Evaluation of the Effect of Heat Exposure on the Autonomic Nervous System by Heart Rate Variability and Urinary Catecholamines

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Abstract: Evaluation of the Effect of Heat Exposure on the Autonomic Nervous System by Heart Rate Variability and Urinary Catecholamines: Shinji YAMAMOTO, et al. Department of Hygiene, Yamaguchi University Graduate School of Medicine—The aim of this study was to investigate the usefulness of heart rate variability (HRV) and urinary catecholamines (CA) as objective indices of heat stress effect. We examined physiological responses, subjective symptoms, HRV and urinary CA to evaluate the effect of heat exposure on the autonomic nervous system. Six healthy male students volunteered for this study. They were exposed on different days to either a thermoneutral condition at wet bulb globe temperature (WBGT) 21°C, or a heated condition at WBGT 35°C for 30 min, while seated on a chair. In the thermoneutral condition, differences of all parameters between the values before and after 30 min exposure were not statistically significant. In the heated condition, heart rate, body temperature and scores for subjective symptoms (feverishness, sweating, mood, and face flushing) significantly increased after 30 min exposure (p<0.05). Also, the high frequency component (HF%) of HRV significantly decreased and the low frequency/high frequency (LF/HF) ratio of HRV significantly increased after 30 min exposure to the heated condition (p<0.05). There were no significant differences between the amounts of urinary CA before and after the 30 min exposures; however, the norepinephrine amount after 30 min exposure to the heated condition was significantly greater than that of the thermoneutral condition (p<0.05). The heat exposure (WBGT 35°C) induced activation of the sympathetic nervous system and a withdrawal of the parasympathetic nervous system. These findings coincide with observed changes of heart rate, body temperature and subjective symptoms. It is suggested that HRV (HF% and LF/HF ratio) and urinary norepinephrine may be useful objective indices of heat stress; HRV seems to be more sensitive to heat stress than urinary CA.

Key words: Heat exposure, WBGT, Autonomic nervous system, Heart rate variability, Urinary catecholamines

In Japan, improvement of working environment and education of labor health have been performed for prevention of heat-related illness in industry. However in recent years, there has been an increasing trend of small accidents, due to heat-related illness, requiring more than 4 d of rest1, 2; moreover, about 20 workers still die of heat-related illness every year3). For preventing heat-related illness, the Japanese Ministry of Labour released the guideline, “Preventive Measures for Heat-Related Illness” in 19964), and its successor, the Japanese Ministry of Health, Labour and Welfare also released another guideline, “Practical Use of WBGT in Preventive Measures for Heat-Related Illness” in 20055). When investigating the actual circumstances of heat-related illnesses, there are many cases which have been caused by insufficient time for breaks, insufficient amounts of water and salt replacement, and lack of information about workers’ health status. Thus, many factors must be considered for decreasing heat-related illness in industries6, 7). Individual difference in heat tolerance capacity is also a major factor7). Though there have been several studies on heat tolerance capacity8), we have few objective biological indices for it at present. If we have simple indices for individual heat tolerance capacity, we could prevent heat-related illness.

An important function of the autonomic nervous system (ANS) is to assist the body in maintaining a constant internal environment (homeostasis). Also, the ANS participates in appropriate coordinated responses to
external stimuli\(^9\)). Accordingly, the response of the ANS to heat exposure may be governed by individual differences in heat tolerance.

In this study, we examined heart rate variability (HRV), urinary catecholamines (CA), physiological responses and subjective symptoms for evaluating the heat exposure effect on the autonomic nervous system, and investigated the usefulness of HRV and urinary CA as objective indices of heat stress.

### Subjects and Methods

The subjects of this study were six healthy male student volunteers (mean age \(\pm SD\), 25.7 \(\pm\) 0.6 years; mean height \(\pm SD\), 171.2 \(\pm\) 2.5 cm; mean weight \(\pm SD\), 70.4 \(\pm\) 6.1 kg; and mean BMI \(\pm SD\), 23.7 \(\pm\) 2.4 kg/m\(^2\)). None of the subjects had a history of disease relating to the autonomic nervous or vascular systems. Written informed consent was obtained from all subjects and the Ethical Review Committee of the Yamaguchi University Graduate School of Medicine approved our protocol.

The subjects wore a T-shirt, long pants and underwear. Each subject participated in two experiments, one with a thermoneutral (air temperature at 25°C and relative humidity at 44%) in the experimental room; wet bulb globe temperature or WBGT 21°C condition, and the other with a heated (air temperature at 37°C and relative humidity at 75%; WBGT 35°C) condition. The WBGT index for indoor conditions is calculated as follows.

\[
WBGT = 0.7 \times T \text{ (wet-bulb temperature)} + 0.3 \times T \text{ (globe temperature)}
\]

In previous studies\(^{10-13}\), only temperature and/or relative humidity were used as indices of the environmental heat condition; however, WBGT is a comprehensive index reflecting air temperature, air humidity (including air velocity) and heat-radiation. The WBGT is recommended by ISO 7243: 1989\(^14\) and is widely used as an index of hot environments for estimating heat stress on working people. Therefore, in this study, we used the WBGT as an index of the experimental heat condition.

The subjects were exposed on different days to either the thermoneutral condition or the heated condition while sitting on a chair with the same tilt angle of the upper body for 30 min. In each experimental session, all subjects urinated approximately 1 h before exposure, then rested in a sitting posture in a thermoneutral condition for 60 min, after which they underwent the experimental exposure. Each subject drank 250 ml of water 1 h and 30 min before exposure (total 500 ml of water).

We measured baseline data and responses to heat exposure just before the exposure and after 30 min of it, respectively. The variables measured were body weight (BWB-800, Tanita, Japan), body temperature: axillary (ET-C202P, Terumo, Japan), sublingual (MC-108L, Omron, Japan), and external auditory canal (IT-500M, Nipro, Japan), heart rate (PC card recorder RD-C2, TEAC, Japan), HRV, and urinary CA (epinephrine and norepinephrine). The 6-min electrocardiographic (ECG) data were recorded during the last 6 min of the resting period and the 6 min after the end of the exposure period. For HRV analysis\(^15\), we used 5-min continuous data of the 6-min ECG data and calculated the power spectral density (PSD)\(^{12,13,16}\) with a fast Fourier transformation (FFT) program (BIMUTAS II, Kissei Comtec, Japan). The sampling time of the ECG data for analog/digital conversion was 1 msec; that of ECG R-R intervals for the FFT analysis was 250 msec. The very low frequency (VLF\%) (0.01–0.04 Hz; indicator of thermoregulation especially in a cold environment\(^13\)), the low frequency (LF\%) (0.04–0.15 Hz; indicator of both sympathetic and parasympathetic nervous activity\(^15\)) and the high frequency (HF\%) (0.15–0.40 Hz; indicator of the parasympathetic nervous activity\(^15\)) components, and the low frequency/high frequency (LF/HF) ratio (indicator of the sympathovagal balance or sympathetic activity\(^15\)) were calculated\(^{17,18}\). Urinary CA was analyzed by the ELISA method (Enzyme-linked Immunosorbent Assay, Epinephrine EIA Kit and Norepinephrine EIA Kit, Immuno-Biological Laboratories, Germany).

Subjective symptoms, i.e., feverishness, thirstiness, sweating, mood, fatigue, face flushing, and dizziness, were investigated before and immediately after exposure. Each subject was asked to rate his subjective symptoms on scales from 1 (none) to 5 (extreme); mood was rated on a scale from 1 (good mood) to 5 (bad mood). All the experiments were carried out between 16:00 and 18:00. During the rest and experimental periods, the subjects watched videos on nature (approximate running time: 60 min). In order to eliminate the experimental order bias, subjects were randomly assigned to two groups of three persons: one group being exposed to the thermoneutral condition first, and the other group being exposed to the heated condition first.

The comparison of parameters within the individual conditions before and after exposure was performed by paired t-test and Wilcoxon signed-ranks test. Results were considered statistically significant when \(p<0.05\).

### Results

The physiological parameters measured before and after exposure are shown in Table 1. In the thermoneutral condition, differences between the values before and after 30 min exposure were not statistically significant. In the heated condition, heart rate and body temperatures significantly increased after exposure (\(p<0.01\)). Under this condition, heart rate increased by an average of about 20 beats/min, and body temperatures increased by about 0.5–0.9°C. In the thermoneutral condition, there were no significant differences of the subjective symptoms.
between before and after exposure (Table 2). In the heated condition, the scores for feverishness, sweating, mood, and face flushing significantly increased after 30 min exposure ($p<0.05$).

The PSD values of VLF, LF and HF components measured before and after exposure are shown in Table 3. In the thermoneutral condition, there were no significant differences between the values measured before and after 30 min exposure. In the heated condition, the PSD values of VLF, LF and HF components significantly decreased after 30 min exposure ($p<0.05$). The differences (pre- and post- exposure) in the heated condition were significantly smaller than those in the thermoneutral condition ($p<0.05$, results not shown).

Table 4 shows the VLF%, LF%, HF% and LF/HF ratio measured before and after exposure. Under the thermoneutral condition, the VLF%, HF% and LF/HF ratio showed no statistically significant differences. Under the heated condition, HF% significantly decreased ($p<0.05$), and the LF/HF ratio significantly increased after 30 min exposure ($p<0.05$), while VLF% and LF% showed no statistically significant changes. The LF/HF ratio after exposure in the heated condition showed a wide deviation.

The urinary catecholamine levels measured before and after exposure are shown in Table 5. Under both conditions, there were no statistically significant differences between the values before and after 30 min exposure. The urinary norepinephrine amount tended to increase after the exposure ($p=0.10$) only in the heated condition. The norepinephrine amount after 30 min exposure under the heated condition was significantly greater than that of the thermoneutral condition ($p<0.05$), whereas before exposure it showed no significant difference between the two conditions. The difference of the norepinephrine amount measured before and after exposure in the heated condition tended to be larger than that in the thermoneutral condition; however it was not statistically significant ($p=0.08$, result not shown).

**Discussion**

In Japan, the WBGT index is used as a heat stress measurement to prevent heat-related illness in working
places with hot environments and in sports. The Japan Society for Occupational Health indicates the Occupational Exposure Limits of heat stress for working people in hot environments. For example, a WBGT index of more than 32.5°C indicates the possibility of increased incidence of heat-related illness and a significantly increased risk of heatstroke, and directs a very light work load (Relative Metabolic Rate, RMR below 1) in work places. RMR is calculated as following:

\[
\text{RMR} = \frac{\text{metabolic energy expenditure during work} - \text{metabolic energy expenditure at rest}}{\text{basal metabolic rate corresponding to the work period}}
\]

In this study, the heated condition (WBGT 35°C) was very severe for the subjects. The scores of subjective symptoms such as feverishness, sweating, mood and face flush increased significantly after 30 min exposure to heat. The heart rate and body temperature after the 30 min heat exposure also increased significantly. These results indicate that high temperature and humidity were responsible for the subjects’ discomfort and that the sympathetic nervous activity was activated by the heat stress.

HF% significantly decreased and the LF/HF ratio significantly increased after 30 min exposure under the heated condition at WBGT 35°C. The PSD values of VLF, LF and HF components significantly decreased after 30 min exposure under the heated condition at WBGT 35°C. The results are shown in Table 3, Table 4, Table 5.

<table>
<thead>
<tr>
<th>Exposure conditions</th>
<th>Thermoneutral environment (WBGT 21°C)</th>
<th>Heated environment (WBGT 35°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>VLF (msec²/Hz)</td>
<td>11,838 ± 7,198</td>
<td>24,885 ± 10,540</td>
</tr>
<tr>
<td>LF (msec²/Hz)</td>
<td>26,547 ± 19,874</td>
<td>31,696 ± 19,350</td>
</tr>
<tr>
<td>HF (msec²/Hz)</td>
<td>26,667 ± 18,140</td>
<td>28,078 ± 25,790</td>
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</tbody>
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WBGT, wet bulb globe temperature. Number of subjects=6. *: p<0.05, versus before (Wilcoxon signed-ranks test).

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</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>VLF%</td>
<td>19.5 ± 10.3</td>
<td>33.9 ± 12.7</td>
</tr>
<tr>
<td>LF%</td>
<td>38.5 ± 7.4</td>
<td>38.0 ± 6.4</td>
</tr>
<tr>
<td>HF%</td>
<td>42.0 ± 14.9</td>
<td>28.2 ± 11.5</td>
</tr>
<tr>
<td>LF/HF</td>
<td>1.11 ± 0.66</td>
<td>1.55 ± 0.65</td>
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</tbody>
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WBGT, wet bulb globe temperature. Number of subjects=6. *: p<0.05, versus before (Wilcoxon signed-ranks test).

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</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>10.9 ± 3.7</td>
<td>19.3 ± 16.7</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>18.8 ± 8.4</td>
<td>25.4 ± 10.0</td>
</tr>
</tbody>
</table>

WBGT, wet bulb globe temperature. Number of subjects=6. *: p<0.05, versus after exposure to thermoneutral environment (paired t-test).
under the same exposure condition. The reason for the decrease of the PSD values after 30 min exposure is thought to have mainly been due to elevation of heart rate\textsuperscript{[3, 16]}

The effects of heat exposure on HRV have been observed only in a few studies. Sollers et al.\textsuperscript{[12]} examined twenty healthy subjects under air temperature at a baseline condition of 22°C and at a heated condition of 35°C with 30 min exposures. The heart rates of the subjects were higher in the hot room condition when compared with the baseline condition, (the heart rates increased significantly by an average of about 4.5 beats/min in the hot room condition) and FFT analysis indicated that HF% decreased and LF% increased. These findings suggest that the increase of heart rate observed in the hot condition, was primarily due to parasympathetic withdrawal\textsuperscript{[12]}.

Brenner et al.\textsuperscript{[13]} investigated seven healthy subjects exposed to thermoneutral (air temperature at 23°C) and heated (air temperature at 40°C, relative humidity at 30%) conditions for 2 h and R-R interval data were collected during the exposure times (25–40 min and 100–115 min). Heart rate increased by an average of about 8 beats/min at 25–40 min in the hot condition; however, the increase was not statistically significant. Heat exposure alone did not significantly change the autonomic balance as analyzed by HRV. In the heated condition, the parasympathetic nervous activity tended to decrease, and the sympathetic nervous activity did not change. The experimental condition (air temperature of 40°C, relative humidity of 30%) was equivalent to the WBGT 30°C. When considering the heart rate, severity of heat stress was speculated to be of a level similar to that in our previous study\textsuperscript{[13]}; however, the HRV indices, HF% and LF/HF ratio changed significantly under the moderately hot condition (air temperature of 35°C and relative humidity of 60%: WBGT 30°C) in the study of Yamamoto et al.\textsuperscript{[18]}. As the humidity in our present study was two times larger than that in the study of Brenner et al.\textsuperscript{[13]}, this may indicate that the severity of heat exposure to the human body might have been largely influenced by humidity.

The severity of heat exposure in our present study was greater than in other studies\textsuperscript{[12, 13, 18]}. In the present study using heat exposure of WBGT 35°C, heart rate increased significantly by about 20 beats/min on average, and HRV indices (HF% and LF/HF ratio) changed significantly. The HRV indices together with heart rate were found to be useful objective indices of heat stress. Besides, the LF/HF ratio after exposure to the heated condition had a large deviation. This might be suggestive of possible individual differences in susceptibility to heat stress.

It is well known that the urinary norepinephrine is a sensitive indicator of the sympathoadrenal medullary response to acute stress and urinary epinephrine is an emotional stress index\textsuperscript{[22, 23]}. The norepinephrine amount after 30 min exposure under the heated condition was significantly greater than that under the thermoneutral condition. Brenner\textsuperscript{[13]} reported that the plasma epinephrine concentration increased, but not significantly, and that the plasma norepinephrine concentration did not change after 2 h heat exposure (blood was sampled at 25 min, 40 min, 100 min and 115 min during exposure). Under the environmental conditions used in Brenner’s study, we previously reported that there was no significant change of urinary CA level\textsuperscript{[19]}. However, urinary norepinephrine may be a useful index of heat stress under severe heat conditions such as WBGT 35°C. Further studies are needed to clarify the response of urinary CA to heat exposure.

**Conclusion**

The influence of severe heat stress was evaluated by non-invasive indices (HRV and urinary CA). From the changes of heart rate, body temperature and subjective symptoms under the heated condition, we conclude that the HRV indices (HF% and LF/HF ratio) and urinary norepinephrine may be useful objective indices of heat stress, and that HRV indices are possibly more sensitive than urinary CA to heat stress.

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