Frequency characteristics of long-term heart rate variability during constant-routine protocol

Naoko Aoyagi,† Kyoko Ohashi,† and Yoshiharu Yamamoto†,‡
†Electrical Physiology Laboratory, Graduate School of Education, University of Tokyo, Bunkyo-Ku, Tokyo 113-0033, and ‡PRESTO, Japan Science and Technology Corporation, Kawaguchi, Saitama 332-0012, Japan

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Aoyagi, Naoko, Kyoko Ohashi, and Yoshiharu Yamamoto. Frequency characteristics of long-term heart rate variability during constant-routine protocol. Am J Physiol Regul Integr Comp Physiol 285: R171–R176, 2003.—The effects of such behavioral factors as physical activity, food intake, and circadian rhythm on long-term heart rate variability (HRV) in humans remain poorly understood. We therefore studied their effects on HRV using a constant-routine protocol that included simultaneous core body temperature (CBT) correction. Seven healthy subjects completed the constant-routine and daily-routine protocols, during which HRV and CBT were continuously monitored. During the constant routine, subjects were kept awake for 27 h in a semirecumbent posture with minimal physical activity; small isocaloric meals were provided every 2 h. During the daily routine, subjects carried on their lives normally. Data were analyzed using generic spectral analysis based on a fast Fourier transform; coarse-graining spectral analysis was also used to eliminate periodicity due to the regular meals for raw HRV and for the CBT-corrected HRV without circadian and/or low-frequency ultradian components. The results showed that 1) the power spectrum of HRV in the constant routine and daily routine had similar power-law scalings at frequencies above ~10^{−3.5} Hz, while 2) below that crossover frequency, HRV was smaller in the constant routine than in the daily routine, with the difference becoming significant (P < 0.05) at <10^{−4} Hz, 3) coarse-graining spectral analysis eliminated diet-induced peaks in generic spectral analysis-based HRV spectra during the constant routine and emphasized the crossover at ~10^{−3.5} Hz, and 4) CBT correction did not alter the results. Below a frequency of ~10^{−3.5} Hz (a period >1 h), HRV is strongly influenced by behavioral factors; above that crossover frequency, HRV is behavior independent, possibly reflecting an intrinsic regulatory system.

Beat-to-beat fluctuations of R-R intervals (RRIs), also known as heart rate variability (HRV), in humans contain oscillations for periods ranging from seconds to hours (24). According to the often-cited report on HRV by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (24), the frequency domain components of long-term (up to 24 h) human HRV fall into four specific frequency bands: high frequency (HF, >0.15 Hz), low frequency (LF, 0.04–0.15 Hz), very low frequency (VLF, 0.0033–0.04 Hz), and ultralow frequency (ULF, <0.0033 Hz).

The division between the VLF and ULF components, i.e., 0.0033 Hz (the period corresponding to ~5 min), seems to follow a report by Bigger et al. (4) showing decreases in the ULF and VLF components of HRV to be more predictive of all-cause mortality in postinfarction patients. In these ULF and VLF bands, the power spectrum of long-term HRV exhibits 1/β-type, power-law scaling (12, 22), and the slope (β) of the scaling was also reported to be a good predictor of patient survival after myocardial infarction (5). Thus the investigation into the origin(s) of the slower fluctuations in HRV is considered important (24).

We believe, however, that such categorization of long-term HRV does not have a firm physiological basis and is, at best, arbitrary. Indeed, long-term HRV is affected by many behavioral factors, including physical activity, food intake, sleep-awake cycles, and circadian and ultradian rhythms. Consequently, it is not known whether the decreases in the ULF and VLF components observed in the previously evaluated patients (4, 5, 24) were due to impaired cardiovascular regulatory mechanisms, reduced physical activity (3, 19) caused by the disease per se, and/or attenuated circadian cardiovascular variability, perhaps resulting from, e.g., co-morbid hypertensive episodes (8).

To facilitate interpretation of past (4, 5, 9, 24) and future results using the spectral characteristics of long-term HRV in humans and to help investigate the physiological origin(s) of slower fluctuations in HRV, we studied the behavioral influences affecting low-frequency HRV. Our specific question was as follows: What would happen to long-term HRV in healthy humans if we eliminated, or at least minimized, known behavioral modifiers of HRV? To address this question, we examined the effect of selected behavioral factors on HRV using a constant-routine protocol (6, 7, 16) that included simultaneous core body temperature (CBT) correction for HRV.

Address for reprint requests and other correspondence: Y. Yamamoto, Educational Physiology Laboratory, Graduate School of Education, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan (E-mail: yamamoto@p.u-tokyo.ac.jp).
METHODS

Subjects. Seven healthy nonsmoking men (21–30 yr of age) with no known sleep problems completed the constant-routine and daily-routine protocols (see Experimental protocols). Each subject gave informed consent to participate in this institutionally approved study after the test protocol was fully described.

Experimental protocols. Originally used in chronobiological research, the constant-routine protocol is a technique designed to investigate the human circadian pacemaker without environmental and behavioral influences (6, 7, 16). Subjects were instructed to keep their regular sleep schedules (0000–0200 and 0700–0900 for sleep onset and awakening, respectively) and to refrain from vigorous exercise or alcohol consumption during the week before the experiment. They then reported to the laboratory at about 0800 after an overnight fast.

After placement of a rectal temperature probe and electrodes for electrocardiography (ECG), data collection was commenced at 0930–1130. During the constant-routine protocol, subjects were kept awake for 27 h in a constant semirecumbent posture with minimal physical activity. They were allowed to work on a laptop computer, read, listen to the radio, and talk to the staff (provided they were not overstimulated) in the controlled laboratory environment (24–25°C, <250 lx light intensity). Isocaloric meals, the caloric contents of which were calculated by dividing the age-estimated daily energy requirement for Japanese (2,300–2,650 kcal) by 12, were provided every 2 h to minimize diet-induced changes in HRV and CBT.

For comparison, HRV and CBT data potentially influenced by moderate and transient physical activity, larger meals, and sleep were collected by having the subjects participate in a daily-routine protocol, which was carried out in the same experimental setting, but in this case the subjects were allowed to continue their normal daily activities, although without vigorous exercise or alcohol consumption. The order of the constant-routine and daily-routine sessions was randomized.

Data collection. Beat-to-beat RRI (from the standard V5 lead of the ECG) and continuous body movements (BMs) were measured using a portable, waist-worn, long-term ambulatory monitor (2). This light-weight (200 g) and small (120 × 65 × 22 mm) device consisted of an amplifier for ECG, two shock sensors with amplifiers measuring trunk acceleration (in the vertical and horizontal axes with resolution of 0.08 g), an eight-bit CPU with 4-MHz frequency, an 8-Mbyte EEPROM, an analog-to-digital converter sampling at 250 Hz, an eight-bit parallel interface for data transfer, and a direct-current power supply from two commercial dry cells. In the CPU, the analog output of the ECG amplifier was band-pass filtered to yield trigger sources corresponding to QRS spikes. The resultant RRI was stored sequentially in the memory. Signals from the shock sensors were recorded as BMs after full-wave rectification and integration over 8 s. In the present study, only BM in the vertical axis was used.

Rectal temperature, serving as an estimate for CBT (one of the standard measures used to assess the temporal characteristics of the human circadian pacemaker), was recorded with resolution of 0.01°C at 1-min intervals using a portable temperature logger (model KMC-604, Gram) (23, 25) or a telemetry device (model WEB-5000, Nihon-Koden).

Any abnormal RRI, caused by BMs or occasional extrasystoles, was corrected by omitting beats (for those <300 ms) or inserting beats (when the RRI was double or triple the length of the preceding intervals). The percentage of the corrected beats was <0.08% of the total of >90,000 beats. Abnormal rectal temperatures caused by probe slips or defecation were corrected by linear interpolation.

Data analyses. To evaluate the frequency characteristics of long-term HRV in the VLF and ULF bands, a new time series with 10-s averaged RRIIs was constructed every second (i.e., at 1 Hz) and split into 20 time-shifted ensembles of 216 data points (216 s = 18.2 h). The lag for the shifted ensembles was determined by dividing the difference between the total length of data for each subject (>97,200 s = 27 h) and the length of the subset by 20. The linear trend of each subset was eliminated by linear regression, and Bingham’s data window was applied before calculation of the power spectral density (PSD) of the subset using a fast Fourier transform. The PSD of the entire HRV record was obtained as an ensemble average of the spectral data for the 20 subsets. This forms the generic spectral analysis (GSA) used in previous studies (4, 5, 9).

Inasmuch as the effects of meals were intentionally periodic and may not have represented the intrinsic frequency character of long-term HRV, we also used coarse-graining spectral analysis (CGSA) (26, 27) to eliminate that periodic component from the total spectral power of HRV. CGSA enables us to discriminate fractal random walks (15) with the 1/ƒα-type, power-law-scaled spectra from simple harmonic motions based on the fact that the original and the rescaled (coarse-grained) time series had random phase relations only with fractal signals (27). The algorithm has been described in detail, and its efficiency in extracting periodic components from mixed harmonic and fractal signals has been demonstrated (27). The same 216 points of 10-s averaged, detrended, and windowed data were used for this analysis, but because of the algorithmic nature of CGSA (27), the lowest frequency of the resultant PSD was twice as high as that obtained with GSA.

Further analysis was conducted to eliminate, or minimize, the effect of circadian and/or low-frequency ultradian rhythms on long-term HRV by using CBT signals as “templates.” For this purpose, cross-correlation coefficients between HRV and CBT were calculated separately for each subject after the data were smoothed using 2-h moving averages (13). Once the lag time with the highest absolute correlation coefficient was obtained, least-squares linear regression was performed for the lagged HRV, with CBT as an independent variable. The residual RRI time series was used as a new HRV series, with less influence of circadian and/or low-frequency ultradian oscillatory components, and analyzed with CGSA as described above.

Statistical analysis. Values are means ± SD. The effects of the constant-routine or daily-routine protocol and frequency on the average log-PSD values for HRV and BM within a bin of log 0.1 Hz were tested by two-way analysis of variance (ANOVA) for the main effects and the interaction. Inasmuch as the BM was recorded at 0.125 Hz and we were interested in the spectral power in the Ulf and VLF bands, the highest frequency of these analyses was set to 0.1 Hz for HRV and BM. When the interaction was significant, this was followed by paired t-tests for the effects of the protocol at the five lowest frequency bins, while Holm's correction was applied to keep the total error of the tests below 5 or 1%. The selection of these five frequency bins was based on the inspections of the log-PSD vs. log-frequency plots (see Figs. 3 and 4).

RESULTS

Figure 1 shows a typical phase relation between HRV and CBT. In this subject, the minimal or maximal
absolute correlation coefficient between HRV and CBT was observed at the lag time of $-104$ min, indicating that the change in HRV preceded that in CBT. For all the subjects, the average lag time was $-85$ min, with the average maximal correlation coefficient (an absolute value) of 0.90, in accordance with the result by Krauch and Wirz-Justice (13), showing that the heart rate was phase advanced to CBT for 1–2 h. Thus the linear regression was performed for the lagged HRV, with CBT as an independent variable, to calculate the residual RRI time series with less influences of circadian and/or low-frequency ultradian oscillatory components.

Results for the representative subject shown in Fig. 1 obtained during the daily-routine and constant-routine protocols are shown in Fig. 2, A and B, respectively. During the former, there were a few episodes of marked tachycardia (decreased RRIs) and hyperthermia (increases in CBT) associated with bursts of BM at $-700$ and $1,500$ min. As expected, BM was substantially reduced during the constant routine, and, together with the absence of sleep effects, CBT exhibited a smooth transition manifesting the existence of circadian rhythm. The transient tachycardia seen in the daily routine was absent in the constant routine, but a substantial level of HRV remained. Also, the time series of HRV during the constant routine showed less nonstationarity over longer time scales, consistent with flatness (whiteness) of the spectrum at the lower frequencies, as described below. CBT correction seemed to reduce a circadian HRV component during the constant routine. However, in the daily routine, the residual RRI time series still resembled HRV before the correction.

When GSA was used (Fig. 3), the group mean PSD of HRV during the daily routine exhibited an almost linear decay on log-log axes, suggesting the existence of power-law scaling (5, 9, 12, 22) for the entire range of frequencies. The ANOVA showed the significant ($P < 0.01$) main effects of frequency and protocol, as well as the “protocol $\times$ frequency” interaction. The mean PSD of HRV during the constant routine showed power-law scaling “roughly” similar to that during the daily routine at frequencies above $-10^{-4}$ Hz. Below that frequency, however, the spectral power of HRV during the constant routine was significantly ($P < 0.05$) lower than that in the daily routine.

The mean PSD of BM in the daily routine also showed the power-law scaling at frequencies above $-10^{-3.5}$ Hz, below which the spectral power was less sloped. The mean PSD of BM during the constant routine was consistently lower than that during the daily routine. There were significant ($P < 0.01$) main effects of protocol and frequency and the protocol $\times$ frequency interaction.

As shown in Fig. 3, there was a small “peak” at $-10^{-3.8}$ Hz in the GSA-based HRV spectra for the constant routine. This frequency corresponds to $-2$ h, i.e., the regular meal interval. When CGSA was used to eliminate the periodic influence of regular meals during the constant routine (Fig. 4, top traces), the peak disappeared, and the similarity in the power-law scalings for the constant routine and daily routine above a crossover frequency of $-10^{-3.5}$ Hz was emphasized. The ANOVA showed the significant ($P < 0.01$) main effects of frequency and protocol and the protocol $\times$ frequency interaction. Because the systematic changes seemed to occur below that crossover frequency, post hoc comparisons for two protocols were made at these frequencies. Consequently, the spectral power of HRV during the constant routine was significantly ($P < 0.05$) lower than that during the daily routine at this range of frequencies (Fig. 4, top traces). Further analysis with the CBT correction did not alter these results (Fig. 4, bottom traces). During the daily routine, however, the mean PSD of HRV remained scaled over the entire range of frequencies, even after minimization of the effects of BM and circadian rhythm.

**DISCUSSION**

Increased attention has recently been paid to the long-term spectral components of human HRV over periods spanning minutes to hours (1, 3–5, 8–11, 17–20, 24). Unlike short-term (e.g., $<10$ min) HRV, the physiological mechanisms of which are relatively well understood (14, 21), the origin(s) of low-frequency fluctuations in HRV remains unclear (24). This is due in part to difficulties in accounting for the numerous behavioral factors, including physical activity, food intake, sleep-awake cycles, and circadian and ultradian rhythms, which, along with short-term autonomic regulation, can affect it.

In the present study, we analyzed the effects of such behavioral factors on long-term HRV in humans using...
a constant-routine protocol, which is a chronobiological research technique used to investigate human biological rhythms while minimizing environmental and behavioral influences (6, 7, 16). With the use of this technique, the effects of physical activity and sleep-awake cycles were substantially reduced, and the effects of “large” meals were minimized by providing subjects with small isocaloric meals every 2 h. Furthermore, residual effects of the regular meals were eliminated by CGSA, and those of circadian rhythm were minimized by subtracting components of HRV covaried with ongoing CBT fluctuations. Thus, after the application of CGSA and the CBT correction (Fig. 4, bottom traces), the PSD of HRV during the constant routine was considered to be virtually free from known behavioral influences.

We found that, for healthy young individuals at frequencies below ~10^{-3.5} Hz (a period longer than ~1 h; dashed-dotted line in Fig. 4), the PSD of HRV in the constant routine was lower than that in the daily routine, indicating that, in this frequency range, HRV is at least in part dependent on behavior. At higher frequencies, by contrast, the power spectra of HRV in the constant routine and daily routine were similar, suggesting that HRV with a periodicity less than ~1 h is relatively independent of the behavioral effects, including those of usual daily activities, possibly reflecting an intrinsic regulatory system.

Comparison of results obtained with different experimental protocols and analytic techniques enabled us to gain insight into the factor(s) affecting HRV at these frequencies, inasmuch as the CBT correction did not substantially affect the results obtained during the constant routine (Fig. 4). Second, the effect of physical activity appears not to be as strong as one might expect, because in the daily routine the mean PSD of BM was less sloped than that of HRV in this frequency range (Fig. 3). If that is the case, the CBT correction would largely eliminate components of HRV covaried with movement-induced, low-frequency hyperthermic episodes (Fig. 2A). Nevertheless, after the CBT correction, the PSD of HRV during the daily routine was still much higher than that during the constant routine.

Fig. 2. Recordings (27 h) for representative subject shown in Fig. 1 during daily-routine (A) and constant-routine (B) protocols. From top to bottom: RRI(s, body movements (BMs), CBT (measured as rectal temperature), and residual RRI time series after CBT correction. AU, arbitrary units.
searchers have recently observed range (Fig. 4). This is a frequency region where re-
and the daily routine were similar in this frequency 
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long-term HRV in cardiac patients at the lowermost 
affecting the sleep-awake differences, on the PSD of 
awake differences in HRV, for instance, were reported 
the effects of sleep-awake cycles are a potential cause
also small (Fig. 3). It is therefore considered likely that 
effects of sleep-awake cycles are a potential cause
of the greater HRV in this frequency range. Sleep-
awake differences in HRV, for instance, were reported
to be smaller in hypertensive patients (8), and the 
effects of co-morbid hypertension, or other co-factors
affecting the sleep-awake differences, on the PSD of
long-term HRV in cardiac patients at the lowermost
frequencies should be studied further.
For HRV at frequencies higher than \( \sim 10^{-3.5} \) Hz, we
need to consider behavior-independent and possibly
intrinsic mechanism(s) of the regulatory system, be-
cause the power-law spectra for the constant routine
and the daily routine were similar in this frequency
range (Fig. 4). This is a frequency region where re-
searchers have recently observed “complex” and/or
“multifractal” HRV dynamics (1, 10, 11). Using the
same dataset used in the present study, Amaral et al.
(1) recently observed that the multifractality in this
frequency range was preserved even during the con-
stant routine; moreover, pharmacological blockade of
vagal and sympathetic influences greatly reduced the
multifractal complexity of HRV. Thus challenges to the
intrinsic autonomic mechanisms might shed light on
HRV dynamics in this frequency range.

The power spectrum of long-term HRV in free-run-
ing humans exhibits 1/\( f^\beta \)-type, power-law scaling, as
shown by the daily-routine data in the present study.
This phenomenon was first described more than a
decade ago (12, 22), and subsequent clinical studies
have revealed that the power in the VLF and ULF bands (4, 5)
and the slope (\( \beta \)) of the scaling (5) are good
predictors of patient survival after myocardial infar-
tion.

More recent studies have focused on a “crossover”
phenomenon in the power-law scaling of long-term
human HRV, seen in log-log plots with multiple linear
relations. For example, by using a detrended fluctua-
tion analysis (18), Feng et al. (17) found such a cross-
over at \( \sim \)10 heartbeats. Inasmuch as we used 10-s
averaged RRI data in the present study, we were un-
able to look at the effects of the constant routine on
this high-frequency crossover; moreover, interpretation
of this phenomenon is difficult because of the existence
of highly periodic fluctuations at around this frequency
(14, 21).

On the other hand, not much attention has been paid
to the possibility of a low-frequency crossover phenom-
emon. We found that the power-law scaling in long-
term human HRV could be categorized into two fre-
cuency components, which were divided at \( \sim 10^{-3.5} \) Hz
and had different physiological meanings. A similar
result was recently reported by Sakata et al. (20).
These investigators found that, when calculated by
CGSA, the power-law behavior in the PSD of 24-h HRV
during the daily routine had a crossover at \( \sim 10^{-3} \) Hz;
they also showed that the slope below that frequency
correlated negatively with age. We therefore believe

Fig. 3. Power spectral density (PSD) for long-term heart rate vari-
ability (RRI) and BM calculated using a fast Fourier transform-
based, generic spectral analysis during constant-routine and daily-
routine protocols. Average log-PSD within a bin of log \( 0.1 \) Hz was
used. Dashed-dotted vertical line indicates frequency of regular
meals during constant routine. Values are means ± SD. †P < 0.05;
‡P < 0.01, constant routine vs. daily routine.

Fig. 4. PSD for long-term heart rate variability before (RRIs; top
traces) and after (bottom traces) CBT correction calculated by coarse-
graining spectral analysis during constant-routine and daily-routine
protocols. Bottom traces are shifted downward by 2 decades for the
purpose of presentation. Average log-PSD within a bin of log \( 0.1 \) Hz
was used. Dashed vertical lines indicate upper boundary frequencies
of very low- and ultralow-frequency bands defined in Task Force
report (24) [VLF(TF) and ULF(TF), respectively]; dashed-dotted ver-
tical line indicates crossover frequency for long-term HRV with a
periodicity of \( \sim 1 \) h. Values are means ± SD. †P < 0.05; ‡P < 0.01,
constant routine vs. daily routine.
that one should be cautious about using a uniform scaling exponent (5, 9) for analyzing long-term HRV.

Finally, our findings might suggest a need for reevaluation of the frequency divisions described in the Task Force report (24). The Task Force division between the VLF and ULF bands is shown in Fig. 4 to emphasize the apparent lack of positive reasons for such categorization; the spectral characteristics around that boundary frequency are quite uniform. Instead, the results of the present study suggested that the division of ULF and VLF components at $-10^{-3.5}$ Hz (a period corresponding to $-1$ h), which potentially probe different physiological mechanisms, would be more physiological and thus suitable for research targeting the origin(s) of low-frequency fluctuations in human HRV.

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