

Original Article

## Sex Difference in the Effect of Whole Body Heating on Cardiovascular Functions

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### Abstract

Cold pressor test (CPT) is a well-known method for evaluating non-baroreflex mediated autonomic cardiovascular functions in humans. It has been reported that autonomic cardiovascular response to CPT differs in males and females and that heat stress attenuates the increase in arterial blood pressure during CPT. Study has also indicated that heat stress attenuates the increase in arterial blood pressure during CPT. The present study assessed the autonomic cardiovascular reactivity in males and females during cold pressor test before and after whole body heating for 40 min. 20 healthy Indian males and 18 females participated in the study. The participants were exposed to 40°C dry bulb temperature and 40% relative humidity in a simulated thermal chamber. They performed CPT before and after heat stress and their beat to beat heart rate and blood pressure were recorded. It was observed that baroreflex sensitivity (BRS) was significantly lower in females during CPT before heat stress ( $p < 0.01$ ) as well as after heat stress ( $p < 0.01$ ). Moreover, following whole body heating, the BRS during CPT increased slightly in females but not in males. Stroke Volume (SV) increased significantly during CPT before heat stress in females from pre-heat baseline (82 ml/beat vs. 101 ml/beat) ( $p < 0.001$ ) as well as during CPT after heat stress from post-heat baseline (75 ml/beat vs. 95 ml/beat) ( $p < 0.01$ ). SV increased significantly in males during CPT after heat stress only ( $p < 0.01$ ) as compared to post-heat baseline (82.5 ml/beat vs. 94.5 ml/beat). Frequency domain analysis of heart rate variability indicated that during CPT, low frequency power in males was higher than females ( $p < 0.05$ ) and high frequency power was higher in females than males ( $p < 0.05$ ). This suggests that autonomic modulation of cardiovascular function during CPT in males is mediated mainly via sympathetic neural system and in females it is mediated via vagal system. LF/HF ratio during CPT was found to be significantly higher in males (2.54) than females (1.27) before heat stress ( $p < 0.01$ ).

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### Introduction

Cardiovascular autonomic reactivity refers to heart rate and blood pressure response to an autonomic neural function challenging task like cold pressor test, head up tilt test, valsalva manoeuvre, hand grip

test, deep breathing test etc. A number of tests like heart rate variability, blood pressure variability, galvanic skin resistance, muscle sympathetic nerve activity are employed to assess the sympatho-vagal balance of the cardiac and vascular functions of the individuals during such challenging task. Study has reported that cardiovascular autonomic reactivity to autonomic task is different between males and females.

The cold pressor test (CPT) is one of the recognized physiological evaluation techniques to assess non-baroreflex mediated autonomic cardiovascular functions (1). In this test, the individual immerses one of his hands into ice cold water (4-6°C) up to wrist for 1-6 min. Blood pressure (BP) and heart rate (HR) response to CPT is recorded continuously. In normal subjects, a vascular sympathetic response is increased during CPT resulting in increased peripheral resistance (2). The HR response to CPT is variable and not homogenous for entire CPT period (3-4).

Study has suggested that the vagal activity as determined from 24 hours of ECG signal was not significantly different between men and women, whereas the spectral indices of low frequency power and low frequency/high frequency ratio, a surrogate measure of sympathetic function, were significantly higher in men (5). Literature has also suggested that heat stress attenuates the increase in arterial blood pressure in response to a non-baroreflex mediated sympathoexcitatory stimulus i.e. the CPT (6). The arterial baroreflex is an important determinant of the neural regulation of the cardiovascular system. The quantitative estimation of the arterial baroreceptor-heart rate reflex (baroreflex sensitivity, BRS) has been regarded as a synthetic index at the sinus-atrial node. Study has also documented a gender wise variation in BRS. Study by Huikuri et al demonstrated that men had a higher BRS than women (7).

We hypothesized that cardiovascular autonomic reactivity to CPT would be different in males and females before and after exposure to whole body heating. The aim of the present study was to examine autonomic control of HR and BP during CPT in

healthy males and females before and after heat stress. The study assessed heart rate variability, baroreflex sensitivity, heart rate and blood pressure in a group of healthy males and females during CPT before and after whole body heating in a simulated environmental chamber.

## Materials and methods

20 healthy males and 18 healthy females in the age range of 21-35 years were selected among post graduate trainees of the Institute as volunteers. They were examined clinically and ruled out that they were not suffering from any disease. They were explained about the protocol of the study in detail and possible consequences of CPT. The ethical committee of the Institute approved the test protocol.

The ECG of the subject was measured during resting sitting posture for 5 min and during cold pressor test for 3 min. The participant was then subjected to heat stress in a simulated environmental chamber for 40 min. The temperature of the chamber was maintained at 40°C and relative humidity at 40%. After exposure to heat stress, the participant was subjected to CPT for 3 min and their HR and BP were measured continuously throughout CPT.

The participant while performing CPT dipped his or her left hand up to wrist into ice cold water at 4°C. CPT was carried out as per the method described by Mourot et al and Cui et al (2, 4). The temperature of the water was maintained at 4-5°C throughout the CPT, by monitoring with a thermometer. A plastic tray of 6 cm height was used for cold water immersion where ice cubes were mixed with normal tap water. The temperature of the laboratory was maintained at about 25°C which was well within the comfort zone of human beings.

The following diagram represents the sequential events of the present study protocol.

The electrocardiography (ECG) was recorded by Procomp Infiniti 5.0 Physiological data recorder (Thought Technology, Montreal, Canada) and various heart rate variability (HRV) indices were then

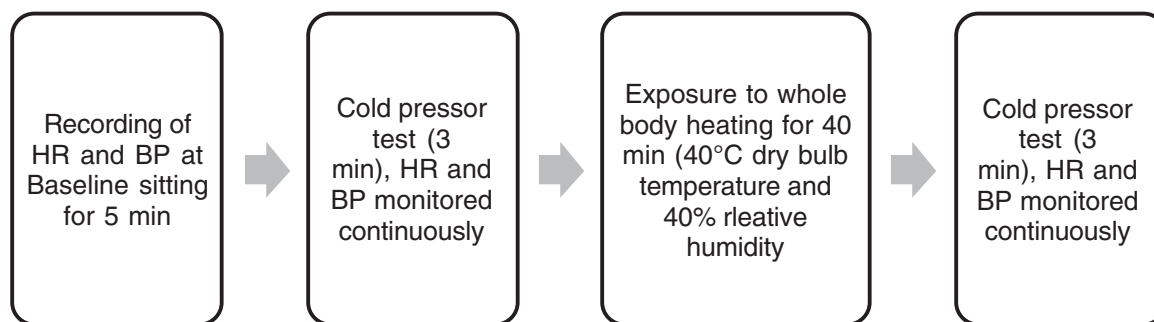


Fig. 1: Sequential events of the present study protocol.

calculated from different periods of ECG data. Three electrodes were placed on surface of the subject's chest, one at just below the right shoulder; other at just below the left shoulder and a third electrode was placed near the umbilicus. Negative and ground terminal of the ECG sensor were connected to right and left electrode respectively. Positive terminal of the ECG sensor was connected to the electrode near the Umbilicus. ECG was recorded at resting sitting and during CPT before heat stress and again after heat stress at resting sitting followed by CPT.

Kubios HRV analysis software, version 2.1, Finland, was used for analyzing the HRV data. ECG data was pre-processed before subjecting it to time domain and frequency domain analysis. Various time domain and frequency domain indices of heart rate variability were analysed conforming to the guidelines of the Task Force of European Society of Cardiology and the North American Society of Pacing and Electrophysiology (8).

For time domain analysis, peak R wave was detected from ECG wave and R-R interval time series was plotted after rejecting the artefacts. ECG data was then interpolated in order to sample the data equidistantly for frequency domain analysis. Power spectral density (PSD) analysis is the most common method of computing frequency domain parameters. PSD estimation provides the basic information of how the power of the signal (i.e., its variability) distributes as a function of frequency. This estimation can be made by two different types of methods: non-parametric and parametric. The non-parametric method is computationally simpler and the results obtained are very similar to the parametric results.

Fast Fourier Transform (FFT) computation, based on Welch's periodogram (9), is the basis of the non-parametric PSD analysis. The Welch's PSD method estimates the power of a signal vs. frequency. This is a method by which a large time-sampled waveform can be frequency-transformed by partitioning the data into shorter segments, transforming each segment, then, averaging the results over all the segments to create a composite frequency-space waveform. Here, original data segment was split up into numerous short data segments and an overlapping between the data segment is allowed to the extent of 50%. The overlapping segments are then windowed by Hamming window.

Various time domain indices of HRV were computed from ECG recording. These included mean HR, standard deviation of N-to-N intervals or RR intervals (SDNN in ms), root mean square of successive differences between adjacent NNs (RMSSD in ms), Number of successive RR interval pairs that differ more than 50 ms (count) (NN50), NN50 divided by the total number of RR intervals (%) (pNN50).

Various frequency domain indices of HRV computed from ECG signal after transforming the ECG signal by Fast Fourier Transformation (FFT) were LF power (Absolute power of low frequency component in the range of 0.04-0.15 Hz- $\text{ms}^2$ ), LF power % (Relative power of LF expressed in percentage of total power), LF power n.u. (Power of low frequency in normalized unit), HF power (Absolute power of high frequency component in the range of 0.15-0.4 Hz- $\text{ms}^2$ ), HF power % (Relative power of high frequency component expressed in percentage of total power), HF power n.u. (Power of high frequency expressed in

normalized unit), Total power ( $\text{ms}^2$ ) Total power of VLF, LF and HF and LF/HF (Ratio between LF and HF band powers).

Arterial blood pressure was measured on a beat to beat basis by arterial tonometer, Finometer Midi (Finapres Medical System, Amsterdam, Netherland) for 5 min at resting sitting and during CPT for 3 min before exposure to heat stress. Recording of blood pressure was then again carried out for 5 min at resting sitting and during CPT for 3 min after heat stress. Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and pulse pressure (PP) were recorded during CPT for 3 min before and after heat stress and also during baseline resting sitting position for 5 min.

Continuous non-invasive measurement of BP was measured by using finger cuff technology. An appropriate sized finger cuff was wrapped around 2<sup>nd</sup> phalanx of the middle finger of left hand. The method of recording continuous blood pressure was based on the volume-clamp technique discovered by the Czech physiologist Jan Peřáz (10-12). The diameter of a finger artery under a cuff was "clamped" i.e. kept at a constant diameter in the presence of the changes in arterial pressure during each heartbeat. Changes in diameter were measured by means of an infrared photo-plethysmograph built into the finger cuff. The finger cuff kept the diameter of the underlying arteries constant by dynamically applying a counter-pressure throughout the cardiac cycle. When, for instance, during systole an increase in arterial volume is detected by the plethysmograph, the cuff pressure is immediately increased by a rapid pressure servo-controller system to prevent the volume changes. Arterial blood pressure was reconstructed from the recorded finger pressure using the Beatscope software of Finapres arterial tonometer. Various scientific studies have revealed that the finger arterial pressure measured by Finapres correlated well with the brachial arterial pressure in clinical as well as experimental settings (13-14).

Baroreflex sensitivity (BRS) was computed by sequence method discovered by Bahjaoui-Bouhaddi et al (15). BRS is defined as change in interbeat

interval in milliseconds per unit change in BP. Sequences of three or more beats in which the SBP and the following pulse interval changed in the same direction (either increasing or decreasing), which reflect the HR response to spontaneous variations in BP, were considered as BRS. BRS was calculated from beat to beat blood pressure data and corresponding interbeat interval of arterial pressure wave. The BRS calculation was carried out using NEVROKARD 9.0.0 BRS analysis software, Slovenia. This method employs ten second duration windows of simultaneous systolic blood pressure and pulse interval data which are interpolated and resampled at 1 s intervals. Each second of time pressure and interval are analyzed for positive cross correlation using time delays between blood pressure and interval from 0 to 5 s. The delay giving the highest correlation is chosen and BRS is computed. When BRS is significant at  $P=0.01$ , it is displayed and entered into the statistics.

Heart rate was derived from the ECG signal that was used for determining various time domain and frequency domain descriptors of HRV. Stroke volume (SV) was analysed by the Model flow technique (16-17). Cardiac output was calculated as the product of HR and SV (18).

The last 3 min of ECG and BP data at resting sitting and 3 min data of CPT before and after whole body heating were considered for HRV and BRS analysis in the study.

Statistical software Statistical 6.0 was used to analyze the data. Data was first checked for the normality by Shapiro Wilks 'W' statistic. Paired and unpaired t-test was carried out for intragroup and intergroup comparison respectively. Level of significance was kept at  $p \leq 0.05$  as significant (S),  $p \leq 0.01$  as moderately significant (MS),  $p \leq 0.001$  highly significant (HS) and  $P > 0.05$  as non-significant (NS).

## Result

Table I shows physical characteristics of the volunteers. Females were having a significantly lower

TABLE I: Physical characteristics of the participants.

Parameters	Male Mean±SD	Female Mean±SD	Level of significance <sup>^</sup> p value
Age (years)	27.6±4.35	29.4±4.96	0.273 (NS)
Body height (cm)	171.2±7.47	158.8±5.84	0.000 (HS)
Body weight (kg)	68.3±9.65	58.5±8.53	0.006 (MS)

<sup>^</sup>Intergroup comparison; Unpaired t-test  
p<0.05 (significant - S); p<0.01 (moderately significant - MS); p<0.001 (highly significant - HS).

body height (p<0.001) and lower body weight (p<0.01) than males.

Fig. 2(a) to (g) shows heart rate, stroke volume, cardiac output and blood pressure responses to CPT in males and females before and after heat stress. HR was significantly higher in females than males at baseline and increased significantly in males during CPT before heat stress. HR was similar during CPT in males and females before heat stress and non-significantly higher in females than males during CPT after heat stress. Stroke volume did not show any significant difference between males and females either at baseline or during CPT. It increased in females during CPT from baseline both before and after heat stress. In males, it increased during CPT after heat stress only. Cardiac output was not significantly different between males and females either at baseline or during CPT. It increased significantly in males and females during CPT both before and after heat stress. At baseline sitting condition, HR was significantly higher in female than male (p<0.01).

SBP showed a significant increase during CPT from baseline sitting both before and after heat stress (p<0.001) in both the genders. DBP increased significantly in males and females during CPT from baseline sitting either before or after heat stress. Also, male value was significantly higher than their female counterparts during CPT before and after heat stress. Mean blood pressure increased significantly during CPT from baseline in males and females before and after heat stress. Pulse pressure increased significantly during CPT from baseline in both males and females before heat stress and only in females after heat stress. PP was also found to be significantly higher in females than males.

Frequency domain indices of HRV are shown in Table 2. LF power was significantly lower in females than males at baseline after heat stress (p<0.05). LF power increased non-significantly in male during CPT from baseline before heat stress. In females, LF power did not show any increment during CPT before heat stress. LF power reduced significantly in males during CPT after heat stress. On the contrary, LF power increased non-significantly in females during CPT after heat stress. LF n.u. (LF power expressed in normalized unit) was found to be significantly and non-significantly higher in males than females during CPT before and after heat stress respectively. HF power was found to be significantly higher in females than males during CPT either before or after heat stress (both being significant at p<0.05). HF power decreased significantly in males during CPT from baseline after heat stress whereas females had an increasing trend either before or after heat stress. HF n.u. (HF power expressed in normalized unit) during CPT was significantly higher in females before heat stress and non-significantly higher after heat stress as compared to males.

Total power was found to have a higher value in males than females during CPT before heat stress (p<0.05). Total power increased non-significantly in males and females during CPT before heat stress as compared to baseline. However, after heat stress, total power decreased significantly (p<0.01) in males and increased non-significantly in females during CPT, as compared to baseline.

LF/HF ratio was found to be significantly higher in males than females during CPT before heat stress and non-significantly higher in males than females during CPT after heat stress. LF/HF ratio increased non-significantly during CPT in females after heat as



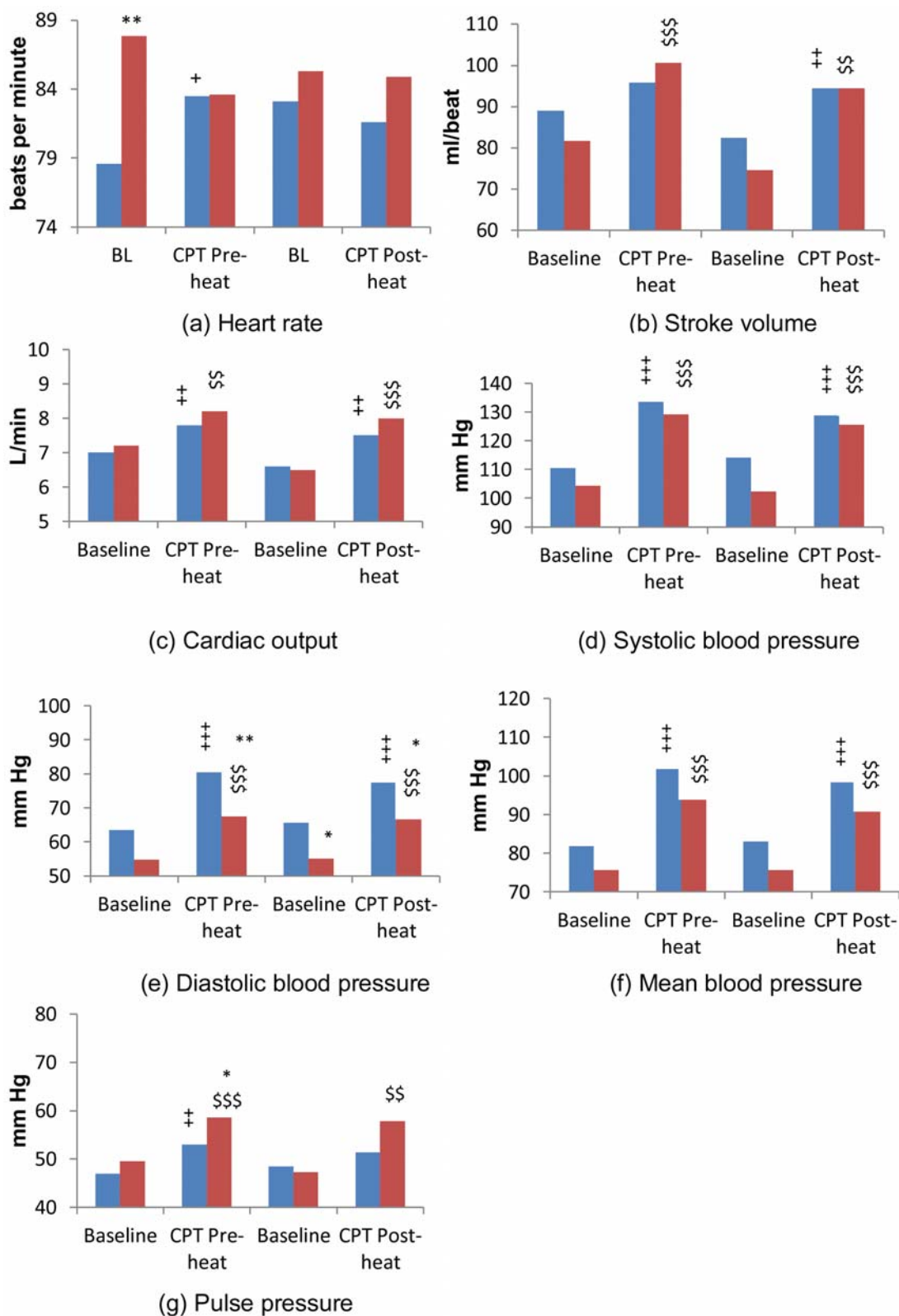


Fig. 2: (a) Heart rate,(b) Stroke volume, (c) cardiac output, (d) systolic blood pressure, (e) diastolic blood pressure, (f) mean blood pressure, (g) pulse pressure in males and females during cold pressor test before and after heat stress. \* indicates significant difference between males and females; \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 and + indicates significant difference in males from baseline; +p<0.05; ++p<0.01, +++p<0.001 and \$ indicates significant difference in females from baseline; \$p<0.05; \$\$p<0.01, \$\$\$p<0.001.

TABLE II : Frequency domain indices of heart rate variability to cold pressor test in males and females before and after heat stress.

		Male Mean±SD	Female Mean±SD	Significance level <sup>^</sup> (p value)
Low frequency component (LF) (ms <sup>2</sup> )	Baseline	1073.6±967.5	856.6±569.7	0.487 (NS)
	Pre-heat CPT	1516.7±1887.7	833.0±698.0	0.240 (NS)
	Baseline	1815.6±1439.3*	889.3±807.0	0.050 (S)
	Post-heat CPT	1046.8±1030.8*	1185.9±1293.7	0.743 (NS)
Low frequency component (LF) (n.u.)	Baseline	62.0±15.02	56.4±19.14	0.358 (NS)
	Pre-heat CPT	62.5±20.74	50.5±16.46	0.049 (S)
	Baseline	58.0±20.73	55.4±15.96	0.711 (NS)
	Post-heat CPT	63.8±19.39	57.9±22.62	0.443 (NS)
High frequency component (HF) (ms <sup>2</sup> )	Baseline	690.6±202.34	616.3±254.6	0.797 (NS)
	Pre-heat CPT	721.7±159.83	1006.3±198.7	0.050 (S)
	Baseline	1099.8±354.25	701.6±251.36	0.05 (S)
	Post-heat CPT	555.4±205.46*	847.8±654.21	0.05 (S)
High frequency component (HF) (n.u.)	Baseline	37.8±14.92	42.0±19.43	0.497 (NS)
	Pre-heat CPT	36.9±20.76	47.7±16.48	0.05 (S)
	Baseline	41.5±20.47	42.3±16.52	0.920 (NS)
	Post-heat CPT	37.8±21.17	40.0±22.69	0.785 (NS)
Total power (ms <sup>2</sup> )	Baseline	1912.8±523.45	1611.2±324.78	0.603 (NS)
	Pre-heat CPT	2363.6±459.86	1939.7±489.36	0.588 (NS)
	Baseline	3168.6±567.89**	1730.6±958.45	0.05 (S)
	Post-heat CPT	1737.2±499.21**	2173.1±789.45	0.451 (NS)
LF/ HF ratio	Baseline	2.03±0.958	1.83±0.547	0.667 (NS)
	Pre-heat CPT	2.54±0.654	1.27±0.568	0.010 (MS)
	Baseline	2.36±0.478	1.52±0.847	0.199 (NS)
	Post-heat CPT	2.60±0.659	2.10±0.458	0.442 (NS)

<sup>^</sup>Intergroup comparison; Unpaired t-test; p≤0.05: S (significant); p≤0.01; MS (moderately significant); p≤0.001: HS (highly significant) and p>0.05: NS (not significant)

\*Intragroup comparison; significantly different from pre-heat baseline, \*p<0.05; \*\*p<0.01; \*\*\*p<0.001

+Intragroup comparison; significantly different from post-heat baseline, +p<0.05; ++p<0.01; +++p<0.001

TABLE III : Time domain indices of heart rate variability to cold pressor test in males and females before and after heat stress.

		Male Mean±SD	Female Mean±SD	Significance level <sup>^</sup> (p value)
SDNN (ms)	Baseline	46.1±14.28	42.9±14.93	0.544 (NS)
	Pre-heat CPT	51.6±20.19	58.8±18.97	0.597 (NS)
	Baseline	58.9±24.07	54.5±22.78	0.612 (NS)
	Post-heat CPT	43.5±15.08	45.2±16.54	0.775 (NS)
RMSSD (ms)	Baseline	37.4±15.13	42.2±21.36	0.459 (NS)
	Pre-heat CPT	44.3±17.93	66.2±20.93**	0.233 (NS)
	Baseline	52.7±32.96*	50.6±31.40	0.863 (NS)
	Post-heat CPT	37.9±17.57	41.0±13.94	0.607 (NS)
NN50 (count)	Baseline	12.3±8.65	12.5±8.12	0.949 (NS)
	Pre-heat CPT	17.9±7.25	19.8±7.58	0.786 (NS)
	Baseline	14.2±8.67	14.6±5.69	0.903 (NS)
	Post-heat CPT	16.1±5.89	14.3±5.45	0.776 (NS)
pNN50 (%)	Baseline	15.5±10.42	14.7±9.68	0.833 (NS)
	Pre-heat CPT	20.0±13.75	23.6±7.42	0.541 (NS)
	Baseline	20.0±10.35	18.8±7.87	0.772 (NS)
	Post-heat CPT	18.0±7.89	19.1±5.55	0.841 (NS)

<sup>^</sup>Intergroup comparison; Unpaired t-test; p≤0.05: S (significant); p≤0.01 MS (moderately significant); p≤0.001: HS (highly significant) and p>0.05: NS (not significant)

\*Intragroup comparison; significantly different from pre-heat baseline, \*p<0.05; \*\*p<0.01; \*\*\*p<0.001

+Intragroup comparison; significantly different from post-heat baseline, +p<0.05; ++p<0.01; +++p<0.001

compared to pre-heat CPT.

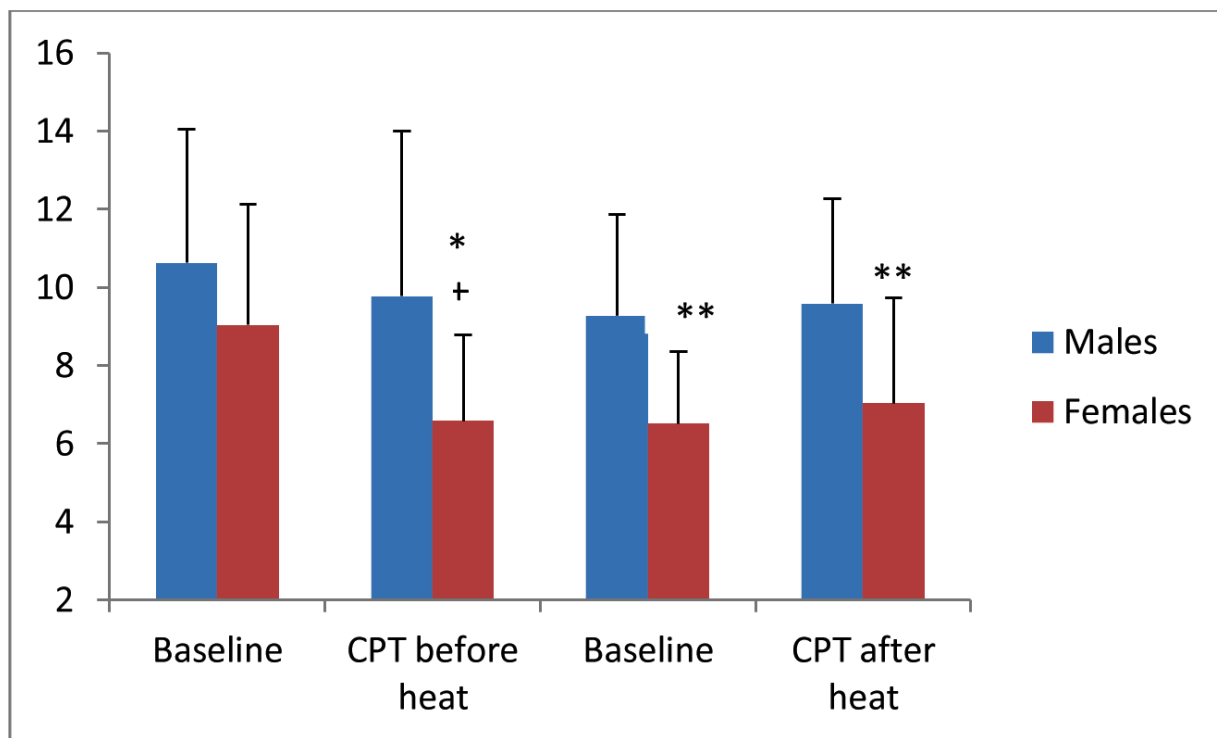
Time domain indices of HRV are shown in Table III. SDNN was found to have a non-significantly lower value in males than females during CPT before and after heat stress. SDNN increased during CPT before heat stress and decreased during CPT after heat stress in both males and females.

RMSSD was non-significantly lower in males than females during CPT before and after heat stress. RMSSD increased significantly in females and non-significantly in males during CPT before whole body heating. After whole body heating, RMSSD decreased non-significantly in both males and females during CPT.

NN50 was non-significantly lower in males than females during CPT before heat stress, but non-significantly higher in males than females during CPT

after heat stress. NN50 increased non-significantly during CPT in males before and after heat stress and increased non-significantly during CPT in females before heat stress and remained similar after heat stress. pNN50% was non-significantly lower in males than females during CPT before and after heat stress. pNN50% increased during CPT in males and females before heat stress and decreased in males and females after heat stress.

Fig. 3 shows BRS of males and females to cold pressor test before and after heat stress. It was observed from the figure that before heat stress, BRS was significantly lower during CPT in females than males (Male: 9.78 ms/mm Hg vs. Females: 6.59 ms/mm Hg). The similar trend was also observed during CPT after heat stress (9.58 ms/mm Hg in male vs. 7.04 ms/mm Hg in female). Baseline resting sitting BRS in female was not significantly different from male before heat stress, but significantly lower after heat stress ( $p < 0.01$ ).



\*Unpaired t-test between males and females. \* $p < 0.05$ ; \*\* $p < 0.01$ , + denotes significantly different in BRS in females as compared to baseline; \* $p < 0.05$ .

Fig. 3: Baroreflex sensitivity (ms/mm Hg) to cold pressor test in males and females before and after heat stress. Values are mean  $\pm$  SD



Result also revealed that BRS significantly reduced in females from pre-heat baseline to CPT before heat stress (9.04 ms/mm Hg to 6.59 ms/mm Hg) ( $p < 0.05$ ) and increased non-significantly during CPT from baseline after heat stress (6.52 ms/mm Hg to 6.89 ms/mm Hg). BRS decreased non-significantly during CPT in males from baseline (10.63 ms/mm Hg vs 9.78 ms/mm Hg) before heat stress and increased non-significantly during CPT after heat stress (9.28 ms/mmHg vs. 9.58 ms/mm Hg).

## Discussion

The present study examined the autonomic cardiovascular functions in males and females during CPT before and after exposure to whole body heating. The study showed that HR increased in males from pre-heat baseline to CPT (78.6 bpm vs. 83.5 bpm). Females did not show this increase in HR during CPT, rather showed an attenuation. Interestingly, HR in males after heat stress showed a non-significant attenuation during CPT. Increase in HR during hand immersion in ice cold water has been reported in a group of male students (19) and in a group of male and female subjects (20). Study has also documented that HR response to CPT varies among males and females (21). A biphasic alteration in HR during CPT, with an initial increase followed by a slow decrease, has been observed by many researchers (22-24). Studies have reported that autonomic modulation of HR during CPT is caused by decreased cardiac vagal outflow along with concomitant involvement of sympathetic activity (25-27). However, contrasting to the previous study results, it has also been reported in the literature, that an increase in vagal activity induced by baroreceptor activation may occur during CPT (28-29).

It has been reported in the literature that autonomic modulation of cardiovascular functions varies among men and women (30). Study has also documented that men had a greater sympathetic activity and women had parasympathetic dominance (31-32). Non-significant increase in HF spectral power (from  $42.0 \pm 19.43$  to  $47.7 \pm 16.48$  n.u.) with simultaneous decrease in LF spectral power (from  $56.4 \pm 19.14$  to  $50.5 \pm 16.46$  n.u.) in females before heat stress is accountable for decreased HR during CPT from

baseline (from  $87.9 \pm 5.87$  to  $83.6 \pm 10.35$  bpm). After heat stress, females had no significant changes in either LF power or HF power and this was reflected in the heart rate value. Slight increase in LF spectral power (LF  $\text{ms}^2$ ) in males before heat might have caused an increase in HR during CPT from baseline (from  $78.6 \pm 10.11$  to  $83.5 \pm 11.28$  bpm). Post heat, reduction in HF spectral power n.u. with concomitant increase in LF power n.u. in females after heat stress probably suggests of shifting of autonomic balance towards sympathetic. Before heat stress, an increase in the LF/HF ratio in males during CPT from baseline (from 2.03 to 2.54) and decrease in LF/HF ratio in females from baseline (1.83 to 1.27) further support the hypothesis of differential activation of sympathetic and vagal system in males and females. Also, this is suggestive of augmentation and attenuation of sympathetic neural system in males and females respectively during CPT. After heat stress, higher value of LF/HF ratio in females is also suggestive of sympathetic dominance over cardiac autonomic function during CPT.

Root mean square of successive differences (RMSSD), a time domain parameters of HRV, has been reported to be associated with vagal mediated control of heart rate (33). A significant increase in RMSSD in females during CPT from baseline was observed (from 42.2 ms to 66.2 ms) ( $p < 0.01$ ). However, after heat stress RMSSD decreased non-significantly in females. RMSSD in males increased non-significantly during CPT from pre-heat baseline and decreased non-significantly during CPT from post-heat baseline (Table III). The decrease in RMSSD value in females during CPT after heat stress is suggestive of shifting of autonomic equilibrium of heart rate control towards sympathetic. On the other hand, increased RMSSD during CPT before heat stress supports the idea of vagal reinforcement of HR control in females. This has also been reflected in HR value of females before heat stress during CPT.

SDNN, standard deviation of all NN intervals, reflects all the cyclic components responsible for variability of heart rate in the period of recording (8). Increased SDNN is considered as hallmarks of parasympathetic prevalence (34). Relatively lower value of SDNN during

CPT after whole body heating suggests autonomic control of heart function in female was shifted towards sympathetic side and before heat it was modulated via vagal system.

SV increased significantly during CPT in females before heat stress from pre-heat baseline ( $p < 0.001$ ) and also during CPT after heat stress from post-heat baseline ( $p < 0.01$ ). SV increased significantly in males during CPT after heat stress ( $p < 0.01$ ) as compared to post-heat baseline. CPT causes a generalized vasoconstriction of peripheral vasculature due to enhanced vascular sympathetic response. This results in shifting of blood from periphery to the central pool, thus, causes an increase in SV. In contrast to the result of the present study, investigators have reported a reduction in SV during last min of CPT from baseline value. Mourot et al in their study have documented that a reduction in SV occur during CPT from 101.0 ml at baseline to 97.6 ml at 3<sup>rd</sup> min of CPT (2). Saab et al also reported a similar finding of reduced SV from 105.0 ml to 82.0 ml during 61-90 s of CPT (19). Notwithstanding, the study has also reported that CPT increases sympathetic activity and blood pressure under normothermic conditions. (6). The researchers have observed that CPT causes an increase in MSNA, along with splanchnic (35), and renal vasoconstriction (36). It is therefore likely that vasoconstriction was intense in females than males during CPT before heat stress.

Whole body heating causes an increase in the skin blood flow by about 5 times and 50% of the cardiac output is channelized to the skin vasculature during heat stress (37). The increase in cardiac output coupled with an attenuated sympathetic activity to the skin vasculature during heat stress was believed to be the reason for attenuated blood pressure response to CPT (6). Increase in MAP during CPT in males and females from baseline before and after heat stress by 16-21 mm Hg was in agreement with the results of the study by Victor et al who reported an increase in MAP by 20 mmHg during last min of 2 min CPT duration (22). Raaof et al documented an increase in MAP by 15 mm Hg (20). MAP increase in the study reported by Kelsey et al was slightly in the lower range and increased by 9 mm Hg in both

males and females (38). Cui et al observed that SBP in healthy male subjects was found to be non-significantly higher at baseline after heat stress as compared to before heat stress (128 vs. 120 mm Hg), DBP reduced significantly (71 mm Hg vs. 66 mm Hg) and MAP remained almost similar (87 mm Hg vs. 86 mm Hg) (6,39). They also observed in another study that whole body heating causes an attenuation in MAP during CPT (40). They observed that during normothermic condition, MAP increased by 28 mm Hg during CPT and after whole body heating the increase in MAP was attenuated to 18 mm Hg during CPT. MAP in the present study was observed to be attenuated in males and females during CPT after heat stress. The larger attenuation was observed in females. Further, females had comparatively lower MAP than males during CPT both before and after heat stress. This possibly points towards the fact that sympathetic mediated vasoconstriction in females was not as stronger as males. Though, SV and CO increased by a larger proportion in females after heat stress, observed attenuated pressor response in females during CPT may be due to diversion of a larger fraction of CO to the skin vasculature and ineffectiveness of skin sympathetic nerve activity to the CPT.

The reason for attenuation in MAP during CPT after heat stress has been explained by Cui et al as an apparent dissociation between muscle sympathetic nerve activity (MSNA) and the corresponding BP response to a vasoconstrictor stimulus (6). Attenuated responsiveness to the sympathetic stimulus compromise blood pressure control, which is commonly observed in heat stressed individuals. The study by Cui et al and others have shown that whole body heat stress attenuates the gain of baroreflex control of MSNA and heart rate (39,41). Baroreflex sensitivity (BRS) in the present study reduced significantly in females during CPT after heat stress from pre-heat baseline (9.04 ms/mm Hg vs. 7.4 ms/mmHg) but not significantly in males (10.63 ms/mm Hg vs. 9.58 ms/mm Hg). Females showed a significant fall in BRS during CPT from baseline before heat stress ( $p < 0.05$ ) and slightly higher value after heat stress. Males also showed a non-significant fall in BRS during CPT before heat stress and slight increase after heat stress. Potts et al observed that

increasing arterial compliance due to heat stress decreases the sensitivity of carotid-vasomotor baroreflex responsiveness (42). The reduction in BRS in females post heat stress as compared to pre-heat stress baseline has been observed in the present study and this has been reflected in higher attenuation in SBP and MAP during CPT in both males and females after heat stress.

### Conclusion

The present study examined the autonomic cardiovascular modulation during cold pressor test in a group of males and females. Results revealed that baroreflex sensitivity reduced significantly in female during CPT. Low frequency spectral power of HRV, a measure of sympathetic activity of autonomic cardiac function, was found to be significantly higher in males than females during CPT before heat stress indicating higher sympathetic dominance. After whole body heating, males had slightly attenuated sympathetic reactivity. High frequency spectral power of HRV was significantly higher in females than males during CPT before heat stress, suggesting that vagal dominance. Total power during CPT was non-significantly higher in males than females before heat stress and lower in males than females after heat stress. LF/HF ratio, a measure of sympatho-vagal balance was found to be significantly higher in

males than females during CPT before heat stress, indicative of higher sympathetic activity in males than females. Heightened LF/HF ratio in females after heat stress is suggestive of switching over of cardiac autonomic function towards sympathetic side.

### Limitations of the present study

The present study did not measure muscle sympathetic nerve activity during CPT before and after heat stress. The MSNA measurement could have given a clear insight of autonomic modulation of cardiovascular responses during CPT in males and females.

### Conflict of interest

Nil

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