Original Paper

Plasma Renalase is Not Associated with Blood Pressure and Brachial-Ankle Pulse Wave Velocity in Chinese Adults With Normal Renal Function

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Key Words

Renalase • Pulse wave velocity • Blood Pressure • Normal kidney function

Abstract

Background/Aims: This study aimed to investigate the association of renalase with blood pressure (BP) and brachial-ankle pulse wave velocity (baPWV) in order to better understand the role of renalase in the pathogenesis of hypertension and atherosclerosis. **Methods:** A total of 344 subjects with normal kidney function were recruited from our previously established cohort in Shaanxi Province, China. They were divided into the normotensive (NT) and hypertensive (HT) groups or high baPWV and normal baPWV on the basis of BP levels or baPWV measured with an automatic waveform analyzer. Plasma renalase was determined through an enzyme-linked immunosorbent assay. **Results:** Plasma renalase did not significantly differ between HT and NT groups ($3.71 \pm 0.69 \mu\text{g/mL}$ vs. $3.72 \pm 0.73 \mu\text{g/mL}$, $P = 0.905$) and between subjects with and without high baPWV ($3.67 \pm 0.66 \mu\text{g/mL}$ vs. $3.73 \pm 0.74 \mu\text{g/mL}$, $P = 0.505$). However, baPWV was significantly higher in the HT group than in the NT group (1460.4 ± 236.7 vs. $1240.7 \pm 174.5 \text{ cm/s}$, $P < 0.001$). Plasma renalase was not correlated with BP levels and baPWV in the entire group. Linear and logistic regression analysis revealed that plasma renalase was

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not significantly associated with hypertension and high baPWV. **Conclusion:** Plasma renalase may not be associated with BP and baPWV in Chinese subjects with normal renal function.

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Introduction

Renalase, first discovered in 2005, is a flavin-adenine-dinucleotide-dependent amine oxidase that is secreted into the blood by the kidneys and is hypothesized to participate in catecholamine metabolism [1]. It is extensively expressed in the kidneys, heart, intestines, liver, skeletal muscles, and blood vessels [2, 3]. In the kidneys, renalase is widely expressed in renal tubule epithelial cells, glomeruli, mesangial cells, and podocytes [4, 5]. Circulating renalase levels markedly increased in patients with end-stage renal disease [6]. Previous evidence revealed that blood renalase levels were also higher in heart transplant recipients with moderate renal dysfunction than in healthy controls and were negatively correlated with estimated glomerular filtration rate (eGFR) [7]. Similar results were observed among 89 prevalent kidney transplant recipients [8]. Therefore, renalase is closely associated with chronic kidney disease (CKD), and blood renalase levels are largely affected by the renal function.

Experimental data provided evidence that recombinant renalase exerted powerful and rapid hypotensive effects on rats and this was suggested to be mediated by circulating catecholamines degradation, which would be expected to decrease cardiac contractility and heart rate [9]. In addition, several renalase polymorphisms appear to be associated with an increased incidence of hypertension [10, 11]. The relationship between circulating renalase and hypertension in humans has been rarely conducted, and findings are conflicting. Plasma renalase was significantly lower in patients with resistant hypertension and hypertensive (HT) patients with aortic coarctation than in normotensive (NT) controls [12, 13]. Conversely, Maciorkowska et al. [14] observed statistically significant higher renalase levels in patients with primary hypertension than in control subjects. Thus, the relationship between plasma renalase and blood pressure, especially in Chinese Han population with normal renal function, has yet to be fully established.

Brachial-ankle pulse wave velocity (baPWV) has been developed as a marker of arterial stiffness that can be measured using a noninvasive automatic device with relative technical simplicity [15]. It has been shown to be associated with intima-media thickness of the carotid artery and aortic PWV, a marker of central arterial stiffness [16]. More importantly, baPWV is a significant and independent predictor of cardiovascular morbidity and mortality in patients undergoing hemodialysis and suffering from acute coronary syndrome [17, 18]. It had been reported that plasma renalase level was correlated with coronary artery disease (CAD), and changes in its level may reflect the degree of coronary artery stenosis [19]. More recently, the first report documented an insignificant correlation between plasma renalase concentration and PWV in patients after repair of coarctation of aorta [12]. Therefore, the relationship between circulating renalase and baPWV remains poorly understood and warrants further investigation.

With these data in consideration, we sought to assess the relationship of renalase with BP and baPWV among Chinese adults with normal kidney function in order to understand the role of renalase in the pathogenesis of hypertension and atherosclerosis

Materials and Methods

Study cohort

In March and April 1987, the cohort of Hanzhong Adolescent Hypertension Study was established on the basis of a baseline survey on 4,623 adolescents aged 6–15 years in over 20 schools in three towns, namely, Qili, Laojun, and Shayan, in Hanzhong, Shaanxi, China [20, 21]. Using an isometric sampling method [22], we randomly selected every tenth subject ($K = 10$) from the large cohort and created a small cohort of 463 subjects.

From May to July of 2013, trained staffs interviewed the participants and collected data on demographics (age, gender, education, occupation, and physical activity), medical conditions, and prescription and nonprescription medication use. Physical examinations were also conducted. baPWV was measured in batches in Hanzhong People's Hospital. Only the participants who completed all the questionnaires and examinations, including physical examination, blood drawing, and BP and baPWV measurements, were included in the study. The exclusion criteria were as follows: (1) secondary hypertension; (2) history of cardiovascular disease, stroke, infectious diseases, abnormal liver function, vascular disease, and chronic obstructive pulmonary disease; (3) serum creatinine $\geq 140 \mu\text{mol/L}$ and/or $\text{eGFR} < 60 \text{ mL/min/1.73 m}^2$ at screening; and (4) inability or unwillingness to participate in all aspects of the study. In the end, 344 subjects were included in our study.

This study was performed in accordance with the Declaration of Helsinki, and the research protocol was approved by the Ethics Committee of the First Affiliated Hospital of Medical School, Xi'an Jiaotong University. Upon admission, the subjects provided their written consent to personal medical data processing.

BP Measurement

BP was measured by three trained staff members using a standard mercury sphygmomanometer, as previously described [23, 24]. In brief, the subjects were instructed to rest in a sitting position for more than 5 min, and their right brachial pressure was measured while they were in a sitting position. Systolic BP (SBP) and Diastolic BP (DBP) were determined as the first and fifth Korotkoff sounds, respectively. Pulse pressure was calculated as follows: $\text{SBP} - \text{DBP}$. The mean arterial pressure (MAP) was determined as follows: $\text{DBP} + (1/3 \times \text{pulse pressure})$. The subjects manifesting a $\text{SBP} \geq 140 \text{ mmHg}$ and/or $\text{DBP} \geq 90 \text{ mmHg}$ or using antihypertensive medications were defined as HT.

baPWV Measurement

baPWV was measured by standard method using tonometry as previously described [23]. Briefly, cuffs with a plethysmographic sensor that acquired waveform data and BP measurements were placed around the arms and ankles bilaterally (BP-203RPEII; Nihon Colin, Tokyo, Japan). The baPWV was calculated as distance/time (cm/s). The average baPWVs from the right and left sides were used for analysis. The subjects with $\text{baPWV} < 1400 \text{ cm/s}$ were considered normal; by contrast, the subjects with $\text{baPWV} \geq 1400 \text{ cm/s}$ were defined as high or atherosclerotic [25].

Biochemical analyses

Overnight fasting blood samples were subjected to biochemical analysis. Total cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), serum creatinine, uric acid, homocysteine, and blood glucose were measured using an automatic biochemical analyzer (model 7600; Hitachi, Ltd., Tokyo, Japan). eGFR was calculated according to Modification of Diet in Renal Disease formula. Plasma renalase levels were assessed by using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Uscn Life Science, Wuhan, China) in accordance with the manufacturer's instructions at a detection range of 3.12–200 ng/mL. The minimum detectable dose of this kit was typically less than 1.38 ng/mL. Five plasma samples were used to evaluate intra- and inter assay coefficients of variation, which ranged from 2.4% to 4.6% and 3.7% to 7.2%, respectively.

Statistical analyses

Data were expressed as means $\pm$ SD for normally distributed values, as median (25th and 75th percentile) for non-normally distributed values, and percentages. In case of normally distributed variables,

Table 1. Demographic and Clinical Characteristics of All, the Normotensive (NT) and Hypertensive (HT) Subjects

Parameters	All	HT	NT	P-value
Age, y	37.0 (35.0-39.8)	37.0 (35.0-41.0)	36.0 (35.0-39.0)	0.028
Sex (M/F)	194/150	78/26	116/124	<0.001
Body mass index (kg/m ²)	24.0 (22.0-26.3)	25.9 (23.3-28.5)	23.3 (21.5-25.5)	<0.001
Alcohol (n, %)	32(12.5)	13(7.9)	19(9.3)	0.336
Smoking (n, %)	146(42.6)	58(55.8)	88(36.8)	0.001
Diabetes mellitus (n, %)	9(2.6)	2(1.9)	7(2.9)	0.455
Systolic blood pressure (mmHg)	122 (114-132)	138 (130-148)	116 (108-123)	<0.001
Diastolic blood pressure (mmHg)	80 (74-90)	96 (90-102)	76 (70-82)	<0.001
Mean arterial pressure (mmHg)	87 (93-104)	109 (105-117)	90 (85-94)	<0.001
plasma renalase (µg/mL)	3.72±0.72	3.71±0.69	3.72±0.73	0.905
Glucose (mmol/L)	4.60±0.87	4.57±0.63	4.61±0.95	0.690
Total cholesterol (mmol/L)	4.33±0.79	4.42±0.81	4.29±0.77	0.162
Triglycerides (mmol/L)	1.36 (1.0-2.0)	1.66 (1.12-2.26)	1.27 (0.95-1.87)	0.001
LDL (mmol/L)	2.40±0.61	2.50±0.64	2.35±0.59	0.034
HDL (mmol/L)	1.67±0.32	1.59±0.28	1.71±0.34	0.002
Homocysteine (µmol/L)	9.2 (7.0-12.1)	9.8 (7.5-14.6)	9.0 (6.8-11.2)	0.006
Serum uric acid (µmol/L)	309.6±83.3	340.9±79.2	296.1±81.5	<0.001
Serum creatinine (µmol/L)	80.5±12.4	84.3±12.6	78.8±12.0	<0.001
eGFR (mL/min/1.73m ²)	89.4±11.5	89.3±11.3	89.4±11.6	0.943
Urine albumin/creatinine (mg/g)	6.25 (4.08-11.37)	7.07 (4.78-13.11)	6.18 (3.85-10.14)	0.043
baPWV (cm/s)	1307.2±219.6	1460.4±236.7	1240.7±174.5	<0.001
Antihypertensive medication				
ACE/ARB inhibitor	36 (10.5%)	36 (34.6%)	—	—
β-blocker	17 (4.9%)	17 (16.3%)	—	—
Calcium channel blocker	42 (12.2%)	42 (40.4%)	—	—
Diuretic	38 (11.0%)	38 (36.5%)	—	—

Abbreviations: HT, hypertensive subjects; NT, normotensive subjects; LDL, low-density lipoprotein; HDL, high-density lipoprotein; eGFR, estimated glomerular filtration rate; baPWV, brachial-ankle pulse wave velocity. ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor. Non-normally distributed variables are expressed as the median (interquartile range). All other values are expressed as mean ± SD or n, %.

Student's *t* test for unpaired samples was performed; Mann-Whitney U test was conducted for non-normally distributed parameters. Chi-square test was carried out to compare categorical variables. Correlations between parameters were determined with Pearson's correlation coefficient if residuals were normally distributed. Otherwise, Spearman's correlation coefficient was determined. To examine the independent relationship among BP, baPWV, and plasma renalase, we constructed multivariate linear and logistic models that included all possible factors: age, gender, body mass index, glucose, total cholesterol, triglycerides, LDL, HDL, homocysteine, serum uric acid, serum creatinine, eGFR, urine albumin/creatinine, alcohol, smoking, and diabetes. Data were appropriately expressed as standardized regression coefficient (β), odds ratios and 95% confidence interval, and probability. Statistical analyses were performed with SPSS version 16.0 for Windows (SPSS Inc., Chicago, IL, USA). Two-tailed $P < 0.05$ was considered significant.

Results

Study Population

344 individuals were included in the final analysis. 104 (30.2) were hypertensive, and 230 (66.8%) were normotensive. Among the 104 hypertensive subjects, 36 (34.6%) were taking an ACE/ARB inhibitor, 17 (16.3%) a β -blocker, 42 (40.4%) a calcium channel blocker, and 38 (36.5%) a diuretic. 7 (6.7%) did not provide information on antihypertensive drug use. Approximately 17.2% of the participants were taking more than one class of antihypertensive medications. The characteristics of the normotensive and hypertensive groups are listed in Table 1. Compared to normotensive counterparts, hypertensive subjects were older,

Fig. 1. Correlations between plasma renalase concentration, systolic blood pressure and diastolic blood pressure in all subjects.

possessed higher BMI, and were more likely to smoke. Triglyceride, LDL, homocysteine, serum uric acid, and serum creatinine were higher in hypertensive subjects than in normotensive subjects, while HDL was lower. baPWV was significantly higher in the HT subjects than in the NT subjects (1460.4 ± 236.7 vs. 1240.7 ± 174.5 cm/s, $P < 0.001$).

Association of plasma renalase levels, hypertension, and BP

Plasma renalase concentration did not significantly differ between HT and NT groups (3.71 ± 0.69 vs. 3.72 ± 0.73 µg/mL, $P = 0.905$, Table 1). Plasma renalase was positively correlated with serum creatinine concentration ($r = 0.109$; $P = 0.043$), but no correlation was observed between plasma renalase and eGFR ($r = -0.103$.

$P = 0.055$). Similarly, plasma renalase concentration did not correlate with SBP ($r = 0.014$, $P = 0.798$), DBP ($r = 0.042$, $P = 0.442$), or MAP ($r = 0.047$, $P = 0.386$) (Fig. 1). Multivariate regression analysis adjusting for age, gender, BMI, glucose, total cholesterol, triglycerides, LDL, HDL, homocysteine, serum uric acid, serum creatinine, eGFR, urine albumin/creatinine, alcohol, smoking, and diabetes demonstrated no association between plasma renalase and hypertension ($OR = 1.041$, $P = 0.825$; Table 2). Likewise, plasma renalase was not associated with hypertension when BP was analyzed as a continuous variable ($\beta = -0.022$, $P = 0.636$ for SBP; $\beta = -0.006$, $P = 0.902$ for DBP; $\beta = 0.006$, $P = 0.906$ for MAP).

Hypertension was reported to be associated with increased urinary albumin excretions [26]. In our study, the HT subjects had a higher urinary albumin excretion [7.07 (4.78 – 13.11) mg/g] than the normotensive subjects [6.18 (3.85 – 10.14) mg/g, $P = 0.043$, Table 1]. Urinary albumin excretion was not associated with renalase ($r = 0.007$, $P = 0.897$, Fig. 3). To confirm our findings, we analyzed their association through multiple linear regression. Indeed, urinary albumin excretion was not correlated with baPWV ($\beta = 0.018$, $P = 0.719$).

Table 2. Effect of Clinical Variables on Risk of Arterial Hypertension Based on Multivariate Logistic Regression*

Variable	OR (95% CI)	P-value
age	0.915 (0.841–0.996)	0.039
BMI	0.782 (0.721–0.849)	<0.001
Gender		
Female	1 (reference)	—
Male	0.315 (0.179–0.554)	<0.001
Renalase	1.041 (0.728–1.488)	0.825

Abbreviations: CI, confidence interval; OR, odds ratio; BMI, body mass index. * Included in the multivariate logistic models: age, gender, body mass index, glucose, total cholesterol, triglycerides, LDL, HDL, homocysteine, serum uric acid, serum creatinine, eGFR, urine albumin/creatinine, alcohol, smoking and diabetes.

Relationship between plasma renalase levels and baPWV

The characteristics of the subjects with normal and high baPWV are listed in Table 3. The subjects with a high baPWV (>1400 cm/s) also had higher SBP, DBP, MAP, BMI, total cholesterol, triglycerides, LDL, serum uric acid, eGFR, and urine albumin/creatinine. They were more likely to be active smoker and hypertensive.

Table 3. Comparison of Demographic and Clinical Characteristics of Subjects with Normal baPWV (baPWV <1400 cm/s) and High baPWV (baPWV ≥1400 cm/s)

Parameters	Normal baPWV	High baPWV	P value
Age, y	37.2±3.0	37.1±3.0	0.88
Sex (M/F)	127/128	67/22	<0.001
Body mass index (kg/m ²)	23.6 (21.7-26.0)	24.8 (22.4-28.0)	0.003
Alcohol (n, %)	24(9.4)	8(9.0)	0.831
Smoking (n, %)	97(38.2)	49(55.1)	0.006
Diabetes mellitus (n, %)	7(2.7)	2(2.2)	0.8
Hypertension (n, %)	48(18.8)	56(62.9)	<0.001
Systolic blood pressure (mmHg)	118 (110-128)	134 (124-148)	<0.001
Diastolic blood pressure (mmHg)	78 (70-86)	90 (80-100)	<0.001
Mean arterial pressure (mmHg)	91 (85-98)	104 (94-115)	<0.001
plasma renalase (μg/mL)	3.67±0.66	3.73±0.74	0.505
Glucose (mmol/L)	4.57±0.79	4.66±1.05	0.406
Total cholesterol (mmol/L)	4.28±0.79	4.48±0.77	0.037
Triglycerides (mmol/L)	1.29 (0.97-1.91)	1.65 (1.06-2.24)	0.009
LDL (mmol/L)	2.35±0.61	2.54±0.56	0.008
HDL (mmol/L)	1.69±0.32	1.62±0.33	0.069
Homocysteine (μmol/L)	9.1 (6.9-11.5)	10.0 (7.3-14.1)	0.113
Serum uric acid (μmol/L)	299.8±83.1	337.7±77.6	<0.001
Serum creatinine (μmol/L)	79.8±12.6	82.6±11.8	0.067
eGFR (mL/min/1.73m ²)	88.5±11.2	91.7±12.2	0.024
Urine albumin/creatinine (mg/g)	6.07 (4.05-10.18)	7.52 (4.26-13.10)	0.021
baPWV (cm/s)	1207.5±120.4	1592.8±186.8	<0.001

Abbreviations: baPWV, brachial-ankle pulse wave velocity; LDL, low-density lipoprotein; HDL, high-density lipoprotein, eGFR, estimated glomerular filtration rate. Non-normally distributed variables are expressed as the median (interquartile range). All other values are expressed as mean ± SD or n, %.

Fig. 2. Correlation between plasma renalase concentration and brachial-ankle pulse wave velocity (baPWV) in all subjects.

Fig. 3. Correlation between plasma renalase concentration and urinary albumin excretion in all subjects.

Plasma renalase did not significantly differ between subjects with and without high baPWV (3.67 ± 0.66 vs. 3.73 ± 0.74 μg/mL, $P = 0.505$). Correlation analysis revealed that baPWV was directly related to BMI, SBP, DBP, MAP, glucose, total cholesterol, LDL, homocysteine, creatinine, triglycerides, and serum uric acid; by contrast, baPWV was not related to plasma renalase concentrations (Fig. 2). Further analyses indicated that eGFR, SBP, gender,

and hypertension status, but not plasma renalase, were independently correlated with baPWV after adjustment for various covariables (Table 4). In multiple linear regression analysis using baPWV as a continuous variable, no correlation between renalase and baPWV was detected ($\beta = 0.026$, $P = 0.515$).

Discussion

In this cohort of Chinese subjects with normal renal function, plasma renalase showed no association with either blood pressure or arterial stiffness as measured by baPWV. To our knowledge, this is the first study examining the relationship of plasma renalase level to blood pressure and arterial stiffness in an Asian cohort.

Only a limited number of clinical studies have examined the relationship between renalase and hypertension, which are summarized in Table 5.

In a clinical study presented as an abstract, Schlaich et al. [13] found that serum renalase was higher in NT controls than in patients with resistant hypertension. Conversely, Maciorkowska et al. [14] argued that patients with primary hypertension showed a significantly higher serum renalase level than NT controls. Schlaich et al. [13] detected renalase concentration through Western blot technique, whereas Maciorkowska et al. [14] used a commercially available ELISA kit. Wybraniec et al. [12] observed lower levels of plasma renalase in HT patients with aortic coarctation than in NT controls. Similar results had been observed in 34 hemodialyzed (HD) patients [27]. However, Koc-Zorawska et al. failed to detect any difference in 60 HD patients with or without hypertension [28]. In these three studies, renalase is estimated using a commercially available kit (USCN Life Science Inc., Wuhan, China) [12, 27, 28]. More recently, Yilmaz et al. [29] used the same method and observed that renalase levels in pregnant subjects with preeclampsia were lower than those in healthy pregnant subjects and non-pregnant controls. Renalase secretion in plasma has been reported to be closely associated with renal functions, including serum creatinine and eGFR, in vivo and in vitro [3]. In the present study, a group of individuals with normal kidney function was recruited from our previous cohort to exclude the effects of renal function on plasma renalase level. Using a commercially available renalase ELISA kit (USCN Life Science, Co., Ltd.), we found that the plasma renalase levels were similar in HT and NT subjects, although baPWV significantly differed between NT and HT groups. We further observed that plasma renalase concentration was not correlated with BP in unadjusted and adjusted analyses. This result is consistent with those reported by Przybylowski et al. [7] and Zbroch et al. [30], who reported that renalase level was not related to BP in heart transplant recipients or patients undergoing peritoneal dialysis. In addition, inconsistent and even contradicting results have also been observed in terms of the relationship between renalase gene polymorphisms and hypertension occurrence. Renalase SNPs, such as rs2296545 and rs2576178 were significantly associated with hypertension [10, 11]. Conversely, other studies have revealed that these SNPs are not associated with hypertension or BP levels [31, 32].

That Western blots and ELISA yield different plasma levels needs further exploration and raises concerns about their proper interpretation, especially in patients with kidney disease. The most relevant issue is that the two antibodies used in the commercial sandwich

Table 4. Effect of Clinical Variables on Risk of High baPWV (≥ 1400 cm/s) Based on Multivariate Logistic Regression*

Variable	OR (95% CI)	P Value
SBP	0.952 (0.928–0.977)	<0.001
eGFR	0.974 (0.949–0.999)	0.04
Gender		
Female	1 (reference)	—
Male	0.532 (0.284–0.994)	0.048
Hypertension		
No	1 (reference)	—
Yes	0.419 (0.194–0.902)	0.026
Renalase	1.081 (0.726–1.61)	0.702

Abbreviations: CI, confidence interval; OR, odds ratio; baPWV, brachial-ankle pulse wave velocity; SBP, systolic blood pressure; eGFR, estimated glomerular filtration rate. * Included in the multivariate logistic models: age, gender, body mass index, glucose, total cholesterol, triglycerides, LDL, HDL, homocysteine, serum uric acid, serum creatinine, eGFR, urine albumin/creatinine, alcohol, smoking and diabetes.

Table 5. Characteristics of human studies on renalase and hypertension

Study ID	Ethnic background	Study design and size	Key findings
Renalase plasma levels are associated with systolic blood pressure in patients with resistant hypertension [#] by Schlaich M et al. 2010 (<i>J Hypertens</i> 2010; 28:e437)	—	Case-control study: Case: patients with resistant hypertension, n = 22 Control: healthy control, n = 4	1) Serum renalase was higher in normotensive controls than in patients with resistant hypertension. 2) Arterial plasma levels of renalase are inversely associated with systolic blood pressure
A novel marker in pregnant with preeclampsia: renalase by Yilmaz ZV et al. 2016 (<i>J Matern Fetal Neonatal Med.</i> 2016;1-6)	Caucasians of Turkish	Case-control study: Case: 1) normotensive pregnancy, n = 40 2) pregnancy with preeclampsia, n = 40 Control: non-pregnancy female, n = 40	1) Healthy pregnant serum renalase levels were significantly higher than non-pregnant controls. 2) Pregnant with preeclampsia had lower renalase levels than healthy controls. 3) Serum renalase levels were inversely associated with blood pressure levels and positively correlated with glomerular filtration rate.
Circulating renalase, catecholamines, and vascular adhesion protein 1 in hypertensive patients by Maciorkowska D et al. 2015 (<i>J Am Soc Hypertens.</i> 2015;9(11):855-64)	Caucasians of Polish	Case-control study: Case: hypertensive subjects, n = 121 Control: healthy control, n = 27	Circulating renalase were significantly higher in patients with hypertension comparing to healthy individuals.
Low plasma renalase concentration in hypertensive patients after surgical repair of coarctation of aorta by Wybraniec MT et al. 2014 (<i>J Am Soc Hypertens.</i> 2014;8(7):464-74)	Caucasians of Polish	Case-control study: Case: patients that receiving Dacron patch repair of aortic coarctation, n = 50 Control: age and gender matched control, n = 50	1) Hypertensive patients exhibit lower levels of renalase than their normotensive counterparts both in case and control groups. 2) Plasma renalase level inversely correlates with serum creatinine, BP and aortic stiffness parameters.
Vascular adhesion protein-1 and renalase in regard to diabetes in hemodialysis patients by Koc-Zorawska E et al. 2012 (<i>Arch Med Sci.</i> 2012 Dec 20;8(6):1048-52)	Caucasians of Polish	Case-control study: Case: hemodialyzed (HD) patients, n = 60 Control: healthy volunteers, n = 16	In HD patients, serum renalase was not significantly different between hypertensive and normotensive patients.
Renalase, stroke, and hypertension in hemodialyzed patients by Malyszko J et al. 2012 (<i>Ren Fail.</i> 2012;34(6):727-31)	Caucasians of Polish	Case-control study: Case: hemodialyzed (HD) patients, n = 34 Control: healthy volunteers, n = 22	In HD patients, serum renalase was significantly lower in hypertensive patients when compared with normotensives.

[#] presented only as an abstract.

ELISA kit (Uscn Life) have not been fully validated. The identity of the antibodies and their epitopes, as well information on their behavior in native western blots, is unknown. For example, the antibodies in the currently available commercial ELISA kit (Uscn Life) may bind epitopes unrecognized by the polyclonal antibodies in the original studies [1, 33]. Alternatively, increased renalase levels by ELISA in advanced CKD may be due to accumulated renalase breakdown products, or to cross-reaction with an unrelated epitope. The polyclonal antibody predominantly detects the dimeric form of renalase (~75 kDa), with an estimated concentration of 4 µg/mL, while the monoclonal antibody preferentially binds

to higher molecular weight species. It is possible that lower GFR might favor the formation and accumulation of higher molecular weight multimers. It is interesting that western blot and ELISA give virtually identical results in subjects with normal renal function. Finally, it is possible that the measurement of plasma renalase activity may be the answer to this issue, as it would provide important insights into the function of renalase in health and disease [3]. Different study populations, sample sizes, and racial differences observed in various studies may also be accounted for contradicting results.

Renalase is expressed not only in the kidney but also in cardiomyocytes, liver, adipose tissues, skeletal muscles, central nervous system, and blood vessels [2, 3]. Cardiovascular risk factors, such as increased BP, may cause endothelial dysfunction and disintegration; as a consequence, small vessels may deteriorate through a condition known as vascular rarefaction, tissue hypoxia, and arteriosclerosis [34]. Serum renalase was found to be associated with endothelial cell injury and inflammation markers, such as von Willebrand factor (vWF), vascular cell adhesion molecule-1 (VCAM-1), and interleukin-6 (IL-6), in kidney and heart transplant recipients [35, 36]. In addition, He et al. [19] reported that the plasma renalase level of the CAD group was significantly lower than that of the control group. They further found that plasma renalase levels of the multi-branch and two-branch stenosis subgroups were significantly lower than those of the normal patients [19]. A recent study, the first in its kind, reported no correlation between plasma renalase PWV in patients after coarctation ($r = -0.23$, $P = 0.11$) [12]. However, the sample size of this study was small ($N=50$), making it difficult to draw firm conclusions. All subjects suffered from aortic coarctation, which potentially led to chronic renal ischemia and influenced plasma renalase levels. Furthermore, this study was designed at measuring the plasma renalase concentrations of patients after surgical repair of aortic coarctation, and was not designed at examining the relationship between serum renalase and PWV. Finally, in calculating the correlation between plasma renalase levels and PWV, PWV was analyzed only as a continuous variable, and not as a categorical variable. In our study, the reference value of baPWV with a cutoff of 1400 cm/s was used as an indicator of atherosclerotic cardiovascular risk and of severity of atherosclerotic vascular damage [25]. We explored the potential association between plasma renalase and arterial stiffness in Chinese adults with normal renal function. We found that plasma renalase was not different between subjects with high baPWV (≥ 1400 cm/s) and normal baPWV (<1400 cm/s). Additionally, plasma renalase levels showed no correlation with baPWV in both unadjusted and adjusted analyses. It is possible that lack of correlation might have resulted from the limitations of the renalase assay (USCN ELISA Kit). Nevertheless, we found no evidence of direct relationship between plasma renalase level and arterial stiffness.

Other limitations in this study should be considered. First, the cohort in this study only consisted of relatively young individuals 31 to 45 years of age. In addition, the method used to assess renalase levels has not been fully validated. No validated methods other than ELISA assay are available; however, the use of ELISA for this purpose has been discussed in detail elsewhere [3]. Finally, the sample size was relatively small and restricted to northern Chinese individuals. Therefore, our results will require replication in other cohorts to determine generalizability to other ethnicities and to populations with different ages.

Conclusion

- a) Plasma renalase may not be associated with the occurrence of hypertension in Chinese subjects with normal kidney function.
- b) Plasma renalase may not play a crucial role in the progression of arterial stiffness.

Disclosure Statement

The authors declare that there is no conflict of interest.

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