Microneurographic characterization of sympathetic responses during 1-leg exercise in young and middle-aged humans

Catherine F. Notarius, Philip J. Millar, Connor J. Doherty, Anthony V. Incognito, Nobuhiko Haruki, Emma O’Donnell, and John S. Floras

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 predetermining peak oxygen uptake, we compared contra-lateral fibular nerve MSNA during 2 min each of mild (unloaded) and moderate (30%-40% of the work rate at peak oxygen uptake, halved for single leg) 1-leg cycling in 18 young (age, 23 ± 1 years (mean ± SE)) and 18 middle-aged (age, 57 ± 2 years) sex-matched healthy subjects. Mean height, weight, resting heart rate, systolic blood pressure, and percent predicted peak oxygen uptake were similar between groups. Middle-aged subjects had higher resting MSNA burst frequency and incidence (P < 0.001) and diastolic blood pressure (P = 0.04). During moderate 1-leg cycling, older subjects’ sympathetic blood pressure increased more (+21 ± 5 vs. +10 ± 1 mm Hg; P = 0.02) and their fall in MSNA burst incidence was amplified (−19 ± 2 vs. −11 ± 2 bursts/100 heart beats; P = 0.01) but because heart rate 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 blood pressure and a larger fall in MSNA.

Key words: aging, microneurography, dynamic exercise.

Résumé : L’activité nerveuse sympathique dans le muscle (« MSNA ») au repos s’accroit avec l’âge. Toutefois, l’influence de l’âge sur la MSNA enregistrée au cours d’un exercice dynamique des jambes n’est pas établie. Nous posons l’hypothèse selon laquelle le vieillissement atténue la réponse sympathico-inhibitrice observée chez de jeunes sujets pédalant d’une jambe à une intensité légère à modérée. Après la détermination de la consommation d’oxygène de pointe, on compare la MSNA du nerf fibulaire contralatéral durant 2 min de pédalage léger (sans charge) et modéré (30-40 % de l’intensité de travail au consommation d’oxygène de pointe divisée en deux pour une seule jambe) d’une seule jambe chez 18 sujets jeunes (23 ± 1 ans, moyenne ± erreur type) et 18 d’âge moyen (57 ± 2 ans) en bonne santé et appariés selon le sexe. Les valeurs moyennes de la taille, de la masse corporelle, du rythme cardiaque de repos, de la pression systolique et du pourcentage prédit de la consommation d’oxygène de pointes similaires d’un groupe à l’autre. Les sujets d’âge moyen présentent des valeurs plus élevées d’incidence et de fréquence des bouffées de MSNA au repos (p < 0,001) et de la pression diastolique (p = 0,04). Au cours du pédalage d’intensité modérée d’une seule jambe, les sujets plus âgés présentent une augmentation plus importante de la pression systolique (+21 ± 5 vs +10 ± 1 mm Hg; p = 0,02); la diminution de leur incidence des bouffées de MSNA est amplifiée (−19 ± 2 vs −11 ± 2 bouffées/100 battements; p = 0,01), mais du fait que rythme cardiaque de repos s’élève moins (+15 ± 3 vs +19 ± 2 bpm; p = 0,03), l’exercice suscite des diminutions similaires de la fréquence de bouffées (p = 0,25). Contrairement à notre hypothèse de départ, l’exercice dynamique d’une jambe à une intensité légère à modérée engendre avec l’âge une plus grande augmentation de la pression systolique et une diminution plus marquée de MSNA. [Traduit par la Rédaction]

Mots-clés : vieillissement, microneurographie, exercice dynamique.

Introduction

Human aging is accompanied by an increase in central sympathetic outflow to skeletal muscle (muscle sympathetic nerve activity; MSNA) (Sundlof and Wallin 1978) without any concurrent diminution of its arterial baroreflex modulation (Ebert et al. 1992; Matsukawa et al. 1996; Rudas et al. 1999). 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).

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C.F. Notarius,* N. Haruki, and J.S. Floras. University Health Network and Mount Sinai Hospital Division of Cardiology, University of Toronto, Toronto General Hospital, University Health Network, 200 Elizabeth St., Toronto, ON M5G 2C4, Canada.

P.J. Millar. University Health Network and Mount Sinai Hospital Division of Cardiology, University of Toronto, Toronto General Hospital, University Health Network, 200 Elizabeth St., Toronto, ON M5G 2C4, Canada; Department of Human Health and Nutritional Sciences, University of Guelph, Guelph, ON N1G 2W1, Canada.

C.J. Doherty and A.V. Incognito. Department of Human Health and Nutritional Sciences, University of Guelph, Guelph, ON N1G 2W1, Canada.

E. O’Donnell. University Health Network and Mount Sinai Hospital Division of Cardiology, University of Toronto, Toronto General Hospital, University Health Network, 200 Elizabeth St., Toronto, ON M5G 2C4, Canada; School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough LE1 3TU, UK.

Corresponding author: Catherine F. Notarius (email: c.notarius@utoronto.ca).

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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-fibre 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 and Mano 1991), dynamic leg exercise of comparable intensity decreases MSNA (Saito et al. 1993; Callister et al. 1994; Ichinose et al. 2008; 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) 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 adjustments to exercise (Proctor and Parker 2006), including a decrease in muscle mass (Frontera et al. 2000), a change in total blood volume (Davy and Seals 1994), and/or changes in venous compliance (Olson and 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 min of such exercise.

### Materials and 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 ± SE; age, 23 ± 1 years; range, 18–28; 6 women) and 18 middle-aged (mean ± SE; 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 an element of a larger study approved by the Research Ethics Boards at both the University Health Network (reference no. 09-0988-AE), the Mount Sinai Hospital (10-0013-E), and the University of Guelph (15J0004). Informed written consent was obtained from all subjects.

#### Procedures

Subjects were studied in a quiet temperature-controlled laboratory at the same time of day, 2 h following the last food intake, on 2 separate days. All participants abstained from caffeine as well as vigorous exercise for 12 h beforehand. On the first day, oxygen uptake at peak exercise (VO₂peak) was determined by open circuit spirometry (Quark CPET system; Cosmed USA Inc., Chicago, Ill., or Moxus Modular VO2 System, AEI Technologies, Pittsburgh, Pa., USA). Subjects performed a graded ramped bicycle ergometer test (17–30 W/min) with both legs until pedal speed could not be maintained above 50 rpm and the respiratory exchange ratio (carbon dioxide production/oxygen uptake) exceeded 1.1. Work increments were individualized, based on physical activity status, to ensure that exercise duration would not exceed 14 min. VO₂peak was expressed as mL/kg/min−1 and as percent of predicted VO₂peak, accounting for age, sex, body weight, and height (Jones et al. 1985). The work rate at 30–40% of VO₂peak was based on 2-legged cycling. This value was halved 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, Fla., USA) at rest and during exercise. In 10 of the young subjects, blood pressure during exercise was assessed during exercise by calculating 30-s averages of beat-by-beat blood pressure (Finometer MIDI; Finapres Medical Systems, the Netherlands), calibrated at rest (BPtru Model BPM-200; BPTRU, Coquitlam, B.C., Canada). Heart rate was derived from lead II of an electrocardiogram. A respiratory belt encircled the abdomen. Multunit recordings of postganglionic MSNA were obtained with a unipolar tungsten electrode inserted selectively into a left peroneal (tibular) 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 heart beats). 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, USA) (Notarius et al. 2015).

#### Protocol

After 10 min of quiet rest, baseline signals were acquired over 7 min 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 min (2 min at zero load and 2 min at 15–20% of the work rate at VO₂peak). Representative MSNA recordings in a young and middle-aged subject at rest and during 1-leg cycling are shown in Fig. 1. Although the target intensity was 20% of the work rate at VO₂peak, 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 min of unloaded 1 leg cycling a resistance comparable to 15–20% of the measured work rate at VO₂peak was applied for a further 2 min. 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 means ± SE. 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 2-factor repeated-measures ANOVA (SigmaStat for Windows, version 3.5;
there was no relationship between resting MSNA and the exercise capacity of both groups was matched. As expected, V˙O₂peak was significantly less in the middle-aged group. However, there was no between-group difference when calculated as a percent of predicted V˙O₂peak (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 V˙O₂peak 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 mm Hg, P = 0.02) whereas diastolic blood pressure did not differ (P = 0.22) (Fig. 2). At the moderate work rate, the 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 W in the young, P = 0.04). Both exercise intensities elicited a fall in blood pressure during moderate cycling was significantly greater in the middle-aged versus younger group (*, P = 0.02) with no difference in DBP between groups (P = 0.21). bt/min, beats/min.

Table 1. Physical characteristics and resting data.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Young group</th>
<th>Middle-aged group</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (women)</td>
<td>18 (6)</td>
<td>18 (6)</td>
</tr>
<tr>
<td>Age, y</td>
<td>22.7±0.7</td>
<td>56.6±1.7***</td>
</tr>
<tr>
<td>Height, cm</td>
<td>172.4±2.2</td>
<td>172.2±2.7</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>67.2±3.8</td>
<td>74.4±3.7</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.4±0.6</td>
<td>25.1±1.0*</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>62±1</td>
<td>64±2</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>112±5</td>
<td>117±3</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>63±1</td>
<td>68±2*</td>
</tr>
<tr>
<td>MSNA, bursts/min</td>
<td>27.3±0.9</td>
<td>44.0±2.0***</td>
</tr>
<tr>
<td>MSNA, bursts/100 hb</td>
<td>44.7±1.5</td>
<td>70.0±3.6***</td>
</tr>
<tr>
<td>VO₂peak, L/min</td>
<td>3.3±0.2</td>
<td>2.3±0.2**</td>
</tr>
<tr>
<td>VO₂peak, ml/(kg-min)-¹</td>
<td>48.5±1.8</td>
<td>30.6±2.2***</td>
</tr>
<tr>
<td>VO₂peak, % predicted</td>
<td>114.7±5.8</td>
<td>110.3±6.5</td>
</tr>
<tr>
<td>Peak work rate, W</td>
<td>232±16</td>
<td>144±17*</td>
</tr>
</tbody>
</table>

Note: Values are means ± SE. BMI, body mass index; hb, heart beat; MSNA, muscle sympathetic nerve activity; VO₂peak, oxygen uptake. Significant difference versus young group: *, P < 0.05; **, P < 0.01; *** P < 0.001.

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), V˙O₂peak was significantly less in the middle-aged group. However, there was no between-group difference when calculated as a percent of predicted V˙O₂peak (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 V˙O₂peak in either age group nor in the group as a whole.
Fig. 3. (A) Muscle sympathetic nerve activity (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 versus young subjects. 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).

![Graph](image)

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. 2014). 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 2 groups.

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 W) and documented also in the median or radial nerve during leg cycling (Saito et al. 1993; Callister et al. 1994; Ichinose et al. 2008; Katayama et al. 2011). However, at higher exercise intensities (>50%–60% of VO2peak and continued to exhaustion), MSNA rose rather than fell (Ray et al. 1993; Ichinose et al. 2008; Katayama et al. 2011).

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; Matsukawa et al. 1996; Rudas et al. 1999; 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 sympatho-inhibitory response to an arterial pressure rise (Studinger et al. 2009). Thus, a 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 years), and older subjects (aged 60 to 69 years), 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 2 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 mechanoreceptor populations. Furthermore, it has been argued that the muscle pump may be less effective in raising preload in older than in younger individuals (Proctor and 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 subjects, 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 metabore-

**Table: Change in MSNA (bursts/min)**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Change in MSNA (bursts/min)</th>
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<tbody>
<tr>
<td>2 min 0 load cycling</td>
<td>-25</td>
</tr>
<tr>
<td>2 min 30-40% cycling</td>
<td>-20 (group effect P = 0.04)</td>
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</table>
flex 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 sympathetic neural activity in older men and women (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 V̇O_{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 findings is the absence of data informing mechanisms. In particular, the effects of the present exercise on cardiac preload in the young and older participants were 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 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.

Conflict of interest statement

The authors have no conflicts of interest to report.

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