Gender difference in age-related changes in muscle sympathetic nerve activity in healthy subjects

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Matsukawa, Toshiyoshi, Yoshiki Sugiyama, Takemasa Watanabe, Fumio Kobayashi, and Tadaaki Mano. Gender difference in age-related changes in muscle sympathetic nerve activity in healthy subjects. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1600–R1604, 1998.—Muscle sympathetic nerve activity (MSNA) was measured directly along with blood pressure at rest in 69 healthy women (20–79 yr old) and 76 age-matched healthy men (16–80 yr old). All were nonobese and normotensive. In the women and men the MSNA was positively correlated with age (women: y = 0.788x – 5.418, r = 0.846, P < 0.0001; men: y = 0.452x + 12.565, r = 0.751, P < 0.0001). The regression intercept of y was significantly lower (P < 0.0001) in the women than in the men, and the regression slope was significantly steeper (P < 0.0001) in the women. The MSNA was lower in women than in men among those <30 (P = 0.0012), 30–39 (P = 0.0126), and 40–49 yr old (P = 0.0462) but was similar in women and men among those 50–59 (P = 0.1911, NS) and ≥60 yr old (P = 0.1739, NS). The results suggest that MSNA increases with age in women and men and that the activity is markedly lower in young women than in men but is markedly accelerated with age.

In this study, to clarify how gender influences the age-related increase in sympathetic nerve activity in humans, we determined the MSNA at rest in groups of healthy, normotensive, nonobese men and women of different ages.

METHODS

Selection of subjects. The subjects were 69 healthy women (20–79 yr old) and 76 healthy age-matched men (16–80 yr old). We divided the women and men into five groups by age: 16 women and 19 men <30 yr old, 11 women and 13 men 30–39 yr old, 14 women and 10 men 40–49 yr old, 13 women and 12 men 50–59 yr old, and 15 women and 22 men ≥60 yr old, and we defined 41–55 yr-old women as the middle-aged women during menopause. Menopausal status was confirmed by menstrual history. Women were considered premenopausal if they had regular menstrual cycle lengths and had not taken hormones orally in the past year. Women were considered postmenopausal if they had not menstruated for ≥12 mo. All were normotensive (blood pressure <140/90 mmHg) and otherwise healthy on the basis of a detailed health questionnaire, physical examination, and resting and maximal exercise electrocardiograms. Because the body fat was demonstrated to affect MSNA in the women and men (8), we selected nonobese subjects; their body weight ranged from −15 to +15% of ideal body weight assessed by body mass index (BMI). They took no medication for ≥2 wk before the study. The study protocols were approved by the Human Research Committee of Research Institute of Environmental Medicine, Nagoya University. Written informed consent was obtained from each subject after a detailed explanation of the purpose of the study, the procedures, and the possible risks.

General procedures. Subjects were examined in the supine position throughout the study session. Systolic, diastolic, and mean blood pressures were determined intermittently every minute using an automatic sphygmomanometer (model BP-203NP, Nihon Colin, Konaki, J apan). The heart rate was monitored by electrocardiogram. MSNA was continuously recorded using the microneurographic method (4, 10–14, 16, 18–20).

MSNA recording. Multunit recordings of MSNA were obtained from a muscle fascicle of the tibial nerve at the popliteal fossa. To record MSNA, a tungsten microelectrode, 100 µm in shaft diameter, with an uninsulated tapered tip of 1–5 µm and impedance of −3–5 MΩ. (no. 26-05-1, Frederick Haer, Brunswick, ME) was inserted manually through the skin. The spike potentials were amplified (model DAM-6A, World Precision Instruments, New Haven, CT), monitored on an oscilloscope (model 5113, Tektronix, Beaverton, OR) and a loudspeaker, and recorded continuously on magnetic tapes, which were later played back to analyze the MSNA. The recorded activity was fed through a band-pass filter (model E3201A, NF, Yokohama, J apan) with a bandwidth of 400–3,000 Hz. The filtered neurogram was passed through an

AN AGE-RELATED INCREASE in sympathetic nerve activity has been established on the basis of earlier observations of age-related increases in plasma norepinephrine concentration as well as increases in directly recorded muscle sympathetic nerve activity (MSNA) using a microneurographic method (4–6, 14–16, 20). However, it is not known how gender influences the age-related increase in sympathetic nerve activity. The available information on this issue is based mainly on plasma norepinephrine concentrations (2, 5, 6, 21). These data have been in part inconsistent and, thus, have failed to provide a clear explanation of the influence of gender on sympathetic activity. Although direct recordings of MSNA would provide a more definitive approach to this issue, few such data are available. Ng et al. (14) measured MSNA in 17 younger subjects (aged 19–30 yr) and 15 older subjects (aged 60–74 yr) and reported an age-related increase in MSNA in resting subjects; they found that the gender difference was an important determinant of MSNA at rest in the younger and older subjects. There have been no studies evaluating the resting MSNA in younger, middle-aged, older, and aged men and in women before, during, and after menopause.
integrated with a time constant of 0.1 s to obtain the mean voltage neurogram of MSNA (4, 10–13). The spikes were identified as MSNA according to the criteria defined in previous studies (10–13): 1) tapping or stretching the muscle and tendon supplied by the impaled fascicle of the tibial nerve elicited afferent mechanoreceptor discharge, whereas stroking the skin in the distribution of the tibial nerve did not, and 2) spikes revealed a characteristic pulse-synchronous "spontaneous" discharge during phases II and III of Valsalva's maneuver.

Experimental protocol. Subjects did not eat for $\geq 3$ h before the experiment. All experiments were performed with the subjects in the recumbent position in a quiet room. After a successful nerve recording had been obtained, MSNA, heart rate, and systolic, diastolic, and mean blood pressures were monitored while the subjects rested quietly for $\geq 30$ min. When all variables were stable, a 10- to 15-min nerve recording was obtained.

Analysis. For the quantitative analysis of MSNA, the mean voltage neurogram of MSNA was displayed together with the electrocardiogram on a multidot thermal recorder (model 8M14, San-ei, Tokyo, Japan). Records were divided into periods of 1-min each, and for each period the amount of nerve activity was determined from the tracing. Sympathetic bursts were identified by inspecting the mean voltage neurogram and are expressed as bursts per minute, according to previous studies (10–13). The values for MSNA, heart rate, and blood pressure obtained over the 10- to 15-min measurement period were averaged. Statistical analysis was performed by ANOVA with the unpaired Student's t-test (see Tables 1 and 4, Figs. 2 and 3). The relationship between age and MSNA in women and men was calculated by linear regression using the least-squares method and by comparisons of the two regression intercepts and two regression slopes (Fig. 1).

Recently, body fat (7, 8) and blood pressure (10, 12, 20) were demonstrated to be closely related to MSNA in humans. Thus a matrix of correlation coefficients between BMI, mean blood pressure, and MSNA was obtained in the women and men (see Table 2). With a partial correlation analysis, the relationships between age and MSNA were examined while adjusting for the BMI or mean blood pressure (see Table 3). Moreover, for comparisons between women and men in MSNA adjusted for BMI or mean blood pressure (see Table 3) and for comparisons between premenopausal and postmenopausal women in MSNA adjusted for age, BMI, or mean blood pressure, an analysis of covariance (ANCOVA) was performed. Statistical significance was defined as $P < 0.05$. Values are means ± SE, correlation coefficient, or $P$ value (except for $n$ and ranges of ages).

RESULTS

Subject characteristics and levels of cardiovascular variables at rest are presented in Table 1. The heights of the women were significantly less than those of the men, and the body weights were significantly lower in the women (except for $\geq 60$ yr). There was no significant difference in BMI between the women and the men. There were no significant differences in systolic, diastolic, and mean blood pressures or the heart rate between men and women, except among those 30–39 yr old. Among those 30–39 yr old, the systolic ($P = 0.0378$), diastolic ($P = 0.0493$), and mean blood pressures ($P = 0.0145$) were higher in the men than in the women, but the heart rate was similar in the two groups.

Table 1. Age, height, weight, BMI, resting SBP, DBP, and MBP, and HR in women and men

<table>
<thead>
<tr>
<th>Age group</th>
<th>n</th>
<th>Age, yr</th>
<th>Height, cm</th>
<th>Weight, kg</th>
<th>BMI, kg/m²</th>
<th>SBP, mm Hg</th>
<th>DBP, mm Hg</th>
<th>MBP, mm Hg</th>
<th>HR, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>$&lt;30$ yr</td>
<td>16</td>
<td>22.4 ± 0.5</td>
<td>157.1 ± 1.4</td>
<td>51.8 ± 2.4</td>
<td>20.9 ± 0.5</td>
<td>105.0 ± 2.1</td>
<td>60.7 ± 1.6</td>
<td>77.5 ± 2.4</td>
<td>58.3 ± 1.2</td>
</tr>
<tr>
<td>Women</td>
<td>19</td>
<td>23.0 ± 0.7</td>
<td>172.3 ± 1.3</td>
<td>67.3 ± 2.0</td>
<td>22.6 ± 0.6</td>
<td>110.4 ± 1.7</td>
<td>63.9 ± 1.3</td>
<td>82.4 ± 1.7</td>
<td>60.8 ± 1.4</td>
</tr>
<tr>
<td>$30–39$ yr</td>
<td>11</td>
<td>34.6 ± 1.0</td>
<td>157.9 ± 1.5</td>
<td>55.1 ± 1.7</td>
<td>22.1 ± 0.6</td>
<td>108.6 ± 1.6</td>
<td>63.6 ± 2.9</td>
<td>79.9 ± 3.3</td>
<td>62.4 ± 1.7</td>
</tr>
<tr>
<td>Men</td>
<td>13</td>
<td>35.7 ± 0.7</td>
<td>170.2 ± 1.4</td>
<td>72.6 ± 2.0</td>
<td>23.3 ± 0.6</td>
<td>119.6 ± 2.4</td>
<td>70.5 ± 1.9</td>
<td>89.9 ± 2.0</td>
<td>64.9 ± 1.7</td>
</tr>
<tr>
<td>$40–49$ yr</td>
<td>14</td>
<td>43.4 ± 0.8</td>
<td>154.4 ± 1.5</td>
<td>54.6 ± 1.8</td>
<td>22.9 ± 0.6</td>
<td>117.4 ± 3.1</td>
<td>68.4 ± 1.9</td>
<td>88.7 ± 2.5</td>
<td>65.5 ± 2.0</td>
</tr>
<tr>
<td>Women</td>
<td>10</td>
<td>43.5 ± 1.0</td>
<td>167.6 ± 1.6</td>
<td>64.3 ± 1.9</td>
<td>22.9 ± 0.4</td>
<td>120.9 ± 2.9</td>
<td>71.2 ± 2.0</td>
<td>92.3 ± 2.5</td>
<td>62.8 ± 1.8</td>
</tr>
<tr>
<td>$50–59$ yr</td>
<td>13</td>
<td>52.6 ± 0.8</td>
<td>152.6 ± 1.4</td>
<td>54.8 ± 2.0</td>
<td>23.3 ± 1.0</td>
<td>118.1 ± 3.6</td>
<td>68.7 ± 1.8</td>
<td>90.6 ± 2.5</td>
<td>63.1 ± 1.0</td>
</tr>
<tr>
<td>Men</td>
<td>12</td>
<td>54.9 ± 0.9</td>
<td>164.2 ± 1.5</td>
<td>62.5 ± 2.1</td>
<td>23.2 ± 0.7</td>
<td>117.7 ± 2.8</td>
<td>68.2 ± 1.8</td>
<td>88.2 ± 2.3</td>
<td>63.2 ± 1.7</td>
</tr>
<tr>
<td>$\geq 60$ yr</td>
<td>15</td>
<td>69.6 ± 1.4</td>
<td>147.9 ± 1.8</td>
<td>49.4 ± 2.0</td>
<td>22.5 ± 0.6</td>
<td>127.3 ± 3.4</td>
<td>68.7 ± 1.8</td>
<td>91.6 ± 2.8</td>
<td>64.2 ± 1.8</td>
</tr>
<tr>
<td>Men</td>
<td>22</td>
<td>69.8 ± 1.5</td>
<td>164.2 ± 1.5</td>
<td>62.5 ± 2.1</td>
<td>23.2 ± 0.6</td>
<td>124.6 ± 2.5</td>
<td>68.6 ± 1.2</td>
<td>90.1 ± 1.4</td>
<td>59.5 ± 1.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; HR, heart rate. *$P < 0.05$ vs. age-matched women.
There were positive relationships between age and MSNA in the women and men (Fig. 1). The MSNA at rest became progressively higher with advancing age in the women (P < 0.0001) and men (P < 0.0001). The regression intercept of y was significantly lower (P < 0.0001) in the women than in the men, and the regression slope was significantly steeper (P < 0.0001) in the women (Fig. 1). There were significant positive correlations between age, BMI, or mean blood pressure and MSNA in the women and men (except between BMI and MSNA in men; Table 2). In addition, there were significant positive correlations between age and MSNA adjusted for BMI (P < 0.0001) and for mean blood pressure (P < 0.0001) with partial correlation analysis (Table 3).

There was a significant gender difference in MSNA; that is, the MSNA was lower in the women than in the men among the subjects <30 (P = 0.0012), 30–39 (P = 0.0126), and 40–49 yr old (P = 0.0462), but it was similar in the women and the men among those 50–59 (P = 0.1911, NS) and ≥60 yr old (P = 0.1739, NS; Fig. 2). For the comparisons between women and men in MSNA adjusted for BMI or mean blood pressure, an ANCOVA was performed (Fig. 3). In MSNA adjusted for BMI and for mean blood pressure, there was also a significant gender difference (Fig. 3). That is, the younger women had lower MSNA values but the older women had MSNA values similar to those of the age-matched men.

In the middle-aged women (41–55 yr) we compared the levels of cardiovascular variables and MSNA at rest before and after menopause (Table 4). There were no significant differences in age, height, weight, BMI, or heart rate between premenopausal and postmenopausal women, but systolic (P = 0.0105), diastolic (P = 0.0256), and mean blood pressures (P = 0.0298) and MSNA (P = 0.0009) were significantly higher in the postmenopausal than in the premenopausal women (Table 4). For the comparisons between women and men in MSNA adjusted for age, BMI, or mean blood pressure, an ANCOVA was performed. The MSNA was significantly higher in the postmenopause than in the premenopausal women for the MSNA adjusted for age (41.3 ± 3.1 vs. 27.9 ± 2.5, P = 0.0040), for BMI (42.9 ± 3.0 vs. 26.8 ± 2.5, P = 0.0005), and for mean blood pressure (43.2 ± 3.5 vs. 26.5 ± 2.8, P = 0.0022).

### Table 2. Matrix of correlation coefficients of age, BMI, MBP, and MSNA in women and men

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>BMI</th>
<th>MBP</th>
<th>MSNA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>0.2392</td>
<td>0.1293</td>
<td>0.1293</td>
<td></td>
</tr>
<tr>
<td>(P = 0.0477)</td>
<td>(P = 0.0080)</td>
<td>(P = 0.0001)</td>
<td>0.2748</td>
<td></td>
</tr>
<tr>
<td><strong>MBP</strong></td>
<td>0.4892</td>
<td>0.0847</td>
<td>0.1709</td>
<td></td>
</tr>
<tr>
<td>(P &lt; 0.0001)</td>
<td>(P = 0.2656)</td>
<td>(P = 0.1709)</td>
<td>0.2748</td>
<td></td>
</tr>
<tr>
<td><strong>MSNA</strong></td>
<td>0.8462</td>
<td>0.4359</td>
<td>0.4903</td>
<td></td>
</tr>
<tr>
<td>(P &lt; 0.0001)</td>
<td>(P = 0.0002)</td>
<td>(P &lt; 0.0001)</td>
<td>0.2748</td>
<td></td>
</tr>
</tbody>
</table>

**Age**, **BMI**, **MBP**, and **MSNA** in women and men after menopause.

### DISCUSSION

In this study we determined the MSNA at rest in groups of healthy, normotensive, nonobese men and women of different ages. There were some key observations. First, the MSNA was significantly increased with age in the women and the men. The regression intercept was significantly lower in the women than in the men, and the regression slope was significantly steeper in the women. Second, the MSNA was lower in the women than in the men among the subjects <30, 30–39, and 40–49 yr old but was similar in the women and the men among those 50–59 and ≥60 yr old. Third, in the middle-aged women the MSNA was significantly higher in the postmenopause than in the premenopausal women of the same age.

The finding of increases in MSNA with advancing age in humans agrees with previous reports (4, 14, 16, 20). In the present study we also confirmed that MSNA is increased with age in women as well as in men. The mechanisms responsible for this increase, however, are not yet clear. Regarding the mechanism involved, the results of a recent investigation by Ebert et al. (4) fail to support the hypothesis that a reduced arterial baroreceptor reflex buffering of sympathetic outflow is responsible for the age-related rise in MSNA. Other hypotheses that remain to be directly tested in humans include...
include decreased cardiopulmonary baroreceptor reflex inhibition of sympathetic outflow (1) and a non-baroreceptor reflex-related elevation in central sympathetic discharge rate (14).

Previous reports on the age-associated increase in MSNA have been based on data obtained only from men (4, 16) or in groups comprised primarily of men (16, 20). Moreover, studies using plasma norepinephrine concentrations as an estimate of the absolute level of sympathetic nervous system activity at rest also failed to provide definitive insight into the potential influence of gender (5, 6, 21). These plasma norepinephrine studies have suggested that sympathetic nerve activity is not different (5, 21), higher (6), or lower (2) in women than in men of similar age. The results of the present study demonstrate that for younger, middle-aged, and older adults humans at rest, the MSNA is significantly lower in women (<50 yr) than in men of the same age but is similar in older women and older men (>50 yr). Recently, Ng et al. (14) measured MSNA in 17 younger subjects (aged 19–30 yr) and 15 older subjects (aged 60–74 yr) and reported progressively higher MSNA values in the young women, young men, older women, and older men, i.e., an age-related increase in MSNA in resting subjects, and that the gender difference was an important determinant of MSNA at rest in the younger and the older subjects. For younger subjects, our results agree with those Ng et al., but there is a difference in results regarding older subjects between our study and theirs. Our present study found that the MSNA was similar between women and men not only at ≥60 but also 50–59 yr old. The reason for the difference in results is not clear but could depend on sociocultural differences between Japanese and American populations (e.g., life history, diet), which could influence MSNA. We consider that MSNA is lower in younger women (<50 yr) than in men but is similar in older women and older men (>50 yr). In the present study, however, the MSNA values tended to be higher in older women rather than similar to those of the age-matched men (Fig. 2). There is a possibility that a larger group of older men and women may reveal a true gender difference; i.e., older women may have a higher MSNA than older men.

In our present study we also found lowered sympathetic nerve activity in younger women and premenopausal middle-aged women and markedly increased activity in postmenopausal middle-aged women and older women. Other investigators demonstrated that the resting levels of plasma norepinephrine were significantly higher in postmenopausal than in premenopausal women (9). However, they did not examine age-matched subjects; i.e., they compared the resting levels of plasma norepinephrine between premenopausal and postmenopausal women (36.7 ± 0.9 vs. 55.7 ± 1.1 yr, P < 0.01). Until now there had been no studies evaluating resting MSNA in younger, middle, older, and aged men and women, including before, during, and after menopause. The mechanisms of lowered sympathetic nerve activity in younger women and premenopausal middle-aged women and markedly increased activity in middle-aged postmenopausal women and older women are not clear, but the following possible mechanism can be offered. Recently, it was suggested that in postmenopausal women (9) and men (3) the sympathetic responses to psychological stress were reduced during the replacement of estrogen. They measured plasma norepinephrine levels before and during psychological stress tasks in postmenopausal women and observed that the increases in plasma norepinephrine levels induced by the stress tasks were reduced after the administration of estrogen compared to placebo. This suggests that estrogen may play a role in the regulation of sympathetic activity. However, the mechanisms underlying these effects are not fully understood and further research is needed to elucidate the specific role of estrogen in the modulation of MSNA.
with after treatment with placebo (3, 9). Moreover, Tollar et al. (17) suggested that progesterone reduced sympathetic tone in men. They measured the plasma norepinephrine concentrations in men before and during the administration of progesterone and showed that plasma norepinephrine levels were reduced during the administration (17). Therefore, in younger and premenopausal women, the existence of two sex hormones, i.e., estrogen and progesterone, may be related to the lowered sympathetic nerve activity in these women. Conversely, in postmenopausal and older women the levels of estrogen and progesterone in blood were significantly reduced, and the reduced levels of the two hormones may be related to the elevation of sympathetic nerve activity in these women.

In summary, we found that 1) in women and men, the sympathetic nerve activity increased with advancing age, and especially in women this activity may be markedly accelerated with age, 2) younger women had lower sympathetic nerve activity than did the younger men, but sympathetic nerve activity was similar in older women and older men, and 3) the sympathetic nerve activity was significantly higher in the postmenopausal than in the premenopausal middle-aged women.

Perspectives

In the present study, to determine how gender influences the age-related increase in sympathetic nerve activity in humans, we observed MSNA at rest in 69 healthy women (20–79 yr) and 76 age-matched healthy men (16–80 yr). The results suggest that MSNA increases with age in women and men and that among women the MSNA is markedly lower in young women than in men but markedly increases with age, implying that MSNA is accelerated during or after menopause. However, we cannot conclude the effects of age, gender, and menopause on sympathetic nerve activity from only the present and previous studies. In the future, larger studies and longitudinal studies, e.g., 10- or 20-yr follow-up studies, are necessary to establish the effects of age, gender, and menopause on sympathetic nerve activity in humans.

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