Gender-related differences in the sympathetic vasoconstrictor drive of normal subjects

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ABSTRACT
The risk of cardiovascular disease has been linked to sympathetic activation and its incidence is known to be lower in women than in men. However, the effect of gender on the sympathetic vasoconstrictor drive has not yet been established. In the present study, we investigated whether there is a gender difference in MSNA (muscle sympathetic nerve activity) and blood flow, and to determine the mechanisms involved. We examined 68 normal subjects, 34 women and 34 men, matched for age, BMI (body mass index) and waist circumference. MSNA was measured as the mean frequency of single units (s-MSNA) and as multi-unit bursts (m-MSNA) from the peroneal nerve simultaneously with its supplied muscle CBF (calf blood flow). Women had lower ($P = 0.0007$) s-MSNA ($24 \pm 2.0$ impulses/100 cardiac beats) than men ($34 \pm 2.3$ impulses/100 cardiac beats), and a greater baroreceptor reflex sensitivity controlling efferent sympathetic nerve activity than men. The sympathetic activity was inversely and directly correlated respectively, with CBF ($P = 0.03$) and CVR (calf vascular resistance; $P = 0.01$) in men only. The responses of an increase in CVR to cold pressor and isometric handgrip tests were significantly smaller in women ($P = 0.002$) than in men, despite similar increases in efferent sympathetic nerve activity. Women had a lower central sympathetic neural output to the periphery, the mechanism of which involved differences in central and reflex control, as well as a lower vasoconstrictor response to this neural output. It is suggested that this may partly explain the observed lower incidence of cardiovascular events in women compared with men.

INTRODUCTION
The level of ABP (arterial blood pressure) and the incidence of cardiovascular disease, including hypertension, in middle-aged populations have been reported to be lower in women than in men [1–6]. However, despite the widely accepted relationship of sympathetic activation to the pathogenesis and cardiovascular complications of hypertension [7,8], the influence of gender on the sympathetic vasoconstrictor drive has not yet been established.

For instance, first, there have been no published reports designed primarily to investigate gender-related differences in the levels of resting MSNA (muscle sympathetic nerve activity). Also, the information that is available from studies in which results have been obtained in men and women have been presented separately showing women to have either lower [9–14] or similar [12,14–17] levels of resting MSNA relative to young and older men respectively.

Secondly, there has been no information regarding gender-related differences in the operation of peripheral sympathetic vasoconstrictor drive as represented by the relationship between MSNA and simultaneously measured muscle CBF (calf blood flow) and CVR (calf vascular resistance). This is important because there are

Key words: autonomic nervous system, gender, muscle sympathetic nerve activity, regional blood flow, vasoconstriction.
Abbreviations: ABP, arterial blood pressure; BMI, body mass index; BRS, baroreceptor reflex sensitivity; BRS-hp/sbp, BRS controlling the heart period; CBF, calf blood flow; CPT, cold pressor test; CVR, calf vascular resistance; IHG, isometric handgrip; MSNA, muscle sympathetic nerve activity; m-MSNA, multi-unit MSNA; s-MSNA, single-unit MSNA; BRS-sna/dbp, BRS controlling s-MSNA.
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reports showing that such a relationship is influenced by local vasoactive, hormonal and structural factors [18–20]. Indeed, it is well known that levels of MSNA do not necessarily correlate with those of ABP between individuals [20–22].

We therefore planned to find out whether the mean frequency of efferent MSNA and CBF in normal women are different from those in matched normal men, and to determine the mechanisms involved. For this purpose, we simultaneously used microneurography directly to quantify efferent MSNA from the peroneal nerve and standard venous occlusion plethysmography to measure CBF during the steady state, and CPTs (cold pressor tests) and IHG (isometric handgrip) exercise tests.

MATERIALS AND METHODS

Subjects
We prospectively examined 68 normal Caucasian subjects, comprising 34 women and 34 men. All had similar sedentary occupational status and dietary habits, including a sodium intake of $\approx 400$ mmol/day, and none was engaged in exercise training. They were screened by history and physical examination, and none was obese or had evidence of hypertension, arrhythmia, neuropathy or chronic conditions that may influence the autonomic nervous system. Twelve of the women were post-menopausal, and six men and seven women had a history of smoking. ABP was defined on the basis of the average of at least three sphygmomanometer readings, and all subjects had normal levels of systolic and diastolic ABP [23]. The subjects of the two groups were matched during recruitment according to age, BMI (body mass index) and waist circumference. The investigation conformed to the principles outlined in the Declaration of Helsinki and was approved by the St. James’s University Hospital Ethics Committee, with all subjects providing informed written consent.

General protocol
Microneurographic and haemodynamic measurements were obtained in an identical manner for all subjects during each session, as reported in detail previously [22,24]. All investigations were performed under similar conditions between 09.00 and 12.00, hours, and subjects were asked to have had a light breakfast and to empty their bladder before commencing the study. They were instructed to avoid nicotine and caffeine products for 12 h, as well as alcohol and strenuous exercise for 24 h prior to the investigation. During each session, the subjects were studied in the semi-supine position when data attained a steady state for at least 30 min. Measurements were made in a darkened laboratory in which the temperature was constant between 22 and 24°C. Resting ABP was measured from the arm using a mercury sphygmanometer. Changes in heart rate and ABP were monitored and recorded using a standard ECG and a Finometer device (FMS; TPD Biomedical Instruments).

Microneurography
Post-ganglionic MSNA was recorded from the right peroneal nerve simultaneously with the other data as described previously [22,24]. The neural signal was amplified ($\times 50000$); then, it was either filtered (bandwidth of 700–2000 Hz) and integrated (time constant 0.1 s) for the purpose of generating bursts representing multi-unit discharge, or left intact to examine raw action potentials. The output of action potentials and bursts from this assembly was passed to a PC-based data-acquisition system (LabView; National Instruments), which digitized the acquired data at 12000 samples/s (16 bits).

MSNA was differentiated from skin sympathetic activity and afferent activity, as described previously [22,25]. Single units (s-MSNA) in the raw action potential waveform were obtained by adjusting the electrode position whilst using fast monitor sweep and an on-line storage oscilloscope to confirm the presence of consistent action potential morphology [22,25]. Only vasoconstrictor units were accepted and examined, the criteria of acceptance being appropriate responses to spontaneous changes in ABP during verification by a preliminary Valsalva manoeuvre and IHG exercise. During the Valsalva manoeuvre, sympathetic nerve activity increased during the latter part of phase-II and/or phase-III and decreased during phase-IV (corresponding to the decrease and increase of ABP respectively). During IHG exercise, performed using a dynamometer (MIE Medical Research), a delayed increase in sympathetic nerve activity was observed. In addition, simultaneous measurement of CVR confirmed the vasoconstrictor function of the observed neural activity.

Analysis was performed independently off-line, using dedicated software based on the LabView system (National Instruments). This allowed electronic superimposition of units from the raw action potentials to establish their same morphology (s-MSNA). Then, an electronic discriminator window was used objectively to count those s-MSNA spikes with consistent morphology, and a threshold discriminator was used to count the R-waves of the ECG. The mean frequency of s-MSNA was quantified over 1 min and over 100 cardiac beats to avoid any interference by the length of the cardiac cycle [26]. The multi-unit bursts of MSNA (m-MSNA) were identified by inspection when the signal-to-noise ratio was $> 3$, and were counted and quantified in a similar manner to s-MSNA. The variability of repeated measurements of 2 min segments of recordings of s-MSNA units and MSNA bursts spanning a period of 30 min or those of two impalements performed within 60 min did not exceed 10%, in terms of twice the 95% confidence intervals around individual differences relative to the mean of the repeated measurements [22].
CBF

CBF was obtained simultaneously with microneurography, using an automated mercury-in-silastic (Whitney) strain-gauge venous occlusion plethysmograph (D. E. Hokanson). The strain-gauge was placed around the widest circumference of the left calf region, and chosen to be 2–3 cm smaller than the calf circumference, such that it was applied under slight tension to the calf. Venous occlusion was effected by inflating a contoured thigh cuff (Model CC-22; D. E. Hokanson), placed around the left thigh, to approx. 60 mmHg or 20 mmHg below the pre-determined diastolic ABP, whichever was the lesser. The DC output from the plethysmograph was passed to a chart recorder (APC Medical) utilizing heat-sensitive paper, so that a graphical recording of the change in limb volume could be produced. During measurement of CBF, the left foot region was excluded by inflating a paediatric cuff placed around the ankle to levels greater than the pre-determined systolic ABP.

CBF was obtained typically at three recordings/min during periods of steady-state conditions. The average of the recordings was expressed in units of ml·100 ml⁻¹·min⁻¹. The intra-observer reproducibility of CBF measurement in this laboratory, obtained as twice the 95% confidence interval of the differences between repeated within-session plethysmography, amounted to 2.4% of the value of the measurement. ABP was measured simultaneously and continuously, and its average value was divided by the average CBF to obtain CVR, which was expressed in arbitrary units.

Other measurements

Responses of haemodynamic variables and sympathetic nerve activity to formal IHG exercise tests and CPTs were measured. The former was performed at 30% of a pre-determined maximal voluntary contraction for 2 min, and the latter by dipping the subject’s hand into cold water with a temperature of less than 4°C for at least 1 min or until discomfort was felt. Baseline and recovery data were taken for 1 min prior to and after each of the two tests. CPT was performed by all women and 32 men, whereas IHG exercise was performed by 33 women and 31 men. The responses of sympathetic nerve activity and its vascular effect were obtained in 33 women and 31 men during CPT, and 30 women and 32 men during IHG exercise; in the remaining subjects, it was not possible to obtain the activity because of interference by changes in leg muscle tension and inability to tolerate cold exposure. Responses to IHG exercise were derived from the differences between data obtained during the second minute of the exercise, when sympathetic activity is known to increase [27], and the average of those obtained during baseline and recovery periods. Responses to CPT were derived as the differences between data obtained during the last 30 s of exposure to cold, when the occurrence of discomfort is known to be accompanied by an increase of sympathetic activity [28], and the average of those obtained during baseline and recovery periods.

BRS (baroreceptor reflex sensitivity) controlling the heart period mainly through vagal effects (BRS-hp/sbp) and that controlling s-MSNA (BRS-sna/dbp) were obtained by a standardized Valsalva manoeuvre and measured independently off-line, using the LabView software. Subjects performed the manoeuvre to between 40 and 50 mmHg for 15 s. BRS-hp/sbp was obtained in all subjects, whereas BRS-sna/dbp was obtained in 28 women and 28 men. To obtain the BRS-hp/sbp, the time interval during which the rise of ABP occurred in stage IV of the Valsalva manoeuvre was identified and each systolic ABP value and its corresponding heart period (phase 0) and the succeeding one (phase 1) were analysed. BRS-hp/sbp was calculated from the slope [29,30] of the best significant linear relationship between the systolic ABP and its heart period (phase 0) or the succeeding one (phase 1). BRS-sna/dbp was calculated in a similar manner by relating the diastolic ABP values to the number of s-MSNA corrected for the length of the heart period over nine beats following each index ABP. A close relationship between sympathetic nerve activity and short-term changes in diastolic ABP has been observed previously [21,31,32].

Statistical analysis

Unpaired Student t tests were used to assess differences in data and absolute responses between the two groups. Percentage responses relative to control values were compared using the Mann–Whitney test. The least-square technique was used for assessing the linear relationship between variables. Values of P < 0.05 were considered statistically significant. Data are means ± S.E.M.

RESULTS

The two groups were matched according to the design of the investigation in respect of age, BMI, waist circumference and heart rate (Table 1). Men were taller and had slightly higher ABP values than women.

Compared with the group of men, women had significantly lower indices of sympathetic nerve activity (Figure 1), lower BRS-hp/sbp and higher BRS-sna/dbp (Figure 2). No significant group differences were found between women and men regarding CVR (P > 0.30; Table 1) or CBF (P > 0.36; Table 1). However, in men, the indices of sympathetic nerve activity were positively correlated with CVR (at least r > 0.38, P < 0.002) and negatively correlated with CBF (at least r < −0.32, P < 0.035), with no such significant correlations in women (at least r < 0.29, P > 0.058; and r < −0.194, P > 0.136 respectively). Figure 3 shows the relationship between these variables in men and women.

The responses to CPT of the two groups are shown in Figure 4. Both groups of women and men had similar
Table 1  Characteristics of the study groups of women and men
Values are means ± S.E.M.  P values as determined using two-tailed unpaired Student t tests; ns, P ≥ 0.05.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Women</th>
<th>Men</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>34</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>43 ± 2.2</td>
<td>45 ± 2.6</td>
<td>ns</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>69 ± 1.7</td>
<td>83 ± 1.6</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165 ± 1.0</td>
<td>179 ± 1.3</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25 ± 0.6</td>
<td>26 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>84.9 ± 1.8</td>
<td>87.2 ± 2.0</td>
<td>ns</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>64 ± 1.2</td>
<td>61 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>Mean ABP (mmHg)</td>
<td>93 ± 1.3</td>
<td>96 ± 1.0</td>
<td>0.04</td>
</tr>
<tr>
<td>Systolic ABP (mmHg)</td>
<td>123 ± 1.8</td>
<td>128 ± 1.6</td>
<td>0.04</td>
</tr>
<tr>
<td>Diastolic ABP (mmHg)</td>
<td>78 ± 1.0</td>
<td>80 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>s-MSNA (impulses/100 cardiac beats)</td>
<td>39 ± 3.6</td>
<td>58 ± 3.8</td>
<td>0.0003</td>
</tr>
<tr>
<td>s-MSNA (impulses/min)</td>
<td>24 ± 2.0</td>
<td>34 ± 2.3</td>
<td></td>
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<tr>
<td>m-MSNA bursts (bursts/100 cardiac beats)</td>
<td>33 ± 3.1</td>
<td>50 ± 3.8</td>
<td>0.0007</td>
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<tr>
<td>m-MSNA bursts (bursts/min)</td>
<td>21 ± 1.8</td>
<td>29 ± 2.3</td>
<td>0.0026</td>
</tr>
<tr>
<td>CBF (ml · 100 ml⁻¹ · min⁻¹)</td>
<td>2.3 ± 0.08</td>
<td>2.4 ± 0.13</td>
<td>ns</td>
</tr>
<tr>
<td>CVR (units)</td>
<td>41 ± 2.0</td>
<td>44 ± 2.4</td>
<td></td>
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<tr>
<td>BRS-hp/sbp (ms/mmHg)</td>
<td>5.3 ± 0.40</td>
<td>7.2 ± 0.55</td>
<td>0.0035</td>
</tr>
</tbody>
</table>

Figure 1  Levels of m-MSNA and s-MSNA expressed per 100 cardiac beats (upper panel) and per min (lower panel) for men and women
Values are means ± S.E.M.  Women have lower sympathetic activity than men.  P values refer to Student’s unpaired t tests when significant.

DISCUSSION

Our present study has demonstrated in normal women and men that there were gender-related differences in the level of MSNA, its vascular effect and its baroreceptor reflex control. The original and salient findings include: (i) a lower central sympathetic output to the periphery in women than in men, brought about by a greater baroreceptor reflex inhibitory effect by ABP; and (ii) the vasoconstrictor effect of the peroneal sympathetic nerve activity was attenuated in women as compared with men, and this occurred both at resting conditions and during sympathetic activation.

Regarding group characteristics of men and women, some of our findings have already been reported previously by others. Thus women have been found to be shorter [5,9,16,17,33–35], to have a lower body weight [9,16,17,33,34,36–38], a slightly lower ABP [2,5,34,37–40] and higher heart rate [5,33,34,38,41] than men.
that were, at least, matched for age. These similarities could be considered to rule out the arguments that our subjects were not representative of other reported studies involving women and men.

Our previous findings and those of others have helped to design our study in respect to avoiding confounding factors that can interfere with sympathetic nerve activity. This was achieved by matching individuals of the two groups. Subjects of both groups were Caucasians and were examined using the same protocol and under similar laboratory conditions whilst avoiding the influence of age, gender, dietary intake, general or regional obesity, large meal or visceral distension; these factors are known to affect sympathetic activity or its control [9,12,14,21,25,26]. Pre- and post-menopausal women were examined, as it has been shown that the onset of menopause does not affect the level of MSNA [14]. The finding of slightly lower levels of ABP in women was associated with a lower, rather than higher, sympathetic nerve activity than in men, a finding that is not consistent with an inhibitory effect through baroreceptor reflex control of sympathetic nerve activity. Also, the differences in sympathetic nerve activity between women and men were greater than the variability attending repeated measurements of this activity [22]. These considerations make it likely that the observed gender differences in sympathetic nerve activity were not solely caused by differences in the levels of ABP or other factors known to affect the sympathetic output.

Our finding of a lower level of efferent sympathetic nerve activity in women than in men is similar to data found in the majority of other reports. For instance, six out of nine studies have reported significantly lower sympathetic nerve activity in young and middle-aged women than men [9–14]. In the remaining three reports, either women were found to have lower sympathetic activity than men when examined at the same ABP [16] or the lower group average of sympathetic activity in women was not significantly different from that in men [15,17]. Of the three studies that examined older men and women, one found lower sympathetic activity in women than in men [9], whereas the other two [20,22] did not. Similarly, resting CBF has been reported to be similar in women and men [15,42]. Our study, which was primarily designed to test the possibility of gender-related differences, has established that non-obese and normal middle-aged women had lower resting efferent sympathetic nerve activity than men.

In both women and men, IHG exercise tests and CPTs caused significant increases in efferent sympathetic nerve activity and ABP. This was not unexpected, because the rise in ABP that brings about a baroreceptor reflex inhibitory effect on efferent sympathetic activity and the rise of this activity in response to IHG exercise tests and CPTs are subject to complex interactions that include central effects [27,31,32,43,44]. Findings reported previously have not been consistent regarding a gender effect on the responses of ABP and sympathetic activity to IHG exercise tests and CPTs are subject to complex interactions that include central effects [27,31,32,43,44]. Findings reported previously have not been consistent regarding a gender effect on the responses of ABP and sympathetic activity to IHG exercise tests and CPTs are subject to complex interactions that include central effects [27,31,32,43,44]. Findings reported previously have not been consistent regarding a gender effect on the responses of ABP and sympathetic activity to IHG exercise tests and CPTs are subject to complex interactions that include central effects [27,31,32,43,44]. Findings reported previously have not been consistent regarding a gender effect on the responses of ABP and sympathetic activity to IHG exercise tests [9,11,36]. In the present study, the responses of ABP to IHG exercise tests were smaller in women, whereas the absolute increases in sympathetic nerve activity were similar to those in men, leading to a greater percentage increase in women. In respect to CPTs, the increases in ABP and sympathetic activity in women were similar to those in men. Similar findings have been reported previously [9,11,13,42].

The mechanisms of the lower resting efferent sympathetic nerve activity in women were shown in the present study to involve a greater baroreceptor reflex inhibitory control of this activity in women than in men. The BRS-sna/dbp in women was steeper than in men and was accompanied by an impaired BRS-hp/sbp
Values are means ± S.E.M. MSNA was positively correlated with CVR and negatively correlated with CBF in men, with no such significant correlations observed in women. P value as determined using an unpaired Student’s t test.

in women relative to men. The latter has been found previously using the Valsalva manoeuvre [39], as in the present study, using neck suction [34] as well as when changing ABP levels by pharmacological agents [40,41]. As such, our method of examining baroreceptor reflex control has yielded similar results to those obtained using other techniques. In respect of the control of efferent sympathetic nerve activity, studies reported previously in animals and humans have indicated that females have a greater central and baroreceptor reflex inhibitory control of this activity than males [45,46]. Our present study has now established that women have a greater baroreceptor reflex inhibitory control of sympathetic activity, and also found a smaller control of heart rate that is mainly mediated by withdrawal of vagal effects. These considerations are consistent with the findings in the present study of a lower resting sympathetic nerve activity and higher heart rate in women than in men.

Another new finding of our present study was the demonstration that the vasoconstrictor effect of the efferent sympathetic activity in women was attenuated relative to that in men. This was apparent in the relationship between the sympathetic activity and either CVR or CBF which was found only in men; greater sympathetic activity was associated with greater CVR and lower CBF in men, but not in women. In addition, women had a smaller increase in CVR than men during augmentation of efferent sympathetic activity by a CPT and an IHG exercise test. Studies reported previously, mainly in animals, have found an attenuated vascular response to sympathetic stimulation in females [46]. The present investigation has now established such gender-related differences in humans.

In the present study, we quantified the central output of sympathetic nerve activity to the periphery and, as such, this could differ from that destined to supply visceral organs [8]. However, in normal subjects, a
correlation has been reported between MSNA and the sympathetic drive to the heart and the kidney, as assessed by the noradrenaline spillover rate [47,48]. Furthermore, it has been reported that men have a predominance of sympathetic effects on the heart interval using spectral analysis of the heart rate [16,37,39,49,50] and higher circulating catecholamine levels than women [11,35,38]. These reported findings are consistent with our results of an existing difference in sympathetic drive between women and men.

Our findings have clinical implications regarding gender differences in respect to conditions related to sympathetic activation. For instance, the level of ABP and the incidence of cardiovascular disease, including hypertension, have been reported to be lower in middle-aged women than in men of similar age [2,3]. Indeed, a lower prevalence of cardiovascular disease has been documented in women than in men [1,4,6]. These reports could be explained on the basis of our present findings of a lower level of sympathetic output and its attenuated vasoconstrictor effect.

In conclusion, our present study has demonstrated that women have a lower central sympathetic nerve activity to the periphery, the mechanism of which involved a greater baroreceptor reflex inhibitory effect on this activity in women than in men. The study has also demonstrated an attenuated vasoconstrictor effect of peroneal efferent sympathetic nerve activity in women than in men. These findings could have implications regarding the lower cardiovascular events observed in women than in men.

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Sympathetic vasoconstrictor drive and gender


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