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Research Article

The Effect of Metabolic Syndrome and Diabetes Mellitus Type 2 on the Arterial Reactivity of Hypertensive Patients during Cold Pressor Test

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Background: Arterial hypertension (AH), metabolic syndrome (MS) and diabetes mellitus type 2 (DM2) are interrelated metabolic disorders. The aim of our study was to evaluate how the coexistence of MS or DM2 with AH influences arterial reactivity during cold pressor test (CPT). *Methods*: We studied 102 patients, 32 with AH (Group A), 38 with AH and MS (Group B) and 32 with AH and DM2 (Group C). All patients underwent full laboratory evaluation and measurement of systolic and diastolic blood pressure (SBP and DBP), heart rate (HR) and carotid-femoral pulse wave velocity (PWVc-f) before and during CPT. *Results*: During CPT PWVc-f, SBP, DBP and HR were increased significantly in all studied groups, but the change of PWVc-f and HR during CPT was significantly greater in group A compared to group C. On the contrary, the coexistence of MS or DM2 with AH does not alter the response of BP to CPT. *Conclusion*: The increase of CV risk resulting from the coexistence of MS or DM2 with AH, is best expressed by PWVc-f, while the change of the former and HR during CPT possibly reflects dysfunction of the autonomic nervous system.

1. Introduction

Cardiovascular disease (CVD) is the principal cause of death globally [1], and arterial hypertension (AH) is the most common and the most serious cardiovascular (CV) risk factor. AH usually coexists with metabolic disorders, such as metabolic syndrome (MS) and diabetes mellitus type 2 (DM2). AH is one of the diagnostic criteria of MS; the prognostic value of the existence of MS in patients with AH is significant. PIUMA study [2], PAMELA study [3], and the study of Pierdomenico et al. [4] showed that MS is an independent risk factor of coronary disease and stroke in patients with essential AH without obvious CVD. Furthermore, AH occurs twice as often in diabetics than the general population. The prevalence of AH in DM patients reaches up to 70–80% [5]. When these two entities coexist, the risk of stroke or CVD (coronary heart disease, cardiac failure, peripheral arterial disease), increases 5 to 6 times compared to hypertensives without DM2.

Large artery stiffness is an independent prognostic factor of both total and CV mortality. The gold standard for evaluating arterial stiffness is pulse wave velocity (PWV) [6], which has been shown to predict CV risk [7]. Specifically, PWV has been established as a powerful and independent prognostic factor of CVD and mortality, especially in patients with end stage kidney disease [8], AH [7], DM2 [9], in the elderly [10], but also in the general population [11], independently of confounding factors, such as age, AH, or the mass of the heart [12].

Cold pressor test (CPT) was introduced in 1932 by Hines and Brown as a standard stimulation for increasing arterial blood pressure (BP) and for estimating reactivity of the CV

system [13]. Since then, there is growing evidence that the degree of CV reactivity to laboratory stressors, such as mental stress and CPT, is predictive for the future development of CV disease [14–16]. Therefore, conversely, it is reasonable to predict that hypertensives with increased CV risk, as are those with MS or DM2, will present different CV responses to sympathoexcitation.

The aim of this study was to estimate whether the worsening of the CV profile because of the coexistence of MS or DM2 with AH affects adversely the elastic properties and the reactivity of vessels to CPT.

2. Methods

2.1. Study Population and Definition. This study was based on the pool of hypertensive patients who attended the outpatient Hypertension Unit of Onassis Cardiac Surgery Center, Athens, Greece. We selected 102 patients, 32 with AH without other major CV risk factors (Group A, mean age 48 ± 10, 19 men), 38 with AH and MS (Group B, mean age 51 ± 12, 22 men), and 32 with AH and DM2 (Group C, mean age 57 ± 9, 18 men).

The diagnosis of AH was made according to the 2007 European Society of Hypertension/European Society of Cardiology guidelines for adults [17], using Dinamap PRO 100 Automated Oscillometric Device. All patients did not take any medicine when the study was conducted. They had either newly diagnosed AH or there had been a wash-out period of 15 days.

For the diagnosis of MS, we used the definition of NCEP/ATP III [18], which demands that three of the following five criteria are met: waist circumference ≥102 cm in men and ≥88 cm in women, BP ≥130/85 mmHg, blood glucose ≥110 mg/dL, plasma triglycerides ≥150 mg/dL, and HDL cholesterol <40 mg/dL in men and <50 mg/dL in women.

For the diagnosis of DM2, we used the definition of ADA [19]: (1) Symptoms of diabetes and a casual plasma glucose ≥200 mg/dL (11.1 mmol/L). Casual is defined as any time of day without regard to time since last meal. The classic symptoms of diabetes include polyuria, polydipsia, and unexplained weight loss. (2) Fasting plasma glucose ≥126 mg/dL (7.0 mmol/L) fasting is defined as no caloric intake for at least 8 h. (3) 2 h plasma glucose ≥200 mg/dL (11.1 mmol/L) during an oral glucose tolerance test. The test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.

Medical history was obtained from all patients, and then they were subjected to physical examination, electrocardiograph, echocardiogram and color Doppler ultrasound of carotid arteries. Accordingly, patients with ventricular arrhythmias, sinus bradycardia or tachycardia or atrioventricular conduction defects, coronary heart disease, secondary hypertension, obstructive arteriopathy, carotid stenosis, aneurysm of the ascending aorta, and any condition that affects the PWV were excluded from the study.

For each subject weight, height, and waist to hip ratio (W/H) were appropriately measured. Waist circumference was measured at the midpoint between the bottom of the rib cage and above the top of the iliac crest in expiration to the nearest 0.1 cm. Hereupon, the body mass index (BMI) was calculated.

In addition, all subjects underwent full laboratory evaluation (lipid profile and kidney function indices). The blood samples were collected from the antecubital vein between 8 a.m. and 10 a.m., in a sitting position, after 12 h of fasting and alcohol absence. The biochemical evaluation was carried out in the same laboratory that followed the criteria of the World Health Organization Lipid Reference Laboratories.

The study protocol has been approved by the Ethics Committee of Onassis Cardiac Surgery Center, Athens, Greece, and informed consent was obtained by all participants in the study.

2.2. Measurement of Arterial Stiffness and Cold Pressor Test. Hemodynamic measurements were conducted in the morning, between 8 and 11 a.m., in a quiet environment, at stable temperature, 24°C. The patients were resting in the supine position for 15 minutes. They were requested to abstain from food, smoking, and caffeine for at least three hours and from alcohol and intense exercise for at least 24 hours before arterial stiffness was measured. SBP, DBP, and HR were measured in the left hand with automatic mercury (Omron M4-I, CE 0197, Netherlands). All participants were subjected to PWV measurement; CPT was performed directly afterwards, in the end of which the second measurement of PWV was conducted. Patients were familiarized with the procedure of measuring PWV, as well as with CPT.

PWVc-f was calculated noninvasively with a validated semiautomated device (Complior System, Colson, Garges Les-Gonesse, France). Complior uses three pressure transducers, which are placed upon superficial arteries, such as the right radial artery, the right common carotid artery, and the right femoral artery, in order for the aorta to be included in the route of the pulse wave. The distances between these three points were measured, and Complior calculated the transit time of the pulse wave. Ten waves were recorded and PWV was obtained from the quotient of the distance between the two points in meters (carotid and femoral artery) to the transit time in seconds which is measured between the two feet of the pulse waves.

As mentioned above, CPT followed immediately after this first measurement of PWV. During CPT patient's hand was immersed from the fingernails to the wrist in cold water (4°C) for three minutes. On the 120th second after the immersion of the hand, BP was measured in the same arm and simultaneously, the second measurement of PWVc-f was taking place. Patients were told to avoid isometric contraction, Valsalva maneuvering, or holding expiration during CPT to avoid affecting hemodynamic parameters.

2.3. Statistical Analysis. The normal distribution of each variable was confirmed by the Kolmogorov-Smirnov normality test. Descriptive statistics and frequencies were estimated for scale and ordinal variables, respectively. Pearson (Spearman) coefficient was used to compute the correlation

of scale variables normally (not normally) distributed. The correlation among ordinal variables was tested by the chi-square test. Comparisons between the baseline characteristics of the three study groups were performed with analysis of variance (ANOVA) and post hoc Tukey tests. The effect of CPT on PWVc-f, SBP, DBP, and HR was checked by the paired t-test. In order to quantify the change of PWVc-f, SBP, DBP, and HR generated by CPT, we used percentage change (Δ) of these parameters, which was defined as 100%× ((the value during CPT−the value before CPT) divided by the value before CPT). The percentage change in the aforementioned parameters was considered more reliable and tangible, because the baseline value (before CPT) was taken into account. The comparison of the % change among the three groups was also carried out by ANOVA and post hoc Tukey tests. Bootstrap sampling was performed on each statistical test to deal with the effect of small sample. For all tests, statistical significance was set at a level of $P < 0.05$. Statistical analysis was performed using SPSS package for Windows version 17.0 (SPSS Inc., Chicago, IL, USA).

3. Results

Table 1 shows the anthropometric characteristics, the laboratory tests, and the hemodynamic parameters of the groups. There were no significant differences in sex, smoking, height, weight, total cholesterol, low density lipoprotein (LDL), creatinine, SBP, and HR among the three groups. Statistically significant differences between groups were found for the following baseline characteristics: age ($P < 0.05$), waist circumference ($P < 0.05$), hip circumference ($P < 0.05$), BMI ($P < 0.001$), W/H ($P < 0.05$), HDL cholesterol ($P < 0.05$), serum triglycerides ($P < 0.001$), serum glucose ($P < 0.001$), HbA1c ($P < 0.001$), DBP ($P < 0.05$), and PWVc-f ($P < 0.05$).

Post hoc tests indicated that group C revealed statistically higher means than group A regarding age ($P < 0.05$), W/H ($P < 0.05$), HbA1c ($P < 0.001$), and serum glucose ($P < 0.001$). Group B also revealed statistically higher means than group A for BMI ($P < 0.001$) and hip circumference ($P < 0.05$), whereas the latter group revealed statistically lower means ($P < 0.05$) than the other two groups (B and C) for serum triglycerides and waist circumference. The opposite stands in the case of HDL ($P < 0.001$ for both of them). Mean values of DBP were found statistically higher in group A compared to group C ($P < 0.05$). In the case of PWC c-f the mean values of group C were statistically higher than the two other groups (A and B) ($P < 0.05$ for both of them).

Pearson correlation was used to examine the correlation between the baseline value of PWVc-f and demographic characteristics or hemodynamic measurements in each group. It seems that PWVc-f is correlated positively with age in group A ($r = 0.583$, $P < 0.001$) and C ($r = 0.492$, $P < 0.05$), but not in B ($r = 0.265$, $P = 0.108$) and with SBP ($r = 0.477$, $P < 0.05$), HDL ($r = -0.353$, $P < 0.05$), and W/H ($r = 0.413$, $P < 0.05$) only in group C.

Mean values of PWVc-f, SBP, DBP, and HR were statistically higher during CPT (Figure 1) in all three groups. All paired t-tests revealed a statistical significance less than 0.001, except for HR ($P < 0.05$) (Table 2). By conducting one-way ANOVA test (Table 3) and post hoc multiple comparisons it was found that group A presented statistically higher mean value of ΔPWVc-f ($F = 3.201$, $P < 0.05$) and ΔHR ($F = 6.317$, $P < 0.05$) compared to that of group C. No statistical significance was found for ΔSBP ($F = 0.214$, $P = 0.807$) and ΔDBP ($F = 2.159$, $P = 0.121$). Group B noted higher ΔSBP values and lower ΔDBP values compared to group A and C. However, mean differences were not statistically significant. The effect of statistically significant baseline characteristics, such as age, on group differences was investigated by univariate procedures. No significant interaction was found on ΔPWVc-f, ΔSBP, ΔDBP, and ΔHR.

4. Discussion

The main results of this study are the following: (a) at rest arterial stiffness is higher in the most metabolically compromised group of hypertensive-diabetic patients (group C), (b) CPT increases significantly PWVc-f and the rest of hemodynamic parameters in all hypertensive groups, and (c) group A showed a more pronounced increase of PWVc-f and HR compared to that of group C, while that difference wasn't noticed in the changes of SBP and DBP.

Comparing the baseline characteristics of the three studied groups, we realize that the noticed differences are expected; some of them by definition of the groups, such as fasting glucose, HbA1c, triglycerides, hip and waist circumference, BMI, and HDL and some of them, such as age, DBP, and PWVc-f, due to pathophysiological mechanisms. In particular, age and PWVc-f are increased, while DBP is decreased from group A to group C. These trends through groups are completely rational, as the observed increase of mean age from group A to group C is accompanied by a decrease in mean DBP, because of the loss of the elasticity of large arteries and the consequent increase in arterial stiffness. However, it is important to highlight that the increase of PWVc-f is not only due to the effect of aging, but also due to the worsening of the CV profile, confirming the prognostic role of PWVc-f in CV events [7]. Our results agree with the study of Tedesco et al. [20], which showed that patients with AH and DM2 have higher arterial stiffness compared to those with one disease or another. To the best of our knowledge our study is the first to compare arterial stiffness in these three groups of patients at rest and during CPT.

It is widely known that CPT is an acute stress test which provokes generalized sympathetic activation and consequently it increases SBP, DBP, and PWV, while the change of HR is not consistent [21–23] in normotensive and hypertensive subjects. In the literature so far, studies compare CV response to CPT in patients with AH [24–26] or with MS [27] or with DM2 [28–30] with healthy controls and not with each other. In our study, all the studied hemodynamic parameters increased during CPT in a statistically significant way in all three groups, as was expected.

But comparing the response of these three groups, we realize that although BP changed proportionally [ΔSBP $P = 0.807$, ΔDBP $P = 0.121$], PWVc-f ($F = 3.201$, $P < 0.05$) and

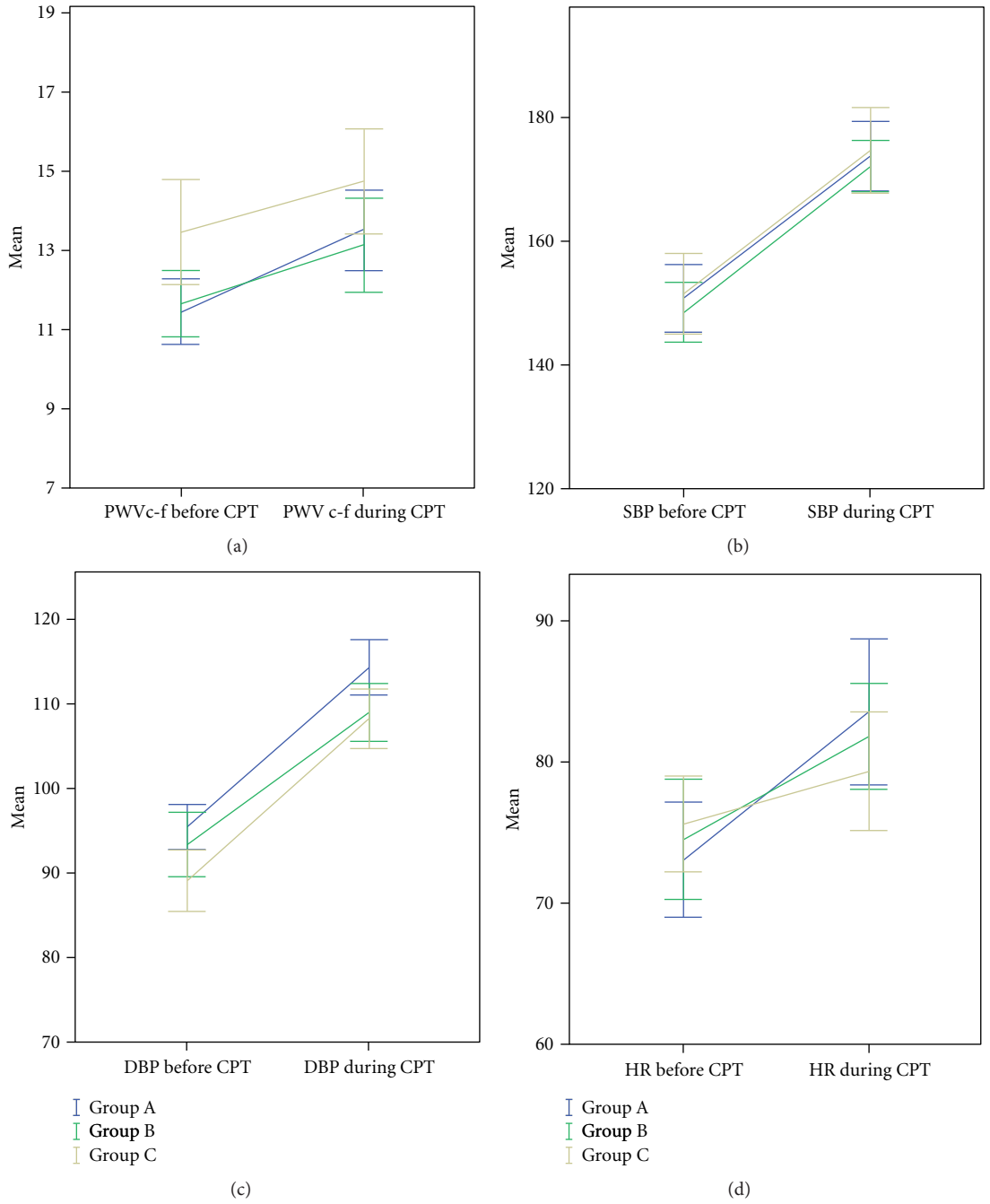

Figure 1: Mean differences of cardiovascular measures before and during CPT.

HR ($F = 6.317$, $P < 0.05$) increased to a greater extent during CPT in group A compared to that of group C. Namely, the most important differences in the change of arterial stiffness and HR are noticed in the metabolic extremes or in other words, between the groups with the lowest (Group A) and the greatest (Group C) CV risk.

CPT can be also used as an index of the integrity of sympathetic function at the efferent level. The different magnitude of the increase of HR during CPT in patients with AH and DM2 in comparison with the other groups may be due to the relation of DM2 to dysfunction of autonomic nervous system (ANS), which is widely known as diabetic cardiovascular autonomic neuropathy (DCAN) [31–33]. DCAN occurs when peripheral autonomic fibers of the CV system are affected, thus resulting in imbalance of sympathetic nervous system (SNS), parasympathetic nervous system (PNS) with relative predominance of SNS and consequently in disturbances of neurohumoral regulation [32]. This imbalance affects the control of HR and the dynamics of central and peripheral vessels [34] and consequently is

Table 1: Baseline characteristics of the study population.

	Group A ($n = 32$)	Group B ($n = 38$)	Group C ($n = 32$)	P value (ANOVA)
Age (years)	48 ± 10	51 ± 12	57 ± 9	0.003
Sex (males)	19 (59.4%)	22 (57.9%)	18 (56.3%)	0.968
Smokers	9 (28.1%)	9 (23.7%)	6 (18.7%)	0.676
Height (cm)	173.8 ± 9.1	171.1 ± 7.7	172.5 ± 9.2	0.418
Weight (kg)	83.5 ± 15.4	90.9 ± 11.2	87.5 ± 12.7	0.065
Waist (W) (cm)	98.8 ± 12.8	107.13 ± 9.6	106.8 ± 8.9	0.002
Hip (H) (cm)	107.2 ± 7.7	113.5 ± 6.9	111.0 ± 8.8	0.004
BMI (kg/m^2)	27.5 ± 3.7	31.1 ± 3.7	29.4 ± 3.6	<0.001
W/H	0.92 ± 0.07	0.94 ± 0.07	0.96 ± 0.05	0.036
Total cholesterol (mg/dL)	203.8 ± 36.4	219.1 ± 36.6	204.9 ± 39.6	0.162
HDL (mg/dL)	54.7 ± 11.8	46.7 ± 13.4	45.5 ± 9.1	0.003
LDL (mg/dL)	130.0 ± 31.6	137.1 ± 38.2	130.5 ± 33.5	0.629
Triglycerides (mg/dL)	95.4 ± 33.9	176.6 ± 66.3	144.4 ± 74.2	<0.001
Fasting glucose (mg/dL)	90.4 ± 10.7	109.4 ± 11.7	129.6 ± 27.6	<0.001
HbA1c (%)	5.12 ± 0.55	5.57 ± 0.48	6.60 ± 1.08	<0.001
Creatinine (mg/dL)	0.94 ± 0.18	0.92 ± 0.19	0.95 ± 0.24	0.794
SBP (mmHg)	150.8 ± 15.5	148.5 ± 2.6	151.5 ± 18.3	0.713
DBP (mmHg)	95.4 ± 7.6	93.4 ± 11.8	89.1 ± 10.3	0.042
HR (bpm)	73.1 ± 11.5	74.5 ± 13.1	75.7 ± 9.6	0.676
PWVc-f (m/sec)	11.5 ± 2.3	11.7 ± 2.6	13.5 ± 3.7	0.012

Values are means ± S.D. or numbers (%).
Group A: patients with arterial hypertension, Group B: patients with arterial hypertension and metabolic syndrome, Group C: patients with arterial hypertension and diabetes mellitus type 2, HDL: high density lipoprotein, LDL: low density lipoprotein, HbA1c: hemoglobin A1c, SBP: systolic blood pressure, DBP: diastolic blood pressure, HR: heart rate, and PWVc-f: carotid-femoral pulse wave velocity.
ANOVA: analysis of variance.

related to decreased HR variability, increased nocturnal BP, and decreased dipping of nocturnal BP and loss of circadian rhythm [32, 35].

In our study population, patients with AH and DM2 present with slightly greater HR before CPT and raise it to a lesser extent during CPT compared to the other groups, possibly because of DCAN. According to the literature, the most important risk factors for DCAN in patients with DM2 are the duration of diabetes and poor glycemic control [36], followed by being male, hypertension, insulin resistance, obesity, high triglyceride concentration, smoking, alcohol consumption, and, more recently suggested, central obesity measured by waist circumference [35, 37, 38]. Consequently, patients of group C and group B secondarily, are very possible to present DCAN, which can explain the reduced change of HR and possibly of PWVc-f too, during CPT compared to patients of group A.

The fact that the three studied groups differed in the change of HR and PWVc-f, but not of SBP and DBP during CPT, indicates that the change of these parameters is influenced by different factors and can be attributed to different mechanisms. These results are in line with the results of another recent study [39], which suggests that the response of PWV to CPT is due to an underlying active mechanism, different from the known reflex mechanism that is responsible for BP's response to CPT. The purpose of the present study, however, was not to reveal the underlying mechanisms for the changes that occurred but simply to define these changes.

Despite the originality of the findings, some limitations of the present study should be acknowledged. First, the pain caused by cold was not standardized with a proper questionnaire in order to relate pain intensity with the observed responses. Physical activity or menstrual phases of women were not recorded; as a result their potential effect on cardiovascular response could not be assessed.

5. Conclusion

This study shows that patients with AH and DM2 have higher arterial stiffness at baseline and during CPT compared to those with AH alone or AH and MS. In addition, the change of HR during CPT was in line with the change of arterial stiffness, while BP was comparably increased in the three studied groups. So, we can conclude that the worsening of the CV profile due to accumulation of metabolic disorders is best expressed by PWVc-f, while the change of the former and HR during CPT possibly reflects the dysfunction of ANS.

Table 2: PWVc-f, SBP, DBP and HR before, and during CPT in each group.

	Before CPT	During CPT	*t* value	*P* value
		Group A		
PWV	11.5 ± 2.3	13.5 ± 2.9	7.642	<0.001
SBP	150.8 ± 15.5	173.7 ± 15.8	14.115	<0.001
DBP	95.4 ± 7.6	114.3 ± 9.3	12.705	<0.001
HR	73.1 ± 11.5	83.6 ± 14.7	7.532	<0.001
		Group B		
PWV	11.7 ± 2.6	13.2 ± 3.7	4.623	<0.001
SBP	148.5 ± 14.8	172.0 ± 12.5	22.138	<0.001
DBP	93.4 ± 11.8	109.0 ± 10.5	15.556	<0.001
HR	74.5 ± 13.1	81.9 ± 11.5	5.204	<0.001
		Group C		
PWV	13.5 ± 3.7	14.8 ± 3.8	5.298	<0.001
SBP	151.5 ± 18.3	174.63 ± 19.5	17.384	<0.001
DBP	89.1 ± 10.3	108.3 ± 9.9	14.501	<0.001
HR	75.7 ± 9.6	79.4 ± 11.9	2.842	0.008

Group A: arterial hypertension, Group B: arterial hypertension and metabolic syndrome, Group C: arterial hypertension and diabetes mellitus type 2, CPT: cold pressor test, SBP: systolic blood pressure, DBP: diastolic blood pressure, HR: heart rate, and PWC c-f: carotid-femoral pulse wave velocity.

Table 3: Comparison of the percentage change of PWVc-f, SBP, DBP, and HR during CPT.

	Group A	Group B	Group C	*P* value
ΔPWVc-f	18.43 ± 13.14	12.54 ± 13.00	10.54 ± 12.94	0.045
ΔSBP	15.46 ± 6.67	16.25 ± 5.63	15.48 ± 5.27	0.807
ΔDBP	20.13 ± 9.94	17.50 ± 8.69	22.25 ± 10.23	0.121
ΔHR	14.53 ± 10.46	10.90 ± 11.94	5.03 ± 9.65	0.003

Group A: arterial hypertension, Group B: arterial hypertension and metabolic syndrome, Group C: arterial hypertension and diabetes mellitus type 2, CPT: cold pressor test, ΔSBP: % change of systolic blood pressure, ΔDBP: % change of diastolic blood pressure, ΔHR: % change of heart rate, and ΔPWC c-f: % change of carotid-femoral pulse wave velocity.

Conflict of Interests

The authors declare that they have no conflict of interests.

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