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Urinary prostasin excretion is associated with adiposity in nonhypertensive African-American adolescents

De-huang Guo[1], Samip J. Parikh[1,2], Julie Chao[3], Norman K. Pollock[1], Xiaoling Wang[1], Harold Snieder[1,4], Gerjan Navis[5], James G. Wilson[6], Jigar Bhagatwala[1,2], Haidong Zhu[1] and Yanbin Dong[1]

BACKGROUND: Metabolic abnormalities in obesity can overstimulate the renal epithelial sodium channel (ENaC) and subsequently lead to blood pressure (BP) elevation. Prostasin, a membrane-bound/secretive serine protease, is thought to activate ENaC via the proteolytic cleavage of the channel. Our specific aim was to explore whether there is a relationship between adiposity and urinary prostasin excretion at the population level.

METHODS: In 271 African-American adolescents, urinary prostasin concentrations were determined by enzyme-linked immunosorbent assay and normalized by urinary creatinine.

RESULTS: Urinary prostasin excretion increased in the overweight/obese group (n = 110, 38.2 ± 4.0 ng/mg) vs. the normal-weight group (n = 161, 20.7 ± 1.2 ng/mg, P = 0.03). Urinary prostasin excretion was significantly correlated with BMI percentiles (r = 0.14, P = 0.02), waist circumference (r = 0.13, P = 0.05), total body fat mass (r = 0.20, P < 0.01), and percentage body fat (r = 0.23, P < 0.01). Urinary prostasin excretion was also correlated with plasma aldosterone (r = 0.11, P = 0.05) and systolic BP (SBP; r = 0.15, P = 0.02), but the significances disappeared after adjustment of any of the adiposity variables.

CONCLUSION: Our data for the first time suggest that adiposity plays a role in urinary prostasin excretion, and its associations with aldosterone and BP appear to be modulated by adiposity. Whether urinary prostasin excretion is a biomarker/mechanism underlying obesity-related hypertension deserves further investigations.

Overweight and obesity in adolescents are continuously on the rise. In a cohort of almost 1,000 adolescents (mean age: 17.6 ± 3.3 y) residing in the southeastern region of the United States, we previously demonstrated that the occurrence of overweight and obesity combined was more common in African-American (39.7%) than in European-American adolescents (28.0%) (1). Obese adolescents are at approximately a threefold higher risk for hypertension than nonobese adolescents (2).

A series of studies conducted by our group and others demonstrate that a significant percentage of African-American adolescents and those with increased adiposity have a diminished natriuretic response (3). Although the precise mechanisms are still being explored, obesity is recognized to increase renal sodium reabsorption and impair pressure natriuresis, possibly via activation of several physiological systems such as the renin–angiotensin–aldosterone system (4). The epithelial sodium channel (ENaC) constitutes the final sodium reabsorption in the kidney, and subsequently regulates extracellular fluid volume and blood pressure (BP) (5). Recently, it has been postulated that metabolic abnormalities in obesity can overstimulate ENaC, which may be a likely cause of hypertension (6). For example, Saha *et al.* (7) showed that ENaC blockage reduced BP in African-American obese hypertensives.

Prostasin, a membrane-bound/secretive glycosylphosphatidylinositol-anchored serine protease, is expressed in a variety of tissues including prostate, colon, liver, ovary, skin, vessels, and proximal and distal tubular cells (8). A series of subsequent studies in cells and animal models have provided compelling evidence that prostasin activates ENaC in the distal tubules by increasing the channel open probability (8). Data on humans, however, are scant. We and others demonstrate that urinary prostasin is detectable in all human subjects regardless of age, gender, and race (9–12). Olivieri *et al.* (10) suggested urinary prostasin as a candidate marker of ENaC activation in European adults. We found that urinary prostasin excretion appeared to be involved in stress-induced pressure natriuresis in African-American normotensive adolescents, indicating that urinary prostasin excretion can be a novel biomarker and/or mechanism underlying salt sensitivity (11). More recently, in a Japanese population consisting of 26 normotensives and 121 hypertensives, Koda *et al.* (9) observed significant correlations of urinary prostasin with urine aldosterone, plasma aldosterone, and urinary Na^+/K^+ ratio, suggesting that urinary prostasin might serve as a surrogate marker for ENaC activation in hypertensive patients.

The first two authors contributed equally to this work.
[1]Department of Pediatrics, Georgia Prevention Institute, Georgia Regents University, Augusta, Georgia; [2]Department of Medicine, Georgia Regents University, Augusta, Georgia; [3]Department of Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston, South Carolina; [4]Department of Epidemiology, Unit of Genetic Epidemiology & Bioinformatics, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; [5]Department of Nephrology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; [6]Department of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, Mississippi. Correspondence: Yanbin Dong (ydong@gru.edu)

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Therefore, we hypothesize that adiposity may enhance the production of prostasin via various pathophysiological pathways, which in turn activates ENaC and subsequently results in BP elevation. In the present study, our primary objective was to explore whether there is a relationship between adiposity and urinary prostasin excretion at the population level. In addition, we tested the associations of urinary prostasin excretion with plasma aldosterone and BP in nonhypertensive African-American adolescents.

RESULTS

Clinical characteristics and their comparisons by BMI percentile group are presented in **Table 1**. Age was similar between the normal-weight group and the overweight/obese group, but the overweight/obese participants were shorter than their normal-weight counterparts. As expected, all the body fat compositions including waist circumference, total fat mass, and percentage of body fat were greater in the overweight/obese group than in the normal-weight group. Levels of plasma renin and aldosterone did not significantly differ between the two groups. Systolic BP (SBP), but not diastolic BP (DBP), was significantly higher in the overweight/obese group vs. the normal-weight group.

Urinary prostasin excretion was significantly higher in the overweight/obese group as compared with the normal-weight group (**Table 1** and **Figure 1**). All these results were adjusted for age and sex. Log-transformed urinary prostasin excretion was significantly correlated with BMI percentile ($r = 0.14$, $P = 0.02$), waist circumference ($r = 0.13$, $P = 0.05$), total body fat mass ($r = 0.20$, $P < 0.01$), and percentage of body fat ($r = 0.23$, $P < 0.01$; **Table 2**). Urinary prostasin excretion was also correlated with plasma aldosterone ($r = 0.11$, $P = 0.05$) and SBP ($r = 0.15$, $P = 0.02$), but the statistical significances disappeared after adjustment of any of the above adiposity variables. We did not find that urinary prostasin excretion was correlated with urinary Na^+ excretion ($U_{Na}V$), K^+ excretion (U_kV), Na^+/K^+ ratio, or urinary albumin. Stratified by BP status (normotensive vs. prehypertensive), prehypertensives ($n = 54$, 31.1 ± 1.8 ng/mg) vs. normotensives ($n = 217$, 22.8 ± 1.2 ng/mg) seemed to have increased urinary prostasin excretion, but the difference did not reach statistical significance ($P = 0.18$). Plasma renin concentration and aldosterone did not differ between normotensives and prehypertensives.

Table 1. Clinical characteristics of the study participants

Variable	Total	Normal weight	Overweight/obese	*P* value
Subject number (*n*)	271	161	57/53	
Sex (M/F)	138/135	89/74	49/61	
Age (years)	16.5 ± 0.1	16.4 ± 0.1	16.6 ± 0.1	0.34
Height (cm)	169.8 ± 0.6	170.8 ± 0.7	168.3 ± 0.9	0.02[a]
BMI percentile	70.3 ± 1.6	54.1 ± 1.8	94.0 ± 0.4	<0.01[a]
Waist circumference (cm)	79.2 ± 0.7	72.7 ± 0.6	88.6 ± 1.1	<0.01[a]
Total body fat mass (kg)	17.7 ± 0.1	11.4 ± 0.1	27.0 ± 0.1	<0.01[a]
% Body fat[b]	23.7 ± 0.7	18.4 ± 0.6	31.4 ± 1.0	<0.01[a]
SBP (mmHg)	112 ± 1	111 ± 1	115 ± 1	<0.01[a]
DBP (mmHg)	60 ± 1	60 ± 1	60 ± 1	0.57
Plasma renin (pg/ml)[b]	12.7 ± 0.5	12.7 ± 0.6	12.7 ± 0.9	0.98
Plasma aldosterone (pg/ml)	106.5 ± 5.1	102.4 ± 6.4	112.4 ± 8.3	0.33
Plasma creatinine (mg/dl)	1.1 ± 0.3	1.0 ± 0.4	1.1 ± 0.3	0.70
$U_{Na}V$ (mEq/h)[b]	7.3 ± 0.4	6.9 ± 0.5	7.7 ± 0.5	0.32
U_KV (mEq/h)[b]	1.7 ± 0.1	1.8 ± 0.2	1.7 ± 0.0	0.39
Urinary creatinine (mg/dl)	117.8 ± 6.2	113.9 ± 7.9	121.7 ± 10.0	0.53
Urinary albumin (μg/ml)	11.1 ± 0.8	10.1 ± 1.0	12.6 ± 1.3	0.13
Urinary prostasin excretion[b] (ng/mg)	27.6 ± 4.0	20.7 ± 1.2	38.2 ± 4.0	0.03[a]

DBP, diastolic blood pressure; SBP, systolic blood pressure; $U_{Na}V$, urinary Na^+ excretion; U_KV, urinary K^+ excretion.

[a]Statistical significance. [b]Analyses were performed based on log-transformed data. Values are mean ± SE. Differences between BMI groups (normal weight vs. overweight/obese) were compared by independent *t*-tests.

DISCUSSION

The principal findings of our study are threefold. First, we observed that overweight/obese vs. normal-weight participants had a greater level of urinary prostasin excretion. Urinary prostasin excretion was higher in obese subjects than overweight and normal-weight subjects combined. Second,

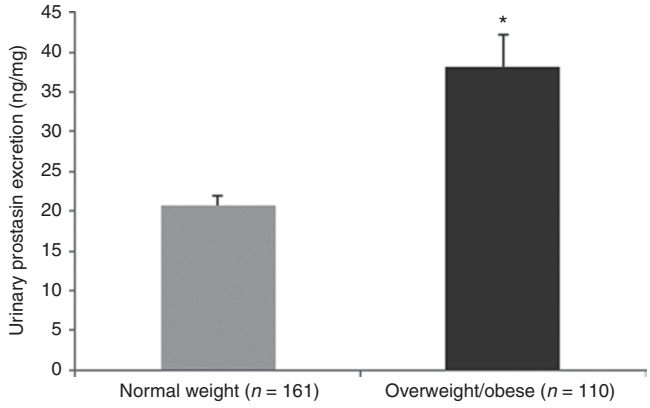

Figure 1. Urinary prostasin excretion in normal weight vs. overweight/obese groups. *$P = 0.03$.

Table 2. Correlations between urinary prostasin excretion and adiposity variables

Variable	*R*	*P* value
BMI percentile	0.14	0.02
Waist circumference	0.13	0.05
Total body fat mass[a]	0.20	<0.01
% Body fat[a]	0.23	<0.01

Values were adjusted for age and sex.

[a]Log-transformed values were used.

urinary prostasin excretion was correlated with adiposity variables including BMI percentile, waist circumference, total body fat mass, and percentage of body fat. Third, associations between urinary prostasin excretion and plasma aldosterone, and between urinary prostasin excretion and SBP, were markedly attenuated when accounting for variations in any of the adiposity variables. These findings suggest that adiposity modulates the relationship of prostasin with aldosterone and BP.

Adipose tissue is not a simple energy-storage organ, but also has an important endocrine and immune function, predominately through releasing adipokines (13). Adiposity-derived inflammatory responses may directly or indirectly cause kidney damage through endothelial injury, increased mesangial matrix synthesis, and tubulointerstitial fibrosis. Inflammation has been hypothesized to have tubular effects by regulating renal sodium transporters and channels, although the data are inconsistent (14–19). Similarly, data concerning the relationship between inflammation and prostasin has been controversial. In mice injected intraperitoneally with bacterial lipopolysaccharide, bladder prostasin mRNA expression was downregulated, whereas the expression of inflammatory factors such as tumor necrosis factor-α and interleukin-6 was upregulated (20). In M-1 cells, transforming growth factor-β1 transcriptionally inhibits prostasin expression by the induction of IkBα and the subsequent inhibition of nuclear factor-κB/Rel activity (21,22). By contrast, we recently found that treatment with interleukin-6 in the M-1 cells increased the mRNA abundance of prostasin by ~30% and the protein expression of prostasin by over 30% (23). The magnitude of the interleukin-6–induced amiloride-sensitive sodium current in the presence of a prostasin inhibitor (aprotinin) dropped by almost 60%. We hypothesized that chronic and low-grade inflammation induced by obesity might upregulate ENaC partly through prostasin, which increases tubular sodium reabsorption. Future studies with the measurement of inflammatory factors may enable us to establish the inflammatory link between adiposity and urinary prostasin excretion.

Emerging data suggest that adipocytes, either directly or indirectly through the release of aldosterone-stimulating factors, may serve as a source of aldosterone (24). Aldosterone increases the rate of tubular sodium reabsorption across epithelia at the distal nephron by enhancing ENaC activity (5). An increase in aldosterone results in the increase of prostasin expression and urinary prostasin excretion in M-1 cells, rats, and humans, althoughwhereas data are inconsistent (25,26). Previously, Narikiyo *et al.* (26) showed that urinary prostasin secretion was substantially increased in patients with primary aldosteronism. These findings suggest that aldosterone can stimulate the production of prostasin. Nevertheless, Wang *et al.* (27) demonstrated that a single injection of adenovirus carrying the human prostasin gene to Wistar rats caused a marked and prolonged (3–4 wk) increase in BP. Elevated plasma aldosterone levels were only detected 3 d after gene transfer before the development of hypertension, indicating that stimulation of mineralocorticoid production is the primary target of prostasin. Furthermore, Maekawa *et al.* (28) found that camostat mesilate, a synthetic serine protease inhibitor decreasing prostasin activity, reduced BP and renal injury in Dahl salt-sensitive rats. These data indicate that prostasin may regulate BP, independent of aldosterone. Indeed, it has been speculated that there might be bi-directional interactions between prostasin and aldosterone (8). The present study showed a correlation between urinary prostasin excretion and plasma aldosterone in African Americans, which is in line with the previous findings in the Japanese population (9). However, the association of urinary prostasin excretion either with aldosterone or with SBP did not persist after adjustment of adiposity in our study. This observation raises the possibility that the relation between urinary prostasin excretion and aldosterone or BP could be explained, in large part, by its association with adiposity. We speculate that adiposity may also increase the production of prostasin via other physiological pathways such as insulin. Insulin, a peptide hormone that regulates glucose metabolism, manifests a stimulatory effect on renal sodium absorption by increasing ENaC activity (6). The immediate natriuretic effect of insulin is attributed to an increase in the open probability of ENaC or an increase in the number of active ENaC at the apical membrane. Whether insulin affects prostasin deserves furthermore investigation. Finally, to be fully functional, prostasin is a serine protease that interacts with matriptase, protease nexin-1, and hepatocyte growth factor activator inhibitor-1B (22,29–31). Whether or not the prostasin complex is associated with adiposity, adiposity-derived factors, and inflammation remains unknown.

We examined healthy normotensive African-American adolescents because (i) healthy normotensive adolescents may not have yet developed significant target organ damage, which might affect the production of urinary prostasin excretion; (ii) the pressure natriuresis concept is particularly appealing due to the greater sensitivity of BP to sodium in African Americans as compared with their Caucasian counterparts (3); and (iiii) urinary prostasin excretion could be used as a possible biomarker to screen adolescents at risk of BP elevation. Nonetheless, there are limitations and weaknesses in this study. First, it was a cross-sectional study. To better understand the relationship between adiposity, urinary prostasin excretion, aldosterone, and urinary Na^+/K^+ ratio as an indirect indicator of ENaC activity, BP elevation, and microalbuminuria as an early marker of chronic kidney disease, longitudinal approaches are needed. Adiposity-related factors such as inflammation, insulin, and glucose should be also studied in this regard. Second, we did not measure urinary aldosterone, which was found to be strongly correlated with urinary prostasin (9). Third, we did not collect 24-h urine samples to determine the relation between urinary prostasin excretion and daily sodium intake. Finally, prehypertensives vs. normotensives merely had a nonstatistically significant increase in urinary prostasin excretion, which could be due to several factors including the early stage of BP elevation and a relatively small sample size of prehypertension. Studies of the level of urinary prostasin excretion in hypertensive populations with a sufficient sample size and ambulatory BP measurements would help to understand the role of prostasin in the etiology of hypertension.

In conclusion, our data for the first time suggest that adiposity likely plays a role in urinary prostasin excretion in humans, and particularly in African-American adolescents, which may in turn activate renal ENaC. This could subsequently lead to the future development of hypertension and cardiovascular disease. Urine is easily and noninvasively collected, accessible for population screening, and represents a medium with a relatively low number of interfering proteins. Whether urinary prostasin excretion could be a biomarker/mechanism underlying the future development of adiposity-related hypertension warrants furthermore investigation.

METHODS

Subject Recruitment and Protocol

The protocol was approved by the Human Assurance Committee of the Georgia Regents University. A sample of 271 apparently healthy, nonhypertensive African-American adolescents was recruited from local public high schools in the Augusta-Richmond County area via school announcements, flyers, handouts, and word of mouth. Written informed parental consent and subject assent were obtained before testing. Data were collected between June 2006 and July 2008. Race (African-American) was identified by self-report of each subject and by parent if the subject was less than 18 y of age. Height, weight, and waist circumference were obtained. Exclusion criteria included any chronic illness, medication use, or a positive pregnancy test. Females were not tested while on their menses, but were tested on the week following completion of their menstrual flow to ensure that all females were tested in the same phase of their menstrual cycle.

Subjects were instructed to relax as completely as possible while lying (supine) on a hospital bed for a 10-min period of time, after which SBP and DBP measurements were taken with a Dinamap monitor (model 1864 SX; Criticon, Tampa, FL) by trained research assistants or nurses. Five readings were made at 1-min intervals and the last three were averaged. For individuals <18 y of age, according to age, sex, and height percentile, prehypertension was defined as an average BP ≥90th and <95th percentile of SBP or DBP, or SBP/DBP ≥120/80 mmHg. For subjects ≥18 y of age, those with SBP/DBP 120–139/80–89 mmHg were considered prehypertensive (32,33). According to the growth charts, BMI between the 5th percentile and 85th percentile was defined as normal weight, BMI between the 85th percentile and 95th percentile was defined as overweight, and BMI of 95th percentile and above was defined as obese (1). Dual X-ray absorptiometry (QDR-4500W; Hologic, Waltham, MA) was used to measure total body fat mass (kg) and percentage of body fat. The urinary albumin in a morning spot urine sample was measured by an enzyme-linked immunosorbent assay according to the manufacturer's instruction using the Albuwell kit (Exocell, Philadelphia, PA). Both intra- and interassay coefficients of variation (CV) were <7%. Creatinine was measured by the ion-selective electrode technique using a NOVA 16 Analyzer (NOVA Biomedical, Waltham, MA). The NOVA has an intra-assay CV of <3% and an inter-assay CV of 4%.

Human Urine Prostasin Measurements

Urinary levels of immunoreactive human prostasin were determined by enzyme-linked immunosorbent assay prepared with a previously described antibody to human prostasin (34). Microtiter plates (96-well) were coated with anti-prostasin immunoglobulin G (IgG; 1 μg/ml, 100 μl per well) overnight at 4 °C. Purified prostasin standards (0.16–10 ng) or samples were added to individual wells in a total volume of 100 μl of phosphate-buffered saline containing 0.05% Tween 20 and 0.5% gelatin (dilution buffer) and incubated at 37 °C for 90 min. Biotin-labeled antihuman prostasin immunoglobulin G was added in each well at a concentration of 1 μg/ml in a total volume of 100 μl and incubated at 37 °C for 60 min. Peroxidase–avidin at a concentration of 1 μg/ml in a total volume of 100 μl was added and incubated at 37 °C for 30 min. The color reaction was performed by adding to each well 100 μl of freshly prepared substrate solution (0.03% 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) and 0.03% H_2O_2 in 0.1 mol/l sodium citrate (pH 4.3)) and incubating the mixture at room temperature for 30 min. The plates were read at 405 nm with a plate reader (Titertek Instruments, Huntsville, AL). On average, the interassay CV was 4.75–5.10%, and the intra-assay CV was 2.30–2.40%. Urinary prostasin excretion was obtained from urinary prostasin concentration normalized by urinary creatinine.

Renin and Aldosterone

Plasma renin concentration and aldosterone were measured by immunoradiometric assay and radioimmunoassay, respectively. Both assays were conducted by using commercial kits from Diagnostic Systems Laboratories (Webster, TX). For renin, the interassay CV was 10.03% at 23.72 pg/ml, and 2.09% at 260.49 pg/ml; and the intra-assay CV was 5.39% at 5.86 pg/ml. For aldosterone, the interassay CV was 14.14% at 54.39 pg/ml and 13.17% at 245.85 pg/ml; and the intra-assay CV was 7.07%.

Statistical Analyses

Descriptive statistics are presented as mean ± SE. Differences in descriptive characteristics between BMI percentile groups (normal weight vs. overweight/obese), and BP groups (normotensives vs. prehypertensives) were compared by independent *t*-tests. Simple bivariate correlations were first conducted, then partial correlations were conducted to adjust for potential confounders such as age, sex, and adiposity variables. Log-transformation was performed to obtain approximation of normal distribution when necessary. A value of $P < 0.05$ was deemed statistically significant. The statistical analyses were performed with SPSS software (version 19.0; SPSS, Chicago, IL).

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