Rapid Communication

Age and Gender Influence Muscle Sympathetic Nerve Activity at Rest in Healthy Humans

Alexander V. Ng, Robin Callister, David G. Johnson, and Douglas R. Seals

Muscle sympathetic nerve activity at rest increases with age in humans. The respective influences of the aging process per se and gender on this increase and whether age and gender effects on muscle sympathetic nerve activity can be identified with plasma norepinephrine concentrations, however, have not been established. To examine these issues, nine young women (aged 24±1 years; mean±SEM), eight young men (aged 26±1 years), seven older women (aged 63±1 years), and eight older men (aged 66±1 years) were studied. All were healthy, normotensive (blood pressure <140/90 mm Hg), nonobese (<20% above ideal weight), unmedicated, nonsmokers engaged in minimal to recreational levels of chronic physical activity. Arterial blood pressure (manual sphygmomanometry, brachial artery), heart rate, muscle sympathetic nerve activity (peroneal microneurography), and antecubital venous plasma norepinephrine concentrations (radioenzymatic assay) were determined during quiet supine resting conditions. Body weight was higher in men, but there were no age-related differences, whereas estimated body fat (sum of skinfolds) was higher in women and in the older groups (p<0.05). Estimated daily energy expenditure, arterial blood pressure, and heart rate were not different among the groups. Both muscle sympathetic nerve activity burst frequency and burst incidence at rest were progressively higher in the young women, young men, older women, and older men (10±1 versus 18±2 versus 25±3 versus 39±5 bursts/min and 16±1 versus 30±4 versus 40±3 versus 61±6 bursts/100 heartbeats, respectively; all p<0.05 versus each other). In contrast, average levels of plasma norepinephrine concentrations were not different among the groups. There was, however, a positive relation between plasma norepinephrine concentrations and muscle sympathetic nerve activity (r=0.65; p<0.0003) when the individual data were pooled. There were no strong or consistent associations between muscle sympathetic nerve activity and any other variable. We conclude that the rise in muscle sympathetic nerve activity with aging in resting humans appears to be independent of age-related differences in ischemic heart disease, obesity, chronic physical activity, or arterial blood pressure, and thus is likely related to some factor associated with the aging process per se. Gender, however, appears to be an important determinant of muscle sympathetic nerve activity at rest in both young and older humans. Finally, the age- and gender-specific influences on muscle sympathetic nerve activity are not necessarily reflected by plasma norepinephrine concentrations. (Hypertension 1993;21:498-503)

Key Words • sympathetic nervous system • aging • gender

In industrialized societies the prevalence of cardiovascular disease increases markedly with advancing age.1-2 The mechanisms responsible for this phenomenon are unknown, but an age-related rise in efferent sympathetic nervous system activity has been postulated as one factor.3 This hypothesis is based largely on experimental observations of age-associated increases in plasma norepinephrine concentration and appearance rate as well as directly recorded sympathetic nerve activity to skeletal muscle (MSNA).4-9 One important but currently unresolved issue is whether the age-related rise in sympathetic activity is caused by the aging process per se. In the overall population, the prevalence of various chronic diseases, obesity, and physical inactivity all increase with age and could influence autonomic nervous system function independent of biological aging processes. If these factors, which have not always been experimentally controlled, rather than the aging process itself were responsible for the increase in sympathetic activity, it is possible that certain interventions could be used to attenuate their effects.

At any age, the incidence of cardiovascular disease is, in general, higher in men than in women.1-2 It is not known, however, whether this relationship is associated with gender differences in sympathetic nerve activity. Current information on this question is based solely on plasma norepinephrine concentrations.4,10,11 These data have been somewhat inconsistent and thus have failed

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to provide a clear understanding of the influence of gender on sympathetic activity. Although direct recordings of MSNA would provide a more definitive approach to this issue, no such data are available.

Accordingly, the primary aim of the present investigation was to provide further insight into whether the human aging process per se results in elevated sympathetic nervous system activity and, if so, whether this aging effect is dependent, at least in part, on gender. A secondary aim was to determine if plasma norepinephrine levels accurately reflect age- and gender-related differences in directly measured sympathetic nerve activity. To accomplish this, MSNA and plasma norepinephrine concentrations were determined during supine rest in groups of healthy, normotensive, nonobese, young and older men and women with similar chronic physical activity levels. Some preliminary results of this study have been reported previously.12

Methods
Subjects
Nine young women (aged 19–30 years), eight young men (aged 22–29 years), seven older women (aged 60–65 years), and eight older men (aged 61–74 years) served as subjects. All were normotensive (arterial blood pressure <140/90 mm Hg), nonobese (<20% above ideal body weight) nonsmokers and were otherwise healthy based on a detailed health questionnaire, physical examination, and resting and maximal exercise electrocardiograms. Four of the older women were taking supplemental estrogen; there were no differences in any of their characteristics or responses compared with the other women in the group. No subjects were taking any other medications that could affect autonomic–cardiovascular function. Subjects ranged from minimally physically active to recreationally active. Signed informed consent was obtained from all subjects. All protocols were approved by the Human Subjects Committee at the University of Arizona.

Measurements
Body density was assessed from skinfold measurements using standard gender-specific procedures; percent body fat was estimated from body density. The Stanford Physical Activity Questionnaire was used to estimate average daily energy expenditure.4 Heart rate was measured from a computer-averaged electrocardiogram, and arterial blood pressure in the left arm by manual sphygmomanometry using American Heart Association guidelines.14 Multiunit recordings of MSNA were obtained from the right peroneal nerve at the fibular head using the microneurographic method as described in detail previously.5 The neural activity was amplified, filtered (bandwidth=700-2,000 Hz), full-wave rectified, and integrated (time constant=100 msec). Plasma norepinephrine concentrations were determined from a 5-mL blood sample drawn from a left antecubital vein catheter. Plasma analysis was performed in duplicate by the single isotope radioenzymatic technique of Peuler and Johnson.15 The sensitivity is ±20–30 pg/mL with coefficients of variation of 4.8% (intra-assay) and 7.8% (interassay).

Results
Subject characteristics and levels of cardiovascular variables at rest are presented in Table 1. There was approximately a 40-year age difference between the younger and older subjects. Within an age group women were shorter than men (p<0.05), and the older subjects were shorter than the younger subjects within the same gender (p<0.05). Men were heavier in each age range (p<0.05), but within the same gender there were no age-related differences. The estimated percentage of body fat was higher in the women and in the older subjects (p<0.05). The estimated daily energy expenditure was similar among the groups. Neither arterial blood pressure nor heart rate at rest was different among the groups.

Both MSNA burst frequency and MSNA burst incidence at rest were progressively higher in the young women, young men, older women, and older men (10±1 [range, 7–12] versus 18±2 [10–31] versus 25±3 [16–35] versus 39±5 [21–69] bursts/min and 16±1 [10–19] versus 30±4 [15–44] versus 40±3 [30–49] versus 61±6 [30–85] bursts/100 heartbeats, respectively, all p<0.05 versus each other; Figures 1 and 2). Although integrated total minute MSNA (total burst area) is influenced by the effect of the recording electrode position on burst amplitude and therefore, cannot theoretically be used for intersubject or intergroup comparisons, these age and gender differences in burst frequency and burst incidence were also observed when MSNA was expressed as total minute activity (i.e., all p<0.05 versus
TABLE 1. Subject Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Younger</th>
<th>Older</th>
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<tbody>
<tr>
<td>n</td>
<td>M</td>
<td>F</td>
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<td>M</td>
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<tr>
<td>F</td>
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<tr>
<td>Age (years)</td>
<td>M</td>
<td>F</td>
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<tr>
<td>M</td>
<td>26±1</td>
<td>66±1*</td>
</tr>
<tr>
<td>F</td>
<td>24±1</td>
<td>63±1*</td>
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<tr>
<td>Height (cm)</td>
<td>M</td>
<td>F</td>
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<tr>
<td>M</td>
<td>182±3</td>
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</tr>
<tr>
<td>F</td>
<td>168±3†</td>
<td>160±2†</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>M</td>
<td>79±3</td>
<td>79±3</td>
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<tr>
<td>F</td>
<td>59±3†</td>
<td>54±3†</td>
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<td>Body fat (%)</td>
<td>M</td>
<td>F</td>
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<tr>
<td>M</td>
<td>16±2</td>
<td>20±2*</td>
</tr>
<tr>
<td>F</td>
<td>20±2†</td>
<td>25±2†</td>
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<tr>
<td>Energy expenditure</td>
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<td>(kcal/kg per day)</td>
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<tr>
<td>M</td>
<td>37±1</td>
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<tr>
<td>F</td>
<td>40±2</td>
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<tr>
<td>Heart rate (bpm)</td>
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<td>F</td>
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<td>M</td>
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<tr>
<td>F</td>
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<td>SAP (mm Hg)</td>
<td>M</td>
<td>F</td>
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<tr>
<td>M</td>
<td>118±2</td>
<td>121±2</td>
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<td>F</td>
<td>115±3</td>
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<tr>
<td>DAP (mm Hg)</td>
<td>M</td>
<td>F</td>
</tr>
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<td>M</td>
<td>67±3</td>
<td>71±2</td>
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<tr>
<td>F</td>
<td>75±2</td>
<td>76±4</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>M</td>
<td>84±3</td>
<td>88±2</td>
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<tr>
<td>F</td>
<td>88±3</td>
<td>94±4</td>
</tr>
</tbody>
</table>

M, male subjects; F, female subjects; bpm, beats per minute; SAP, resting systolic arterial blood pressure; DAP, resting diastolic arterial blood pressure; MAP, resting mean arterial pressure. Values are mean±SEM.

*Significant (p<0.05) pairwise difference between younger and older subjects within a gender.
†Significant (p<0.05) pairwise difference between men and women within an age range.

In the subjects studied twice, there was no difference in the average burst frequency between the two trials (p=0.85). The Pearson correlation coefficient was R=0.93, and each subject had the same relative ranking to one another in the two trials.

In contrast, antecubital venous plasma norepinephrine concentrations were not different among the groups (201±24 [range, 120–269] versus 189±35 [85–337] versus 218±40 [132–399] versus 338±77 [98–707] pg/mL; all NS versus each other; Figure 2). The somewhat higher average value and variation for the older men were due to relatively high levels of 593 and 707 pg/mL in two of the subjects; the latter man had the highest MSNA burst frequency (69 bursts/min) and burst incidence (85 bursts/100 heartbeats) of all subjects, whereas the former exhibited levels (40 bursts/min and 62 bursts/100 heartbeats) close to his group average. The older men’s average level of MSNA was significantly higher than the other three groups even when the data from these two men were deleted.

When the individual data from all four groups were pooled, there was a positive relationship between plasma norepinephrine concentrations and both MSNA burst frequency and MSNA burst incidence (r=0.65 and 0.58, respectively; both p<0.002; Figure 2). Neither MSNA burst frequency, MSNA burst incidence, nor plasma norepinephrine concentrations were significantly related to subject height, weight, percent fat, heart rate, or diastolic or mean arterial pressure. MSNA burst frequency, but not MSNA burst incidence or plasma norepinephrine concentrations, was weakly related to systolic arterial pressure (r=0.35; p<0.05) and estimated daily energy expenditure (r=0.36; p<0.05).

Discussion

The results of the present study provide experimental support for at least three new conclusions regarding the level of sympathetic nervous system activity in humans under resting conditions. First, the elevated levels of MSNA in older people do not appear to be the consequence of age-associated differences in overt ischemic heart disease, obesity, chronic physical activity, or as reported recently and confirmed here, resting levels of arterial blood pressure. Second, gender appears to be an important determinant of the absolute level of MSNA in both young and older humans. Third, these age- and gender-specific influences on MSNA are not always reflected by venous plasma norepinephrine concentrations.

Increases in MSNA with advancing age have been reported previously in humans. The factors and mechanisms responsible for this rise, however, have not been established. Because our older subjects were normotensive, were not obese, were not heavier than their gender-matched younger control group, had equivalent levels of daily energy expenditure, and were free of overt ischemic heart disease, the present findings suggest that the age-related elevation in MSNA is likely due to some factor associated with the aging process per se. With regard to the mechanisms involved, the results of a recent investigation by Ebert and colleagues fail to support the postulate that reduced arterial baroreceptor reflex buffering of sympathetic outflow is responsible for the age-related rise in MSNA. This is in contrast to prior reports in anesthetized animals indicating impaired arterial baroreceptor reflex control of renal and splanchnic sympathetic nerve activity with senescence. Other hypotheses that remain to be directly tested in humans include decreased cardiopulmonary baroreceptor reflex inhibition of sympathetic outflow and a non-baroreceptor reflex-related elevation in central sympathetic discharge rate.

Previous reports on the age-associated rise in MSNA have been based on data obtained only from men or in a group comprised primarily of men. Moreover, studies using plasma norepinephrine concentrations as an estimate of the absolute level of sympathetic nervous system activity at rest also have failed to provide definitive insight into the potential influence of gender. One such investigation reported significantly (25%) higher supine plasma norepinephrine levels in women versus men with an average age of 41 years, whereas another study reported no differ-
FIGURE 1. Representative tracings from mean voltage neurograms demonstrate an increased muscle sympathetic nerve activity (MSNA) at rest with age and higher levels in men compared with women of the same age. Bottom neurogram, from an older man, represents the highest activity recorded in our subjects. BF, mean burst frequency (bursts per minute) averaged over 5 minutes of rest; BI, mean burst incidence (bursts per 100 heartbeats) averaged over 5 minutes of rest; AP, arterial pressure (brachial artery in millimeters of mercury).

ences in groups of men and women with an average age of approximately 30 years. In a retrospective study using a compilation of data from several previous investigations, Goldstein and colleagues\(^4\) reported no significant differences in venous plasma norepinephrine concentrations in men versus women with subjects of all ages pooled or when separated into ranges of <40 and >40 years of age; when separated by decade, however, they did find a significantly higher average plasma norepinephrine level in the 25–34-year-old women versus men. Taken together, these plasma norepinephrine data would suggest that sympathetic activity is either not different or is higher in women compared with men of similar age. In contrast, the results of the present study demonstrate that for both younger and older adult humans at rest, MSNA is significantly lower in women compared with men of the same age. Whether this gender-related difference extends to sympathetic outflows to regions or tissues other than skeletal muscle and the nature of the mechanisms involved are issues that remain to be examined.

In the present study, we found a statistically significant, positive correlation between MSNA and venous plasma norepinephrine concentrations at rest when the data from all subjects were pooled (Figure 2, bottom right); MSNA accounted for approximately 40% of the variance in plasma norepinephrine levels. This is in agreement with previous data on normotensive young, middle-aged, and older men and women.\(^8\)\(^–\)\(^22\) Our findings indicate, however, that despite this modest overall correlation, the average group plasma norepinephrine concentrations failed to reflect the age- and gender-associated differences in MSNA. This is consistent with the findings of Goldstein et al,\(^4\) who found a statistically significant, albeit weak (\(r=0.29\)), correlation between venous plasma norepinephrine concentrations and age in normotensive humans. However, the decade-by-decade average levels did not increase consistently with increasing age in either men or women. In another report,\(^10\) plasma norepinephrine concentrations were found to increase with age only in white men. Moreover, several laboratory investigations,\(^23\)\(^–\)\(^24\) including our own,\(^23\) have found no differences in plasma norepinephrine levels in younger and older humans under resting conditions. In contrast to these findings, Yamada et al\(^8\) reported age-associated increases in the average levels of both MSNA and plasma norepinephrine in groups of young, middle-aged, and older normotensive adults. Taken together, the findings to date indicate that plasma norepinephrine concentrations do not always reflect age- and gender-related differences in sympathetic nervous system activity to skeletal muscle, even in normotensive,
FIGURE 2. Graphs show that muscle sympathetic nerve activity (MSNA) at rest, measured in bursts per minute (frequency) or bursts per 100 heart beats (incidence), increases with age and is higher in men than in women. Plasma norepinephrine (PNE) at rest is not significantly different among the four groups, although individual levels are moderately correlated with MSNA. Line of identity is drawn in the scatterplot at the bottom right. YW, younger women; YM, younger men; OW, older women; and OM, older men. *Significant difference (p<0.01) between any pairwise comparison. Bars are mean±SEM.

References

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