INTRODUCTION

The analysis of heart rate variability (HRV) has been reported as a method of quantitatively detecting autonomic modulations which are related to mental stress (Kamada et al., 1992; Guinjoan et al., 2007; Bhattacharyya et al., 2008; Vaccarino et al., 2008; Lackschewitz et al., 2008; Shinba et al., 2008). The HRV analysis has been generally performed by means of the electrocardiography (ECG), where the spectral power of low frequency (LF) component, that of high frequency (HF) component and the rate of each component (LF/HF) of HRV are used for the assessment. An increased LF, increased LF/HF or decreased HF component is interpreted as the domination of the sympathetic activity or a decrease in the vagal tone (Cohen et al., 2000a,b; Chen et al., 2009).

At the sites of work in Japan, the increase of depressive workers who cannot help taking leave from their work is becoming a crucial problem (Ministry of Health, Labour and Welfare, 2006 and 2008). Because of this social background, an appropriate tool to assist evaluating mental stress is now required at the workplace. In response to this requirement, we have been examining the usefulness of the acceleration plethysmography (APG) which has the potential of being utilized as a replacement of...
ECG at the workplace (Takada et al., 2008a,b). The APG system is a newly developed system in Japan, which comprises a very small portable sensor and a software (Takada et al., 1997; Takada et al., 2004). The system has several advantages: it can be used at any place as it can analyze HRV in the short time using merely one finger, without removing one’s clothes and in the sitting position, and it does not need any specific professional technique for measurement. The HRV is sensitively influenced by individual mental and physical conditions and the environment of the measurement, because basically the HRV is in the background of autonomic activity (Rimoldi et al., 1990; Malliani et al., 1991; Kamath and Fallen, 1993). Therefore, to clinically use the short-term HRV derived from APG for mental health, ‘stationarity’ of the measurements of HRV in resting state must be justified as a fundamental premise. The stationarity of the limited time series is that the statistic of the mean value and the correlation coefficient, etc. is stable in a constant measurement condition.

There are several research studies on reliability of short-term HRV or long-term HRV measured by ECG (Kowalewski and Urban, 2004; Sandercock et al., 2004; Kristiansen et al., 2005; Sztajzel et al., 2008). However, there are few studies relating to stationarity of HRV measured by the plethysmography (Giardino et al., 2002; Bolanos et al., 2006; Koskinen et al. 2009), and no studies yet by applying APG.

In this research, we investigated stationarity of the short-term HRV derived from APG measured under the rest condition in daily life (under normal circumstances), in order to confirm that the obtained data of the short-term HRV can be reliable. The ultimate purpose is to examine whether there is agreement in the two measurements in the repetitive determination of the HRV parameter. In this regard, the short-term measurements by APG were repeated under a rest condition in the same time zone of the different days.

METHODS

Sample design

All subjects participated in the repeated measurements of HRV by the APG system. The sample size was determined through the following processes by referring to the results in our previous research (Takada et al., 2005). Our previous research has reported the HRV parameters in pre-exercise and post-exercise measurements for 124 male subjects of age 30-59. In the pre-exercise measurements, the mean of the proportion of the LF power in total power (LF%) was 36.9 % and the standard deviation (SD) of the LF% was 17.7 %, while the mean of the proportion of the HF power in total power (HF%) was 21.1 % and SD of the HF% was 12.5 %. In the post-exercise measurements, the mean of the LF% was 48.0 % and SD of the LF% was 19.3 %, while the mean of HF% was 12.9 % and SD of the HF% was 8.3 %.

In the first process, the LF% and the HF% were employed as end points (Takada et al., 2004). According to the previous results, where the LF% was assumed to be an end point, SD of the LF% was set as 18.5 %, and its increase caused by an exercise was set as 11.1 %. Therefore, using the standard equation $N = 2(\frac{\alpha^2}{2} + \frac{\beta^2}{2}) \left(\frac{SD^2}{\Delta^2}\right)$, the sample size was calculated based on “$\alpha = 0.05$ and $\beta = 0.20$” as shown below.

$N = 2(1.96+0.84)(18.5 / 11.1)^2 = 43.7$

Similarly when the HF% was assumed to be an end point, the SD of the HF% was set as 10 %, and its decrease caused by an exercise was set as 8.2 %. The sample size was again calculated by using the above standard equation based on “$\alpha = 0.05$ and $\beta = 0.20$” as follows.

$N = 2(1.96+0.84)(10 / 8.2)^2 = 23.3$

In the second process, by comparing the above two results, a reasonable sample size of 44 for this study was determined.

Subjects

The subjects were 44 healthy university male students (age 21.2±1.5) who signed a document
agreement to participate in the research. The profiles of the subjects comprised their body mass index, pulse rate (PR), blood pressure and the sleeping time (Table 1). The PR and blood pressure were measured twice. The daily sleeping time was self-reported. The study procedure was reviewed and approved by the Ethics Committee of Suzuka University of Medical Science, where the study was conducted.

**Equipment**

The APG system has been newly developed in Japan, which consists of a very small portable sensor and a software (U-MEDICA, Inc.) (Figure 1). In the system, the result of the frequency analysis on HRV is shown in the monitor promptly after recording APG. The sensor is an optical reflection-type with a centre wavelength of 940nm. The output maximum amplitude is ±3.3V. The time constant of the direct current cut is 1.5 sec. The sampling frequency for the analog to digital exchange is 1000Hz. The resolution ability is 3.23m V/digit.

Basically, the APG system offers the second derivatives of the plethysmograms. By differentiating a plethysmogram obtained twice, the baseline of waves becomes steady and hence more precise measurement becomes possible. The APG waveform consists of five systolic waves: the a-wave which is definitely positive; the b-wave (definitely negative); the c-wave (definitely positive); the d-wave (definitely negative); and the e-wave (definitely positive) (Takada et al., 1997) (Figure 2). The a-a intervals on APG, which are the intervals of the consecutive a-waves, correspond to the R-R intervals on ECG (Takada et al. 2004; Takada et al. 2008a). Immediately after recording the APG waves, the a-a intervals are measured and they give PR. Simultaneously, the a-a intervals are analyzed by using the maximum entropy method (MEM) of the frequency analysis. The MEM is an appropriate method to analyze the short length of data. In the MEM analysis, the resolution ability of spectrum was set as 0.001 Hz. The power spectra were quantified at 0.02-0.15 Hz as the low frequency area, and 0.15-0.5 Hz as the high frequency area. From HRV spectra, the power of the LF and HF spectral components of HRV was represented on the monitor as absolute values (ms²), or as the proportion in total power (0-100%) (Figure 3).

**Procedure**

The HRV measurements of 44 healthy male subjects were done twice during a one-week period duration by the APG system. On the day of measurements, subjects were directed to avoid smoking, drinking and exercise until the measurements started. In the two measurements for subjects, a resting state was maintained for 30 minutes before a measurement in order to keep their rest condition. A resting state in this research represents the state of sitting on a chair. All measurements were executed on the right index finger in the sitting position. The 100 beats of APG were recorded for each measurement. Every measurement time zone was set from 11:00 AM to 12:30 PM, and ambient temperature and relative humidity in the room was controlled at 24-28°C and 40-50% in every measurement.

<table>
<thead>
<tr>
<th>Table 1. Descriptive data of the subjects (n=44).</th>
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</thead>
<tbody>
<tr>
<td>Age (y.o.)</td>
</tr>
<tr>
<td>Body mass index</td>
</tr>
<tr>
<td>Pulse rate (beat/minute)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Systolic pressure (mmHg)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Diastolic pressure (mmHg)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Sleeping time (hours/day)</td>
</tr>
</tbody>
</table>

SD: standard deviation; Body mass index = [body weight (kg)] / [body height (m)]²
condition. Breathing was not adjusted before the measurement, to avoid unnecessary tension for the subjects.

Immediately after APG waves were recorded, the a-a intervals were analyzed, to offer the LF% and the HF% by the APG system. The autonomic balance is often assessed by the relation between LF and HF components represented as normalized units (0-100 n.u.), or by the ratio of the LF to HF component (LF/HF). “The normalized unit” of each component was often derived from the ratio of

Fig. 1. Measurement of the HRV by the APG system. (ARTETT, U-medica Inc., Osaka).

Fig. 2. The a-a intervals on APG waves. The upper line represents APG waves and the lower one shows original pulse waves.
absolute power of each component to the total absolute power (0-100) (LF/[LF+HF] and HF/[LF+HF])(Malliani et al., 1991; Yamasaki et al., 1996; Petelenz et al., 2004). Therefore, both the components represented in the form of proportion (LF%, HF%) and the components represented as normalized units (LFn.u., HFn.u.) were examined in this study. Obviously, it can also be calculated from LF%/LF%+HF% and HF%/LF%+HF%.

Statistical analysis

With regard to the LF%, the HF%, the LFn.u., the HFn.u. and the new parameter of the logarithm of the LF/HF ratio (Log10 [LF/HF]), each distribution in two measurements was examined by using the Q-Q plot and the Shapiro-Wilk normal test.

For each parameter in two measurements, the mean, SD and the 95% confidence interval (95% CI) were calculated. Furthermore, the geometric mean and the 95% CI of the LF/HF were derived from the antilogarithms of the Log10 [LF/HF]. Homogeneity of variance in the two measurements for each parameter was examined with F-test. Subsequently, Paired t-test was carried out to compare the mean values in two measurements for each parameter, based on the limitation of Type-I error and Type-II error as defined in the study design. Moreover, to examine the level of variation in two measurements in the individual person, the internal consistency reliability analysis for each parameter was carried out using Cronbach’s α coefficient.

RESULTS

As shown in the Q-Q plots in Figure 4, in each measurement the LF%, the HF%, the LFn.u., the HFn.u. and the Log10 [LF/HF] were normally distributed.

Table 2 shows the mean and SD of the measurement for each parameter. The 95% CIs of two measurements were as follows: [45-55% and 42-53%] for the LF%; [27-36% and 27-37%] for the HF%; [56-67 and 54-65] for the LFn.u.; [34-44 and 35-46] for the HFn.u.; and [0.12-0.34 and 0.08-0.32] for the Log10 [LF/HF]. The 95% CI for the LF/HF was calculated as [1.3-2.2 and 1.2-2.1]. According to the results of F-tests, the F-value was 0.795 (p=0.378) for the LF%, <0.0005 (p=0.996) for the HF%, 0.320 (p=0.575) for the LFn.u. and the HFn.u., and 0.181 (p=0.672) for the Log10 [LF/HF]. As mentioned above, the F value of each parameter was small and a significant difference
Fig. 4. Distribution of the HRV parameters represented by the Q-Q plots (n=44).

LF% (first)

Expected Normal Value

Observed Value

p=0.612 with Shapiro-wilk normal test.

LF% (second)

Expected Normal Value

Observed Value

p=0.463 with Shapiro-wilk normal test.

HF% (first)

Expected Normal Value

Observed Value

p=0.085 with Shapiro-wilk normal test.

HF% (second)

Expected Normal Value

Observed Value

p=0.585 with Shapiro-wilk normal test.

LFn.u. (first)

Expected Normal Value

Observed Value

p=0.132 with Shapiro-wilk normal test.

LFn.u. (second)

Expected Normal Value

Observed Value

p=0.208 with Shapiro-wilk normal test.

Fig. 4. Distribution of the HRV parameters represented by the Q-Q plots (n=44).
Table 2. Comparison between the first and the second measurements of the HRV parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>SD</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LF%</td>
<td>First</td>
<td>51</td>
<td>16</td>
<td>45-55</td>
</tr>
<tr>
<td>Second</td>
<td>48</td>
<td>18</td>
<td>42-53</td>
<td></td>
</tr>
<tr>
<td>HF%</td>
<td>First</td>
<td>32</td>
<td>15</td>
<td>27-36</td>
</tr>
<tr>
<td>Second</td>
<td>32</td>
<td>16</td>
<td>27-37</td>
<td></td>
</tr>
<tr>
<td>LFn.u.</td>
<td>First</td>
<td>61</td>
<td>18</td>
<td>56-67</td>
</tr>
<tr>
<td>Second</td>
<td>60</td>
<td>19</td>
<td>54-65</td>
<td></td>
</tr>
<tr>
<td>HFn.u.</td>
<td>First</td>
<td>39</td>
<td>18</td>
<td>34-44</td>
</tr>
<tr>
<td>Second</td>
<td>40</td>
<td>19</td>
<td>35-46</td>
<td></td>
</tr>
<tr>
<td>Log10 [LF/HF]</td>
<td>First</td>
<td>0.23</td>
<td>0.36</td>
<td>0.12-0.34</td>
</tr>
<tr>
<td>Second</td>
<td>0.20</td>
<td>0.39</td>
<td>0.08-0.32</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Geometric mean</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>LF/HF</td>
<td>1.7</td>
</tr>
<tr>
<td>Second</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Significant differences between the first and second measurements were examined with Paired t-test. SD: standard deviation, 95% CI=95% confidence interval. The geometric mean on LF/HF was derived from the antilogarithms of the Log10 [LF/HF].
was not seen between two measurements. Therefore, the homogeneity of variance in two measurements for each parameter was suggested. In addition, a significant difference was not seen between two measurements for every parameter with Paired \( t \)-test. The equality of the mean values in two measurements was suggested.

The internal consistency reliability as evaluated by Cronbach’s \( \alpha \) of 0.6-0.7 was acceptable for every parameter. Each the HF\%, the LF\( \text{n.u.} \), the HFn\( \text{u.} \) and the Log\( \text{10} \) [LF/HF] appeared to have ‘good’ internal consistency, Cronbach’s \( \alpha >0.7 \). However, only the LF\% indicated 0.63 for Cronbach’s \( \alpha \) (Table 3).

Table 3. The Cronbach’s \( \alpha \) coefficients of the HRV parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cronbach’s ( \alpha )</th>
</tr>
</thead>
<tbody>
<tr>
<td>LF%</td>
<td>0.634</td>
</tr>
<tr>
<td>HF%</td>
<td>0.732</td>
</tr>
<tr>
<td>LF( \text{n.u.} )</td>
<td>0.702</td>
</tr>
<tr>
<td>HF( \text{n.u.} )</td>
<td>0.702</td>
</tr>
<tr>
<td>Log( \text{10} ) [LF/HF]</td>
<td>0.703</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Much research has reported the validity of the HRV analysis by using ECG for evaluating autonomic modulations (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). In clinical use, ECG offers much information of cardiac disease in addition to HRV. On the other hand, the amount of information by APG could be regarded inferior as compared with the one provided by ECG, although APG offers information of both the autonomic nerve balance and arteriosclerosis (Takada H et al., 1997; Takada et al., 2004). If HRV is measured at a working site, however, measurement equipment which is simple to operate is highly desirable. In terms of operation, APG has several advantages over ECG: it can measure HRV even in a narrow office because of its portable type; it does not need a professional for the measurement because it does not require any special knowledge; a person neither needs to take off clothes nor requires to take a lying position, therefore the person does not leave the seat for a long time. Furthermore, the APG system is less expensive than the ECG equipment. To this end, in view of cost, promptness of frequency analysis and the easiness in its operation, we have been examining the possibility of APG as a tool that replaces ECG to analyze HRV at a working site (Takada et al., 2008a,b).

Regarding the measurement time, the guidelines of HRV suggested that the HF element needs at least one minute, and that the LF element requires at least two minutes to obtain reliable results (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Another recent research reported that the LF/HF and the HF measured within 50 sec could be reliable to monitor mental stress (Salahuddin et al., 2007). We attempted various measurement time units, and found that it was difficult to maintain a constant posture in a resting state for two minutes. Two minutes or more measurement was clinically inappropriate for making the measurement stable. On the other hand, in clinical use in Japan, the measurement of 100 beats by ECG has been often used to evaluate the autonomic nerve function. On that occasion, coefficient of variation of RR intervals (CVRR) is used to evaluate diabetic neuropathy and so on. The reason that the 100 beats for the measurement has been applied is because the number of the rate intervals should be constant to compare data of persons. Therefore, the 100 beats were adopted as a suitable time for the measurement in this study. For the purpose of confirming the agreement in two measurements of HRV parameters derived from APG, the sample size was determined as a research design on the
basis of the level of \( \alpha = 0.05 \) and \( \beta = 0.20 \) that limits the type I error and type II error.

As shown in the results, each parameter of HRV, the LF\%, the HF\%, the LFn.u., the HFn.u. and the \( \log_{10}[\text{LF/HF}] \) showed the normal distribution. The mean values of the first and the second measurements for each parameter were mostly equal, and homogeneity of variance in the two measurements for each parameter was shown. Moreover, at the comparison of the mean values in two measurements, significant difference was not seen for each parameter in this designed sample (\( \alpha = 0.05, \beta = 0.2 \)). Furthermore, the parameters such as the HF\%, the LFn.u., the HFn.u. and the \( \log_{10}[\text{LF/HF}] \) indicated more than 0.7 for Cronbach’s \( \alpha \). Those values are ‘good evaluation’, but only the LF\% indicated a little smaller Cronbach’s \( \alpha \). Despite different measurement days, an acceptable internal consistency within an individual subject was observed.

From the above results, it can be considered that the HRV remains stationary at the measurement of 100 beats regardless of the measurement day, though it is sensitively influenced by individual mental or physical conditions. However, it is physiologically natural that some variations of autonomic activity exist within an individual subject. Actually, in the sampled subjects of this research, the ranges of each the LF\% and the HF\% were estimated as 42-55\%, and 27-37\%. The ranges of the LFn.u. and the HFn.u. were also estimated as 54-67, and 34-46. Thus, the range of the LF/HF became 1.2-2.2. In other words, for healthy young males, the HRV parameters derived from APG by 100 beats measured in the same time zone in daily life are fairly stable within a certain range.

For healthy young males, the short-term HRV in a resting state in daily life might offer information of a baseline of the autonomic activity in the same time zone, if uncommon activities such as long resting, sleeping or a state of excessive excitation can be avoided before the measurement (Kleiger et al., 1991; Van Hoogenhuyze et al., 1991). However, for more precise clinical evaluation, especially in mental health, the establishment of the reference intervals of HRV parameters is needed. Further study which examines a large population in several age groups is required. The stationarity of the short-term HRV, suggested in this research, might contribute to the process of deciding the reference intervals of HRV in future.

In conclusion, for healthy young males, the parameters of short-term HRV derived from APG measured in a resting state in the same time zone in daily life remain stationary regardless of the measurement day.

REFERENCES


