Masked Hypertension and Atherogenesis: The Impact of Apelin and Relaxin Plasma Levels

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Recent evidence demonstrates that masked hypertension (MH) is a significant predictor of cardiovascular disease, while apelin and relaxin are two novel factors with a significant role in vascular regulation. Apelin is an adipokine that elicits endothelium-dependent vasorelaxation and reduces arterial blood pressure, while relaxin is a protein hormone that induces the production of nitric oxide and vascular endothelial growth factor and inhibits endothelin and angiotensin II. This study aimed to investigate whether apelin and relaxin plasma levels are affected in patients with MH and compare the findings with those of healthy normotensives. One hundred-thirty (60 men, 70 women) healthy patients with a mean age of 45±12 years who had clinic blood pressure <140/90 mmHg were studied. The whole study population underwent 24-hour ambulatory blood pressure monitoring (ABPM). According to the ABPM recordings, 24 individuals (8 men, 16 women) had MH and the remaining 106 patients (52 men, 54 women) had normal ABPM recordings. Apelin and relaxin plasma levels were determined in both groups (enzyme-linked immunosorbent assay method). The apelin (220±121 vs 315±147 pg/mL, P=.001) and relaxin (35.2±6.7 vs 56.8±13.6 pg/mL, P<.001) plasma levels were significantly lower in the masked hypertensive group compared with normotensive controls. Our findings suggest that patients with masked hypertension have significantly lower apelin and relaxin levels. This observation may have prognostic significance for future cardiovascular events in patients with MH and needs further investigation. *J Clin Hypertens (Greenwich).* 2013;15:333–336. ©2013 Wiley Periodicals, Inc.

The phenomenon of masked hypertension (MH) is defined as a clinical condition when patient office blood pressure (BP) is <140/90 mm Hg but ambulatory or home BP readings are in the hypertensive range.[1,2] Different BP thresholds have been proposed to define MH, making it difficult to compare results from various studies. Indeed the prevalence of MH in the general population could be as high as 10%, while data obtained in several cross-sectional studies have demonstrated large differences with prevalence rates from a low of 8% to a high of 49%.[3–6] A body of evidence indicates that MH is a significant predictor of cardiovascular disease. Data obtained from several cross-sectional studies reported that MH is associated with increased left ventricular mass index[7–9] and carotid intima-media thickness.[10] Furthermore, in longitudinal studies, MH was a strong predictor of cardiovascular outcome,[11] mortality[12] and target organ damage.[13,14]

Apelin, a recently described adipokine, although synthesized outside adipose tissue, exists in at least 3 forms, consisting of 13, 17, or 36 amino acids, all originating from a common 77-amino-acid precursor. In the cardiovascular system, apelin elicits endothelium-dependent, nitric oxide–mediated vasorelaxation and reduces arterial BP. In addition, apelin demonstrates potent and long-lasting positive inotropic activity, which is preserved even in injured myocardium and is not accompanied by myocardial hypertrophy. Apelin synthesis in adipocytes is stimulated by insulin, and plasma apelin level markedly increases in obesity associated with insulin resistance and hyperinsulinemia.[15] In addition to regulating cardiovascular function, apelin inhibits water intake and vasopressin production.

Relaxin is a protein hormone first described in 1926 by Frederick Hisaw. Humans have 7 members, relaxin-1, -2, and -3 and insulin-like (INSL) peptides 3, 4, 5, and 6. It is an offshoot of the large insulin superfamily. Each member consists of 2 chains, commonly referred to as A and B, which are held together by 2 interchain disulfide bonds and another intrachain disulfide bond present within the A chain. The cysteines residues present in each chain, together with the distinctive disulfide bonding pattern, are conserved across all members of the superfamily.[16–18]

The aim of our study was to investigate whether plasma levels of apelin and relaxin are affected in patients with MH and compare the findings with those of healthy normotensive patients.

METHODS

This is a consecutively recruited cohort. A total of 285 patients who attended the hypertension clinic of our

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hospital were screened. All patients were taking no antihypertensive medication or other medication that interferes with parameters measured and were nonsmokers. A total of 145 of 285 patients met these criteria. Five of 145 patients who were initially enrolled were excluded from the study because of inadequate ABPM recordings. Ten more patients from the normotensive group were excluded because of inadequate blood samples. Finally, the study was performed in 130 (60 men, 70 women) Greek patients with a mean age of 45±12 years who had clinic BP <140/90 mm Hg and attended the hypertension clinic of our hospital. The whole study population underwent 24-hour ambulatory BP monitoring (ABPM). According to the ABPM recordings, 24 individuals (8 men, 16 women) with a mean age of 46±7 years and body mass index (BMI) 25.9±2.1 kg/m^2 had MH (19%) (daytime systolic BP [SBP] ≥135 mm Hg or daytime diastolic BP [DBP] ≥85 mm Hg [group A]) and the remaining 106 patients (52 men, 54 women) with a mean age of 44±6 years and BMI 25.5±2.4 kg/m^2 had normal ABPM recordings (group B). The demographic characteristics of the participants are presented in Table I.

All patients were following a standardized diet before sampling and none had any thyroid functional abnormality. Alcohol consumption was determined by a questionnaire that asked for daily consumption of wine, liquor, and beer; alcohol intake was expressed in grams per day. Information concerning physical activity was obtained from questionnaires that have been previously described.[20] Before the study, written informed consent was obtained from each participant, which was approved by the hospital review committee. At the base-line examination, all participants underwent a physical examination with a medical history, laboratory assessment of risk factors for cardiovascular disease and routine electrocardiogram. The BMI was calculated as weight/height^2 (kg/m^2).

TABLE I. Demographic Characteristics and Laboratory Assessment of the Study Population

	Group A (n=24)	Group B (n=106)	*P* Value
Age, y	46±7	44±6	.2
Sex, male/female	11/13	49/57	.7
BMI, kg/m^2	25.9±2.1	25.5±2.4	.42
SBP clinic, mm Hg	125±8	124±7	.6
DBP clinic, mm Hg	80±3	79±4	.18
Total cholesterol, mg/dL	234±26	232±25	.75
HDL, mg/dL	44±7	41±5	.41
LDL, mg/dL	155±25	152±24	.52
Triglycerides, mg/dL	99±31	102±32	.7
Fasting glucose, mg/dL	106±41	97±33	.3
Hemoglobin A_{1C}	5.6±1.0	5.3±0.5	.16

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure.

Measurement of BP and Laboratory Assessment

Measurements of SBP and DBP were made on the right arm to the nearest mm Hg with the use of a sphygmomanometer. All measurements were made in the supine position after the patient had rested for 15 minutes. Results are the average of measurements obtained on at least 3 separate occasions, which were performed by the same trained nurse, who was not aware of the history of the patients.

The recruitment of MH patients was made according to the European Society of Hypertension Working Group on Blood Pressure monitoring guidelines, which define individuals with MH as those who have clinic BP <140/90 mm Hg and daytime SBP >135 mm Hg or daytime DBP >85 mm Hg.[19,20] BP measurements consisted of clinic BP (see above), home BP (average of morning and evening measurements on a semiautomatic device), and ABPM with Spacelabs 90207 (Spacelabs Healthcare, Issaquah, WA), which recorded BP every 20 minutes during the daytime (between 10 AM and 8 PM) and 40 minutes during nighttime (between midnight and 6 AM) for 24 hours.[3] Patients recorded a daily action profile from which information about the precise times of sleeping and waking were obtained.

Venous blood samples were collected without stasis after 10 minutes of supine rest. Participants were instructed to avoid strenuous physical activity and not to smoke tobacco during the hour preceding this examination, which took place between 8 AM and 9 AM. All patients fasted for at least 12 hours. Serum cholesterol and triglyceride levels were determined by an enzymatic method and low-density lipoprotein was calculated according to the Friedwald formula, because no patient had a triglyceride level higher than 400 mg/dL.

Plasma apelin levels were quantified by a radioimmunoassay (Phoenix Pharmaceuticals Inc, Burlingame, CA) with intraassay and interassay coefficients of variation of 5.9% and 8.2%, respectively. A Quantikine Human Relaxin Immunoassay (DRL200, R&D Systems Inc, Minneapolis, MN) was used according to the manufacturer's protocol to determine the relaxin of the samples. All samples were run in triplicate and serum relaxin concentration values were calculated based on the standard curve, including the zero dose standards. The intraassay and interassay coefficients of variation for relaxin levels were 4.7% and 10.2%, respectively.

Statistical Analysis

Values are expressed as means±standard deviations. Differences between the two groups were analyzed by one-way analysis of variance. The variables that showed significant differences were compared afterward between each group by use of Bonferroni test for multiple comparisons. A *P* value <.05 was accepted as statistically significant.

RESULTS

Clinic and ambulatory BP values are presented in Table I. Apelin and relaxin levels were determined in 26 patients with confirmed MH and 104 healthy normotensives. The 2 groups were not different with respect to age, sex, BMI, smoking status, and lipid profile (Table I). No differences were observed between the two groups regarding physical activity, alcohol consumption, and menopausal status (data not shown). The apelin and relaxin levels were significantly lower in the masked hypertensive group compared with the normotensive control group (Table II).

DISCUSSION

The results of our study have shown that MH is associated with decreased apelin and relaxin plasma levels compared with patients with normal BP. This finding may potentially contribute to an increased incidence of cardiovascular events in this group.

The phenomenon of MH is defined as a clinical condition in which a patient's office BP level is <140/90 mm Hg but ambulatory or home BP readings are in the hypertensive range.[21] The prevalence of MH in the general population could be as high as 10%.[5] The prevalence of MH in our study group was found in 19% of patients, which is in the range reported in the general population.[4,21]A significant body of evidence indicates that MH is a significant predictor of cardiovascular disease and outcome.[22–24]

Apelin is a novel endogenous peptide detected in a variety of tissues and organs, including the heart and vessels, which has been shown to play a role in the physiology and pathology of the cardiovascular system. By activating the G-protein–coupled receptor APJ, apelin produces vasodilation with a subsequent BP reduction via a nitric oxide–dependent mechanism, enhances diuresis by antagonizing arginine vasopressin, and exerts positive inotropic action on the myocardium.[25–28] Apelin production is also regulated by factors such as fasting and refeeding, insulin, hypoxia, growth hormone, and tumor necrosis factor α.[29] Hypertension, with its hemodynamic alterations and cardiac complications, might be an interesting field of exploration; however, the activity of the apelinergic system in this entity has not yet been extensively investigated. Indeed apelin levels were significantly lower in the hypertensive patients compared with the control patients,[30] while hypertensive patients without concomitant diseases that affect cardiovascular functions demonstrated lower plasma apelin levels than controls.[31] Our results have shown that MH is associated with decreased apelin plasma levels and are in accordance with the above-mentioned results indicating the strong correlation between masked hypertension and adipose tissue.

Relaxin is an ovarian hormone secreted by the corpus luteum during gestation in rodents and humans. Its role in the reproductive system has been well documented. More recent studies have shown it to be a pleiotropic hormone, capable of also targeting numerous nonreproductive organs of the cardiovascular, nervous, respiratory, tegumental, and excretory and digestive systems.[32,33] Moreover, relaxin levels are shown to be affected by factors such as fasting and obesity, pregnancy, heart failure, and stress.[34,35]

In the treatment of heart failure, the effects of relaxin include the production of nitric oxide, inhibition of endothelin, inhibition of angiotensin II, production of vascular endothelial growth factor, and production of matrix metalloproteinases.[35] In aged hypertensive rats, it has been demonstrated that relaxin is potent in mediating reversal of arterial remodeling and improving arterial structural compliance.[36] In humans, relaxin levels were significantly lower in hypertensive patients as compared with controls, which are in accordance with our results.[37]

TABLE II. Apelin and Relaxin Levels and Results of ABPM Between Groups A and B

Parameters	Group A (n=24)	Group B (n=106)	*P* Value
Apelin, mg/dL	220±121	315±147	<.001
Relaxin, mg/dL	35.2±6.7	56.8±13.6	<.001
Mean daytime SBP, mm Hg	134±5	121±8	<.01
Mean daytime DBP, mm Hg	88±4	77±4	<.01

Abbreviations: DBP, diastolic blood pressure; SBP, systolic blood pressure.

STUDY LIMITATIONS

To our knowledge, this is the first study to investigate the levels of apelin and relaxin in a patient population with MH. The small number of patients is a potential limitation of this study. Larger studies are needed to establish the potential role of apelin and relaxin in masked hypertensive patients.

CONCLUSIONS

Serum apelin and relaxin levels were decreased in patients with MH. This observation may have prognostic significance for future cardiovascular events in patients with MH. A circadian variation pattern in the levels of apelin and relaxin could possibly explain the variations in BP observed in patients with MH. Further studies are needed to elucidate this issue.

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