

Effect of sex, prehypertension, and baseline LF HRV power on blood pressor response to cold pressor test

Abstract

Cold pressor test (CPT) is used as sympathoexcitatory maneuver. HRV is a useful tool for assessment of ANS activity. Purpose of this study was to compared baseline HR, blood pressure, LF and HF HRV power, and cardiovascular reactivity to CPT between normotensive and prehypertensive men and women and to find predictors of blood pressor responses to CPT.

Baseline HR, SBP, DBP, LF, and HF HRV power were recorded in 307 normotensive and prehypertensive young men and women. Immersion of the right hand into ice cold water for one minute was performed to compare cardiovascular reactivity to CPT between these groups. General linear model was used to test for effect of sex and prehypertension on baseline cardiovascular and HRV variables and to compare cardiovascular response to stress. Stepwise multiple linear regression analysis was used to reveal predictors of Δ SBP and Δ DBP.

Blood pressure response to CPT, negatively associated with resting blood pressure and positively associated with BMI, was less in prehypertensive men compared with normotensive men and prehypertensive women. Sex-based differences of Δ SBP and Δ DBP may be explained by differences in blood pressure and height and by negative association between Δ SBP and resting LnLF which was less in women.

Cardiovascular system, Cold pressor test, sex-based differences, prehypertension, Heart Rate Variability

Introduction

Until now, hypertension remains a major global health issue, it leads to severe complications and raises a risk of cardiovascular disease, brain stroke, chronic kidney disease, and premature death [1, 2]. That is why an early diagnostic and awareness of hypertension is crucial for controlling and prevention from further its development. However, there is very little literature showing specific cardiovascular and HRV characteristics of subjects only developing hypertension [3-7].

It has been suggested that the dysfunction of autonomic nervous system is a cornerstone of hypertension pathophysiology which underlies the initiation and development of hypertension [1, 3]. The cold pressor test (CPT) is commonly used as a sympathoexcitatory maneuver and standard laboratory method to evoke stress response and to evaluate a cardiovascular reactivity to stress [8-11]. The data regarding the differences in blood pressure (BP) response to CPT between normotensives and hypertensives are highly controversial. Such that some of previous reports have demonstrated that increase of blood pressure during CPT was significantly higher in subjects having family history of hypertension [10], in hypertensive [8], and atherosclerotic patients [12]. These studies provide information that cardiovascular hyperreactivity to cold stress is an important risk factor for the development of hypertension and that cold pressor test may be used as an indicator of the severity of hypertension and atherosclerosis. However, there is a series of other studies which did not find any effects of hypertension or positive family history of hypertension on BP response to CPT [7, 13, 14]. Moreover, some authors found that sympathoneural responses to the cold pressor test in individuals with essential hypertension and in those genetically predisposed to hypertension was reduced and did not support "pressor reactor" hypothesis of hypertension development [9]. Some other reports demonstrated that cold pressor test caused an increase of the blood pressure less in the hypertensive than in the normotensive subjects [5, 15, 16].

The heart rate variability (HRV) is a potentially useful noninvasive tool for the evaluation of autonomic nervous system activity and its effect on the cardiac function [17]. The high frequency HRV power (HF) is widely used as an index of cardiovagal activity, however interpretation of the low frequency HRV power (LF) is more controversial. It was presumed that LF power, especially, expressed in normalized units, is a marker of sympathetic modulation [18] or reflects both sympathetic and vagal activity [19], that LF oscillations of HRV are determined mainly by the parasympathetic nervous system, and although the final pathway is predominantly vagal, LF power provides information about the modulation of vasomotor tone [20]. The other reports suggested that LF power is not an index of sympathetic tone but rather of the baroreflex modulation of cardiac autonomic outflows [21, 22]. On the base of previous reports and our own investigations [6], the

interpretation of LF HRV power extended observations in that LF power is an index of the baroreflex modulation of cardiac autonomic outflows [21] and accepted that LF oscillations of HRV represent sympathoinhibitory arm of baroreflex [6].

The objectives of this study were to: 1) analyze the effect of sex and prehypertension on baseline HR, systolic blood pressure (SBP), diastolic blood pressure (DBP), LF and HF HRV power, and cardiovascular reactivity to CPT; 2) identify cardiovascular and HRV predictors of the blood pressure response to cold pressor test.

Materials and Methods

A total of 307 subjects were enrolled from V.N. Karazin Kharkiv National University student population. The participants were subdivided into four groups according to sex and blood pressure according to JNC-7 classification [23], referred thereafter to as the normotensive (NT) and prehypertensive (PHT) groups (Table 1). No one prehypertensive subject had ever been treated for hypertension. According to their medical history and examination of resting ECG, subjects with cardiac rhythm abnormalities, respiratory, or metabolic diseases were excluded from the study. None of the participants routinely took medicine. The subjects were fasted > 8 hours, and were asked to refrain from alcohol, caffeine, tobacco, abstaining from recent exercise >20 hours. None of the subjects participated in sports at a competitive level. All of them gave written informed consents.

Table 1. *Participants anthropometric and resting characteristics.*

	Men		Women	
	NT	PHT	NT	PHT
n	79	76	95	57
Age, years	20.23±0.19	20.17±0.20	20.04±0.18	20.12±0.23
Height, m§§§	1.77±0.01***	1.75±0.01***	1.60±0.01†	1.63±0.01
BMI, kg/m ² ‡‡	22.43±0.43†	24.01±0.43	22.16±0.39	23.4±0.50

Data are expressed as means±SE; NT, normotensive; PHT, prehypertensive; n, number of participants; *** $P<0.00$, vs. women of similar blood pressure; † $P<0.05$, vs. prehypertensives of the same sex; §§§ $P<0.00$, significant main effect of sex; ‡‡ $P<0.0$, significant main effect of PHT; || $P<0.0$, significant sex*PHT interaction; were found from general linear model [GLM] two-way MANOVA.

The ECG was continually recorded during 5-minutes resting and CPT stages following a stabilization period. Cold pressor test was performed by immersion of the right hand into ice cold water for one minute. HR was obtained by digital 2-channel ECG amplifier with a sampling

frequency 1000 Hz (CardioLab 2010, XAI-Medica, Ukraine), [4]. SBP and DBP were recorded at the end of resting and CPT stages by means the automatic blood pressure digital sphygmomanometer (Nissei WS-1011, Nihon Seimitsu Sokki Co., Ltd, Japan).

The nonparametric method of fast Fourier transformation was used to represent the signal in frequency domain during the resting stage. The low frequency and high frequency HRV power were expressed in the absolute units. For their interpretation we proceeded from the assumption that HF power received from 0.15 to 0.40 Hz oscillations is a measure of the cardiovagal outflow [17, 20, 21], LF power computed from low frequency oscillations ranging from 0.04 to 0.15 Hz is the sympathoinhibitory baroreflex-mediated index of cardiac autonomic regulation [6].

Data are presented as means \pm SE. All tests of statistical significance were 2-tailed, $P < 0.05$ was considered significant (unless otherwise specified). Two-way MANOVA was used to compare age, height, BMI, resting HR, SBP, DBP, LnLF, LnHF HRV power and also responses of HR, SBP, DBP to CPT by sex and presence of prehypertension. To meet the MANOVA assumptions the outcome variables that were not normally distributed were naturally log-transformed (LF and HF power). Three-way repeated measures MANOVA was used to test for possible effects of sex, PHT, immersion of the hand into ice cold water, and their interaction on HR, SBP, and DBP. The BMI and height were used as covariates in Two-way MANCOVA and Three-way repeated measures MANCOVA.

The stepwise multiple linear regression models were constructed to assess the relative importance of sex, BMI, height, resting BP (SBPrest and DBPrest), LnLF power (LnLFFrest), LnHF power (LnHFFrest), HR (HRrest), and Δ HR in SBP and DBP responses (Δ SBP and Δ DBP). The two-way and three-way interactions were included in the models to improve their overall fit. To avoid multicollinearity, the explanatory variables were centered before inclusion in the stepwise regression models. Statistical analysis was performed with the SPSS statistical program, version 22.

Results

Baseline hemodynamic and HRV characteristics. There was a significant main effect of sex and PHT on the resting heart rate (Fig. 1). The testing of simple effects revealed that HRrest was significantly less in normotensive men compared with women and compared with prehypertensive men.

The resting systolic blood pressure (SBPrest) was higher in men than in women, and diastolic blood pressure (DBPrest) was higher in men as a whole (significant main effect), but this differences was not significant between normotensives (Figs. 2A and 2B).

In the current study there was a significant main effect of sex on the resting LnLF HRV power and no significant sex-based and/or PHT-based-differences in the resting LnHF HRV power between groups (Figs. 3A and 3B). The testing of simple effects revealed that LnLFrest was greater in normotensive men compared with women but sex-based differences in LnLFrest between prehypertensives did not reach significant level.

When BMI was used as a covariant all the sex-based and/or PHT-based-differences in resting indices remained at the same or increased significant level. However, when height was taken into account the sex-based differences in HRrest were no longer significant, the sex-based differences in LnLFrest still were found but at lower significant level (for main effect, $P=0.001$ and $P=0.021$, without covariates and with a height as a covariate, respectively) and the sex-based differences in blood pressure increased (not shown).

Hemodynamic response to cold pressor test. The immersion of the right hand into ice-cold water for 1 minute led to an increase of HR, SBP, and DBP ($P<0.001$ for all variables) in all groups (not shown).

There was a significant main effect of sex and PHT on the increment of SBP and DBP, such that both SBP and DBP increased more in women than in men and prehypertensives demonstrated the less increment of blood pressures than normotensives (Figs. 4A and 4B). The further analysis of simple effects has shown that both Δ SBP and Δ DBP was less in prehypertensive than in

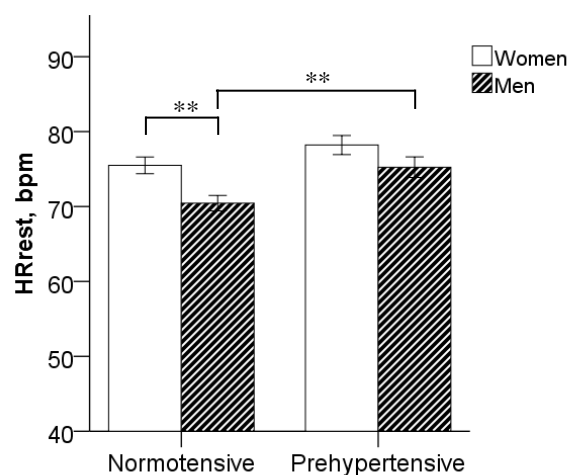


Figure 1. Effect of sex and prehypertension on HR at rest, $**P<0.01$. Sex: $P=0.001$. Prehypertension: $P=0.002$. Sex* Prehypertension: $P=0.393$.

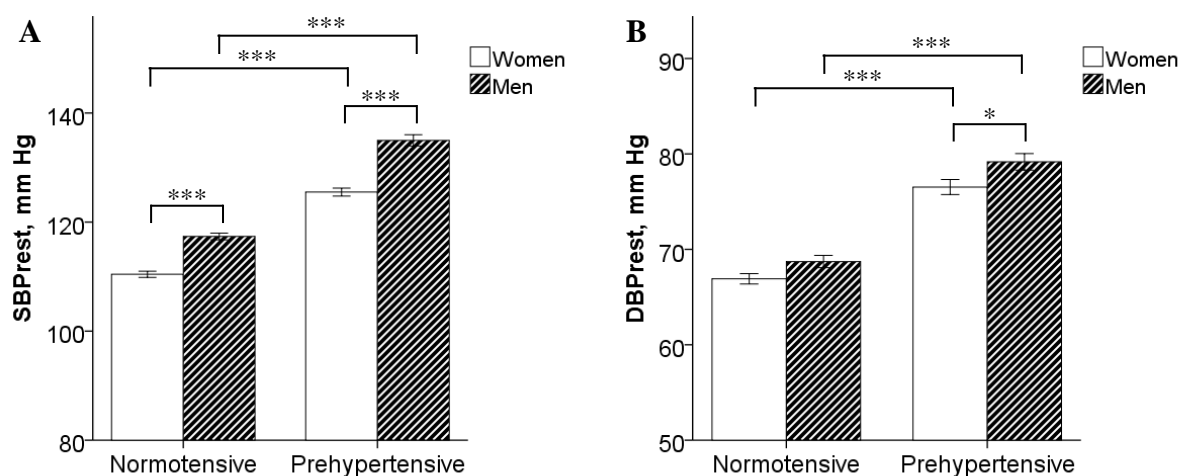


Figure 2. Effect of sex and prehypertension on resting SBP (A) and DBP (B). $*P<0.05$; $***P<0.001$. For SBP, Sex: $P<0.001$; Prehypertension: $P<0.001$; Sex* Prehypertension: $P=0.096$. For DBP, Sex: $P=0.002$; Prehypertension: $P<0.001$; Sex* Prehypertension: $P=0.557$.

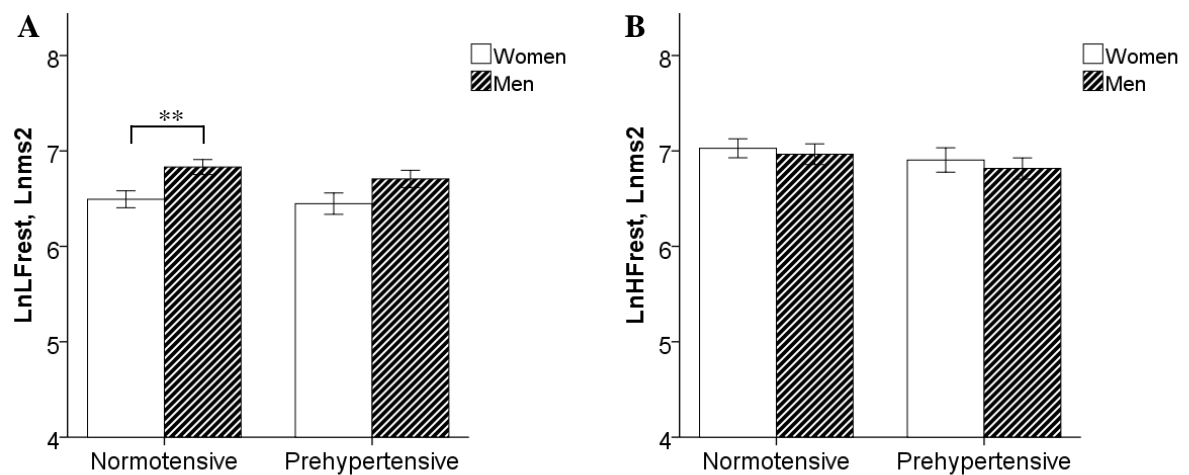


Figure 3. Effect of sex and prehypertension on resting LnLF power (A) and LnHF power (B). ** $P < 0.01$. For LnLFrest, Sex: $P = 0.001$; Prehypertension: $P = 0.359$; Sex* Prehypertension: $P = 0.674$.

normotensive men. However, the differences in the increment of BPs between normotensive and prehypertensive women did not reach significant level (Figs. 4A and 4B).

The increment of SBP and DBP was less in prehypertensive men compared with prehypertensive women (Figs. 4A and 4B). As a result, despite resting DBP was higher in prehypertensive men than in women (Fig. 2B), sex-based differences in DBP among prehypertensives were no longer significant during the immersion of hand into ice cold water (not shown). The analysis of simple effects revealed that sex-based differences in the increment of blood pressure between normotensive did not reach significant level (Figs. 4A and 4B).

No statistically significant sex-based and/or prehypertension-based differences were found in the increment of HR.

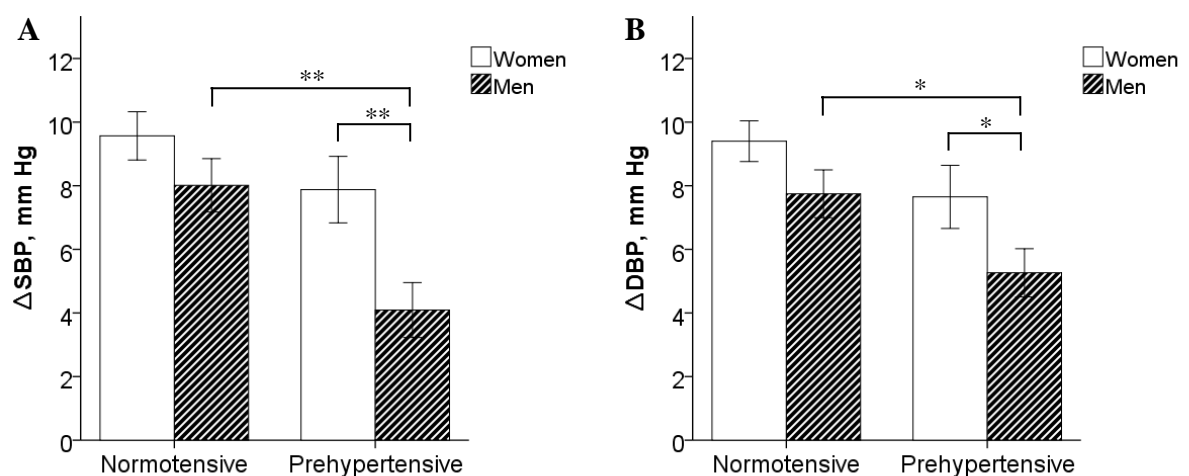


Figure 4. Effect of sex and prehypertension on SBP (A) and DBP (B) response to cold pressor test. * $P < 0.05$; ** $P < 0.01$. For Δ SBP, Sex: $P = 0.002$; Prehypertension: $P = 0.001$; Sex* Prehypertension: $P = 0.204$. For Δ DBP, Sex: $P = 0.010$; Prehypertension: $P = 0.007$; Sex* Prehypertension: $P = 0.637$.

When BMI was taking into account by means MANCOVA, the significance level of sex- and PHT-based differences in SBP, and DBP responses was increased. However, when height was also taking into account, the sex-based differences in Δ SBP and Δ DBP were no longer significant (for main effect, $P=0.002$ and $P=0.010$ without covariates, and $P=0.086$ and $P=0.076$ with height as a covariate, for Δ SBP and Δ DBP, respectively). At this time, PHT-based differences in SBP, and DBP responses did not change (not shown).

Table 2. Predictors of SBP and DBP responses to cold pressor tests. Final stepwise regression coefficients of the Models including centered variables, two-way and three-way interactions. The reference category was normotensive men.

Models	Unstandardized		Standardized	P
	Coefficients		Coefficients	
	B	Std. Error	Beta	
Model 1. Dependent variable: ΔSBP, $R^2=0.235$				
(Constant, mm Hg)	7.513	0.401		<0.001
Δ HR, bpm	0.582	0.076	0.394	<0.001
SBPrest, mm Hg	-0.171	0.036	-0.254	<0.001
BMI, kg/m ²	0.245	0.108	0.121	0.024
LnLFrest*Height, ms ² *m	-14.097	4.845	-0.169	0.004
HRrest*Height, bpm*m	-0.757	0.372	-0.119	0.043
Δ HR*BMI, bpm*kg/m ²	0.042	0.021	0.102	0.045
Model 2. Dependent variable: ΔDBP, $R^2=0.211$				
(Constant, mm Hg)	7.542	0.350		<0.001
Δ HR, bpm	0.339	0.100	0.262	0.001
DBPrest, mm Hg	-0.215	0.045	-0.253	<0.001
BMI, kg/m ²	0.317	0.094	0.187	0.001
Δ HR*Women, bpm	0.299	0.134	0.172	0.027

Predictors of SBP and DBP responses to cold pressor test. The immersion of the hand into ice-cold water caused an increment of SBP and DBP positively associated with Δ HR and BMI and negatively associated with SBPrest and DBPrest, respectively (Table 2). The SBP response to cold stress also was reduced in subjects with high values of resting LnLF power, and this effect dependent on their height. The association between Δ DBP and Δ HR was stronger in women.

Discussion

The main findings of our study are the following: The SBP and DBP response to CPT was less in prehypertensive men compared with normotensive men and prehypertensive women. The LnLFrest was significantly lower in normotensive women than in men. The Δ SBP was negatively associated with LnLFrest and this effect dependent on the height. In addition to negative association between Δ BP and resting blood pressure, Δ SBP and Δ DBP were positively associated with Δ HR and BMI.

CPT as a sympathoexcitatory maneuver. The fact that the increment of blood pressure was positively associated with Δ HR supports the notion that CPT commonly is used as a sympathoexcitatory maneuver to provoke an increment in both arterial pressure and heart rate [6-10, 13-15, 23].

The positive association of Δ DBP with Δ HR was even stronger in women. This fact may indicate that HR determines DBP *per se* and/or contribution of the sympathetic nervous system into DBP response to CPT is more in women than in men.

Baseline blood pressure as a predictor of BP response to CPT. Although the results of this study showing that the increment of SBP and DBP during cold pressor test was less in prehypertensives as a whole (significant main effect), prehypertensive men demonstrated significantly lower BP responses to cold pressor test than normotensives (significant simple effect) and prehypertensive women tended to have lower BP increments than normotensives women, are in contrast with some previous findings [8, 10, 23, 24], they are in agreement with other studies which did not find any effects of hypertension or positive family history of hypertension on hemodynamic functions during the cold pressor test [7, 14] or also find that cold pressor test produced an increase of the arterial pressure significantly lower in the hypertensive than in the normotensive subjects [5, 9, 13, 15, 16]. The inconsistency of our data and result from some previous reports may be due to difference in ethnicity, age, stage of hypertension, and also the fact that gender, BMI, height as well as LnLFrest was not taken into account.

Sex-based differences of BP response to CPT. These results showed that the increment of SBP and DBP during CPT in women group, as a whole, was greater than in men are in congruence with some previous studies [24]. Also, as it was shown previously by Benetos and Safar, blood pressure elevation during CPT is a very common reaction in young normotensive subjects especially in those that have the lowest mean blood pressure [16]. Since in the current study women had blood pressure less than men, the increment of blood pressure was more. In addition, when height was used as a covariate, the sex-based differences in Δ SBP and DBP were no longer significant. Also, the multiple linear regression revealed that the increment of SBP was negatively associated with LnLFrest by Height interaction, such that the shorter was a participant the less LnLF power

reduced increment of SBP. Also, Δ SBP was negatively associated with HR_{rest} by Height interaction, the shorter was a participant with high HR, the more was increment of SBP. Thus, the sex-based differences of Δ SBP and Δ DBP in women may be partially explained by the lower blood pressure and short stature of women.

BMI as a predictor of BP response to CPT. As it was shown in the current study, the increment of SBP and DBP during CPT was positively associated with BMI but negatively associated with presence of prehypertension (Table 2). This finding and the fact that obesity is a major risk for essential hypertension [25] may partially explain results from some previous reports indicating higher reactivity to CPT in hypertensive subjects. However, despite the importance of obesity in the pathogenesis and progression of hypertension due at least in part to increases in sympathetic nerve activity, increased SNS activity in various tissues appears to be differentially controlled in obesity [25]. In addition, an obesity paradox was described, in that overweight and obese patients have a more favorable prognosis than leaner patients [26]. A promising mediator of obesity-induced SNS activation and hypertension development, is leptin [25]. However, obesity may induce selective leptin resistance, and furthermore, humans with loss-of-function mutations in leptin gene or leptin receptor have low BP despite severe obesity [27]. Thus, taking into account these findings, we cannot identify effects of prehypertension and obesity on CPT responses and it is not surprising that obesity and hypertension differently effect cardiovascular response to cold stress.

Resting LnLF HRV power as a predictor of BP response to CPT. Despite LF HRV power is widely used as a measure of cardiovagal activity, interpretation of LF HRV is highly controversial. It was suggested that LF HRV power provides information about the sympathetic or/and parasympathetic outflow, baroreflex modulation of cardiac autonomic outflows, vasomotor tone modulation, and mechanisms of central autonomic outflow [19, 20, 21, 28]. According to our hypothesis based on ours and others previous investigations, LF power is an index of the baroreflex modulation of cardiac autonomic outflows, specifically, of the sympathetic limb of the negative feedback controlling mechanism, that is sympathoinhibitory arm of baroreflex [6]. In this study the increment of SBP was negatively associated with resting LnLF power dependently on height. This finding may indicate that in the young subjects the baseline sympathoinhibitory influences prevent from the increase of blood pressure during sympathoexcitatory maneuver - cold pressor test.

conclusion : the SBP and DBP response to CPT, negatively associated with resting blood pressure and positively associated with BMI, was less in prehypertensive men compared with normotensive men and prehypertensive women. The sex-based differences of Δ SBP and Δ DBP may be partially explained by the lower blood pressure and short stature of women and by the fact

that Δ SBP was negatively associated with resting LnLF which was less in women and that this effect dependent on the height.

Limitations of the Study

One of limitations of this study is that women were not controlled for menstrual cycle. Taken into account a large sample size of participants it is suggested that there would be minimal effect of sex hormones on the data.

We did not control for socioeconomic status. However, all participants were recruited from the student population of the local University; they had similar education level and lifestyle.

We did not control for a positive family history of hypertension. Although to control for a positive family history of hypertension is ideal, the level of family history hypertension awareness was very low and even participant's prehypertension awareness was inadequate and low.

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