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Research Article

Sex Differences in the Response of Left Ventricular Functions to Sympathetic Activation by Cold Pressor Test in Normotensive Young Adults

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ABSTRACT

Background: Reactivity to sympathetic stimulation can be used to predict future cardiovascular events. However, vascular reactivity, like other cardiovascular events, is expected to exhibit sexual disparity. This study was designed to assess the sex differences in the response of left ventricular function to sympathetic stimulation via cold pressor test (CPT).

Methods: Left ventricular (LV) function was assessed in fifty-one (26 males; 25 females) young adult (18-35 years) consenting participants. Blood pressure, heart rate and left ventricular functions were assessed before and after sympathetic stimulation via CPT.

Results: Baseline levels of systolic blood pressure (SBP), end diastolic volume (EDV), end systolic volume (ESV) and stroke volume (SV) were higher in males than in females. Post CPT, cardiac output (CO), SBP, diastolic blood pressure (DBP) and mean arterial blood pressure (MABP) increased in both male and female participants when compared with the baseline values. The post CPT values of EDV and ESV were only elevated in the male participants while CPT had no effect on ejection fraction (EF) and SV in both male and female participants. The post CPT values of CO, EDV, ESV, SV, SBP and MABP were higher in the male participants.

Conclusion: The response of left ventricular functions to sympathetic stimulation via the cold pressor test CPT exhibit sexual dimorphism with greater magnitude of reactivity in the male participants.

INTRODUCTION

Autonomic dysfunction is a hallmark of hypertension and several cardiovascular diseases.[1] Hence, reactivity to autonomic activation such as sympathetic stimulation is usually used to predict future cardiovascular events.[2,3] However, cardiovascular physiology, risk factors and pathophysiology exhibit sexual differences in humans [4] and in animals.[5] Considering the importance of elevated BP as an underlying risk factor for several cardiovascular pathologies,[6] it is possible that several consequent cardiovascular diseases could also exhibit sexual dimorphism in their pathophysiology and severity.

Cardiovascular functions are known to be affected by temperature and other environmental stressors.[3] The Cold Pressor Test (CPT) is a standardized test used to characterize sympathetic nervous activity.[7] The test is known to cause global sympathetic activation and result in significant arteriolar constriction followed by an increase in blood pressure.[7]It has been used clinically as a stress test to assess left ventricular function.[7-9] Several studies have indicated that the CV response to the cold pressor test can predict the future development of hypertension and cardiovascular events. For instance, studies in black and white adults as well as children have indicated that black participants, who are at increased risk for developing early hypertension, show stronger vascular reactions to the cold pressor test than do white participants.[10]However, the influence of sex in the hyperreactive response of the LV functions to sympathetic stimulation elicited via CPT is not clear. Considering the sexual dimorphic nature of several cardiovascular pathologies and the importance of sympathetic stimulation in these cardiovascular pathologies, we sought to investigate the role of sex in the response of LV functions to sympathetic stimulation elicited by cold pressure test in normotensive young adults.

MATERIALS AND METHODS

Fifty-one (51) normotensive young participants (aged 23.55 ± 0.51 years, 26M:25F) took part in the study after informed consent was obtained from each of them. Ethical approval (LREC/10/06/590) was granted by the Health Research and Ethics Committee of the Lagos State University Teaching Hospital (LASUTH), Ikeja. The procedure was carried out in accordance with the latest revision of the 1975 Declaration of Helsinki.[11] Participants were enrolled using a systematic sampling method viz: all the staff and students who volunteered haven been made aware of the expectations of the study were given numbers after dividing them into male and female groups. Thereafter, every 4th individual, each group was enrolled in the study beginning with the fourth number from each group, until the desired number of participants who fitted into the Inclusion Criteria for the study had been reached.

To be included in the study, the participants had to be healthy young adults, aged 18years to 35years, and normotensive with a resting blood pressure <140/90 mmHg.[12] The participants were non-obese (BMI <30kg/m2). They were required to have no history of smoking, chest pain or use of cardiovascular or respiratory medications. As described in our earlier study,[8]individuals were excluded if their resting blood pressure was <110/60 mmHg; baseline plasma norepinephrine was above the normal range or their end-diastolic volume and end-systolic volume below the normal range for age. Menstruating females, pregnant women and ladies on oral contraceptives were excluded. Trained athletes were also excluded from this study, because they tend to have increased muscle mass.[13]To reduce confounders, participants were asked to refrain from caffeine-containing beverages for at least three hours; and alcohol beverages and exercise for at least four hours prior to the experiments.[14]

Upon arrival in either laboratory, participants were allowed to rest for 10minbefore the commencement of the study. Thereafter, the participant was connected to a Comen C80 patient monitor system (Shenzhen Comen Medical Instrument Co. Ltd, China) for the measurement of baseline blood pressure (BP) using an automatic cycling non-invasive BP monitor and the standard oscillometric method. Heart rate (beat/minute) was determined from Lead II of the12-lead ECG cable connected to the Patient Monitor after recording for one minute at a speed of 25mm/sec.

Two-Dimensional Echocardiographic Assessment

Left ventricular function was assessed using a Vivid q Cardiovascular ultrasound machine (General Electric Medical System, Horten, Norway). Participants were placed in the left lateral decubitus position and a phased-array transducer with a frequency of 3.5MHz was connected to the 2-Dimensional transthoracic echocardiography machine and placed over the left border of the sternum. The image of the heart was reflected on a gray scale picture on the monitor of the echocardiography machine. Left ventricular function (LVF) was measured by using the motion-mode (M-mode) technique obtained at the left parasternal long axis view (the first echocardiography view). An M-mode cursor was then placed through the septal and posterior left ventricular walls just beyond the tip of the mitral leaflets. The internal left ventricular dimensions were measured between the endocardial border of the septum and the endocardial border of the posterior wall in systole and diastole.[15] Left ventricular internal diameter in diastole and left ventricular end-diastolic volume (LVEDV) were used as indices of preload.[16] Ejection fraction was used as an index of systolic function.[16]All ultrasound assessments were made by REA.

Exposure of participants to the cold pressor test

The participants were instructed to avoid performance of Valsalva maneuver or hyperventilating during the test. They were then asked to immerse their right foot up to the ankle for one minute in ice slurry maintained at 4oC.[17] With the foot still in the ice slurry, BP, HR, and left ventricular function measurements were repeated.

Stroke volume (ml) was calculated as LV end-diastolic volume (ml) minus LV end-systolic volume (ml). Cardiac output, (CO) (l/min) was calculated as the product of stroke volume, SV (ml) and heart rate, HR (beat/minute).

Statistical analyses were carried out using GraphPad Statistical software, Prism 5 for Windows (GraphPad Software, San Diego, California, USA). Data were expressed as mean ± Standard Error of Mean, (SEM).Test for normality of distribution was carried out using the Shapiro-Wilk test and where the test failed, the Mann-Whitney U test was carried out to detect differences between the non-parametric data. Differences between means were compared using Student's paired t-test. Statistical significance was accepted at 95% confidence interval.

RESULTS

The characteristics of the participants are as shown in Table 1. The participants were age-matched. However, the females weighed slightly more than the males and their body mass index (BMI) was also significantly higher.

Sex differences in the baseline blood pressure and left ventricular function

As shown in Table 2, at baseline, systolic blood pressure (SBP) of the male participants was significantly higher ($p = 0.02$) compared with the female participants. However, diastolic blood pressure (DBP) and mean arterial blood pressure (MABP) were similar between the two groups. End diastolic volume (EDV), end systolic volume (ESV) and stroke volume (SV) were significantly higher ($p=0.04$; $p = 0.005$; $p = 0.04$) in males when compared with the female participants respectively (Table 3). However,cardiac output (CO) and ejection fraction (EF) were similar among the two groups.

Sex differences in the magnitude of blood pressure response to the cold pressor test

In both male and female participants, CPT significantly elevated the SBP, more so in males. Although DBP was significantly higher in the male participants, post CPT, there was no significant difference in the magnitude of response of DBP to CPT between the two groups. Also, among both male and female participants, CPT increased the MABP significantly when compared with the baseline values. There was no significant difference in the response between the male and female participants. Likewise, the magnitude of response of MABP to CPT was similar in both male and female participants (Table 2).

Sex differences in the magnitude of left ventricular function response to cold pressor test

After exposure to the CPT, cardiac output (CO) in both sexes and end diastolic volume (EDV) in males only, were significantly higher when compared with the baseline values. However, CO as well as the magnitude of response of the CO to CPT were higher in males ($p = 0.02$; $p = 0.005$ respectively) when compared with the female participants (Table 3). Sex had no influence on the effect of CPT on ejection fraction

(EF). In the male participants, CPT significantly increased the EDV when compared with the baseline values. Stroke volumes post CPT was significantly higher (p = 0.004) in males when compared with female participants though there was no significant difference in the magnitude of response of SV to CPT between the two groups (Table 3).

Table 1: Characteristics of participants

	X ± SD (n = 26) (range)	**X ± SD (n = 25) (range)**	**p**
Age (year)	23.55 ± 3.82 (18-34)	22.05 ± 2.82 (18-34)	>0.5
Weight (kg)	62.23 ± 8.36 (45 – 80)	66.07±2.14 (48-84)	0.047
Height (m)	1.68 ± 0.08 (1.52 – 1.86)	1.66±0.03 (1.52-1.86)	>0.5
BMI (kg m^{-2})	21.9 ± 2.5 (16.3 -26.3)	23.6±0.5 (17.3-29.2)	0.039

Table 2: Sex differences in the magnitude of blood pressure response to the cold pressor test

Parameters		**Male (n = 26)**	**Female (n = 25)**	**p**
SBP (mmHg)	Before CPT	120 ± 1	116 ± 2	**0.02 ***
	After CPT	141 ± 3	132 ± 2	**0.008***
	%Δ	17 ± 2	14 ± 2	0.15 NS
DBP (mmHg)	Before CPT	70 ± 1	71 ± 1	0.40NS
	After CPT	92 ± 3	87 ± 2	**0.04***
	%Δ	31 ± 3	23 ± 3	**0.03***
MABP (mmHg)	Before CPT	88 ± 1	87 ± 1	0.26NS
	After CPT	108 ± 3	99 ± 4	**0.04***
	%Δ	23 ± 2	14± 5	0.05

KEY: n = number of participants; CPT = cold pressor test; NS = not significant; %Δ = magnitude of change; SBP = systolic blood pressure; DBP = diastolic blood pressure; MABP = mean arterial blood pressure.

Table 3: Magnitude of left ventricular function response to the cold pressor test

Parameters		**Male (n = 26)**	**Female (n = 25)**	**p**
CO (l/min)	Before CPT	4.23 ± 0.19	4.42 ± 0.17	0.23 NS
	After CPT	5.98 ± 0.26	5.08 ± 0.26	0.009 *
	%Δ	47.55 ± 8.63	15.92 ± 6.39	0.003*
EF (%)	Before CPT	61.23 ± 1.13	62.61 ± 1.20	0.20 NS
	After CPT	59.96 ± 1.43	62.32 ± 1.21	0.11 NS
	%Δ	1.43 ± 2.65	0.12 ± 2.18	0.35 NS
EDV (ml)	Before CPT	105.30 ± 3.46	97.57 ± 2.71	0.04*
	After CPT	113.30 ± 3.79	6.62 ± 3.23	0.0007 **
	%Δ	8.61 ± 2.97	0.64 ± 4.33	0.07NS
ESV (ml)	Before CPT	40.78 ± 1.403	5.40 ± 1.61	0.005*
	After CPT	44.85 ± 2.21	36.61 ± 2.12	0.005*
	%Δ	9.87 ± 4.01	3.58 ± 3.92	0.13 NS
SV (ml/beat)	Before CPT	65.23 ± 2.60	59.44 ± 1.65	0.03*
	After CPT	67.35 ± 2.68	53.72 ± 2.38	0.0002*
	%Δ	5.31 ± 4.51	8.70 ± 4.34	0.30 NS
HR (beats/min)	Before CPT	67.96 ± 1.63	73.68 ± 2.35	0.07NS
	After CPT	89.42 ± 2.52	94.20 ± 3.15	0.25NS
	%Δ	32.62 ± 3.09	27.92 ± 2.88	0.39NS

KEY: n = number of participants; CPT = cold pressor test; NS = not significant; EF = Ejection Fraction; EDV = End Diastolic Volume; ESV = End Systolic volume; SV = Stroke Volume; HR = Heart Rate; %Δ = magnitude of change

DISCUSSION

In the present study, we assessed sex disparity in sympathetic stimulation by means of the cold pressor test (CPT), on blood pressure parameters and left Ventricular (LV) functions in normotensive young adults. We observed that: 1). The baseline systolic blood pressure (SBP), end diastolic volume (EDV), end systolic volume (ESV) and stroke volume (SV) were higher in males. 2). Sympathetic stimulation via CPT elevated cardiac output (CO), SBP, diastolic blood pressure (DBP) and mean arterial blood pressure (MABP) in both male and female participants. 3). CPT elevated EDV and ESV only in the male participants. 4). CPT had no effect on ejection fraction (EF) and SV in both male and female participants. 5). The post CPT values of CO, EDV, ESV, SV, SBP and MABP were higher in male participants. 6). The magnitude of response of EDV, ESV, SV, and SBP to CPT were similar in both groups. However, the magnitude of response of CO, DBP and MABP to CPT was higher in the male participants.

That the baseline SBP in male was higher compared to that in the females in this study is consistent with findings from other studies and this been largely attributed to the role of sex hormones as testosterone has been reported to play a role in the development of high BP while oestrogen is protective.[18] From puberty onwards, BP parameters are usually higher in males when compared with age-matched premenopausal females.[4] In adolescents, BP is lower in boys compared with girls mainly due to lower SV in girls.[19] With respect to CVDs, men are at greater risk, and the incidence of hypertension in men is greater than for premenopausal women of the same age.[19,20]

Likewise, the higher basal EDV values that was observed in male in the present study is consistent with previous findings that reported a higher EDV in males compared with females with no difference in EF.[21]

The elevation in cardiac output (CO), SBP, diastolic blood pressure (DBP) and mean arterial blood pressure (MABP) in both male and female participants after CPT support the sympathetic stimulating effect of CPT. Stimulation of the sympathetic nervous system has been shown to increase heart rate, cardiac contractility, venous capacitance as well as constricting the resistance vessels.[22]

The higher magnitude in the response of CO, DBP and MABP to CPT in the males suggest sexual dimorphism in the response to sympathetic stimulation by CPT. The concept that males and females use the two arms of the baroreflex system differently (4), further sheds more light on the higher magnitude of response of in males. For instance, at all ages, women have been reported to have more reduced sympathetic activities and enhanced parasympathetic activity as male have higher plasma norepinephrine levels than women.[23]

The cold pressor test is used clinically to evaluate arterial and LV functions (3) as well as predict future cardiovascularevents.[24] The increased magnitude of response to cardiovascular parameters in males when compared with the females in this study, is consistent with the higher susceptibility and prevalence of hypertension and CVD in males when compared with age-matched premenopausal women.[20,25]

Our result show that women responded with a higher change in cardiac output following exposure to CPT. This is consistent with previous report that sowed men responding to stress by increasing vascular resistance due to increased vasoconstriction and increased sympathetic discharge to the blood vessel whereas women increased heart rate thereby, increasing cardiac output. Therefore, BP increase in females is usually due to CO increase while it is due to elevated systemic vascular resistance in males,[2,19] suggesting a fundamental sex disparity in basic mechanism of BP regulation in males and females.

The elevated post CPT values of EDV and ESV observed only in the male participants is consistent with the reported higher sympathetic discharge in males when compared with female as activation of LV sympathetic function is associated with activation of the sympathetic nervous system.[26] The higher post CPT values of CO, EDV, ESV, SV, SBP and MABP of males participants when compared with that of the female participants in this study is further consistent with the sex disparity in cardiovascular functions as a whole and the LV function specifically. Left ventricular geometry differs between men and women [27] and this imparts on LV systolic function.[4] Women have been reported to have smaller LV chambers and lower stroke volumes but a higher resting HR which accounts for the similar cardiac output with men.[28,29]

We conclude that the response of LV functions to sympathetic stimulation via CPT exhibit sexual dimorphism with greater magnitude of vascular reactivity in the male participants.

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