Calibrated variability of muscle sympathetic nerve activity during graded head-up tilt in humans and its link with noradrenaline data and cardiovascular rhythms

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Marchi A, Bari V, De Maria B, Esler M, Lambert E, Baumert M, Porta A. Calibrated variability of muscle sympathetic nerve activity during graded head-up tilt in humans and its link with noradrenaline data and cardiovascular rhythms. *Am J Physiol Regul Integr Comp Physiol* 310: R1134–R1143, 2016. First published March 23, 2016; doi:10.1152/ajpregu.00541.2015.—Muscle sympathetic nerve activity (MSNA) variability is traditionally computed through a low-pass filtering procedure that requires normalization. We proposed a new beat-to-beat MSNA variability computation that preserves dimensionality typical of an integrated neural discharge (i.e., bursts per unit of time). The calibrated MSNA (cMSNA) variability technique is contrasted with the traditional uncalibrated MSNA (ucMSNA) version. The powers of cMSNA and ucMSNA variabilities in the low-frequency (LF, from 0.04 to 0.15 Hz) band were computed with those of the heart period (HP) of systolic and diastolic arterial pressure (SAP and DAP, respectively) in seven healthy subjects (age, 20–28 years; median, 22 years; 5 women) during a graded head-up tilt. Subjects were sequentially tilted at 0°, 20°, 30°, 40°, and 60° table inclinations. The LF powers of ucMSNA and HP variabilities were expressed in normalized units (LFnu), whereas all remaining spectral markers were expressed in absolute units. We found that 1) the LF power of cMSNA variability was positively correlated with tilt angle, whereas the LFnu power of the ucMSNA series was uncorrelated; 2) the LF power of cMSNA variability was correlated with LF powers of SAP and DAP, LFnu power of HP and noradrenaline concentration, whereas the relationship of the LFnu power of ucMSNA variability to LF powers of SAP and DAP was weaker and that to LFnu power of HP was absent; and 3) the stronger relationship of cMSNA variability to SAP and DAP spectral markers compared with the ucMSNA series was confirmed individually. The cMSNA variability appears to be more suitable in describing sympathetic control in humans than traditional ucMSNA variability.

Heart rate variability; arterial pressure variability; MSNA; catecholamines; autonomic nervous system; cardiovascular control

SPECTRAL ANALYSIS OF SPONTANEOUS cardiovascular variability allows the indirect, noninvasive, inference of the autonomic function in humans (1, 20). The suitability of the spectral indexes derived from the beat-to-beat variations of the heart period (HP), of systolic and diastolic arterial pressure (SAP and DAP, respectively) has been validated by assessing their correlation with spectral indexes derived from direct, invasive, microneurographic recordings of muscle sympathetic nerve activity (MSNA) (5, 11, 28) during sympatho-inhibitory and/or sympatho-excitatory maneuvers (4, 10, 18, 21, 26). The link between MSNA and arterial pressure (AP) is widely recognized with MSNA burst rate increasing when AP falls and MSNA becoming silent when AP rises as a result of an operating baroreflex that inhibits sympathetic drive (29), even though central mechanisms such as the entrainment of a central oscillator by pulse-synchronous baroreceptor nerve activity cannot be excluded (2). Baroreflex-mediated modifications of the sympathetic drive are responsible for the changes in HP, and they partially explain the link between MSNA and HP variations (7). Traditionally, validation of the link between MSNA and modifications of HP and AP (mainly SAP and DAP in cardiovascular variability studies) was carried out after applying a suitable low-pass filtering procedure to the MSNA signal that retains the range of frequencies typical of cardiovascular variability (i.e., from 0 to 0.5 Hz) (4, 10, 18, 21, 26). Because the power of the MSNA signal in the low-frequency (LF, from 0.04 to 0.15 Hz) band is significantly and positively correlated with the LF power of HP variability when expressed in normalized units and with the LF power of SAP variability when expressed in absolute units, this suggests that HP and SAP spectral indexes, when expressed in suitable units, may provide insight into autonomic regulatory mechanisms (21).

Because the spectral indexes that describe MSNA variability are traditionally computed directly from a low-pass-filtered version of the MSNA signal (4, 10, 13, 18, 21, 26, 27, 31), these markers quantify the magnitude of changes in burst amplitude and area about their mean value more than the variations in their rate of occurrence. In other words, the variability series of MSNA, as currently derived from the MSNA signal, has the same physical dimension of the MSNA signal (i.e., mV) and does not have the physical dimension of a neural discharge (i.e., bursts per unit of time). As a consequence, the spectral indexes derived from MSNA variability depend on numerous factors including number of active fibers, proximity to the recording electrode to the bundle, operator’s experience in picking the nerve up, acquisition system settings (e.g., the gain of the neural traffic amplifier), and level of noise superimposed on the MSNA signal. These factors increase
the within- and between-subject variability, and their influence is usually mitigated by normalization procedures that take the form of division by the variance of the MSNA variability in baseline condition (27) or by the variance minus the power in the very-low-frequency (VLF) band (i.e., <0.04 Hz) (21).

We hypothesize that the traditional low-pass-filtered MSNA variability signal has limited power in tracking the magnitude of the response of the cardiovascular control to a sympatho-excitatory stimulus, it is intrinsically exposed to influences of noise, and it blurs physiological relationships with more traditional cardiovascular variabilities that, conversely, might be highlighted by exploiting a different definition of MSNA variability.

In this study we propose a new approach, referred to as calibrated MSNA (cMSNA) variability, to obtain a beat-to-beat MSNA variability from the integrated MSNA signal. In contrast to the method currently in use, referred to as uncalibrated MSNA (ucMSNA) variability, cMSNA has the desirable characteristic of directly assessing the modulations of the neural discharge signal (i.e., number of bursts per unit of time). The LF power derived from cMSNA variability was computed during a graded head-up tilt protocol that induces a sympathetic activation and vagal withdrawal according to the inclination of the tilt table in healthy volunteers (3, 4, 9, 10, 13, 14, 16, 17, 23). In this study we evaluated the relationship of the activity of the baroreceptor reflexes (16, 17, 23). In this study we evaluated the relationship of the LF power of the cMSNA variability to 1) tilt table angles; 2) plasma noradrenaline (NA) concentration and spillover; 3) traditional indirect noninvasive spectral indexes derived from HP, SAP, and DAP series; and 4) the LF power of the ucMSNA variability.

MATERIALS AND METHODS

Experimental protocol and data acquisition. The study involved seven healthy subjects [age, 20–28 yr (median 22 yr), body mass index, 21–29 kg/m² (median 24 kg/m²), 5 women]. The study protocol was approved by the Alfred Hospital Ethics Review Committee (No. 144/06) and conformed to the relevant guidelines of the National Health and Medical Research Council of Australia and principles of the Declaration of Helsinki for medical research involving humans. All subjects provided written informed consent. The head-up tilt protocol was fully described elsewhere (14). Briefly, all subjects were tested in the morning, after a light breakfast. Caffeine and alcohol intake was excluded after 7:00 PM on the evening before the study. The radial artery was cannulated percutaneously (3-F, 5 cm, Cook catheter) to enable AP monitoring and blood sampling for catecholamine measurement. A lead III ECG recording was taken. Respiration movements were monitored via piezoelectric device (ADI Instruments, Australia). Multuniitary sympathetic nerve firing rates in postganglionic fibers distributed to the skeletal muscle vasculature were recorded by using clinical microneurography as previously described (14). After the common peroneal nerve was located, a tungsten microelectrode (FHC, Bowdoinham, ME) was inserted percutaneously and adjusted until satisfactory spontaneous MSNA was observed in accordance with previously described criteria (14). After instrumentation, subjects were allowed to rest for at least 30 min. Subjects were then sequentially tilted to 0°, 20°, 30°, 40°, and 60° (T0, T20, T30, T40, and T60, respectively) for 10 min at each angle. The head-up tilt test started from the horizontal position and occurred incrementally with respect to the previous tilt table inclination, thus allowing us to maintain the positioning of the microelectrodes in the same subject and to preserve the invariable quality of the MSNA recordings over the entire experimental session. AP, ECG, respiratory rate, and MSNA were measured at each tilt angle. The raw MSNA signal was band-pass filtered (700–2,000 Hz), amplified, rectified, and integrated (time constant of 0.1 s) to obtain integrated MSNA, AP, ECG, respiratory movements, and integrated MSNA were digitized at 1.000 Hz using a PowerLab data acquisition system (ML785/8SP; ADI Instruments, Australia), and recordings were stored for off-line analysis. Blood samples were taken at the end of each head-up tilt session including T0, and all other parameters were collected continuously. Plasma NA concentration and NA appearance rate (i.e., spillover) were determined as previously described (14). No subject exhibited signs of presyncope during the orthostatic challenge.

Extraction of the beat-to-beat HP and AP variability series. The R-wave peaks on the ECG were located with minimal jitters of their positions using parabolic interpolation. The temporal distance between two consecutive parabolic apexes was taken as an HP approximation. The maximum of AP inside the i-th HP was defined as the i-th SAP value, where i is the cardiac beat counter. The i-th DAP was computed as the minimum of AP following the i-th SAP. The respiratory signal provided an estimate of the respiratory rate. The occurrences of R-wave peaks and SAP and DAP fiducial points were carefully checked to avoid erroneous detections or missed beats. The beat-to-beat series of HP = [HP(i), i = 1, . . . , N], SAP = [SAP(i), i = 1, . . . , N], and DAP = [DAP(i), i = 1, . . . , N] were extracted, where N is the series length. Sequences with N = 256 consecutive measures were selected at random in each experimental session. The series was linearly detrended. If nonstationarities were evident despite the linear detrending, such as very slow drifting of the mean or sudden changes in the variance occurred, the random selection was carried out again. The means and variances of HP, SAP, and DAP were indicated as HPmean, HPvar, SAPmean, SAPvar, DAPmean, and DAPvar, respectively, and expressed, respectively, as ms, ms², mmHg, mmHg², mmHg, and mmHg². From the MSNA signal we computed the burst frequency and incidence, indicated as bfMSNA and biMSNA, and expressed as bursts/min and bursts/100 beats, respectively.

MSNA beat-to-beat series extraction. The i-th ucMSNA value was obtained by dividing the integral computed over the integrated MSNA signal between the (i-1)-th and i-th DAP values by the interdiastolic interval (21). Extraction of cMSNA variability requires detection of the MSNA bursts from the integrated MSNA signal. Sympathetic bursts were detected by an adaptive thresholding in which the burst detection threshold was updated on a beat-to-beat basis to follow baseline wandering and physiological variations of the MSNA burst amplitude (6). The MSNA peaks overcoming the adaptive threshold were considered as MSNA bursts. To account for the latency from the AP sensing to the possible sympathetic response (12, 32), the MSNA burst was searched in a temporal window ranging from 0.9 to 1.7 s starting from the R-wave peak of the first R-wave peak delimiting the current HP (6). The cMSNA signal was obtained from the integrated MSNA signal (Fig. 1C) by counting the number of MSNA bursts inside a moving time window of 5 s, thus obtaining a step-wise count MSNA signal (Fig. 1D), and by low-pass filtering the step-wise count MSNA signal (Fig. 1E) with a finite impulse response filter with a cutoff frequency of 0.5 Hz (8,000 coefficients), thus exclusively retaining the frequency range of cardiovascular variability. As expected from the physiological relationship between MSNA and AP (29), the step-wise count MSNA signal (Fig. 1D) and its filtered version (Fig. 1E) are in phase opposition with the LF oscillations of AP, clearly visible as the modulation of SAP and DAP values over time (Fig. 1B). Finally, the low-pass count MSNA signal was down-sampled once per cardiac beat at the occurrence of the first R-wave peak delimiting the i-th HP as detected from the ECG (Fig. 1A). The resulting time series was expressed in bursts/s by dividing the count MSNA values by the length of the time window. As a consequence of these definitions the beat-to-beat variability of both cMSNA = [cMSNA(i), i = 1, . . . , N] and ucMSNA = [ucMSNA(i), i = 1, . . . , N] were synchronous with the beat-to-beat variability series of HP, SAP, and DAP.
power of the HP series (HF<sub>HP</sub>) expressed in absolute units (i.e., ms<sup>2</sup>), considered to be a marker of vagal modulation directed to the heart (1); 3) the LF<sub>HP</sub>/HF<sub>HP</sub> ratio, a dimensionless index obtained by dividing the LF power by the HF power, both computed over the HP series, deemed to be an indicator of the sympathetic-vagal balance to the heart (20); and 4) LF powers of SAP and DAP (LF<sub>SAP</sub> and LF<sub>DAP</sub>), expressed in absolute units (i.e., mmHg<sup>2</sup>), considered to be indexes of sympathetic modulation directed to the vessels (20). The LF power of the cMSNA series (LF<sub>MSNA</sub>) expressed in absolute units (i.e., bursts<sup>2</sup>/s<sup>2</sup>) was taken as a direct invasive index of sympathetic modulation. The LF power of ucMSNA series expressed in normalized units (LFnu<sub>ucMSNA</sub>), defined as the LF power of the ucMSNA variability multiplied by 100 and divided by the σ<sup>2</sup><sub>ucMSNA</sub> minus the power of the ucMSNA variability in the VLF band, was computed as a direct invasive index of sympathetic modulation (21). It was necessary to apply the normalization procedure to the LF power of the ucMSNA variability because the ucMSNA series had the same dimension as that of the MSNA signal (i.e., mV), and the amplitude of its oscillations was arbitrary and depended on factors largely independent of the burst rate.

Figure 2 shows an example of ucMSNA (Fig. 2, A and B) and cMSNA (Fig. 2, E and F) variability derived from the same subject during T0 (Fig. 2, A and E) and T60 (Fig. 2, B and F) and the corresponding autoregressive power spectral density (Fig. 2, C and D and Fig. 2, G and H, respectively). In both ucMSNA and cMSNA variabilities the amplitude of the dominant oscillation increased during T60 compared with T0. The power spectral density of both ucMSNA and cMSNA variabilities exhibited a dominant peak in the LF band that became sharper during T60 compared with T0. The cMSNA variability is less noisy at higher frequencies (i.e., close to 0.5 Hz) than the ucMSNA series.

Fig. 1. Example of ECG, invasive arterial pressure (AP), integrated muscle sympathetic nerve activity (MSNA), step-wise count MSNA, and its low-pass-filtered version during a 60° tilt (T60). A: ECG (gain factor = 1.000). B: invasive AP. C: integrated MSNA (gain factor = 1.000). D: step-wise count MSNA derived from the integrated MSNA by counting the number of bursts occurring in a moving window of ΔT = 5 s. E: low-pass filtered version of step-wise count MSNA obtained using a finite impulse response filter with a cutoff of 0.5 Hz. A slow rhythm with a period of about 12 s is visible in D and E. The respiratory cycle in this example is about 4 s. Signals were recorded in the same subject during T60.

Autoregressive power spectral analysis. The power spectrum was estimated according to a univariate parametric approach fitting the series with an autoregressive model (20). Autoregressive spectral density was factored into components, each of them characterized by a central frequency. A spectral component was labeled as LF if its central frequency lay in the LF band, whereas it was classified as high frequency (HF) if its central frequency was between 0.15 and 0.5 Hz (30). The LF and HF powers were defined as the sum of the powers, each of them characterized by a central frequency. A spectral component was labeled as LF if its frequency density was factored into components, each of them characterized by a central frequency. The following spectral indexes were computed: 1) LF power of the HP series expressed in normalized units (LFnu<sub>HP</sub>), defined as the LF power of the HP series multiplied by 100 and divided by the σ<sup>2</sup><sub>HP</sub> minus the power of the HP series in the VLF band, deemed to be an index of sympathetic modulation directed to the heart (20); 2) HF power of the HP series multiplied by 100 and divided by the index of sympathetic modulation directed to the heart (20); and 3) the LF<sub>HP</sub>/HF<sub>HP</sub> ratio, a dimensionless index obtained by dividing the LF power by the HF power, both computed over the HP series, deemed to be an indicator of the sympatho-vagal balance to the heart (20); and 4) LF powers of SAP and DAP (LF<sub>SAP</sub> and LF<sub>DAP</sub>), expressed in absolute units (i.e., mmHg<sup>2</sup>), considered to be indexes of sympathetic modulation directed to the vessels (20).
Results of time domain analyses of HP, SAP, and DAP series; MSNA signals; and NA concentration and spillover values are summarized in Table 1. With the increase in intensity of the orthostatic stimulus, $\mu_{\text{HP}}$ was significantly decreased as early as during T20 compared with T0 and it progressively declined with tilt table inclination. Conversely, $\mu_{\text{SAP}}$ remained stable and $\mu_{\text{DAP}}$ increased only during T60. We found that $\sigma^2_{\text{HP}}$ was highly sensitive to the orthostatic challenge, being decreased during T20, T40, and T60. Conversely, both $\sigma^2_{\text{SAP}}$ and $\sigma^2_{\text{DAP}}$ increased only at the highest tilt table inclination (i.e., during T60). Whereas tonic sympathetic activity (expressed as $b_{\text{fMSNA}}$) increased as early as T30 and remained higher during T40 and T60, $b_{\text{MSNA}}$ was unaffected by the orthostatic challenge. Although NA concentration increased during T40 and T60, NA spillover remained stable.

Table 1. Time domain parameters and NA data

<table>
<thead>
<tr>
<th>Index</th>
<th>T0</th>
<th>T20</th>
<th>T30</th>
<th>T40</th>
<th>T60</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu_{\text{HP}}, \text{ms}$</td>
<td>1.003 ± 0.152</td>
<td>0.945 ± 0.145*</td>
<td>0.901 ± 0.134*</td>
<td>0.807 ± 0.122*</td>
<td>0.688 ± 0.120*</td>
</tr>
<tr>
<td>$\sigma^2_{\text{HP}}, \text{ms}^2$</td>
<td>5.239 ± 0.1071</td>
<td>2.824 ± 0.1002*</td>
<td>4.541 ± 0.2187</td>
<td>3.266 ± 0.1803*</td>
<td>2.498 ± 0.939*</td>
</tr>
<tr>
<td>$\mu_{\text{SAP}}, \text{mmHg}$</td>
<td>124.2 ± 16.2</td>
<td>122.7 ± 17.1</td>
<td>125.0 ± 17.1</td>
<td>122.3 ± 14.4</td>
<td>122.3 ± 16.7</td>
</tr>
<tr>
<td>$\sigma^2_{\text{SAP}}, \text{mmHg}^2$</td>
<td>11.2 ± 8.8</td>
<td>9.4 ± 6.2</td>
<td>13.4 ± 8.4</td>
<td>22.2 ± 16.5</td>
<td>35.5 ± 22.2*</td>
</tr>
<tr>
<td>$\mu_{\text{DAP}}, \text{mmHg}$</td>
<td>68.8 ± 9.4</td>
<td>68.8 ± 10.9</td>
<td>69.4 ± 12.4</td>
<td>74.0 ± 11.1</td>
<td>75.5 ± 14.8*</td>
</tr>
<tr>
<td>$\sigma^2_{\text{DAP}}, \text{mmHg}^2$</td>
<td>11.3 ± 8.5</td>
<td>9.3 ± 4.1</td>
<td>12.1 ± 6.1</td>
<td>15.5 ± 7.9</td>
<td>23.4 ± 10.9*</td>
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<tr>
<td>$b_{\text{fMSNA}}, \text{bursts/min}$</td>
<td>19.0 ± 6.0</td>
<td>22.0 ± 5.9</td>
<td>26.1 ± 5.4*</td>
<td>29.6 ± 4.9*</td>
<td>26.3 ± 9.1*</td>
</tr>
<tr>
<td>$b_{\text{MSNA}}, \text{bursts/100 beats}$</td>
<td>31.1 ± 9.6</td>
<td>33.5 ± 6.4</td>
<td>38.3 ± 5.5</td>
<td>39.0 ± 3.3</td>
<td>29.3 ± 8.9</td>
</tr>
<tr>
<td>NA concentration, pg/ml</td>
<td>129.7 ± 53.1</td>
<td>153.7 ± 76.1</td>
<td>214.3 ± 85.2</td>
<td>251.6 ± 116.2*</td>
<td>243.2 ± 252.1*</td>
</tr>
<tr>
<td>NA spillover, ng/min</td>
<td>335.0 ± 165.3</td>
<td>329.0 ± 129.7</td>
<td>437.8 ± 132.2</td>
<td>497.7 ± 241.0</td>
<td>529.0 ± 529.4</td>
</tr>
</tbody>
</table>

HP, heart period; DAP, diastolic arterial pressure; MSNA, muscle sympathetic nerve activity; NA, plasma noradrenaline; SAP, systolic arterial pressure; $\mu_{\text{HP}},$ HP mean; $\sigma^2_{\text{HP}},$ HP variance; $\mu_{\text{SAP}},$ SAP mean; $\sigma^2_{\text{SAP}},$ SAP variance; $\mu_{\text{DAP}},$ DAP mean; $\sigma^2_{\text{DAP}},$ DAP variance; $b_{\text{fMSNA}},$ MSNA bursts frequency; $b_{\text{MSNA}},$ MSNA bursts incidence; T0, T20, T30, T40, and T60, head-up tilt at 0°, 20°, 30°, 40°, and 60°. Values are expressed as means ± SD. *$P < 0.05$ vs. T0.
Results of frequency domain analyses are given in Table 2. The LFnuHP power significantly increased during T40 and T60 compared with T0, whereas the LFHF/HHF ratio was augmented only during T60. Conversely, HFHF power was significantly decreased as early as T20 and progressively declined with tilt table angle. LFSAP and LFDAHP powers rose significantly only at the highest tilt table angle (i.e., during T60). LFMSNA power was significantly increased during T40 with respect to T0, whereas the LFnuMSNA index was unaffected by the orthostatic challenge.

Figure 3 shows individual values (open circles) for LFMSNA (Fig. 3A) and LFnuMSNA (Fig. 3B) powers as a function of tilt table inclination. A significant positive association of LFMSNA power with tilt table angle ($r = 0.377, P = 2.79 \times 10^{-2}$) was found. On the contrary, LFnuMSNA power was uncorrelated with tilt table inclination.

Figure 4 shows the individual values (open circles) for LFSAP (Fig. 4A), LFDAHP (Fig. 4B), LFNuHP (Fig. 4C), and HFHF (Fig. 4D) powers as a function of tilt table inclination. All indirect, noninvasive, spectral indexes of the autonomic control were significantly correlated with tilt table inclination, but whereas LFSAP, LFDAHP, and LFNuHP powers were positively associated with the intensity of the orthostatic stimulus ($r = 0.43, P = 1.11 \times 10^{-2}$, $r = 0.434, P = 1.03 \times 10^{-2}$ and $r = 0.486, P = 3.59 \times 10^{-3}$, respectively), the HFHF marker was negatively correlated ($r = -0.769, P = 1.08 \times 10^{-7}$).

Figure 5 shows results of the linear correlation analysis between LFnuMSNA power and spectral indexes derived from HP, SAP, and DAP series. The degree of association was computed after pooling together data from all subjects regardless of experimental condition. We found that the LFnuMSNA marker exhibited a positive correlation with LFSAP (Fig. 5A) and LFDAHP (Fig. 5B) indexes ($r = 0.428, P = 1.16 \times 10^{-2}$ and $r = 0.402, P = 1.86 \times 10^{-2}$, respectively), but it was uncorrelated with LFNuHP (Fig. 5C) and HFHF (Fig. 5D) powers.

Figure 6 shows the results of linear correlation analysis between LFMSNA power and spectral indexes derived from HP, SAP, and DAP series. The degree of association was computed after pooling together data from all subjects regardless of experimental condition. The correlation between the LFMSNA marker and LFSAHP index (Fig. 6A) was positive and significant ($r = 0.69, P = 6.28 \times 10^{-7}$). The same result held for LFDAHP power (Fig. 6B, $r = 0.74, P = 5.75 \times 10^{-7}$). LFMSNA power was positively correlated with the LFnuHP marker (Fig. 6C, $r = 0.417, P = 1.4 \times 10^{-2}$) as well. Conversely, it was uncorrelated with HFHF power (Fig. 6D).

Table 3 shows the results of correlation analysis of catecholamine data (i.e., NA concentration and spillover) on spectral variability indexes. Pearson product-moment correlation coefficient; $r$; and type I error probability, $P$, are reported. NA concentration was uncorrelated with LFNuHP and LFHF/HHF indexes; it was positively correlated with LFSAP, LFDAHP, LFNuMSNA, and LFnuMSNA powers; and negatively correlated with the HFHF marker. NA spillover was unrelated with all spectral variability indexes.

Table 4 displays data from those subjects whose data featured a squared correlation coefficient, $r^2$, larger than 0.5 between traditional spectral indexes and MSNA markers computed in the LF band over cMSNA (i.e., LFMSNA and ucMSNA; i.e., LFnuMSNA) variabilities. Correlation analysis was carried out on a case-by-case basis provided that the association between the same variables computed after pooling together data from all subjects was significant over both cMSNA and ucMSNA series. This situation occurred only when the
LFSAP or LFDAP markers were considered: indeed, the HFHP power was uncorrelated with either the LFnuucMSNA or LFcMSNA index, and the LFnuHP power was significantly correlated with the LFcMSNA marker but uncorrelated with the LFnuucMSNA power, thus suggesting that the relationship of the LFnuHP power to the LFcMSNA index was tighter than that to the LFnuucMSNA power. The fraction of subjects with $r^2$ larger than 0.5 between the LFSAP power and LFnuucMSNA marker was lower than that between the LFSAP power and LFcMSNA index (i.e., 2 vs. 5 out of 7, respectively). Similar fractions were found between the LFDAP power and LFnuucMSNA marker and between the LFDAP power and LFcMSNA index (i.e., 1 vs. 5 out of 7, respectively). This finding indicates a better suitability of the cMSNA variability in describing the LF oscillations of sympathetic activity responsible for slow SAP and DAP fluctuations compared with the ucMSNA series.

Fig. 4. Linear regression analysis of the LF power of systolic and diastolic arterial pressures (LFSAP, and LFDAP, respectively), LF power of the heart period series expressed in normalized units (LFnuHP), and high-frequency (HF) power of the heart period series (HFHP) indexes on tilt table angles during graded orthostatic challenge. Individual values (open circles) for LFSAP (A), LFDAP (B), LFnuHP (C), and HFHP (D) indexes are shown as a function of tilt table inclination (i.e., T0, T20, T30, T40, T60). Linear regression analysis is carried out and, if the slope of the regression line is significantly larger than 0 with $P < 0.05$, the linear regression (solid line) and its 95 percent confidence interval (dotted lines) are plotted as well.

Fig. 5. Linear regression analysis of the LFSAP, LFDAP, LFnuHP, and HFHP indexes on the LF power of ucMSNA series expressed in normalized units (LFnuucMSNA) during graded orthostatic challenge. Individual values (open circles) for LFSAP (A), LFDAP (B), LFnuHP (C), and HFHP (D) indexes on LFnuucMSNA power after pooling data from all subjects regardless of experimental condition. Linear regression analysis is carried out and, if the slope of the regression line is significantly larger than 0 with $P < 0.05$, the linear regression (solid line) and its 95 percent confidence interval (dotted lines) are plotted as well.
with NA concentration; and normalized LF power of the ucMSNA series are correlated.

<table>
<thead>
<tr>
<th>Type of Analysis</th>
<th>Fraction of subjects exhibiting $r^2 &gt; 0.5$ between SAP or DAP spectral markers and MSNA variability indexes in the LF band during graded head-up tilt protocol</th>
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<tbody>
<tr>
<td>LF$<em>{SAP}$ vs. LF$</em>{nuMSNA}$</td>
<td>2/7</td>
</tr>
<tr>
<td>LF$<em>{SAP}$ vs. LF$</em>{MSNA}$</td>
<td>5/7</td>
</tr>
<tr>
<td>LF$<em>{DAP}$ vs. LF$</em>{nuMSNA}$</td>
<td>1/7</td>
</tr>
<tr>
<td>LF$<em>{DAP}$ vs. LF$</em>{MSNA}$</td>
<td>5/7</td>
</tr>
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</table>

$r$, Pearson product-moment correlation coefficient; $P$, type I error probability. *$P < 0.05$. 

DISCUSSION

The findings of the present study can be summarized as follows: 1) we propose a new procedure for deriving MSNA variability (i.e., the cMSNA series) that preserves the dimensionality of a neural discharge signal by directly assessing the modulation of the burst frequency; 2) the LF power of the cMSNA series is positively related with tilt table angle, whereas the normalized LF power of the ucMSNA series is unrelated to it; 3) the LF power of the cMSNA variability is positively associated with the LF powers of SAP and DAP series and with the normalized LF power of HP series; 4) the association of the normalized LF power of the ucMSNA variability with the power of SAP and DAP series is weaker and even absent with the normalized LF power of HP series; 5) the LF power of cMSNA variability and the normalized LF power of the ucMSNA series are unrelated to the HF power of the HP series; 6) the LF power of cMSNA variability and the normalized LF power of the ucMSNA series are correlated with NA concentration; and 7) individual regression lines confirm a stronger link between the cMSNA index in the LF band and the SAP and DAP spectral markers compared with the link computed over the ucMSNA variability as a likely result of the better suitability of the cMSNA series in describing the variability of the sympathetic discharge.

cMSNA variability is more suitable than its ucMSNA version in describing sympathetic control. This study proposes a new procedure for the extraction of MSNA variability (i.e., the cMSNA series), validates it using a graded head-up tilt protocol, and correlates spectral indexes extracted from it with those derived from traditional HP, SAP, and DAP variability series. The LF power of the cMSNA variability increased gradually with tilt table inclination. This finding suggests that the amplitude of variations in the neural discharge about its mean value rises with the magnitude of the orthostatic stimulus and stresses that, in addition to a tonic increase in the mean sympathetic activity (here confirmed by the increase in NA concentration), the modulation of the sympathetic discharge increases with the magnitude of the orthostatic stressor. This finding is not surprising given that the correlation between MSNA variability and tilt table angles is well known using a more traditional definition of MSNA variability, but here it was detected as early as during T40 (4, 10, 13). This finding is in agreement with those linking MSNA variability in the LF band with the
intensity of the sympathetic activation induced by graded lower body negative pressure protocol (26) or by modifications of AP values (21, 27).

In contrast to the traditional definition of MSNA variability, the cMSNA series allows us to preserve the dimensionality of the neural discharge signal (i.e., bursts per second) because cMSNA variability is directly related to the modulation of the burst frequency. As a consequence of its physical dimensionality, it does not require normalization procedures to compare data from different subjects. Conversely, normalization procedures are mandatory when using traditional low-pass-filtered MSNA variability because factors that have nothing to do with neural traffic but that can alter the amplitude and area of the MSNA bursts (such as the position of the electrode, its proximity to the bundle, number of active units, amplification gain, and effects of noise superposed to the MSNA signal) might profoundly influence conclusions. The reduction in the effect of these factors is responsible for the less-noisy nature of cMSNA variability compared with traditional low-pass-filtered MSNA variability and contributes to keeping within- and between-subject variability under control, thus improving the statistical power of the analysis. LF power of cMSNA variability was significantly related to tilt table inclination, whereas LF power of the ucMSNA series was unrelated to it, and the association of LF power of the cMSNA series with traditional noninvasive spectral indexes of sympathetic modulation was stronger than that found using ucMSNA variability regardless of whether the analysis was carried out after pooling data from all subjects or on a case-by-case basis.

Traditional cardiovascular variability indexes in time and frequency domains during graded head-up tilt. Head-up tilt is one of the most utilized maneuvers to induce sympathetic activation and vagal withdrawal. They are driven by a reduction in venous return and central blood volume, and consequent baroreflex unloading (4, 19, 24). Here, we confirm the traditional findings relevant to time and frequency domain analyses of HP, SAP, and DAP series (3, 4, 9, 10, 13, 14, 16, 17, 23, 26). More specifically, the HP mean decreased significantly and the HP variance declined as well. Conversely, as expected, the SAP mean did not vary, whereas SAP variance, DAP mean, and DAP variance were significantly increased at the highest tilt table inclination (i.e., during T60). Spectral indexes exhibited expected trends with tilt table angles as well. More specifically, the LF powers of SAP and DAP series, the normalized LF power of HP variability, and the ratio of LF-to-HF power computed over the HP series increased, whereas the HF power of HP variability were steadily decreased.

cMSNA and ucMSNA variability indexes in time and frequency domains during graded head-up tilt. Time domain indexes derived from the MSNA signal indicated that our head-up tilt protocol elicited the expected tonic sympathetic activation (3, 4, 9, 10, 13, 14, 26). Indeed, burst frequency was significantly increased, and the same trend was observed with burst incidence even though the increase did not reach statistical significance. This finding was also confirmed by the rise in NA concentration at tilt table angles larger than or equal to 40°. The LF power of cMSNA variability gradually increased with tilt table angle, thus suggesting that head-up tilt increases the amplitude of changes in the sympathetic discharge (i.e., the variation in sympathetic tonic activity about its mean value). Because this modification occurred in the LF band, this finding is likely to be unrelated to respiratory modulation of MSNA signal and more likely to be related to slower AP regulatory mechanisms (4, 10, 13, 18, 21, 26, 27). Contrary to expectations, the normalized LF power of ucMSNA variability was unrelated to tilt table angles. Because a significant relationship with tilt table angle is expected (4, 10, 13, 21, 26), this lack indicates that the normalized LF power of ucMSNA variability cannot manage the smallness of the sample size and the low intensity of the orthostatic stimulus (i.e., subjects did not undergo orthostatic challenges with tilt table inclinations steeper than 60°). Conversely, the significant correlation of LF power of the cMSNA series with tilt table angles indicates that this index is more powerful in tracking modifications to sympathetic control during graded head-up tilt, especially when sample sizes are small and the intensity of the stimulus is limited (i.e., at small tilt table angles). It is worth noting that both indexes (i.e., the LF power of cMSNA variability and the normalized power of ucMSNA series) were correlated with NA concentration and unrelated to NA spillover.

Comparing the relationship of cMSNA and ucMSNA spectral indexes to indirect, noninvasive, autonomic markers derived from HP, SAP, and DAP series. The LF power of SAP and DAP series was proposed as an indirect, noninvasive marker of sympathetic modulation directed to the vessels because it was significantly associated with the LF power of ucMSNA variability (21). Ryan et al. (26) confirmed the strong correlation between the LF power of SAP series and LF power of ucMSNA series during sympathetic activation induced by graded lower body negative pressure test. However, Ryan et al. (26) found that when the analysis was carried out on individual subjects, this relationship disappeared, thus indicating a poor within-subject reproducibility during the same recording session and across days. As a consequence, Ryan et al. (26) concluded that the amplitude of LF oscillations in SAP and DAP could not be used as noninvasive surrogates for direct, invasive measurements of modulation of sympathetic activity as derived from MSNA variability. This conclusion might be the consequence of the use of a ucMSNA series and a lack of application of any normalization procedure to the MSNA parameters being both factors that increase within-subject variability. These factors might be responsible for the disagreement observed between the LF marker derived from ucMSNA variability and the LF power of SAP and DAP variabilities reported by Taylor et al. (31) as well.

One of the major findings of this study is that the associations of LF power of cMSNA variability with spectral indexes in the LF band derived from HP, SAP, and DAP series are much stronger than those computed when the normalized LF power of ucMSNA variability was considered. The association of LF power of cMSNA variability with spectral markers in the LF band derived from SAP and DAP series was found in a larger fraction of subjects compared with the association of normalized LF power of ucMSNA variability with the same spectral indexes. We suggest that at least part of the disagreement observed by Ryan et al. (26) and by Taylor et al. (31) might be attributable to factors that are independent of the physiology of cardiovascular control and, importantly, may affect any traditional definition of ucMSNA variability based on the low-pass filtering procedure. We hypothesize that if
ucMSNA variability is substituted for a cMSNA series, conclusions might be different. In addition, we note that the LF power of cMSNA expressed in absolute units remained unrelayed to the HP power of the HP series, thus stressing the independence of this index from vagal modulation.

Interpretation of the correlation between cMSNA spectral parameters and indirect, noninvasive, autonomic markers derived from HP, SAP, and DAP series. The global correlation of LF power of cMSNA variability with LF indexes of SAP and DAP series, confirmed even individually in the majority of subjects, suggests that the LF power of SAP and DAP series can be used as a noninvasive marker of sympathetic modulation directed to vessels (21). This conclusion was supported by the correlation of NA concentration with SAP and DAP spectral indexes. The correlation of LF power of cMSNA variability with the normalized LF power of HP series supports the use of this index as a marker of sympathetic modulation directed to the heart (21). Because LF power (when expressed in absolute units) is under sympathetic and vagal controls (22) and the proportion of these influences is unknown, this result might be surprising, especially because in our experimental protocol the magnitude of the orthostatic challenge is limited and, as a consequence, vagal modulation in the LF band might predominate over sympathetic contribution. Normalization of LF power might be responsible for the observed significant link with the LF power of cMSNA variability. However, one factor deserves attention. The absence of a correlation between the LF power of the cMSNA series and the HF power of the HP series challenges the existence of a sympatho-vagal balance in that the rise in sympathetic control implies, by necessity, a withdrawal of vagal regulation and vice versa (15). Indeed, if the concept of sympato-vagal balance held in our experimental protocol, the two indexes should be negatively correlated. Since the normalized LF power of the HP series in conjunction with the normalized HP power of the HP series was designed to quantify the concept of sympato-vagal balance [because when the normalized LF power rises the normalized HF power declines and vice versa (15, 20) given that their sum is 100 (8, 23)], the correlation of the normalized LF power of the HP series with the LF power of cMSNA variability could misrepresent the underlying physiology because it leads necessarily to a correlation of LF power of cMSNA variability with the normalized HF power of the HP series. This same reasoning applies to the ratio of LF-to-HF power computed over the HP series. This conclusion is supported by the lack of correlation of NA concentration with either normalized the LF power or the LF-to-HF power ratio computed over the HP series.

Limitations of the study and future developments. Even though correlations are statistically significant according to the adopted level of significance, they are weak in absolute terms. We advocate future studies to test the results on a larger database, perhaps an ad hoc one through a joint project involving several laboratories in different countries, to obtain the optimal sample size, and to check whether more subjects could lead to expected improvements in the squared correlation coefficient. In addition, an extension in the range of intensity of the orthostatic stimulus might be helpful to test whether the observed rise in the LF power of cMSNA variability with tilt table inclination continues or whether saturation occurs.

In addition to the limited size of the sample, this study might suffer from the peculiarity of having used an incremental head-up tilt test that did not randomize the challenge (i.e., the tilt table inclination) and did not allow subjects to return to the horizontal position. This choice was helpful because it minimized the likelihood of losing the positioning of the micro-electrodes during MSNA recordings, but it has the drawback of introducing a time dependency in the measures that is not directly tackled by our statistical analysis. Future studies should test the possibility of monitoring cMSNA variability with an adequate sample size in protocols in which the challenge is randomized and subjects are returned to the horizontal position.

Perspectives and Significance

The study employed a new way to obtain cMSNA variability data by preserving the dimensionality of a neural discharge (i.e., bursts per second). The defined cMSNA variability limits the effect of factors that directly affect the quality of the MSNA recordings by altering amplitude and area of the bursts. The validation, carried out over a protocol that induces a progressive sympatho-excitation, suggests that the LF power of the cMSNA series can track the increase in sympathetic modulation associated with the increased intensity of the orthostatic stimulus. The important association of the spectral indexes derived from cMSNA series with those derived from spontaneous fluctuations in cardiovascular variables in the LF band noninvasively derived from ECG and AP recordings, found both globally (i.e., over the entire population) and individually in a significant proportion of subjects, stresses the usefulness of indirect, noninvasive, spectral indexes in assessing autonomic function. In addition, it suggests that part of the disagreement observed between traditional MSNA variability indexes and HP, SAP, and DAP spectral markers in the LF band might be due to the intrinsic weakness in ucMSNA variability more than to the physiology of cardiovascular control. Therefore, this study proposes exploitation of the cMSNA series instead of the more traditional ucMSNA series in studies that model cardiovascular variability interactions (25) and integrate direct measurements of sympathetic activity in suitable descriptions of cardiovascular control (9).

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


