

DISCUSSION

The aim of this study was to compare the MSNA responses of healthy young and middle-aged, individuals to short duration mild and moderate intensity dynamic leg exercise. At rest MSNA burst frequency and incidence were higher in the middle-aged participants and similar to values previously published (Notarius *et al.*, 2015) (Millar *et al.*, 2015) (Davy *et al.*, 1998;Houssiere *et al.*, 2006;Hart *et al.*, 2015). Our hypothesis was that aging would attenuate the contralateral sympatho-inhibitory response observed when young subjects perform mild to moderate 1-leg cycling. However, we observed the opposite response during both mild and moderate dynamic leg cycling: for a similar relative workload and a trend towards a higher perceived exertion, the fall in MSNA burst incidence, representing central sympathetic outflow, was greater in the older group. As anticipated from prior literature describing age-related decreases in chronotropic reserve (Fleg *et al.*, 1994;Correia *et al.*, 2002;Ferrari *et al.*, 2003;Fisher *et al.*, 2010), heart rate increases during cycling were blunted in our middle-aged subjects. As a consequence, reductions in the frequency of nerve firing were similar in the two groups.

Ray and colleagues (Ray *et al.*, 1993) were the first to report a decrease in MSNA burst frequency (fibular nerve) during mild and moderate dynamic leg extension in young healthy seated subjects. Because this response was absent when exercise was performed supine, the sympatho-inhibition observed was attributed to loading of the cardiopulmonary baroreflex by muscle pump-induced elevations in central venous pressure during upright rhythmic leg exercise (Ray *et al.*, 1993). That initial observation was subsequently confirmed but restricted to low work rates only (10-20 watts) and documented also in the median or radial nerve during leg cycling (Saito *et al.*, 1993;Ichinose *et al.*, 2008;Callister *et al.*, 1994;Katayama *et al.*, 2011). However, at higher exercise intensities (>50-60% of VO2 peak and continued to exhaustion), MSNA rose rather than fell (Ray *et al.*, 1993;Katayama *et al.*, 2011;Ichinose *et al.*, 2008).

In the present series, the novel age-related effect on the central sympathetic response elicited by the onset of mild and moderate dynamic leg exercise invites speculation as to potential causal mechanisms. Since perceived exertion tended to be greater in middle-aged subjects, it is more likely that the greater reduction in burst incidence observed reflects age-related differences in autonomic reflex input and central network processing rather than attenuated central command.

Arterial baroreflex modulation of heart rate is impaired in older humans (Monahan, 2007), but its control of sympathetic outflow to skeletal muscle vasculature is preserved

(Ebert *et al.*, 1992;Rudas *et al.*, 1999;Matsukawa *et al.*, 1996;Monahan, 2007). As anticipated from the literature, the systolic blood pressure response to moderate cycling in the middle-aged group was double that of younger participants (Fleg *et al.*, 1994;Correia *et al.*, 2002;Ferrari *et al.*, 2003;Fisher *et al.*, 2010). Greater arterial stiffness with increasing age may have contributed to this effect (Redfield *et al.*, 2005). Interestingly, Studinger et. al. observed, in older subjects, an accentuated sympathoinhibitory response to an arterial pressure rise (Studinger *et al.*, 2009). Thus, one plausible interpretation of the present findings is that the augmented sympatho-inhibition of older subjects reflects primarily an appropriate and proportional arterial baroreceptor reflex response commensurate with this 2-fold greater rise in systolic pressure.

Interactions with sympatho-inhibitory cardiopulmonary baroreceptor reflexes, stimulated by muscle-pump induced increases in preload also should be considered. When studied, in isolation, using head down tilt, the reflex sympatho-inhibitory response to an increase in central venous pressure appears preserved with age (Tanaka *et al.*, 1999). However, the same group also reported, with aging, an augmented reflex sympathetic excitatory response to combined unloading of low and high pressure baroreceptors via graded lower body negative pressure (they did not evaluate the inhibitory response to concurrent increases in cardiac filling and systemic pressures) (Davy *et al.*, 1998). In an earlier comparison of young (aged 18 to 36), and older subjects (aged 60 to 69), in which reflex increases in sympathetic outflow were inferred from the magnitude of forearm vasoconstriction in responses to both selective and combined unloading of carotid and cardiopulmonary mechanoreceptors, the slope of the relationship between changes in forearm vascular resistance and central venous pressure was similar in young and older subjects but the additive summation of the two responses observed in young participants was absent in the older group (Shi *et al.*, 1996). From the latter finding it was concluded that aging alters the central integration of afferent neural input from these mehanoreceptor populations. Furthermore, it has been argued that the muscle pump may be less effective in raising preload in older than in younger individuals (Proctor D.N. & Parker, 2006). Thus, when such evidence is considered in aggregate, the anticipated net response, in the context of the present protocol, would be an attenuated, rather than augmented central sympathetic response to exercise.

The present levels and duration of 1-leg cycling are unlikely to activate the muscle metaboreflex in young healthy subejcts, but whether this also is the case for middle-aged adults is uncertain as the literature concerning aging and the muscle metaboreflex is inconsistent. Some investigators report preserved muscle metaboreflex responses with age (Ng *et al.*, 1994;Greaney *et al.*, 2013;Sidhu *et al.*, 2015), whereas others have observed an impaired MSNA reflex response (Houssiere *et al.*, 2006;Markel *et al.*, 2003). Importantly, in the context of the present discussion, is the observation of preserved interaction, with age, between the arterial baroreflex and the muscle metaboreflex regulation of sympathetic outflow (Ng *et al.*, 1994;Greaney *et al.*, 2013).

Limitations

The World Health Organization defines aging for statistical purposes as 60 years and older, which is slightly higher than the mean age of our middle-aged cohort (World Health Organization, 2013). Subjects in the present study were matched for relative VO_2peak, expressed as the percent of predicted value based on age and body size and of note, these were above population averages. Thus, relative fitness levels were similar and would not explain the observed difference in sympathetic responses. These results are limited to the mild and moderate work rates studied and may not be representative of higher exercise intensities.

The principal limitation to the interpretation of this study's key finding is the absence of data informing mechanisms. In particular, the effects of the present exercise on cardiac preload in the young and older participants was not determined.

Summary

The present study is the first to directly examine differences in the contralateral muscle sympathetic response to early onset, non-steady state dynamic leg exercise in middle-aged and young subjects. Contrary to our initial hypothesis, the drop in MSNA burst incidence during short duration mild and moderate intensity cycling was augmented in the older participants. We attribute this finding to an age-related effect on autonomic reflexes engaged by exercise and in particular amplified arterial baroreflex-mediated sympathoinhibition, induced by the 2-fold greater increase in systolic blood pressure elicited when older subjects exercise.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to report.

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Figure Legends

Figure 1: Representative 30 second traces of MSNA and HR in a young and middle-aged subject during the second minute of pre-cycle, 0 load cycle and moderate load cycle. MSNA, muscle sympathetic nerve activity; HR, heart rate in beats per minute.

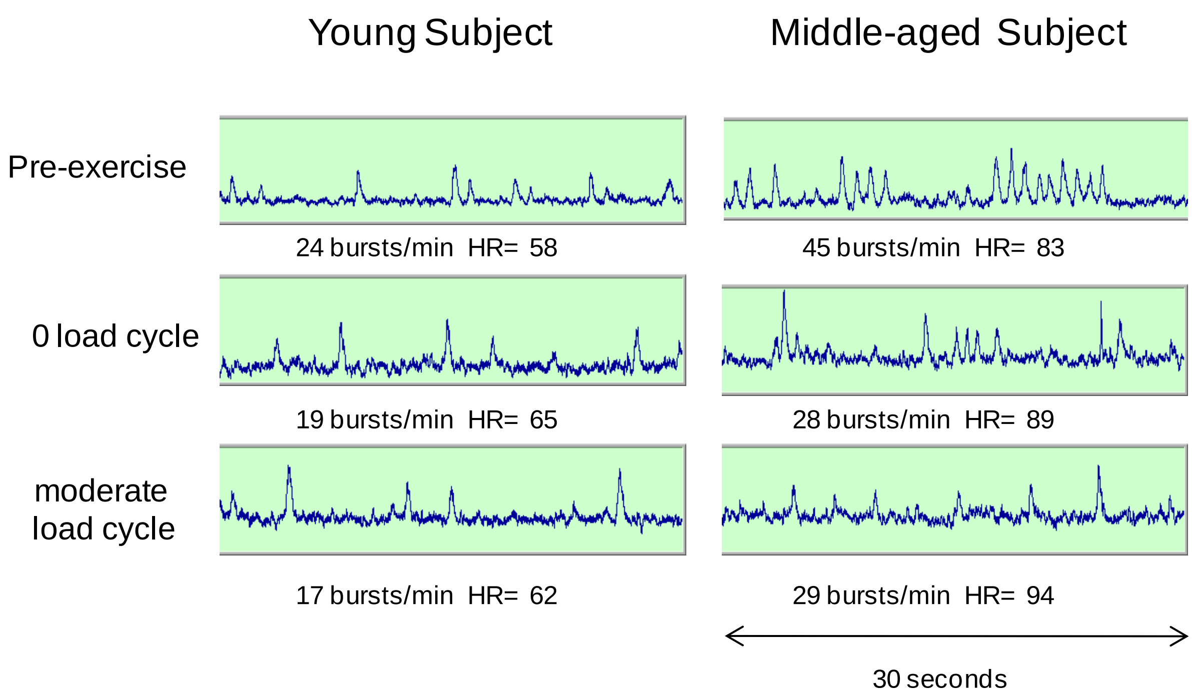
Figure 2: Change in heart rate (HR), systolic (SBP) and diastolic (DBP) blood pressure from baseline during moderate 1-leg cycling in middle-aged (open bars n=12 for BP and 18 for HR) and young (closed bars n=18) subjects. The mean change in HR is lower (+, P=0.03), whereas the change in SBP is significantly higher in the middle-aged vs younger group (*, P=0.02) with no difference in DBP between groups (P=0.21).

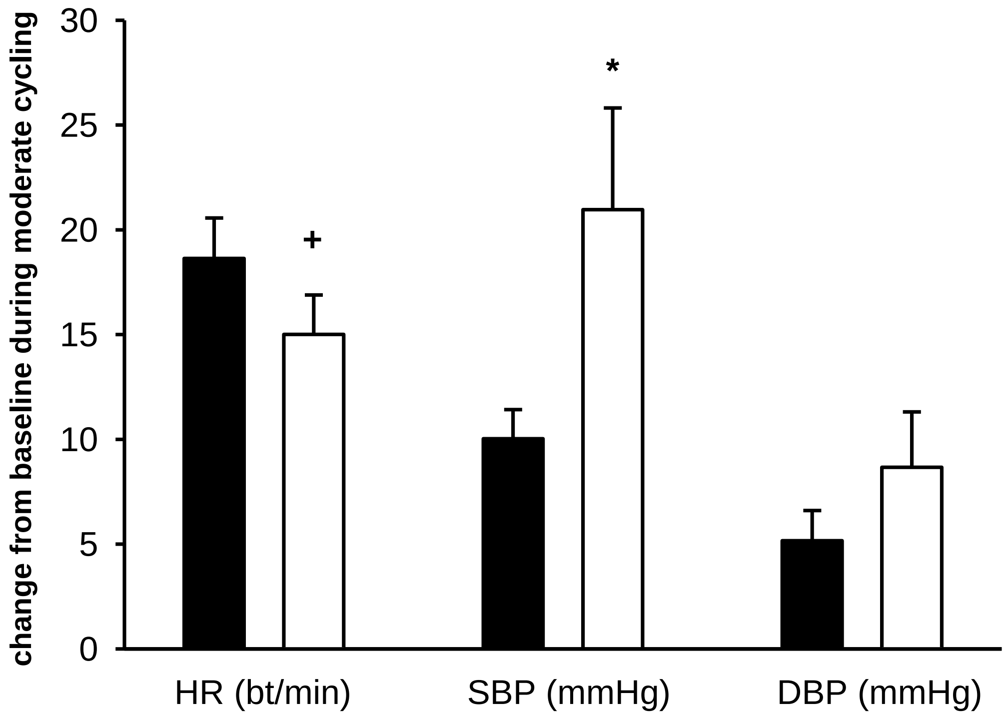
Figure 3: **A:** MSNA burst incidence decreases progressively during 1-leg cycling, more in middle-aged (open bars, n=18) than in young subjects (closed bars, n=18) (group effect P=0.01; exercise intensity effect P=0.04). * P=0.03 vs young subjects. MSNA, muscle sympathetic nerve activity; hb, heart beats. **B:** MSNA burst frequency decreases to a similar extent in both cohorts (group effect P=0.25) with no intensity effect (P=0.87) or interaction (P=0.33).

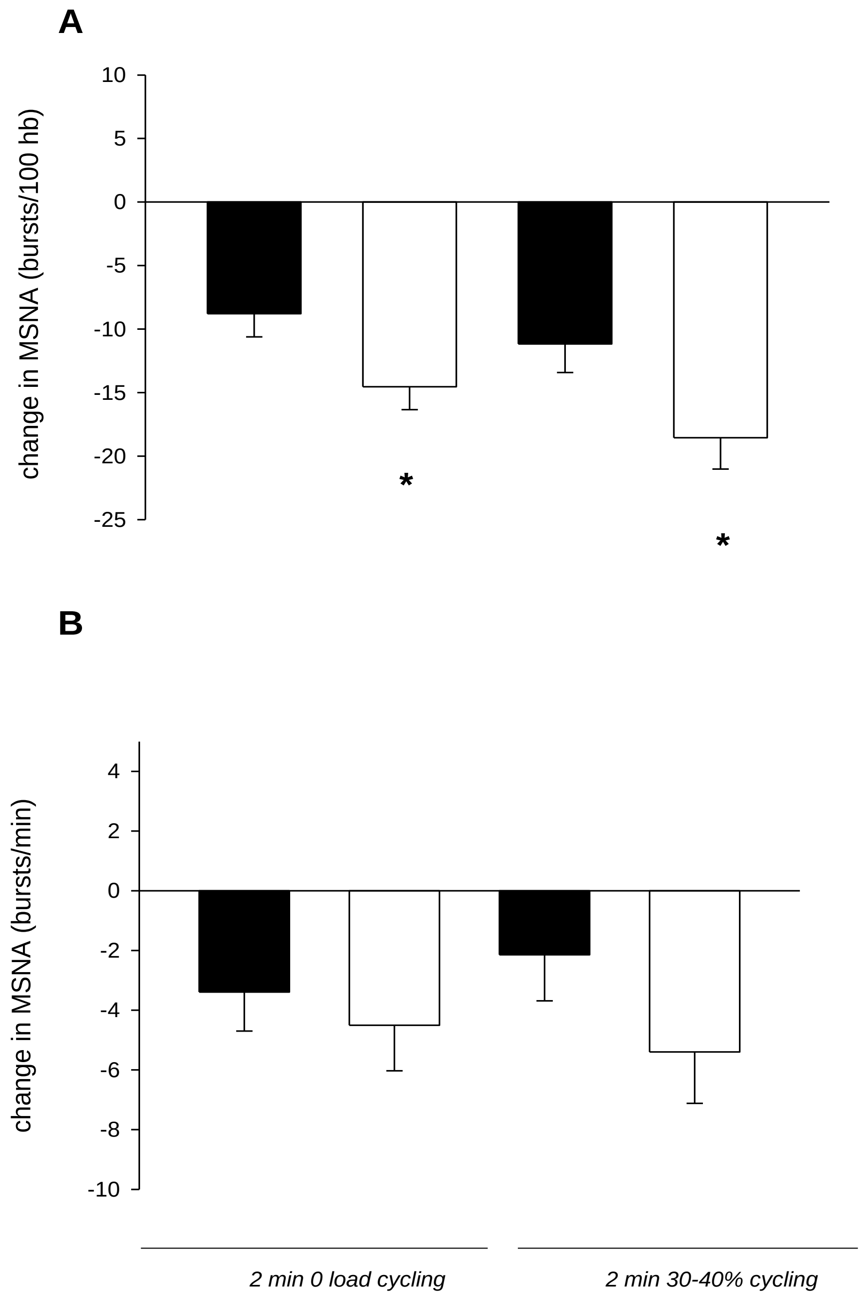
Table 1.Physical Characteristics and Resting Data.

Variable	Young group	Middle-aged group
Number (women)	18 (6)	18 (6)
Age (years)	22.7 ± 0.7	56.6 ± 1.7 *
Height (cm)	172.4 ± 2.2	172.2 ± 2.7
Body Weight (kg)	67.1 ± 2.8	74.4 ± 3.7
BMI (kg/m^2)	22.4 ± 0.6	25.1 ± 1.0+
Heart Rate (bpm)	62± 2	64±3
Systolic Blood Pressure (mmHg)	112±5	117±3
Diastolic Blood Pressure	63± 1	68± 2+
MSNA (bursts/min)	27.3±0.9	44.0 ± 2.0*
MSNA (bursts/100 hb)	44.7± 1.5	70.0 ± 3.6*
VO2peak (L/min)	3.3± 0.2	2.3 ± 0.2**
VO2peak($ml/kg·min^{-1}$)	48.5± 1.8	30.6 ± 2.2*
VO2peak (% predicted)	114.7 ± 5.8	110.3 ± 6.5
Peak Work Rate (watts)	232 ± 16	144 ± 17+

Table 1. Mean± SE. *,P<0.001; vs. young group; **, P<0.01; vs. young group; +, P<0.05; BMI, body mass index; MSNA, muscle sympathetic nerve activity; hb, heart beat; VO2, oxygen uptake.


Young Subject
Middle-aged Subject
Pre-exercise
24 bursts/min HR= 58
45 bursts/min HR= 83
0 load cycle
19 bursts/min HR= 65
28 bursts/min HR= 89
moderate load cycle
17 bursts/min HR= 62
29 bursts/min HR= 94
30 seconds


change from baseline during moderate cycling
30
25
20
15
10
5
0
+
*
HR (bt/min)
SBP (mmHg)
DBP (mmHg)


A
change in MSNA (bursts/100 hb)
10
5
0
-5
-10
-15
-20
-25
*
*
B
change in MSNA (bursts/min)
4
2
0
-2
-4
-6
-8
-10
2 min 0 load cycling
2 min 30-40% cycling