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HEART RATE REPRODUCIBILITY ASSESSED BY SURROGATE DATA ANALYSIS

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ABSTRACT

Purpose: Combinations of head up tilt (HUT) and lower body negative pressure (LBNP) have been used to study syncope. Since HUT invokes initial, partly transient cardiovascular responses, in this work we employed the HUT phase (5 min in all subjects) to arrive at a state of orthostatic loading to be used as a reference load for subsequent added LBNP phases. We investigated the pattern of cardiovascular responses during the LBNP phase across four runs for test subjects using surrogate data analysis. Methods: Ten healthy young males were subjected to HUT + LBNP to achieve a presyncopal end-point in four runs, each separated by ≥2 weeks. Beat to beat continuous hemodynamic variables were measured and analyzed. Results: Expected heart rate increases, in response to the decreases in stroke volume induced by graded LBNP, were observed. Heart rate was the only variable that showed reproducibility between subjects across four runs. Conclusion: During LBNP phase of the combined HUT+ LBNP protocol, heart rate was the only reproducible variable, thus confirming its central role during added LBNP in upright tilted men. Surrogate data analysis is useful tool to differentiate physiological responses from chance events in repeated runs.

Keywords: *lower body negative pressure, presyncope, hemodynamics, stroke volume, blood pressure*

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OCENA REPRODUCIBILNOSTI SRČNEGA UTRIPA Z NADOMESTNO ANALIZO PODATKOV

IZVLEČEK

Za raziskovanje sinkope so bile uporabljene kombinacije testa z nagibanjem (HUT) in testa z negativnim tlakom spodnjega dela telesa (LBNP). Ker test HUT sproži najprej deloma začasne odzive krvožilnega sistema, smo v tem projektu uporabili fazo HUT (5 minut za vse preiskovance), da smo dosegli stanje ortostatične obremenitve, ki se uporablja kot referenčna obremenitev za nadalje dodane faze LBNP. Preiskali smo vzorec odzivov krvožilnega sistema med fazo LBNP tekom štirih ponovitev na preiskovancih, pri čemer smo uporabili nadomestno analizo podatkov. Na desetih zdravih mladih moških smo izvedli testa HUT in LBNP, da bi dosegli presinkopo v štirih poskusih, med vsakim poskusom sta minila najmanj dva tedna. Izmerili in analizirali smo kontinuirane hemodinamične spremenljivke utripa. Ugotovili smo, da pričakovani srčni utrip naraste kot odziv na zmanjšanje obsega udarcev, kar sproži postopni test LBNP. Srčni utrip je bil edina spremenljivka, ki je pokazala reproducibilnost med preiskovanci v času vseh štirih ponovitev. Med fazo LBNP v okviru kombiniranega protokola HUT in LBNP je bil srčni utrip edina reproducibilna spremenljivka, kar potrjuje njegovo osrednjo vlogo med dodajanjem LBNP pri moških, ki so med testom nagibanja obrnjeni vertikalno. Nadomestna analiza podatkov je uporabno orodje za diferenciranje psiholoških odzivov od priložnostnih dogodkov med ponovitvami postopkov.

Ključne besede: *negativni tlak spodnjega dela telesa, presinkopa, hemodinamika, obseg udarcev, krvni tlak*

INTRODUCTION

Like hypergravity (induced by centrifuge) (Evans et al, 2006), head up tilt (HUT) combined with lower body negative pressure (LBNP) challenges the maintenance of blood pressure stability and can force the control system to exploit the full spectrum of cardiovascular compensatory mechanisms, revealing characteristics of individual blood pressure control that might go unnoticed with conventional orthostatic stress. The combination of HUT followed by LBNP (HUT + LBNP) induces cardiovascular and neuroendocrine changes, which are dependent on stress intensity and duration (Al-Shamma & Hainsworth1987; el-Bedawi, & Hainsworth, 1994).

Orthostatic tolerance has been typically studied using either graded LBNP or HUT alone or combination of both. When HUT + LBNP is employed, extensive central hypovolemia leading to presyncope is seen in most test subjects underlining the fact that HUT+LBNP represents a significant challenge to the cardiovascular system (Hainsworth, & el-Bedawi, 1994). There exists a considerable body of evidence regarding hormonal and hemodynamic changes due to HUT and LBNP in presyncopal state (Howden et al., 2001; Lelorier et al., 2003; Lightfoot et al., 2001) but little is known about the individual stability of orthostatic tolerance in subjects. Wide variance in inter-individual reproducibility but high intra-individual reproducibility of responses to orthostatic tolerance has been previously observed in our test subjects, induced by HUT + LBNP across multiple runs for test subjects (Evans et al., 2006; Goswami et al., 2009b).

We have previously shown that heart rate and stroke volume responses are reproducible across HUT+ LBNP (Goswami et al., 2009c). Since HUT invokes initial, partly transient cardiovascular responses, in this work we used the HUT phase (5 min in all the subjects) to arrive at a state of orthostatic loading that acts as a reference load for the following consecutively added LBNP phases. While the data used here are from the study (Goswami et al., 2009c) [which used data of the entire HUT + LBNP phases], in this paper we focus solely on the cardiovascular responses during the graded LBNP phase ("hypergravity" or added orthostatic loading phase) in subjects who were tilted upright at 70 degrees. While we observed reproducibility in heart rate and stroke volume during the entire HUT + LBNP protocol (Goswami et al., 2009c), in this paper we examined only the LBNP phase to investigate whether this reproducibility was present across the four runs. It was not the aim to identify the underlying physiological mechanisms causing a given response but to analyze the measured data by statistical means. To our knowledge, we are not aware of any study that has examined only the LBNP phase of a combined HUT + LBNP paradigm to investigate the reproducibility of hemodynamic responses across several runs. We believe that this is important, as the added orthostatic loading caused by increasing LBNP would provide useful information on how the physiological systems behave during extreme (or what the body is not normally used to) stress and across repeated runs. Indeed, reproducibility of heart rate and stroke volume during the LBNP phase would indicate that the system behaves the in the same manner under added orthostatic loading as under normal head up tilt.

METHODS

Subjects

Because gender and age may affect orthostatic and stress responses (reviewed in Goswami et al., 2008), we focused on young healthy men whose physical characteristics were homogeneous. The study was done in healthy, non-obese, non-medicated, non-smoking males who were free from any somatic or mental symptoms or diseases. Ten men of age 25±3 years, weight 75±12 kg, height 179±6 cm, and with a supine heart rate of 69±10 bpm met these criteria. They were advised to keep their fluid and salt intake according to their usual dietary habits and to refrain from alcohol during any part of the study period. Subjects were familiarized with the test protocol and gave written informed consent to participate in the study. The study was approved by the Medical University of Graz Ethics Board, and was performed in accordance with the 1989 WMA Declaration of Helsinki.

Protocol

The measurements were conducted on fasting subjects. Experimental tests were carried out in a semi-dark, quiet room with a temperature maintained at 23–24 °C, and humidity between 50–55%, between the hours of 9–11 am. Each subject was tested four times with a minimum of a two-week interval between test runs.

Each experimental run started with a 30 min supine rest period to acquire cardiovascular steady state conditions. At minute zero of the stress protocol, the tilt table was brought to 70° head-up position. After five more minutes, -20 mmHg LBNP was added. LBNP was increased by 10 mmHg every 3 minutes, particularly as we wanted to drive the subjects to presyncope quickly. We chose this protocol, as LBNP stress duration influences stress responses and we also wanted to minimize the possible effects of rapid adaptation. As soon as presyncopal signs or symptoms occurred, the table was brought back to 0° and LBNP was stopped at once. The criteria of presyncope (Grasser et al., 2008a) were when all/either of the following occurred: a) Blood pressure drop below systolic 80 mmHg or by ≥25 mmHg/min, diastolic by ≥15 mmHg/min, and /or heart rate decrease by ≥15 bpm; b) Lightheadedness, dizziness, visual disturbances, nausea, stomach awareness, clammy skin, excessive sweating, or skin pallor.

During the test the subjects were instructed to avoid undue movements of the lower limbs and to breathe normally. Test subjects were secured and had access to an emergency shutdown (automatic return to supine and pressure neutralization) at all times.

Test Apparatus

The test was carried out at the Institute of Adaptive and Spaceflight Physiology (www.meduni-graz.at/iap/AHST.htm) using a combined tiltable-LBNP chamber device equipped with a footrest. Care was taken to maintain the sealing at the iliac crest, as sealing position has been shown to affect hemodynamic responses (Goswami et al., 2009a). A transition from supine to upright position as well as negative pressure buildup was complete within 5 seconds. Suction was provided using a commercially available vacuum cleaner located in an adjacent room. The execution of the pre-programmed test protocol and synchronous recording of all data from the cardiovascular monitoring system was done by LabView®.

Measurements

Electrodes were placed at the neck and thoracic regions, the latter specifically at the midclavicular line at the xiphoid process level. Continuous hemodynamic monitoring included systolic and diastolic blood pressure, heart rate (3-lead ECG), and thoracic impedance using the Task Force Monitor®, CNSystems, Graz, Austria. This monitor estimates arterial pressure using finger cuffs (the principle of vascular unloading technique was used to estimate the arterial blood pressure (Penaz principle)) and regular calibration to standard arm cuff measurements (by Task force monitor). Mean arterial blood pressure (MAP) was calculated from diastolic (DBP) and systolic pressures (SBP), respectively: MAP = DBP + 1/3 (SBP - DBP). Stroke volume was calculated from the impedance data.

Data Analysis and Interpretation

Sample Size

Using typical cardiovascular changes during orthostatic loading from previous studies (Evans et al., 2001; Gao et al., 2008; Grasser et al., 2008b), error probability (α) of 0.05, power (1-ß) of 0.80, we estimated the number of subjects required to be 10.

Data Preparation

Cross-correlation (CC) analysis was used to assess reproducibility of heart rate and stroke volume responses as a function of time, as well as heart rate to stroke volume relationships comparing consecutive runs per person using Matlabs XCORR algorithm. This algorithm produces an estimate of the correlation between two random sequences:

$$C(m) = E[A(n+m)*conj(B(n))] = E[A(n)*conj(B(n-m))]$$

XCORR, an unbiased estimator, normalizes the sequence so that the autocorrelations at zero lag are equal to unity (MatLab R2007a, The MathWorks Inc.).

To characterize the non-linear dynamic features of the protocol by appropriate statistical means, data sets were created from the original data by computing the Fourier transform and randomizing the imaginary part in the frequency domain. Following this randomization in the frequency domain, data were then transformed back to the time domain by inverse Fourier transformation. Such data have the same mean, standard deviation, and power spectrum as the original data (Theiler et al., 1992) and can be used as so-called surrogate data for statistical analysis. For every original data set 100 such data sets were calculated and correlated with the original signal. The 95th percentile of the correlation coefficient of this comparison was then used to reject the null hypothesis, namely that there was no difference between a random signal produced by the original run and a signal from another run. Comparisons were done using a paired t-test. Thus, if the correlation coefficient of the 95th percentile (taking in account alpha error $p<0.05$) of the randomized signal was lower than the correlation coefficient in another run, the differences between runs were more likely to be due to physiological mechanisms than to the randomization itself.

For each subject in a run, the period between the commencement of LBNP and the maximum heart rate was used to assess cardiovascular reproducibility. During this period, cross correlation of HR and SV was also done (Table 1). To compare the runs, we used the shorter time of the two respective pairings, to calculate the CC between the pairs and to calculate the surrogate data (see Table 2 & 3).

RESULTS

Figure 1 shows the relationship of MAP, HR and SV changes across the entire combined protocol (supine rest, HUT and graded LBNP). That is, the influence of HUT application on the subsequent LBNP induced hemodynamic changes.

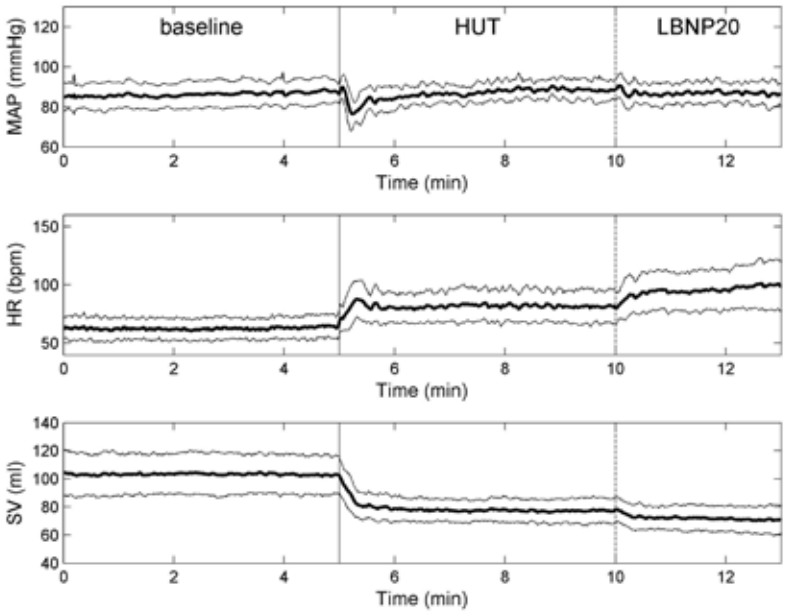

Figure 1: The influence of HUT application on the subsequent LBNP induced hemodynamic changes (MAP, HR and SV). The values indicated here are the means (SD) of all the 10 subjects across the four runs. LBNP20 represents only the initial phase of the graded LBNP. Legend: MAP: Mean arterial pressure; HR: Heart rate; SV: Stroke volume.

Overall cardiovascular effects of the HUT +LBNP are presented elsewhere (Goswami et al., 2009c).

The correlations of HR and SV during increasing LBNP phase for 10 subjects (A–J) are shown in the Table 1.

Table 1. Summary of cross correlation (CC) between heart rate and stroke volume in all subjects (A to J). # Refers to machine failure and shut down during run 1.

Subject	Heart rate : Stroke volume			
	$CC_{1,1}$	$CC_{2,2}$	$CC_{3,3}$	$CC_{4,4}$
A	-0.922	-0.915	0.903	-0.843
B	-0.934	-0.941	-0.925	-0.901
C	-0.649	-0.802	-0.684	-0.632
D	-0.901	-0.927	-0.932	-0.942
E	-0.617#	-0.633	-0.351	-0.533
F	-0.918	-0.913	-0.946	-0.946
G	-0.870	-0.870	-0.909	-0.924
H	-0.823	-0.845	-0.939	-0.942
I	-0.895	-0.906	-0.861	-0.793
J	-0.417	-0.668	-0.630	-0.719

Correlation between paired runs was significantly higher for HR data when compared to the corresponding surrogate data (Table 2).

*Table 2. Cross correlation of heart rate across runs (1–4). Paired T-test between Cross Correlation Coefficents of runs 1,2,3,4 (C1,2,C1,3, ... C3,4) to runs and surrogate (C1,surrogate, C2,surrogate, C3,surrogate) ; * ... p<0.05; ** ... p<0.01; *** ... p<0.001.*

	HR_2	HR_3	HR_4
HR_1	$C_{1,2}$ = 0.725 ± 0.153 **	$C_{1,3}$ = 0.728 ± 0.181***	$C_{1,4}$ = 0.740 ± 0.137 **
	$C_{1,surrogate}$ = 0.587 ± 0.108	$C_{1,surrogate}$ = 0.593 ± 0.134	$C_{1,surrogate}$ = 0.600 ± 0.123
HR_2		$C_{2,3}$ = 0.743 ± 0.206 **	$C_{2,4}$ = 0.707 ± 0.195 *
		$C_{2,surrogate}$ = 0.546 ± 0.131	$C_{2,surrogate}$ = 0.557 ± 0.119
HR_3			$C_{3,4}$ = 0.804 ± 0.109***
			$C_{3,surrogate}$ = 0.537 ± 0.094

Table 3. Cross correlation of stroke volume across runs (1–4). Designations as in Table 2.

	SV_2	SV_3	SV_4
SV_1	$C_{1,2}$ = 0.575 ± 0.264	$C_{1,3}$ = 0.578 ± 0.291	$C_{1,4}$ = 0.582 ± 0.229
	$C_{1,surrogate}$ = 0.533 ± 0.131	$C_{1,surrogate}$ = 0.549 ± 0.126	$C_{1,surrogate}$ = 0.552 ± 0.131
SV_2		$C_{2,3}$ = 0.624 ± 0.269	$C_{2,4}$ = 0.586 ± 0.241
		$C_{2,surrogate}$ = 0.499 ± 0.135	$C_{2,surrogate}$ = 0.495 ± 0.130
SV_3			$C_{3,4}$ = 0.584 ± 0.260
			$C_{3,surrogate}$ = 0.443 ± 0.162

However, no differences in the CC were seen for SV data when compared to the corresponding surrogate data (Table 3).

Blood pressures, particularly diastolic and systolic, showed poor correlation between runs. Therefore, no differences, or an even higher correlation, to the corresponding surrogate data was found. For example, we calculated the CC of run 1 vs 2 and run 1 vs surrogate data, etc. See Table 2 for details.

DISCUSSION

The application of LBNP, to subjects who are already in HUT position, is an additional significant orthostatic challenge. We observed that heart rate responses were reproducible for the four runs, thus underlying the central role of heart rate in blood pressure regulation in subjects undergoing graded LBNP.

To maintain blood pressure and adequate cerebral perfusion during central hypovolemia (Mosqueda-Garcia et al., 2001) caused by HUT, LBNP or hemorrhage, reflex increases in heart rate and total peripheral resistance (el-Bedawi & Hainsworth, 1994; Hainsworth & el-Bedawi, 1994) occur. In our study, the increases in heart rate as well as the decreases in stroke volume during the LBNP phase were comparable to what has been reported by others (Convertino & Sather, 2000), and are reported elsewhere (Goswami et al., 2009c).

The major finding of this study was that physiological reproducibility in heart rate differed from the "surrogate" data across the runs. By comparing surrogate data with original ones across runs, we conclude that the correlation between runs was higher than between run and surrogate data from the run. This confirmed that physiological reproducibility in heart rate differed from the simulated "surrogate" data across the runs thus suggesting that these represented real physiological responses rather than one obtained by chance (Blaber et al., 1995).

Blood pressure data, on the other hand, showed poor correlation across runs. Furthermore, no differences, or an even higher correlation, to the corresponding surrogate data was found. This could be attributed to the close range in which the blood pressure is maintained across the entire graded LBNP stress. This is consistent with the observation that blood pressure is the primary regulated variable during stress (Julius, 1988), and therefore, operates within a narrow range.

Limitations

It is also possible that the initial HUT phase (not used in this study) of the combined HUT+LBNP could have resulted in varying levels of central volume at the beginning of the LBNP phase, which could affect the response patterns during the LBNP phase (Figure 1). For example,regulatory features might be called upon in persons with lower functional central volume reserve, induced by the HUT, to maintain blood pressure during graded LBNP. That is, such persons might more fully employ the possible range of cardiac and peripheral-vascular response patterns available in order to compensate for limited central volume reserve.

CONCLUSIONS

Young healthy males subjected to graded lower body negative, while in upright tilted position, showed only heart rate reproducibility across the four runs. This is in contrast to both heart rate and stroke volume reproducibility observed in the same subjects, when the entire HUT +LBNP protocol was used. This confirms that heart rate plays a central role in maintaining arterial blood pressure (Convertino & Sather, 2000), both under HUT (similar to everyday standing) and graded LBNP (so called "unknown stress"). Finally, surrogate data analysis is a useful tool to differentiate physiological responses from chance events in repeated runs.

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