

# Prehypertensive African-American Women Have Preserved Nitric Oxide and Renal Function but High Cardiovascular Risk

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## Key Words

Pre-hypertension • Nitric oxide • High-sensitivity C-reactive protein • African-Americans

## Abstract

**Aims:** African-Americans, in particular women, exhibit disproportionate levels of hypertension, inflammation, and oxidative stress compared to other ethnic groups. The relationship between prehypertension, renal function, inflammation, and oxidative stress was examined. **Methods:** Twenty-eight African-American women ( $53.5 \pm 1.1$  years) followed an AHA diet and then underwent 24-hour ambulatory BP (ABP) monitoring. Urinary albumin (uAlb), serum and urinary creatinine, glomerular filtration rate (GFR), 24-hour urinary  $\text{Na}^+$  excretion, plasma superoxide dismutase, total antioxidant capacity (TAC), urinary (uNOx) and plasma (pNOx) nitric oxide levels, and high-sensitivity C-reactive protein (hsCRP) were measured. **Results:** When the group was divided by average 24-hour ABP into optimal and non-optimal groups, a significant difference existed between the groups for uNOx ( $p = 0.001$ ; nonoptimal:  $933.5 \pm 140.4$ , optimal:  $425.0 \pm 52.6 \mu\text{mol/gCr}$ ), and for hsCRP ( $p = 0.018$ , non-optimal:  $3.9 \pm 0.7$ , optimal:  $1.9 \pm 0.6 \text{ mg/l}$ ). Significant in-

verse relationships existed between hsCRP and uNOx and between uAlb and pNOx in the non-optimal group, between GFR and pNOx in the entire group, and positive association existed between TAC and uNOx in the optimal group. **Conclusions:** These results suggest that in African-American women as BP levels rise toward hypertension, the NO/NOS balance may be associated with renal function, and may have implications for CV risk based on their hsCRP levels.

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## Introduction

Overall, African-Americans have a 40% greater prevalence of hypertension (HTN), a higher rate of multiple cardiovascular disease (CVD) risk factors, a 4- to 6-fold greater incidence of end-stage renal disease (ESRD), and the highest overall coronary disease mortality rate of any ethnic group in the United States [1]. In particular, middle-age African-American women are 2–3 times as likely to have HTN as Caucasian women, and HTN is an important causative factor in the lifetime risk for developing heart failure and ESRD [2, 3]. Furthermore, oxidative stress, and inflammation have also been associated with



HTN [4–6]. Recently, plasma levels of high-sensitivity C-reactive protein (hsCRP), an acute-phase hepatic protein, have been related to impaired renal function and HTN [7, 8]. On the other hand, results from independent studies on the endogenous vasodilator nitric oxide (NO) conflict because some studies suggest a deficiency in NO with ESRD and HTN [9], while others report upregulated NO production [10]. NO serves as a potent vasodilator that is produced through oxidation of L-arginine to L-citrulline and released from the endothelial layer of blood vessels [11]. Vascular NO levels serve to maintain endothelial function either mechanically through augmented smooth muscle relaxation, or chemically through the rapid inactivation of the superoxide radical ( $O_2^-$ ) [12]. In fact, within the renal system, NO exerts a powerful influence on the regulation of glomerular filtration rate (GFR) and sodium ( $Na^+$ ) excretion [13].

Pre-hypertension (PHTN) is known to be a predictor for the future development of HTN. PHTN is a blood pressure (BP) classification defined by the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7) as systolic BP between 120 and 139 mm Hg and diastolic BP between 80 and 89 mm Hg [14]. Individuals with PHTN are likely to develop HTN within 5 years, yet the factors involved in this transition remain unclear. The impact of PHTN in African-American women on the development of ESRD and subsequent CVD progression may be substantial, since African-Americans are more likely to develop PHTN at younger ages and its presence is associated with future atherosclerosis [15]. Data from the Women's Health Initiative Study suggests that women with PHTN are at increased risk for CVD events [16]. Recently, a few population-based studies have reported that the risk of renal disease may extend to PHTN, but defining the BP levels at which this risk exists needs to be determined [17–19]. Furthermore, the physiologic derangements associated with impaired renal function as seen in HTN may occur earlier during the period of PHTN and are likely to involve increased oxidative stress and inflammation as seen in frank HTN. Despite this plausibility, the relationship between BP levels in the PHTN range, renal function, inflammation, and oxidative stress remains to be fully established.

It has been suggested that using 24-hour ambulatory BP (ABP) monitoring better predicts the risk of morbid events than clinical BP measurements because a 24-hour average considers the pressure load on the blood vessel system over an entire 24-hour period [20]. From this, BP values have been suggested that define the upper limits of ABP averages. 'Optimal' 24-hour average BP values are

<125/75, 'normal' 24-hour average BP values are <130/80, and 'abnormal' 24-hour average BP values are >135/85 [21]. The PHTN classification range of BP by JNC 7 (systolic BP 120–139 mm Hg or diastolic BP 80–89 mm Hg) includes a larger range of BP values than those that fall primarily in the 'normal' 24-hour average BP range. Considering this, we used average 24-hour ABP values obtained through ABP monitoring to classify participants into BP groups in order to better compare potential effects that a PHTN BP may have on renal function and oxidative stress markers in African-American women.

The purpose of our study was to compare several indices of renal function with urinary (uNOx) and plasma (pNOx) NO levels, the plasma antioxidant biomarkers superoxide dismutase (SOD) and total antioxidant capacity (TAC), and the inflammatory marker hsCRP, in African-American women with both optimal and nonoptimal average 24-hour ABP levels.

## Materials and Methods

This study included pre- and post-menopausal African-American women ( $n = 28$ ) between the ages of 40–75 years ( $53.5 \pm 1$ ) who were sedentary, nonsmokers, nondiabetics, not on lipid-lowering medications, had an average BMI of  $32.1 \pm 1$ , were on no more than one antihypertensive medication, and were without any end-organ damage. Participants responded to media advertisements and underwent a telephone interview to assess their initial eligibility. The study was approved by the Institutional Review Board of Temple University, Philadelphia, Pa., USA. All participants provided their written, informed consent during their first laboratory visit. None of the women were on a hormone replacement therapy regimen. Medical histories were reviewed on the first laboratory visit to ensure they met the study inclusion criteria listed above.

A 12-hour overnight fasting blood sample was drawn for blood chemistry, complete blood count, lipid profiles, serum creatinine (SCr), and hsCRP levels. Glomerular filtration rate (GFR) was calculated using the 4-variable MDRD equation specific to African-Americans:  $GFR (ml/min/1.73 m^2) = 186 \times (SCr^{-1.154}) \times (age^{-0.203}) \times (1.21) \times (0.742 \text{ if female})$  [22]. Participants then underwent a physical examination and a physician-supervised echocardiogram bicycle stress test to screen for any cardiovascular, pulmonary, or other chronic diseases. All qualified participants then underwent 6 weeks of dietary instruction (1 h each session) with a registered dietician instructing them on how to maintain the American Heart Association low-fat (<30% total calorie intake) and low-sodium (<3 g/day) diet. Participants had to follow this prescribed diet continuously throughout the dietary instruction period and be weight stable before undergoing any testing. Under close supervision of the study physician, any woman using one antihypertensive medication was tapered off the medication and remained off of their medication for the duration of the study. At the completion of the 6 weeks, a submaximal treadmill (TM) exercise test was performed to measure the volume of oxygen con-



sumption ( $\text{VO}_2$ ). The TM test was terminated when participant reached 75–80% of their estimated heart rate reserve. Regression analysis using data collected by indirect calorimetry was used to predict  $\text{VO}_{2\text{max}}$  levels.

#### *Measurement of Plasma (pNOx) and Urinary Nitrates/Nitrites (uNOx)*

Levels of NO end-products were measured using a modified Griess assay. Blood samples were collected in  $\text{K}_2$  EDTA tubes, centrifuged at 2,000 g for 20 min at 4°C, and then the plasma was frozen at –80°C until assay. On the day of assay, plasma samples were ultrafiltered through a 10,000 MWCO Amicon Ultra filter (Millipore) by micro-centrifuge at 14,000 g for 30 min at 4°C. Urine was aliquoted from the total-volume 24-hour urine collection and frozen at –80°C until assay. All urine samples were diluted 1:10 in reaction buffer (HEPES based). Briefly, the assay involves an enzymatic conversion of nitrate to nitrite by nitrate reductase (*Aspergillus* species) followed by measurement of nitrite through formation of a magenta-colored azo dye as a product of a Griess reaction. The Griess reagents used were N-(1-naphthyl)ethylenediamine in 2 M hydrochloric acid and sulfanilamide in 2 M hydrochloric acid. Absorbance was read at 540 nm using a SpectraMax Microplate Reader (Molecular Devices, Sunnyvale, Calif., USA). All reagents used were obtained from Assay Designs (Ann Arbor, Mich., USA). Inter-assay and intra-assay CVs were 7.6 and 10.6%, respectively.

#### *Measurement of Plasma Superoxide Dismutase*

Superoxide dismutase (SOD) activity was determined using a commercially available kit (Cayman Chemical, Ann Arbor, Mich., USA). Blood samples were collected in sodium-heparin tubes, centrifuged at 2,000 g for 20 min at 4°C, and then the plasma was frozen at –80°C until assay. Plasma samples were diluted 1:5 in sample buffer (50 mM Tris-HCl, pH 8.0). SOD activity was measured by utilizing a tetrazolium salt radical detector solution, diluted in assay buffer (50 mM Tris-HCl, pH 8.0, containing 0.1 mM diethylenetriaminepentaacetic acid and 0.1 mM hypoxanthine), to detect superoxide radicals generated by hypoxanthine and xanthine oxidase. One unit of SOD activity is defined as the amount of enzyme needed to exhibit a 50% dismutation of the superoxide radical. Absorbance was read at 450 nm using a SpectraMax Microplate Reader (Molecular Devices). The detection limit of the kit was 0.025 U/ml. Inter-assay and intra-assay coefficients of variation were 5.9 and 12.4%, respectively.

#### *Measurement of Total Antioxidant Capacity*

Total antioxidant capacity (TAC) activity was determined using a commercially available kit (Cayman Chemical). Blood samples were collected in sodium-heparin tubes, centrifuged at 2,000 g for 20 min at 4°C, and then the plasma was frozen at –80°C until assay. Plasma samples were diluted 1:20 in assay buffer (5 mM potassium phosphate, pH 7.4, containing 0.9% sodium chloride and 0.1% glucose). TAC measurement was based on the ability of antioxidants in the plasma to inhibit the oxidation of ABTS® (2,2'-azino-di- to ABTS by metmyoglobin). The capacity of the antioxidants in plasma to prevent ABTS oxidation is compared with that of a water-soluble vitamin E analogue, Trolox. Absorbance was read at 750 nm using a SpectraMax Microplate Reader (Molecular Devices), and TAC activity quantified as mil-

limolar Trolox equivalents. The detection limit of the kit was 0.044 mM. Inter-assay and intra-assay coefficients of variation were 6.7 and 9.2%, respectively.

#### *Ambulatory BP Monitoring and Urine Collection*

Participants underwent ABP using a noninvasive monitor (SpaceLabs Medical Inc., Model 90219, Redmond, Wash., USA) beginning on the morning of a typical day, with the exclusion of Friday through Sunday. The BP cuff was fitted to the participant's non-dominant arm with cuff size determined by upper arm circumference. BP measurements were obtained at 30-min intervals during the day (6.00 a.m. to 10.00 p.m.) and 60-min intervals at night (10 p.m. to 6 a.m.). Participants were instructed not to exercise prior to or during the 24-hour ABP monitoring period and to pause momentarily and maintain their body position during each BP measurement. From this, we used the average 24-hour ABP values to classify participants in our study into BP groups. Due to the fact that some of the participants were initially on one antihypertensive medication which was stopped before inclusion into the study, we defined the BP groups for this study based on ABP categories. Any participant with 24-hour average ABP <125/75 were placed into the 'optimal' group, while any participant with 24-hour average ABP >125/75 was placed into a 'nonoptimal' group. This was in order to compare potential BP effect on renal function, inflammation, and oxidative stress levels in African-American women.

Total volume of urine collected over the same 24-hour period was measured and recorded. Samples of 24-hour urine were then aliquoted and sent to Quest Diagnostics for measurement of urinary creatinine (UCr), urinary sodium (Na), and urinary albumin (uAlb) levels. From this data, creatinine clearance (CrCl) ratio was calculated:  $\text{CrCl (ml/min)} = (\text{UCr} \times 24\text{-hour urine volume}) / \text{SCr} \times 24 \times 60 \text{ min}$ .

#### *Statistical Analysis*

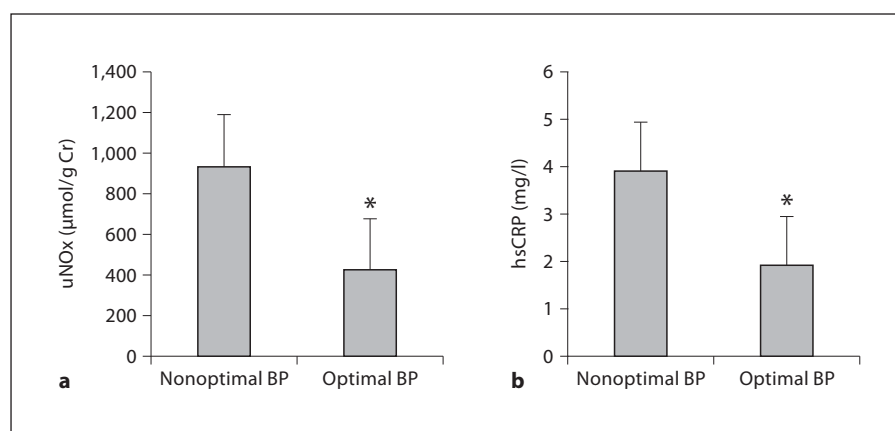
Data are presented as means  $\pm$  SE and significance was set at  $p < 0.05$ . The distribution of all variables was examined using the Shapiro-Wilk test of normality, and homogeneity of variances was determined using Levene's test. Variables that were found to fail the normality test were log adjusted for any statistical analysis, but true physiological values of any variable are reported. Independent t-tests and ANOVA were used to determine if there were significant differences between BP groups (optimal and non-optimal). Pearson correlation was used to determine if there were relationships between variables. All variables with  $p < 0.05$  in the Pearson correlation analyses were examined by linear regression analysis. Data was further analyzed using MANOVA and covarying for menopause status, BMI, age, and prior BP medication usage. All statistical analyses were performed using SPSS version 17.0 (SPSS Inc., Chicago, Ill., USA).

## **Results**

Twenty-eight African-American women, age  $53 \pm 1$  years, participated in this study. Table 1 shows subject characteristics divided by BP group. HDL-C was significantly different between groups, with the optimal group



**Fig. 1.** Blood pressure group differences in uNOx (a) and hsCRP (b). uNOx = Urinary nitric oxide metabolites; hsCRP = high-sensitivity C-reactive protein. Values are mean  $\pm$  SE. \*  $p < 0.05$  between blood pressure groups.



**Table 1.** Subject characteristics by blood pressure group

|                                | Optimal<br>(n = 14) | Nonoptimal<br>(n = 14) | p<br>value |
|--------------------------------|---------------------|------------------------|------------|
| Age, years                     | 53.9 $\pm$ 1.6      | 53.0 $\pm$ 1.7         | 0.69       |
| BMI                            | 32.4 $\pm$ 1.5      | 31.9 $\pm$ 1.4         | 0.79       |
| Total cholesterol, mg/dl       | 195.3 $\pm$ 6.7     | 191.0 $\pm$ 7.6        | 0.67       |
| HDL-C, mg/dl                   | 71.0 $\pm$ 4.6      | 57.0 $\pm$ 2.9         | 0.02*      |
| LDL-C, mg/dl                   | 108.9 $\pm$ 8.1     | 114.4 $\pm$ 6.8        | 0.61       |
| Triglycerides, mg/dl           | 75.8 $\pm$ 5.4      | 97.3 $\pm$ 11.8        | 0.11       |
| VO <sub>2max</sub> , ml/kg/min | 25.3 $\pm$ 1.5      | 24.3 $\pm$ 0.9         | 0.59       |
| Fasting glucose, mg/dl         | 95.0 $\pm$ 3.1      | 93.1 $\pm$ 2.1         | 0.61       |
| WBC count, million/ $\mu$ l    | 5.4 $\pm$ 0.4       | 5.2 $\pm$ 0.5          | 0.65       |
| hsCRP, mg/l                    | 1.9 $\pm$ 0.57      | 3.9 $\pm$ 0.72         | 0.02*      |
| Average 24-hour BP, mm Hg      |                     |                        |            |
| Systolic BP                    | 116.3 $\pm$ 1.7     | 133.8 $\pm$ 1.9        | 0.00*      |
| Diastolic BP                   | 71.5 $\pm$ 1.9      | 81.3 $\pm$ 1.5         | 0.00*      |

Data are presented as mean  $\pm$  SE. n = Sample size; BMI = body mass index; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein; VO<sub>2max</sub> = maximum oxygen consumption; WBC = white blood cell; hsCRP = high-sensitivity C-reactive protein; BP = blood pressure.

\* Denotes significant difference between groups.

having higher levels (71.0  $\pm$  4.6 compared to 57.0  $\pm$  2.9,  $p = 0.02$ ). No significant differences existed between groups for any of the other characteristics, except for hsCRP which was higher in the nonoptimal BP group (3.9  $\pm$  0.72 compared to 1.9  $\pm$  0.57,  $p = 0.02$ ), as seen in figure 1b. Table 2 shows the oxidative stress and renal function variables for the participants divided by BP group. The only variable that was significantly different between groups was uNOx, as seen in figure 1a. The women with nonoptimal BP had significantly higher levels of uNOx

**Table 2.** Oxidative stress and renal characteristics by blood pressure group

|  | Optimal<br>(n = 14) | Nonoptimal<br>(n = 14) | p<br>value |
|--|---------------------|------------------------|------------|
| SOD, U/ml                              | 4.9 $\pm$ 1.1       | 3.7 $\pm$ 0.7          | 0.75       |
| TAC, mM                                | 2.3 $\pm$ 0.5       | 2.6 $\pm$ 0.4          | 0.62       |
| uNOx, $\mu$ mol/g Cr                   | 425.0 $\pm$ 52.6    | 933.5 $\pm$ 140.4      | 0.001*     |
| pNOx, $\mu$ mol/l                      | 22.6 $\pm$ 3.7      | 22.6 $\pm$ 3.9         | 0.97       |
| TV, ml                                 | 1,644.3 $\pm$ 287.3 | 1,947.7 $\pm$ 173.9    | 0.38       |
| Na, mmol/g Cr                          | 83.2 $\pm$ 9.7      | 102.6 $\pm$ 9.2        | 0.16       |
| Urinary creatinine, g/24 h             | 1.6 $\pm$ 0.1       | 1.4 $\pm$ 0.1          | 0.39       |
| Urinary albumin, mg/24 h               | 9.2 $\pm$ 1.8       | 14.4 $\pm$ 4.0         | 0.26       |
| Serum creatinine, mg/dl                | 0.85 $\pm$ 0.03     | 0.83 $\pm$ 0.04        | 0.72       |
| GFR, ml/min/1.73 m <sup>2</sup>        | 91.2 $\pm$ 3.3      | 96.8 $\pm$ 5.7         | 0.41       |
| CrCl ratio, ml/min/1.73 m <sup>2</sup> | 129.5 $\pm$ 10.9    | 124.6 $\pm$ 12.8       | 0.77       |

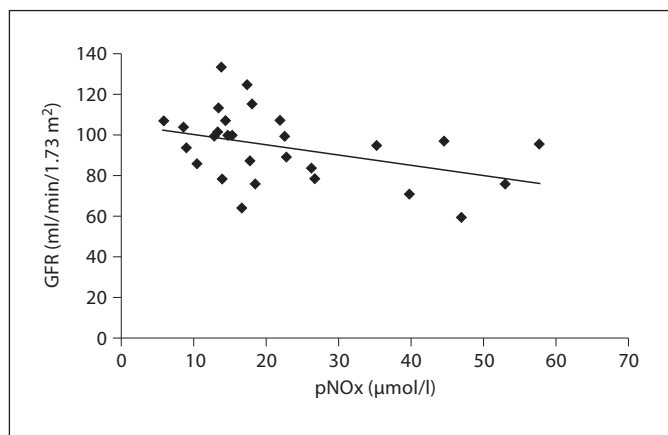
Data are presented as mean  $\pm$  SE. n = Sample size; SOD = superoxide dismutase activity; TAC = total antioxidant capacity; uNOx = urinary nitric oxide metabolites; pNOx = plasma nitric oxide metabolites; TV = total 24-hour urine volume; Na = urinary sodium; GFR = glomerular filtration rate; CrCl = creatinine clearance ratio.

\* Denotes significant difference between groups.

(933.5  $\pm$  140.4  $\mu$ mol/g Cr compared to 425.0  $\pm$  52.6  $\mu$ mol/g Cr,  $p = 0.001$ ).

Correlation and regression analyses showed several significant associations between oxidative stress and renal function markers. For the entire group, a significant inverse relationship existed between GFR and pNOx ( $r = 0.411$ ,  $p = 0.030$ ), as seen in figure 2. Sub-analysis of the nonoptimal BP group revealed significant inverse relationships between hsCRP and uNOx ( $r = 0.660$ ,  $p = 0.019$ ), and between uAlb and pNOx ( $r = 0.566$ ,  $p = 0.035$ ) (fig. 3).





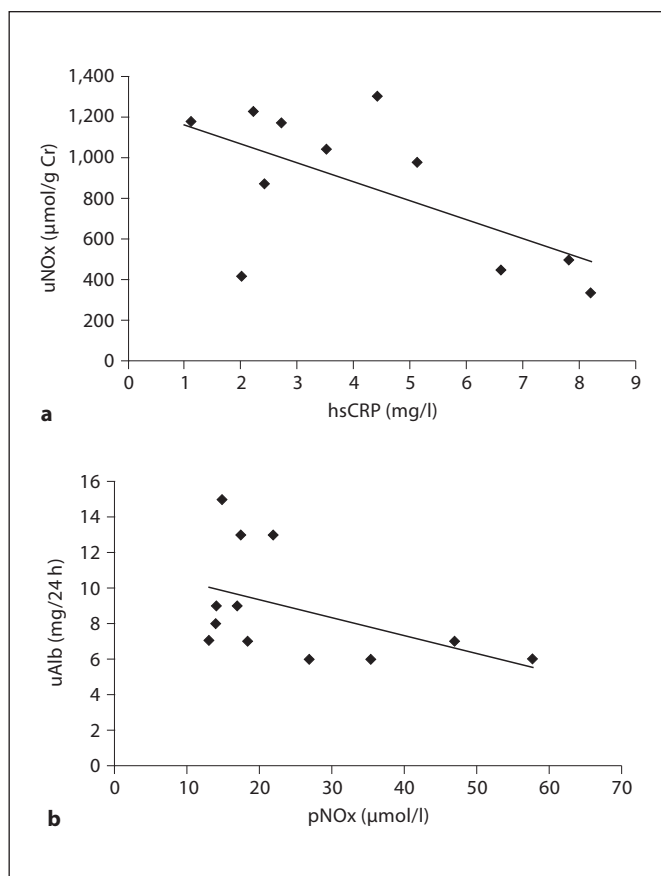
**Fig. 2.** Regression analysis between GFR and pNOx ( $r = 0.411$ ,  $p = 0.030$ ). GFR = Glomerular filtration rate; pNOx = plasma nitric oxide metabolites.

Finally, as shown in figure 4, there was a significant positive association between TAC and uNOx ( $r = 0.549$ ,  $p = 0.042$ ) in the participants with optimal BP.

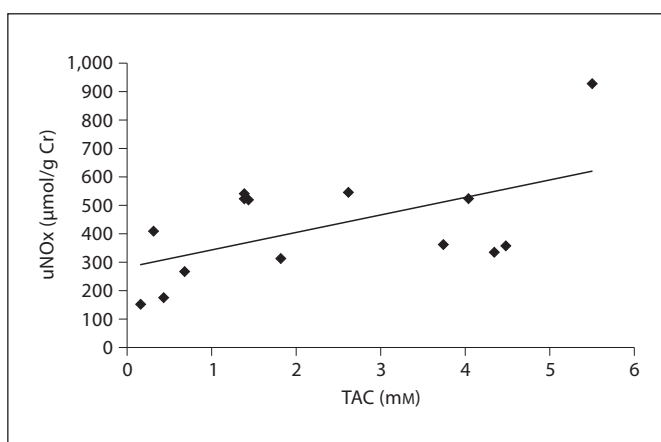
## Discussion

To the best of our knowledge, this study is the first to report increased uNOx levels in a group of African-American women with nonoptimal BP levels, similar to PHTN BP levels, compared to women with optimal BP levels. Our results confirm in humans what has been established by molecular studies in animal models. We found a significantly higher level of uNOx in women with elevated BP levels. We found no significant difference in pNOx levels, SOD activity, or TAC between groups. Our results suggest that a NO/NOS imbalance may exist within the renal system in African-American women with elevated BP, but that these BP levels may not elicit an imbalance in pNOx or plasma antioxidant activity.

Data on the relationship between renal function, HTN, and NO production are conflicting. Although studies have reported depressed NO production with essential HTN or with renal impairment, others have suggested that an increase in NO activity occurs. For example, Schmidt and Baylis [23] reported that uNOx levels were lower in chronic renal dialysis patients than in healthy individuals. Likewise, Kim et al. [24] found a significantly lower uNOx production in the kidneys of rats with chronic renal failure but found no significant difference in pNOx levels between the renal failure rats and normal



**Fig. 3.** Regression analyses in the non-optimal blood pressure group. **a** hsCRP with uNOx ( $r = 0.660$ ,  $p = 0.019$ ). **b** uAlb with pNOx ( $r = 0.566$ ,  $p = 0.035$ ). hsCRP = High-sensitivity C-reactive protein; uNOx = urinary nitric oxide metabolites; uAlb = urinary albumin; pNOx = plasma nitric oxide metabolites.



**Fig. 4.** Optimal blood pressure group regression analysis between TAC and uNOx ( $r = 0.549$ ,  $p = 0.042$ ). TAC = Total antioxidant activity; uNOx = urinary nitric oxide metabolites.



rats. In contrast, other research supports increased NO production. Lyamina et al. [25] found that uNOx levels were increased in young individuals with BP in the PHTN range ( $137 \pm 3.2/88 \pm 1.9$  mm Hg), leading authors to suggest that elevations in uNOx levels may be a compensatory response to the rising BP. This result is similar to our findings.

Generation of NO occurs by three nitric oxide synthase (NOS) enzyme isoforms. Two, endothelial NOS (eNOS) and neuronal NOS (nNOS), are constitutive and continuously produce low levels of NO. The third NOS isoform is inducible (iNOS) and activated in response to cellular stress, toxins, and cytokines [26]. When iNOS activity is increased, it produces close to 1,000 times more NO than eNOS or nNOS, and this cellular iNOS-mediated NO production can continue for hours [27, 28]. Impaired NO production variably affects endothelial function in systemic and renal blood vessels depending on the NOS isoform affected. Further supporting the view that NO production may be augmented during the early stages of HTN are several recent molecular biology studies that provide mechanistic evidence by showing overall increased NO production in SHR. Vaziri et al. used young SHR in order to attempt to discern the effects of PHTN on NO metabolism and reported significant increases in uNOx, eNOS, and iNOS [29]. Kumar et al. reported an overproduction of iNOS in the cortex, inner medulla, and proximal tubules of SHR [30]. They also separately showed that eNOS and nNOS expressions were decreased in the inner medulla and renal cortex [31]. The authors concluded that an imbalance in NO/NOS system exists in the SHR kidney. Likewise, Suzuki et al. [32] used the one-clamp model in adult Wistar-Kyoto rats and Dubey et al. [33] used one-kidney, one-clip HT rats, and both reported that increased NO production accompanied the early rise in BP. These studies indicate that the delicate balance of NO production from specific NOS isoforms may affect the quantitative measurement of the total circulating NO metabolites. Furthermore, they also showed that early rises in BP, such as that seen with PHTN, may have different effects on NO production than that found in established essential HTN. Research in humans with varying levels of BP is necessary to elucidate the role of NO metabolism in the transition from normal BP to PHTN and then to HTN.

Another important finding from our study was that the African-American women with nonoptimal BP levels had significantly higher hsCRP levels compared to those with optimal BP levels. The American Heart Association and the Centers for Disease Control define hsCRP risk

categories as  $<1$  mg/l is low risk,  $1-3$  mg/l is average risk, and  $>3$  mg/l is high risk [34]. Our nonoptimal group had hsCRP levels that fall in the high-risk category with an average of 3.9 mg/l. The biomarker hsCRP has consistently been associated with HTN, CVD and atherosclerosis [35–38]. Our data extends this further by suggesting that hsCRP may have association with BP levels in the PHTN range. In particular, in relation to women, Ridker et al. [39] found that hsCRP was a strong significant predictor of risk for future cardiovascular events in women. And furthermore, African-American women have been reported to have the highest levels of hsCRP compared to other ethnic groups [40]. Interestingly, in our study, within group analysis revealed inverse relationships between hsCRP and uNOx levels in both groups, but the correlation was only significant in the nonoptimal, higher BP, group. Other studies have provided evidence confirming such an association. Verma et al. [41] used cultured endothelial cells to show that high concentrations of hsCRP reduces eNOS activity, decreases eNOS mRNA stability, and quenches NO production. Separately, Venugopal also tested the effect of CRP on eNOS expression and activity and found a direct reduction in eNOS activity [42]. Our results support these findings by reporting that African-American women with non-optimal BP levels exhibit an inverse association between hsCRP and uNOx. Taken together, despite significantly higher levels of both uNOx and hsCRP in women with nonoptimal BP levels, it seems plausible that the high hsCRP levels may be affecting eNOS stability and thus creating an inverse association within this group.

We also found an inverse correlation between uAlb excretion and pNOx within both groups, again with significance found only in the nonoptimal BP group. Increased uAlb excretion is a key marker for diagnosing renal dysfunction and has been associated with increased risk of CVD [43]. Although both groups had uAlb excretion levels within normal limits, higher uAlb levels were significantly correlated with lower pNOx in the women with non-optimal BP levels. This correlation suggests that African-American women with nonoptimal BP levels, suggestive of PHTN, who have higher uAlb levels and therefore tend towards renal dysfunction have lower pNOx activity. It has been previously reported that elevated uAlb levels in healthy adults were associated with impaired arterial vasodilatory capacity as measured by pNOx [44]. Our study provides support for this in African-American women.

GFR, as estimated by the 4-variable MDRD equation, continues to be a key diagnostic marker of renal function.



Measuring the volume of fluid filtered through the renal cavity per unit time, GFR gives a relative indicator of filtering capacity in the kidneys. While the average GFR for our nonoptimal group was higher than that of the optimal group, the difference between groups was not statistically different. When the entire group was combined, there was a significant inverse correlation between pNOx and GFR. It is speculated that participants with lower GFR levels may have some compensatory mechanism leading to higher pNOx levels.

Participants in the present study were tapered from their anti-hypertensive medication and were studied after a minimum of 3 weeks without medication use. However, long-term use of anti-hypertensive medication may affect factors involved in BP and renal regulation. In addition, albuminuria and GFR may influence BP responses to antihypertensive drug therapy [45]. It is recognized that some antihypertensive medications are effective at improving endothelial function as well as reducing BP, yet evidence is limited whether the pharmacologically induced improvement is transient or permanent [46, 47]. Interestingly, in our study, SCr ( $p = 0.035$ ) and GFR ( $p = 0.04$ ) were significantly different between BP groups after covarying for prior antihypertensive medication usage, indicating a potential interaction between GFR and BP responses to long-term antihypertensive medication use. Further research needs to be conducted to examine the potential long-term effects of antihypertensive medication on renal function.

Finally, our within-group analyses showed that in the optimal BP group, plasma TAC was significantly and positively correlated with uNOx. It has been shown that HTN individuals have lower antioxidant activity [48, 49], in particular a reduction in TAC [50]. This may extend to PHTN BP levels and may explain why no correlation was observed in our nonoptimal group.

It must be noted that there are some limitations to our study. First, our sample size is small, but this was due to the exclusion of diabetics, smokers, women on more than one antihypertensive medication, and women with cardiovascular disease. This was intentionally done in order to create as homogenous group as possible and to ensure lack of confounding variables that may influence renal function or oxidative stress measures. Second, only associations are reported, not causations; however, we provide strong support for each of our findings from mechanistic studies in animal or cell models. Finally, only antioxidant variables were included in this study. Future research should include other oxidative stress biomarkers to better understand the physiological association between NO levels and renal function.

In conclusion, our data report that African-American women with nonoptimal BP levels, representative of PHTN BP levels, have higher uNOx production, higher levels of hsCRP, and exhibit significant inverse correlations between renal function measures and NO metabolism when compared to African-American women with normal BP levels, who had a positive association between TAC and NO production. Together, these results suggest that in African-American women the NO/NOS balance may be associated with renal function, and that African-American women with BP levels in the PHTN range may have significant cardiovascular risk.

### Acknowledgements

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