Spontaneous fluctuations in the peripheral photoplethysmographic waveform: roles of arterial pressure and muscle sympathetic nerve activity

Gregory S. H. Chan,1 Azharuddin Fazalbhoy,2,7 Ingvars Birznieks,3,6,7 Vaughan G. Macefield,5,7 Paul M. Middleton,1,8 and Nigel H. Lovell4

1School of Electrical Engineering and Telecommunications, 2Prince of Wales Clinical School, and 3School of Medical Sciences, Faculty of Medicine, and 4Graduate School of Biomedical Engineering, University of New South Wales, Sydney, New South Wales; 5School of Medicine and 6School of Biomedical and Health Sciences, University of Western Sydney, Sydney, New South Wales; 7Neuroscience Research Australia, Sydney, New South Wales; and 8Discipline of Emergency Medicine, University of Sydney, Sydney, New South Wales, Australia

Submitted 6 October 2011; accepted in final form 14 November 2011

Chan GS, Fazalbhoy A, Birznieks I, Macefield VG, Middleton PM, Lovell NH. Spontaneous fluctuations in the peripheral photoplethysmographic waveform: roles of arterial pressure and muscle sympathetic nerve activity. Am J Physiol Heart Circ Physiol 302: H826–H836, 2012. First published November 23, 2011; doi:10.1152/ajpheart.00970.2011.—Assessment of spontaneous slow waves in the peripheral blood volume using the photoplethysmogram (PPG) has shown potential clinical value, but the physiological correlates of these fluctuations have not been fully elucidated. This study addressed the contribution of arterial pressure and muscle sympathetic nerve activity (MSNA) in beat-to-beat PPG variability in resting humans under spontaneous breathing conditions. Peripheral PPG waveforms were measured from the fingertip, earlobe, and toe in young and healthy individuals (n = 13), together with the arterial pressure waveform, electrocardiogram, respiration, and direct measurement of MSNA by microneurography. Cross-spectral coherence analysis revealed that among the PPG waveforms, low-frequency fluctuations (0.04–0.15 Hz) in the ear PPG had the highest coherence with arterial pressure (0.71 ± 0.15) and MSNA (0.44 ± 0.18, with a peak of 0.71 ± 0.16 at 0.10 ± 0.03 Hz). The normalized midfrequency powers (0.08–0.15 Hz), with an emphasis on the 0.1-Hz region, were positively correlated between MSNA and the ear PPG (r = 0.77, P = 0.002). Finger and toe PPGs had lower coherence with arterial pressure (0.35 ± 0.10 and 0.30 ± 0.11, respectively) and MSNA (0.33 ± 0.10 and 0.26 ± 0.10, respectively) in the LF band but displayed higher coherence between themselves (0.54 ± 0.09) compared with the ear (P < 0.001), which may suggest the dominance of regional vasomotor activities and a common sympathetic influence in the glabrous skin. These findings highlight the differential mechanisms governing PPG waveform fluctuations across different body sites. Spontaneous PPG variability in the ear includes a major contribution from arterial pressure and MSNA, which may provide a rationale for its clinical utility.

photoplethysmography; cardiovascular variability; power spectrum analysis; sympathetic nervous system; microneurography

The photoplethysmogram (PPG) waveform is widely applied in pulse oximetry for the estimation of arterial O2 saturation, based on the relative absorption of red light and infrared light by circulating blood in the underlying tissues. Recently, there has been interest in a more extensive utilization of the infrared PPG waveform signal for cardiovascular assessment, given the availability of pulse oximeters in most clinical settings and their ability to provide noninvasive and continuous patient monitoring using cost-effective technology (1, 28, 37, 41). Infrared light (with a wavelength of ~900 nm) penetrates deep into the skin and subcutaneous tissue and is similarly absorbed by oxygenated and deoxygenated blood; thus, it is relatively insensitive to blood oxygenation (1, 15, 21). The resultant infrared PPG waveform reflects the intravascular blood volume change at a peripheral site (such as the fingertip, ear, or forehead) under the combined influences of central perfusion pressure and local vascular control mechanisms. A comparison of the PPG and arterial pressure pulse showed that both waveforms underwent similar changes during vasoactive drug intervention and thus may reflect similar vascular mechanisms (30).

An emerging application is the use of beat-to-beat blood volume fluctuation in the peripheral PPG waveform to infer cardiovascular function in patients. For example, in patients undergoing mechanical ventilation, the magnitude of the respiratory variation in PPG is regarded as a useful clinical sign for predicting the capacity of cardiac output to increase during fluid therapy (12, 13). Spontaneous low-frequency (LF) oscillations have also been identified in the PPG, with a similar frequency (0.04–0.15 Hz) to the autonomic fluctuations in other cardiovascular signals, such as heart rate variability and blood pressure variability (24, 35). These slow waves have been found to be potentially useful for a range of clinical applications, including risk stratification of sepsis (29, 36) and acute coronary syndromes (26), and the identification of patients with low systemic vascular resistance (27). In relation to sepsis and acute coronary syndromes, it has further been found that within the LF band of the ear PPG waveform, a specific spectral region (0.08–0.15 Hz), termed the midfrequency (MF) band, may provide particularly useful diagnostic information (26, 29).

Despite this clinical interest, the physiological mechanisms underlying spontaneous PPG waveform variability (PPGV) in the LF and MF bands remain incompletely understood. Early work has attributed the LF waves in PPGV to sympathetic nerve activity (SNA), given that their relative strength increased with active standing (5) and decreased with anesthesia (20, 38). The important role of skin SNA in the control of skin vascular tone has been well established, with an early study (6) reporting the occurrence of transient vasoconstrictor waves in the finger PPG subsequent to skin SNA bursts. Recent studies (9, 29, however, have found that fluctuations in the PPG are not identical across body sites (e.g., fingertip and earlobe) and therefore cannot be solely explained by a common sympathetic
influence in the skin vasculature. In fact, the cold pressor test has been shown to induce a marked drop in the finger PPG pulse amplitude (indicating skin vasoconstriction) with no apparent effect on the ear pulse (3). Conversely, the ventilator-induced variation and pulse amplitude of the ear PPG have been found to be much better indicators of central blood volume compared with the finger PPG (12, 25). These findings raise the possibility that differential patterns in PPGV across different sites of the body may be due to the relative contribution of central hemodynamic versus local neurally mediated vasoconstrictor activities. As suggested by human studies (3, 12, 25), the ear PPG appears to be more responsive to central influences and less sensitive to sympathetic modulation of the local skin vasculature. The implication is that ear PPGV may, to a large extent, reflect the passive effect of systemic arterial pressure and sympathetic vascular activity that has a dominant role in blood pressure control, i.e., muscle sympathetic nerve activity (MSNA), which can be recorded directly via tungsten microelectrodes inserted percutaneously into a peripheral nerve (11, 18, 31, 46). The finger PPGV, on the other hand, may be more indicative of local vasomotor fluctuations, including those driven by neural (and non-neural) mechanisms.

The aim of this study was, therefore, to address the extent to which arterial pressure and MSNA contribute to peripheral PPGV in resting and spontaneously breathing humans in an attempt to better understand the physiological mechanisms behind the waveform fluctuation patterns found to be useful for the diagnosis of critically ill patients in previous studies (26, 29, 36). PPGV was measured from the fingertip, earlobe, and toe, and its relationship with arterial pressure and MSNA in the frequency domain was assessed by cross-spectral coherence analysis. Moreover, regression analysis was performed to determine whether spectral measures of PPGV reflect both inter-individual differences in MSNA and its oscillatory pattern, given the previously demonstrated relationship between MSNA and systemic cardiovascular variability (34). It was hypothesized that spontaneous PPGV measured from the ear included an important contribution from the underlying variability in systemic arterial pressure and, related to this, the underlying variability in MSNA. This hypothesis may provide a rationale for the novel diagnostic value of the ear PPGV in pathological conditions such as sepsis and acute coronary syndromes (26, 29), which have been shown to be associated with alterations in MSNA (14, 40).

METHODS

A total of 13 healthy subjects (9 men and 4 women) were studied, with their baseline characteristics (means ± SD) shown in Table 1. The ear tympanic temperatures of these subjects were within the normal range based on tympanic measurements (35.4–37.8°C) (44). This study was approved by the Human Research Ethics Advisory Panel of the University of New South Wales and conformed with standards set forth by the Declaration of Helsinki. Written informed consent was obtained from all participants, and, before the experiment, subjects were requested to provide information about their physical conditions. None reported any history of cardiovascular, respiratory, endocrine, or neural disease. Subjects were also advised to refrain from food and caffeine beverages for at least 4 h before the study and not to consume alcohol or undergo intensive exercise within 24 h before the experiment. None of the subjects were smokers.

Experimental protocol. Measurements were made in a quiet room at an ambient temperature of ~23°C. Subjects were seated comfortably in a semirecumbent position on a reclining chair, with their legs supported in the extended position. A baseline blood pressure measurement was performed using a clinically validated oscillometric blood pressure device (NIBP 7000, Colin) with the cuff placed around the left arm over the brachial artery. Ear tympanic temperature was measured from the tympanic membrane using an instant ear thermometer (Omron Healthcare, Kyoto, Japan). The ECG was acquired from the lead II configuration using surface electrodes through a bioamplifier (AD Instruments, Sydney, NSW, Australia). PPG waveforms were measured from the earlobe, the tip of the left middle finger, and the tip of the big toe by infrared sensor probes using light at 950 nm (MLT1020EC, MLT1020FC, and MLT1020PPG, AD Instruments), and all PPG sensors operated in the reflection mode. Finger arterial pressure was recorded beat by beat using an inflatable cuff placed on the right middle finger (Portapres model-2, Finapres Medical System, Amsterdam, The Netherlands). The respiratory rate was measured using a nasal temperature probe (AD Instruments). MSNA was recorded from fascicles of the common peroneal nerve supplying the ankle and toe extensor and foot evertor muscles via tungsten microelectrodes (FHC, Bowdoinham, ME) inserted percutaneously at the level of the fibular head. Multunit neural activity was amplified (gain: 20,000; bandpass: 0.3–5.0 kHz) using an isolated amplifier (Neuro-Amp EX, AD Instruments). The ECG, PPG waveforms, finger arterial pressure, respiration, skin temperature, and MSNA signals were continuously recorded using a Powerlab data-acquisition system (AD Instruments) at a sampling rate of 10 kHz for MSNA, 1 kHz for the ECG, and 200 Hz for other signals. After the completion of the experimental setup, a resting period of at least 10 min was allowed, followed by a continuous 5-min period of data acquisition. Finger arterial pressure and PPG signals were acquired with the subject’s forearms supported by armrests maintained at close to the heart level. Subjects were advised to maintain normal and stable spontaneous breathing and to refrain from body movement during the recording process.

Data analysis. Analysis was performed on the 5-min data recorded from each subject, and all signal processing and feature extraction were implemented in Matlab (Natick, MA). The R waves of the ECG and the cardiac pulses of the PPG and arterial pressure signals were identified using a set of programming routines involving low-pass filtering, high-pass filtering, and threshold-based peak detection. Zero-phase low-pass filtering (14 Hz cutoff) was performed on the PPG and arterial pressure signals before pulse detection. The mean arterial pressure (MAP) time series was used to represent perfusion pressure, whereas the beat-to-beat mean value of PPG was used to indicate the peripheral blood volume change. MAP and PPG mean values were obtained from each cardiac cycle by averaging the signals between successive troughs, as shown in Fig. 1.

MSNA bursts were detected using semiautomated computer algorithms similar to previously reported methods (11, 16) and then
verified by manual inspection. The raw nerve signal was subjected to band-pass filtering (700–2,000 Hz), rectification, moving integration (time constant: 0.1 s), and removal of very slow trends (40-s Hanning window moving average with a 0.02-Hz cutoff) to obtain the MSNA signal. The sympathetic bursts were detected from the MSNA signal based on two primary criteria: 1) synchronization with the ECG R waves with a reflex latency of \( \pm 1.25 \pm 0.35 \text{ s} \) and 2) a signal-to-noise ratio of \( \sim 2.5:1 \) when the burst amplitudes were compared with the background noise amplitudes. As the strength of the nerve signal varies among subjects due to the position of the microelectrode tip within a fascicle and its impedance, the MSNA signal was normalized by dividing by the largest sympathetic burst occurring during the measurement period (18, 45). The start and finish of a burst were identified as the local minima on either side of the detected peak from the low-pass filtered MSNA signal (4-Hz cutoff); integration of the prefiltered MSNA signal over this burst period yielded the burst area. The computed MSNA intensity measures included burst frequency (number of bursts/min), burst incidence (number of bursts/100 heart beats), and total MSNA (number of bursts/min multiplied by the mean burst area). For spectral and cross-spectral analysis, the continuous MSNA signal (after normalization) was used (2, 11).

Fig. 1. Tracings of physiological signals for two different subjects (A and B). The top four panels show the finger photoplethysmogram (PPG), toe PPG, ear PPG, and arterial blood pressure (BP). Beat-to-beat mean values of PPG and arterial pressure waveforms are shown as the darker lines. The bottom panel shows respiration (Resp) as the lighter line at the top (from nasal temperature, with downward deflection corresponding to inspiration), and muscle sympathetic nerve activity (MSNA) as the darker line at the bottom. The PPG waveforms, respiration, and MSNA are displayed in arbitrary units (au). Note that the occurrences of dips and the subsequent rises in ear PPG and arterial pressure that coincide with the MSNA bursts (with a period of \( \sim 10 \text{ s} \)); also note the similar fluctuations in finger and toe PPGs.
Beat-to-beat sequences of R-R interval, MAP, and ear/finger/toe PPG mean values (Ear, Finger, and Toe, respectively) were initially interpolated to evenly spaced samples in time (4 Hz) based on a previously described method (4) and then further downsampled to 1 Hz (after antialiasing low-pass filtering) to extract the variability signals, given that only the spectral content at < 0.5 Hz was analyzed.

The MSNA and respiration signals were also downsampled to 1 Hz after low-pass filtering. The very-LF trend (75-s Hanning window moving average with a 0.01-Hz cutoff) was subtracted from the variability signal before spectral analysis, given that the spectral band of interest was 0.04–0.45 Hz. The power spectral density (PSD) of each signal was computed by the Welch method. This involved dividing the 5-min data into smaller sections of 80 s with 75% overlap, multiplying each section with a Hanning transform, squaring and scaling the magnitude to give the auto power spectrum, and finally averaging the spectra of all segments to obtain the PSD. Cross-spectral analysis was also performed on the pairs of variability signals using the Welch method.

With the auto power spectra of signals x and y defined as $P_x(f)$ and $P_y(f)$, respectively (where $f$ is frequency) and the cross power spectrum of x-y defined as $P_{xy}(f)$, the transfer function of x-to-y $H(f) = P_{xy}(f)/P_x(f)$, and the magnitude and phase angle of the transfer function was obtained accordingly. The magnitude squared coherence function $\gamma^2(f)$ was defined as follows: $\gamma^2(f) = |H(f)|^2/P_x(f)P_y(f)$; the coherence assesses the linear correlation between spectral components in the two signals, with a value ranging from 0 (lack of linear correlation) to 1 (perfect linear relationship).

The power spectra of the variability signals, including R-R interval, MAP, Ear, Finger, Toe, and MSNA were divided into LF (0.04–0.15 Hz) and high-frequency (HF) bands (0.15–0.45 Hz). The HF band encompasses the typical breathing rate of most human subjects and is thus dominated by the effect of respiration. The LF band reflects the impact of various regulatory influences; for heart rate variability, these would include cardiac sympathetic and vagal nerve activities, whereas for MAP and PPGV, these would be mainly sympathetic vasomotor activities and other non-neural vascular mechanisms, such as autoregulation (35). In addition, the MF subband (0.08–0.15 Hz) was intersected at 0.15 Hz, the frequency that defines the separation of the LF and HF bands. The spectral powers in the LF, MF, and HF bands were calculated by integration of the power spectrum over the specified frequency range. Note that the absolute spectral powers of PPGV were not used for intersubject comparison given their dependency on the PPG signal strength, which varies with the optical properties of the measurement sites (1, 21).

The transfer function of x-to-y $H(f) = P_{xy}(f)/P_x(f)$ and the magnitude squared coherence function $\gamma^2(f)$ was defined as follows: $\gamma^2(f) = |H(f)|^2/P_x(f)P_y(f)$; the coherence assesses the linear correlation between spectral components in the two signals, with a value ranging from 0 (lack of linear correlation) to 1 (perfect linear relationship).

The power spectra of the variability signals, including R-R interval, MAP, Ear, Finger, Toe, and MSNA were divided into LF (0.04–0.15 Hz) and high-frequency (HF) bands (0.15–0.45 Hz). The HF band encompasses the typical breathing rate of most human subjects and is thus dominated by the effect of respiration. The LF band reflects the impact of various regulatory influences; for heart rate variability, these would include cardiac sympathetic and vagal nerve activities, whereas for MAP and PPGV, these would be mainly sympathetic vasomotor activities and other non-neural vascular mechanisms, such as autoregulation (35). In addition, the MF subband (0.08–0.15 Hz) was intersected at 0.15 Hz, the frequency that defines the separation of the LF and HF bands. The spectral powers in the LF, MF, and HF bands were calculated by integration of the power spectrum over the specified frequency range. Note that the absolute spectral powers of PPGV were not used for intersubject comparison given their dependency on the PPG signal strength, which varies with the optical properties of the measurement sites (1, 21).

The transfer function of x-to-y $H(f) = P_{xy}(f)/P_x(f)$ and the magnitude squared coherence function $\gamma^2(f)$ was defined as follows: $\gamma^2(f) = |H(f)|^2/P_x(f)P_y(f)$; the coherence assesses the linear correlation between spectral components in the two signals, with a value ranging from 0 (lack of linear correlation) to 1 (perfect linear relationship).

The results are as follows: $P$ = 0.04–0.45 Hz. The power spectral density (PSD) of each signal was computed by the Welch method. This involved dividing the 5-min data into smaller sections of 80 s with 75% overlap, multiplying each section with a Hanning transform, squaring and scaling the magnitude to give the auto power spectrum, and finally averaging the spectra of all segments to obtain the PSD. Cross-spectral analysis was also performed on the pairs of variability signals using the Welch method.

With the auto power spectra of signals x and y defined as $P_x(f)$ and $P_y(f)$, respectively (where $f$ is frequency) and the cross power spectrum of x-y defined as $P_{xy}(f)$, the transfer function of x-to-y $H(f) = P_{xy}(f)/P_x(f)$, and the magnitude and phase angle of the transfer function was obtained accordingly. The magnitude squared coherence function $\gamma^2(f)$ was defined as follows: $\gamma^2(f) = |H(f)|^2/P_x(f)P_y(f)$; the coherence assesses the linear correlation between spectral components in the two signals, with a value ranging from 0 (lack of linear correlation) to 1 (perfect linear relationship).

The results are as follows: $P$ = 0.04–0.45 Hz. The power spectral density (PSD) of each signal was computed by the Welch method. This involved dividing the 5-min data into smaller sections of 80 s with 75% overlap, multiplying each section with a Hanning transform, squaring and scaling the magnitude to give the auto power spectrum, and finally averaging the spectra of all segments to obtain the PSD. Cross-spectral analysis was also performed on the pairs of variability signals using the Welch method.

With the auto power spectra of signals x and y defined as $P_x(f)$ and $P_y(f)$, respectively (where $f$ is frequency) and the cross power spectrum of x-y defined as $P_{xy}(f)$, the transfer function of x-to-y $H(f) = P_{xy}(f)/P_x(f)$, and the magnitude and phase angle of the transfer function was obtained accordingly. The magnitude squared coherence function $\gamma^2(f)$ was defined as follows: $\gamma^2(f) = |H(f)|^2/P_x(f)P_y(f)$; the coherence assesses the linear correlation between spectral components in the two signals, with a value ranging from 0 (lack of linear correlation) to 1 (perfect linear relationship).

RESULTS

Cross-spectral analysis. Waveform signals from two subjects with coherent LF fluctuations in MSNA, MAP, and ear PPG are shown in Fig. 1; the power spectra of one subject who had a spectral peak at ~0.1 Hz in MSNA, MAP, and ear PPG are shown in Fig. 2. Cross-spectral analysis in the LF range revealed significant differences ($P < 0.0001$) between the average coherence of MSNA with MAP and PPG signals at various sites. Overall, MSNA was most coherent with MAP ($0.56 ± 0.15, P < 0.05$, MSNA-MAP vs. MSNA-Ear and $P < 0.001$, MSNA-MAP vs. MSNA-Finger/Toe), followed by ear PPG ($0.44 ± 0.18, P < 0.001$, MSNA-Ear vs. MSNA-Finger). Finger and toe PPG had lower coherence with MSNA ($0.33 ± 0.10$ and $0.26 ± 0.10$, respectively). The distribution of MSNA coherence across the whole frequency band is shown in the group average curves shown in Fig. 3, which highlight the presence of a characteristic peak at close to 0.1 Hz that was particularly prominent in MAP and ear PPG. The actual frequency of the LF peak varied across individuals but had an average frequency of 0.1 Hz (Table 2). Apart from one subject, who had an average respiratory rate within the LF and MF range (0.13 Hz), all other subjects had a mean breathing rate within 0.15–0.45 Hz and were thus without a significant respiratory influence on the LF peak. This can be demonstrated by the group average curves (Fig. 3), which showed an increasing coherence of cardiovascular signals with respiration toward 0.2–0.3 Hz, the typical breathing rate of these subjects. It is noteworthy that the MSNA and respiration coherence curves intersected at ~0.15 Hz, the frequency that defines the separation of the LF and MF bands.

A significant difference ($P < 0.0001$) was also found between the average LF coherences of MAP with PPG at different sites and between the coherences of the PPG signals themselves. Of the three sites, ear PPG showed the highest coherence with MAP ($0.71 ± 0.15, P < 0.001$, MAP-ear vs. MAP-finger/toe) compared with toe and finger PPGs ($0.35 ± 0.10$ and $0.30 ± 0.11$, respectively). Comparisons between the PPG signals showed that finger-toe coherence ($0.54 ± 0.09$) was significantly higher than ear-finger coherence ($0.31 ± 0.10, P < 0.001$) and ear-toe coherence ($0.30 ± 0.11, P < 0.001$). Group average curves (Fig. 4) showed that the average MAP-ear coherence was >0.5 across all frequencies, with a plateau in the LF band (>0.6 at 0.04–0.15 Hz), which was in sharp contrast with the low coherence of MAP-finger. Finger and toe PPG, however, had a relatively high coherence between themselves (>0.5 below 0.1 Hz), with a peak of 0.8 at 0.07 Hz (Table 2). The similarity between the LF fluctuations of finger and toe PPG is shown in Fig. 1.

The average transfer gain and phase curves of MAP-ear (which displayed sufficiently high coherence and input power of MAP in the LF range) are shown in Fig. 5, with the maximum gain normalized to 1 and the phase expressed in
MSNA LFnu was positively related to Toe LFnu (Table 3). This result, with PPG leading MAP, was consistent with previous findings that finger LFnu, although with a much lower coherence, was positively related to Finger MFnu (27), but its relationships with Finger MFnu (0.23), Toe MFnu (0.45), and MAP MFnu (0.40) were not significant. The significant relationships remained significant after the exclusion of one subject with a respiratory rate within the LF and MF range.

**Regression analysis.** The spectral powers of various physiological signals in the subject group are shown in Table 3. MSNA LFnu was positively related to Toe LFnu ($r = 0.63$, $P = 0.02$), but its relationships with Ear LFnu ($r = -0.29$, $P = 0.34$), Finger LFnu ($r = 0.21$, $P = 0.48$), and MAP LFnu ($r = -0.01$, $P = 0.97$) were not significant. MSNA MFnu was positively related to Ear MFnu ($r = 0.77$, $P = 0.002$), as shown in Fig. 6, but its relationships with Finger MFnu ($r = -0.36$, $P = 0.23$), Toe MFnu ($r = -0.26$, $P = 0.40$), and MAP MFnu ($r = 0.45$, $P = 0.12$) were not significant. The significant relationships remained significant after the exclusion of one subject with a respiratory rate within the LF and MF range.

Absolute MSNA spectral powers in all frequency bands were correlated with total MSNA ($r = 0.62$, $P = 0.02$, for LF; $r = 0.66$, $P = 0.01$, for MF; and $r = 0.70$, $P = 0.007$, for HF) but not with burst frequency. With respect to the relationship between MSNA intensity and the normalized spectral powers, only Toe MFnu was negatively related to MSNA burst frequency ($r = -0.62$, $P = 0.02$). However, it was noted that MAP MFnu had a parabolic relationship with total MSNA with $R^2 = 0.58$ (Fig. 7A). A similar relationship was seen in Ear MFnu, although with a much lower $R^2$ of 0.27 (Fig. 7B). Both of these fits were better than the linear models ($R^2 = 0.02$ and $R^2 = 0.07$, respectively).

**DISCUSSION**

Despite exhibiting a potential value for clinical diagnosis, the physiological mechanisms behind the beat-to-beat variability in peripheral PPG waveforms are not well understood, particularly in relation to the roles of arterial pressure and SNA in the genesis of these fluctuations. This study is the first to use direct recording of MSNA by microneurography to gain insights into the physiological meaning of spontaneous blood volume fluctuations in PPGV. The major finding was that LF oscillations in the ear PPG include major contributions from MSNA and MAP and that the dominance of the 0.1-Hz rhythm in the ear (as indicated by MFnu) reflects a similar pattern in the MSNA spectrum. In contrast, finger and toe PPG signals show a lower coherence with MSNA and MAP compared with the ear, but are closely related to each other in the LF range, suggesting the dominance of regional vasomotor responses and the existence of a common influence that is specific to these two sites (i.e., sympathetic vasoconstrictor drive to the glabrous skin). These findings have demonstrated that the relative contribution of systemic arterial pressure could explain the apparent differences between PPG waveform fluctuations at various sites (29). Moreover, they have shown that spontaneous PPGV in the ear includes an important contribution from both arterial pressure and MSNA and could reflect the relative dominance of the 0.1-Hz oscillation in MSNA. This may provide a physiological rationale for its potential clinical utility, as previously demonstrated in sepsis and acute coronary syndromes (26, 29).

**Relationship of ear PPGV with MAP and MSNA.** The infrared PPG waveform largely reflects blood volume fluctuation in the underlying skin and subcutaneous tissue, including significant contributions from the small arteries and arterioles (1, 15, 21). Typical measurement sites include the finger, ear, forehead, and toe, where infrared light may easily penetrate into the small blood vessels. The PPG waveform measured from the earlobe showed a higher coherence with MAP, with close to zero phase shift in the LF range, meaning that changes in central perfusion pressure are being passively transmitted to
this vascular region, inducing a variation in intravascular volume. Conversely, the ear PPG fluctuation had a low coherence with finger PPG, which was known to be strongly modulated by skin SNA (3, 6); thus, it was unlikely to be mediated by sympathetic control of the local skin vasculature. These results were in agreement with previous findings showing that the ear PPG may convey information about the central circulation more effectively than the finger PPG, possibly due to the more limited sympathetic innervations in the earlobe compared with the fingertip skin, leading to a greater contribution from passive hemodynamic changes (3, 12, 25). The heterogeneity in the regional PPG responses mirrors that observed from the multisite measurement of skin microvascular blood flow using laser-Doppler flowmetry, which revealed a higher dependency of skin blood flow on arterial pressure fluctuation in certain skin sites (such as the facial region) compared with others (such as the palm and sole) (22).

It is of interest to note that the MAP-ear transfer gain function resembles a high-pass characteristic in the LF range. A possible reason could be the derivative (dP/dt) operation introduced by the vascular compliance properties, which emphasizes HF fluctuations when converting MAP to capacitive fluctuations.
blood flow in distensible vessels (8). However, as PPG is more directly related to blood volume than flow (1), the conversion of flow to volume via integration may cancel out some of the positive phase introduced by the derivative operation \((+0.5\pi\text{ rad})\), resulting in a phase shift being close to zero.

Given the high coherence and zero phase displayed between MAP and ear PPG, the relationship of ear PPG with MSNA oscillation appears to be secondary to the passive influence of MAP, which is known to be coupled with MSNA via the arterial baroreflex (18, 31, 46). This MSNA-related oscillation evidently arises from the passive response of the local vasculature, as there is no skeletal muscle in the earlobe and hence no direct influence of MSNA on the local vascular tone. Since this relationship is indirect and is likely to be driven by arterial pressure, which is regulated not only by MSNA but also SNAs in other organs depending on the specific conditions, the ear PPGV should not be taken as equivalent to MSNA variability in general. In addition, the relationship between MSNA and arterial pressure may vary depending on the responsiveness of \(\alpha\)-adrenergic receptors to neural stimulation.

Nevertheless, the present results have clearly shown that among individuals, the relative dominance of the 0.1-Hz oscillation in the ear circulation could indicate the presence of a similar oscillation in MSNA. This oscillatory pattern of SNA has been observed at the same frequency as vasomotor waves of arterial pressure, which are often referred to as the “Mayer waves” (31). Given the important role of arterial pressure in ear PPG fluctuation, there is a physiological reason to explain why this dominant rhythm can be detected using the spectral power of ear PPGV. The spectral measure MFnu (Fig. 6), which indicates the percent contribution of oscillations at the frequency range of 0.08 – 0.15 Hz relative to the overall power, was used to depict this particular spectral pattern. Inspection of the frequency distribution of MAP-MSNA and ear-MSNA coherence (Fig. 3A) showed a distinct peak at close to 0.1 Hz. This represented a specific frequency range at which MSNA

<table>
<thead>
<tr>
<th>Table 2. Peak coherence between physiological signals and its corresponding frequency in the LF band (0.04 – 0.15 Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peak Coherence</strong></td>
</tr>
<tr>
<td>MAP-MSNA</td>
</tr>
<tr>
<td>Ear-MSNA</td>
</tr>
<tr>
<td>Finger-MSNA</td>
</tr>
<tr>
<td>Toe-MSNA</td>
</tr>
<tr>
<td>Finger-Toe</td>
</tr>
</tbody>
</table>

Values are means ± SD, with ranges in parentheses. LF, low frequency; MAP, mean arterial pressure; Ear, Finger, and Toe, photoplethysmogram (PPG) waveform variability measured from the ear, finger, or toe, respectively.
oscillation was most apparent in the cardiovascular signals, which was emphasized using the MF subband.

The association between ear PPGV and a specific oscillatory pattern of SNA offers a plausible explanation for the utility of Ear MFnu in patient risk stratification, particularly in those suffering from sepsis and acute coronary syndromes (26, 29). In these studies, the superiority of Ear MFnu over LFnu as well as spectral measures derived from finger PPGV in the classification of disease has been emphasized. In the sepsis study, severe sepsis patients with elevated blood lactate levels were found to have significantly lower Ear MFnu compared with the less severe systemic inflammatory response syndrome patients (29). In the acute coronary syndrome study, high-risk patients with elevated cardiac troponin levels (indicating structural damage to the myocardium) were found to have significantly higher Ear MFnu compared with those patients without troponin elevation (26). Endotoxemia, which has similar cardiovascular effects to sepsis, is known to have suppressive effects on MSNA (40). On the other hand, acute coronary syndromes represent a pathophysiological continuum, with the most severe stage of acute myocardial infarction associated with greatly elevated MSNA compared with the less severe stage of unstable angina (14). Given the involvement of MSNA in the pathophysiology of both sepsis and acute coronary syndromes, it is conceivable that patient-specific differences in the spectral measures of the ear PPGV could be attributed to the oscillatory patterns of MSNA. In fact, altered spectral patterns in MSNA have been implicated in heart failure (2), providing further support to the notion that these sympathetic oscillations may reflect the underlying status of neural circulatory control associated with disease.

Despite the frequency domain association with MSNA, MFnu should not, however, be simply taken as a measure of sympathetic tone given the nonlinear relationship with the actual level of SNA (Fig. 7). It appears that the MFnu of MAP and Ear increases with MSNA across subjects in the lower range but then decreases toward the upper range, such that the maximum 0.1-Hz oscillation is attained within a medium range of MSNA. This nonlinear relationship may be due to the fact that MSNA bursts are not only gated by the LF and MF rhythms but also by higher frequency rhythms, including respiration (23). In subjects with high MSNA, if the augmented intensity level was largely attributed to MSNA bursts gated by respiration rather than the MF rhythm, the value of MFnu could remain low. This would mean that Ear MFnu cannot not be used to indicate the exact level of MSNA and that its previously identified value in the risk stratification of patients with pathological conditions (26, 29) was not because of its direct relationship with the intensity of sympathetic nerve firing. Rather, the clinical utility of PPGV, as demonstrated by these studies, may arise from its ability to detect a specific oscillatory pattern of MSNA that is characterized by the dominance of a 0.1-Hz rhythm (i.e., high MFnu). Although the physiological

### Table 3. Spectral powers of the physiological signals in the subject group

<table>
<thead>
<tr>
<th>Signal</th>
<th>LF Power</th>
<th>HF Power</th>
<th>LFnu</th>
<th>MFnu</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-R interval</td>
<td>1.234 ± 1.208 ms²</td>
<td>1.217 ± 1.604 ms²</td>
<td>58 ± 18</td>
<td>30 ± 12</td>
</tr>
<tr>
<td>MAP</td>
<td>5.6 ± 2.7 mmHg²</td>
<td>0.5 ± 0.3 mmHg²</td>
<td>91 ± 7</td>
<td>27 ± 11</td>
</tr>
<tr>
<td>MSNA</td>
<td>610 ± 342 au²</td>
<td>1,359 ± 637 au²</td>
<td>31 ± 8</td>
<td>23 ± 6</td>
</tr>
<tr>
<td>Ear PPG</td>
<td>35 ± 525 au²</td>
<td>5 ± 5 au²</td>
<td>89 ± 7</td>
<td>37 ± 11</td>
</tr>
<tr>
<td>Finger PPG</td>
<td>601 ± 514 au²</td>
<td>14 ± 9 au²</td>
<td>97 ± 2</td>
<td>20 ± 7</td>
</tr>
<tr>
<td>Toe PPG</td>
<td>397 ± 351 au²</td>
<td>16 ± 14 au²</td>
<td>96 ± 2</td>
<td>22 ± 8</td>
</tr>
</tbody>
</table>

Values are means ± SD. LF, low frequency (0.04–0.15 Hz); HF, high frequency (0.15–0.45 Hz); LFnu, normalized LF power; MFnu, normalized midfrequency (0.08–0.15 Hz) power.
significance of this rhythm remains to be completely understood, it is well accepted that its genesis involves the engagement of the autonomic nervous system and neural reflex mechanisms (31, 35) and possibly reflects a compensatory response of SNA mediated by autonomic reflexes in response to hemodynamic challenges, such as that seen in central hypovolemia (11). It is believed that the differences in Ear MFnu between patients in various stages of pathological conditions, such as sepsis and acute coronary syndromes (26, 29), may similarly reflect the activation of compensatory reflex pathways in the sympathetic nervous system in relation to the diseases (14, 40).

The extraction of useful information from the PPGV spectrum for intersubject comparison relies on the derivation of normalized spectral power, given that the absolute spectral power would depend on the PPG signal strength, which varies with the optical properties of the measurement sites for different subjects (1, 21). As the normalized power refers to the power of a given band divided by the total power, its interpretation would require consideration of not only the mechanism that governs the frequency band of interest but also the whole spectral range. This may pose a limitation in using this approach for inferring the change mediated by one specific mechanism [such as sympathetic or vagal nerve activity in heart rate variability (7)] during physiological stimuli. However, the normalized power is useful for describing the distribution of power within the frequency spectrum, for example, a spectrum with a dominant peak in the 0.1-Hz range (relative to the fluctuations in the whole spectral band) can be characterized by a high value of normalized MF power (Fig. 2). The relative dominance of LF or MF power in PPGV, which appears to be indicative of pathological states (26, 27, 29, 36), cannot be depicted simply by assessing the absolute spectral power in one particular band and would be best represented by the normalized power.

**Physiological mechanisms of finger and toe PPGV.** Finger and toe PPG signals did not demonstrate high coherence with MSNA, MAP, and ear PPG in the LF range, suggesting a dominance of regional vasomotor activity that leads to dissociation between central perfusion pressure and peripheral blood flow. The relatively high coherence between finger and toe PPG at <0.1 Hz, however, points to the existence of a common regulatory influence that is specific to these two sites. The glabrous skin circulation of the fingers and toes comprise an abundance of arterioles and arteriovenous anastomoses (AVAs) that are under strong influence of the cutaneous SNA (39). The coherent blood flow variations in these vascular structures may well be driven by the fluctuations in skin SNA (6, 10, 33, 42). Notably, one study (22) has actually identified synchronous blood flow fluctuations (0.03–0.08 Hz) in the glabrous skin of the palm and sole that were not apparent in other skin regions. These fluctuations have been largely attributed to the sympathetic modulation of skin AVAs.

Apart from the sympathetic control of the vasculature, the presence of AVAs that shunt blood directly from the arterioles to the venules may enhance the influence of venous pressure in finger and toe PPGs (32), which may also lead to reduced coherence with arterial pressure. Another possible reason for the low coherence with arterial pressure could be the presence of non-neurally mediated changes in the local skin vasculature, including autoregulation or the myogenic response (22, 43, 47). It needs to be emphasized, however, that these findings were based on the measurement of skin capillary blood flow using laser-Doppler flowmetry, and the question of whether similar autoregulatory responses are influential in the PPG, which reflects blood volume change at a deeper level (15, 21), remains to be clarified. Notwithstanding the generally low coherence with MSNA in the LF band, there were specific frequency components (~0.1 Hz) at which finger and toe PPGs still displayed considerable coherence (>0.5) with MSNA (Table 2), and Toe LFnu was positively related to MSNA LFnu. Hence, the effect of MSNA on the finger and toe circulation cannot be totally disregarded.

**Limitations.** The present results were based on interindividual relationships between MSNA and cardiovascular signals at rest and therefore should not be extrapolated to the case of within-subject changes in sympathetic tone under physiological stimuli. As this study was designed to address the contributions of MAP and MSNA in relation to spontaneous PPG oscillations under resting conditions (in a similar condition as how data are recorded clinically) and how they may explain the differing spectral patterns of PPGV among individuals, measurements were performed in a group of subjects who exhibited a range of MSNAs. This was done to examine the cross-spectral coherence relationship between PPGV and the variability of MAP/MSNA as well as the interindividual relationships of the spectral patterns derived from these signals. The present results, however, cannot infer the association between the changes in PPG, MAP, and MSNA variabilities within an individual under a physiological stimulus, which will be a separate question that needs to be addressed by further studies. Recently, it has been suggested that the ability of LF spectral power to reflect changes in SNA may depend on the specific mechanism of sympathetic activation (19).

Moreover, as the study was performed on a limited number of young and healthy subjects, further studies with a larger cohort that includes elderly subjects and unhealthy patients will be desirable, to better understand whether similar relationships still hold with variations in age and pathological condition. Given that the ability of MSNA to cause vasomotor changes in the peripheral circulation relies on the responsiveness of α-adrenergic receptors to neural stimulation, it is anticipated that reduced vascular responsiveness in elderly subjects and in certain patients with autonomic dysfunction may weaken the relationship between MSNA and circulation signals (17).

The use of a normalization procedure to facilitate the comparison of total MSNA between subjects has its limitations, although it is believed that performing intersubject comparisons based on normalized total MSNA is worthwhile, given the incorporation of information from both burst amplitude and frequency, which may better reflect the actual intensity level of sympathetic nerve firing. Nevertheless, results from both total MSNA and burst frequency are presented.

**Conclusions.** In summary, this study examined the relationship between spontaneous fluctuations in MSNA, arterial pressure, and peripheral PPG waveforms measured from the fingertip, earlobe, and toe in healthy individuals. Cross-spectral coherence analysis revealed that LF variability in the ear PPG was more strongly related to MAP and MSNA compared with finger and toe PPGs and that relative dominance of the 0.1-Hz oscillation in the ear PPG reflected a similar pattern in MSNA. Finger and toe PPGs displayed a higher coherence between
themselves, suggesting a common influence in the glabrous skin vasculature. These findings highlight the differential mechanisms that govern regional PPG waveform fluctuations and provide evidence that the ear PPGV may reflect a specific oscillatory pattern of MSNA, which may offer an explanation for its diagnostic value, as demonstrated in a range of clinical conditions.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: G.S.C., A.F., and I.B. conceived and designed the research; G.S.C., A.F., and I.B. performed experiments; G.S.C. analyzed data; G.S.C., A.F., and I.B. drafted manuscript; G.S.C. and A.F. revised manuscript; G.S.C., A.F., and I.B. interpreted results of experiments; G.S.C. prepared figures; G.S.C. and A.F. edited and revised manuscript; G.S.C., A.F., and I.B. analyzed and approved final version of manuscript.

REFERENCES

39. Roddie IC. Circulation to skin and adipose tissue. In: Handbook of Physiology. The Cardiovascular System. Peripheral Circulation and Or-


