

Gender and ethnic differences in urinary stress hormones: the population-based Chicago Health, Aging, and Social Relations Study

Christopher M. Masi,^{1,2} Edith M. Rickett,³ Louise C. Hawley,¹ and John T. Cacioppo^{1,3}

¹Institute for Mind and Biology, ²Department of Medicine, and ³Department of Psychology, University of Chicago, Chicago, Illinois 60637

Submitted 9 March 2004; accepted in final form 10 May 2004

Masi, Christopher M., Edith M. Rickett, Louise C. Hawley, and John T. Cacioppo. Gender and ethnic differences in urinary stress hormones: the population-based Chicago Health, Aging, and Social Relations Study. *J Appl Physiol* 97: 941–947, 2004. First published May 14, 2004; 10.1152/jappphysiol.00256.2004.—Gender and ethnic disparities in cardiovascular disease and mortality have spurred interest in the epidemiology of stress hormone production. Greater disease burden among men and blacks raises the possibility of gender and ethnic differences in stress hormone production. The purpose of this study was to determine whether urinary stress hormones were higher among men and blacks in a population-based sample. Urinary hormone analysis permits a time-integrated assessment of the stress response system. However, differences in collection and standardization strategies have led to inconsistent findings. Subjects were an ethnically diverse population-based sample of 229 men and women aged 50–67 yr who provided an overnight urine specimen. Urine concentration was standardized using a traditional creatinine-based approach as well as a new method that accounts for muscle mass. With the use of creatinine standardization, no gender or ethnic differences were noted in epinephrine or cortisol production. Norepinephrine levels were higher among women compared with men ($P = 0.001$), however. After accounting for muscle mass, we found that both epinephrine ($P = 0.018$) and norepinephrine ($P = 0.033$) levels were higher among men compared with women. No significant differences in cortisol production were found by gender or ethnicity. The consistency of these results with previous studies of 24-h urine samples suggests muscle mass should be accounted for when comparing overnight urinary hormone values across gender and ethnicity.

African American; blacks; male; norepinephrine; epinephrine; cortisol; creatinine

HEART DISEASE MORTALITY VARIES by gender and ethnicity in the United States. Among those aged 55–64 yr, heart disease mortality among men is twice that of women and 1.9 times higher among blacks compared with whites (2). The causes of gender and ethnic disparities in heart disease are likely multifactorial, including differences in genetic predisposition, diet, access to medical care, and quality of medical care. Differential exposure to stressors and varying patterns of stress response may also contribute to disparities in heart disease. Because stress hormones fluctuate diurnally or in response to stressors or physical activity, measurement of urinary cortisol and catecholamines permits a time-integrated assessment of the stress response system (15).

Currently, two primary strategies exist for collecting urine samples and calculating urinary hormone levels. The first is collecting a 24-h urine sample and determining hormone levels per urine volume (19). The second is collecting an overnight urine sample and calculating hormone values per amount of urinary creatinine (4, 18, 19). Each approach has advantages and disadvantages (see Table 1). A third approach, which accounts for urine flow, has been used when neither 24-h nor overnight sampling is practical (8).

Twenty-four-hour urine sample collection is preferred because it provides an integration of hormone production over a longer period of time. However, compliance with this approach is poor and often not practical (12). Overnight urine sampling is less onerous, but this approach also has drawbacks. For example, overnight urine production is influenced by hydration status. Dehydrated individuals produce urine of lower volume and higher concentration. Well-hydrated individuals produce a higher volume of urine and more dilute hormone concentrations. Dividing urinary hormone concentration by urinary creatinine concentration removes the unit of volume from the results and is the most common method of correcting for the effects of hydration status (8).

Unfortunately, this approach also presents problems. The first is that creatinine excretion is influenced by muscle mass, and muscle mass varies as a function of individual differences that are independent of hormone production. Gender differences are a case in point. In a study of 468 men and women aged 18–88 yr, Janssen et al. (11) found that men had significantly more skeletal muscle mass compared with women both in absolute terms (33 vs. 21 kg) and relative to body mass (38.4 vs 30.6%). Other studies have shown greater urinary creatinine among men compared with women over 24 h (10) and in spot samples (14). When urinary hormone concentration is divided by creatinine concentration, the larger values of creatinine concentration among men reduce the hormone-to-creatinine ratio. This effectively underestimates urinary hormone values among men. Using a nationally representative data set, Mattix et al. (14) demonstrated that using a single cutoff value (30 $\mu\text{g}/\text{mg}$) of urinary albumin-to-creatinine ratio led to a significant underestimation of microalbuminuria prevalence in men (6.0%) compared with women (9.2%). This was attributed to the significantly higher urinary creatinine excretion among men (152 mg/dl) compared with women (108 mg/dl). The authors concluded that gender-specific albumin-to-creatinine

Address for reprint requests and other correspondence: C. M. Masi, Dept. of Medicine, Univ. of Chicago, 5841 S. Maryland Ave., M/C 2007, Chicago, IL 60637 (E-mail: cmasi@medicine.bsd.uchicago.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Table 1. Comparison of urinary hormone collection and reporting methods

24-hour Sample	Overnight Sample
Advantages 1. Less influenced by sleep-wake cycle. 2. Not influenced by timing of collection initiation.	Advantages 1. Compliance less difficult. 2. Hormone concentration can be standardized using urinary creatinine concentration.
Disadvantages 1. Compliance more difficult. 2. Values may be influenced by body size.	Disadvantages 1. Hormone production influenced by sleep-wake cycle and physical activity. 2. Values influenced by timing of specimen collection. 3. Creatinine production is affected by diet, exercise, lean muscle mass, kidney function, and possibly ethnicity. 4. Creatinine standardization underestimates hormone production among those with greater muscle mass.

ratio cutoff values should be used to account for differences in creatinine excretion when screening for microalbuminuria.

A second problem with creatinine standardization is that creatinine excretion varies by ethnicity. James et al. (10) found that blacks excrete 5% more urinary creatinine per weight compared with whites. This difference was attributed to greater muscle mass among blacks compared with whites. A subsequent study found 30% higher urinary creatinine per weight among blacks compared with whites (7). Ethnic differences in muscle mass, muscle metabolism, and dietary protein were offered as possible explanations. Although gender differences in muscle mass are well established, ethnic differences in muscle mass are less clear. Gallagher et al. (5) found no black-white muscle mass difference among men but a 10% greater muscle mass among black women compared with white women (5). As for muscle metabolism, Ama et al. (1) found that the activity of muscle enzymes (creatinine kinase, hexokinase, and lactate dehydrogenase) was 30–40% greater among blacks compared with whites. Others have suggested that increased urinary creatinine among blacks may be related to higher levels of testosterone. Testosterone promotes the synthesis of creatine, a creatinine precursor (7).

The effects of creatinine standardization on test results can be seen by comparing studies that report 24-h urinary hormone levels with those that report creatinine-corrected hormone levels. Souza et al. (17) reported greater 24-h excretion of both epinephrine and norepinephrine among men compared with women. Gerlo et al. (6) also found higher 24-h urinary epinephrine, norepinephrine, and dopamine levels among men compared with women. In that study, correcting for urinary creatinine excretion reversed the gender effect and resulted in higher creatinine-corrected norepinephrine and dopamine among women compared with men. The creatinine-corrected epinephrine remained higher among men, but the gender difference was reduced. Correcting for creatinine excretion, Hansen et al. (9) found higher 24-h urinary epinephrine among men but no gender differences in urinary excretion of norepinephrine or cortisol. That study did not report raw hormone values. Another study noted higher creatinine-corrected overnight epinephrine among women compared with men and some evidence of lower epinephrine and norepinephrine among blacks compared with whites (15). These results suggest that creatinine correction leads to underestimation of urinary hormone values among men and may affect comparison of urinary hormones by ethnicity.

The first aim of the present study was to examine gender and ethnic differences in overnight urinary catecholamines, cortisol, and creatinine in a population-based sample of older adults

in an urban setting. A related aim was to investigate whether inconsistencies in the prior literature might be due to the effects of gender and ethnic differences in creatinine excretion on creatinine-corrected hormone values. Finally, we tested a new method of analysis that permits calculation of urinary hormone concentrations while accounting for gender and ethnic differences in creatinine excretion due to muscle mass.

MATERIALS AND METHODS

Subjects

A population-based sample of 229 English-speaking blacks (44 women, 37 men), Hispanics (33 women, 33 men), and non-Hispanic whites (43 women, 39 men) from Cook County, Illinois, aged 50–67 yr, served as participants in the first year of the Chicago Health, Aging, and Social Relations Study, a longitudinal, population-based study. A multistage probability sampling design was used to select respondents. Additional information on the sampling and recruiting strategies is available from the authors on request. Informed consent was obtained, and participants were paid \$126 for participating in the first year. Table 2 provides demographic information about the sample.

Procedures

Before their scheduled test day in the laboratory, participants were mailed instructions and a container (prefilled with 25 ml of 50% glacial acetic acid as a preservative) for an overnight urine sample. Participants were instructed to thoroughly void, but not into the container, before going to bed on the night before the laboratory tests. The provided container was used for any nighttime voiding and for the first morning void the next day. Participants were also instructed to mix the contents of the container.

Participants arrived at the laboratory at ~8:30 AM, whereupon the urine sample volume was measured and aliquots of urine were frozen at -80°C and batched for later testing. After providing informed consent, participants began a day of assessments that included demographic evaluation (age, education, occupation), self-report surveys, interviews, lunch, and a cardiovascular protocol that is not directly related to this study.

Measures

Urinary analyses. Analyses of urinary creatinine and urinary free hormones (epinephrine, norepinephrine, and cortisol) were conducted using high-performance liquid chromatography. The acidity of each urine sample was adjusted as necessary to achieve optimal extraction of constituents. Inter- and intra-assay variability (i.e., coefficient of variation) for these high-performance liquid chromatography analyses is 4–7% for epinephrine, 3–5% for norepinephrine, 6–10% for cortisol, and 2% for creatinine. Although 229 participants enrolled in the study, the number of participants who provided urinary samples for

Table 2. Demographic profile of participants

	All	White	Black	Hispanic	Statistical Significance
Subjects					
Total	229	82	81	66	
Men	109	39	37	33	
Women	120	43	44	33	
Mean age \pm standard deviation	57.4 \pm 4.5	58.2 \pm 4.3	58.2 \pm 4.8	55.6 \pm 3.7	
Overall ANOVA					$F(2,228) = 8.09$ $P < 0.001$
Education					
Less than high school	31	6	12	13	
High school graduate/general educational development	72	18	28	26	
Some college	52	18	20	14	
College graduate	33	14	11	8	
Completed graduate school	39	26	9	4	
Missing information	2	0	1	1	
Overall Pearson χ^2					$\chi^2(8) = 25.51$, $P < 0.01$
Income					
Less than \$5,000	4	0	3	1	
\$5,001–\$7,500	2	0	1	1	
\$10,001–\$15,000	13	3	8	2	
\$15,001–\$20,000	15	3	7	5	
\$20,001–\$30,000	21	9	6	6	
\$30,001–\$40,000	21	4	9	8	
\$40,001–\$50,000	31	8	12	11	
\$50,001–\$75,000	42	16	13	13	
\$75,001–\$100,000	35	17	9	9	
\$100,001–\$200,000	25	15	6	4	
Over \$200,000	7	5	1	1	
Missing information	13	2	6	5	
Overall Pearson χ^2					$\chi^2(20) = 27.52$, $P = 0.121$

which hormone and creatinine levels could be determined varied from 198 to 221 depending on the constituent.

Weight, body mass index, and fat-free mass. Participants were weighed and measured on a standard medical scale (Detecto, Pro-Med Products, Atlanta, GA). Body mass index (BMI) was calculated as weight (kg)/height (m²). Body composition was measured by using bioelectrical impedance analysis (RJL Systems, Clinton Township, MI). Using this system, two pairs of electrodes are attached to specific anatomic landmarks on the ankle and wrist of the subject. An electrical current of 50 kHz is applied across the distal source electrodes, and resistance to the current is detected by the proximal detecting electrodes. As the current travels through the body, it experiences a slight delay due to living cells. This delay, compared with the reference signal, provides a reactance reading that reflects intracellular volume and permits the calculation of body cell mass and intracellular water. Relative to the reference signal, the nondelayed signal provides a magnitude (resistance) reading that permits the calculation of extracellular water and extracellular mass. Fat-free mass is calculated as the sum of body cell mass and extracellular mass.

Demographic Variables

Gender, ethnicity, age, education, and income were the demographic variables of interest. Gender and ethnicity were treated as categorical variables, whereas the other variables were treated as continuous. Education was obtained by using a 5-point scale, ranging from "1 = less than high school" to "5 = completed graduate school." Income was obtained by using a 11-point scale, ranging from "1 = less than \$5,000" to "11 = over \$200,000." See Table 2 for the distribution of education and income.

Data Reduction and Statistical Analysis

Three methods for calculating overnight urinary hormone levels were used. In the first and simplest, we controlled for volume by dividing total urinary hormone by the volume of urine produced. In

the second, we controlled for urine concentration by dividing the hormone concentration by the urinary creatinine concentration.

Finally, we developed a new method of standardization for urine concentration that removes the variance in creatinine levels attributable to differences in muscle mass. The proposed method utilizes a partial regression approach. In essence, the first step involves calculating residualized scores for creatinine levels using fat-free mass as the predictor. That is, each individual has a residual score that represents the difference between his or her creatinine level (the "observed value") and the predicted value on the basis of the regression of urinary creatinine levels (the dependent variable) on fat-free mass (the predictor variable). These residual scores represent creatinine values that do not differ as a function of fat-free mass (see Fig. 1). By eliminating the effects of muscle mass on creatinine values, these residual creatinine scores can be used to adjust for urine concentration without biasing against those with larger muscle mass.

In the second step, urinary hormone values were corrected for variations in urine concentration. Rather than simply dividing the hormone levels by residual creatinine levels, we conducted a second regression analysis, but this time using residual creatinine scores as the predictor and the overnight urinary hormone of interest (epinephrine, norepinephrine, and cortisol) as the dependent variable (see Fig. 2). The residual scores for the urinary hormone levels reflect each individual's hormone levels independent of urine concentration and do not overcorrect for other sources of creatinine differences. Results are expressed as the mean difference in hormone value between the group (i.e., men, women, black, white, Hispanic) and the value predicted by the regression of hormone concentration on residualized creatinine level.

Analysis of variance was used to assess creatinine and hormone differences in the categorical variables, gender and ethnicity. We also tested for the presence of interaction effects to determine whether gender differences varied as a function of ethnicity. Ordinary least squares regression models were used to test the effects of age, education, and income on creatinine and hormone levels. For each

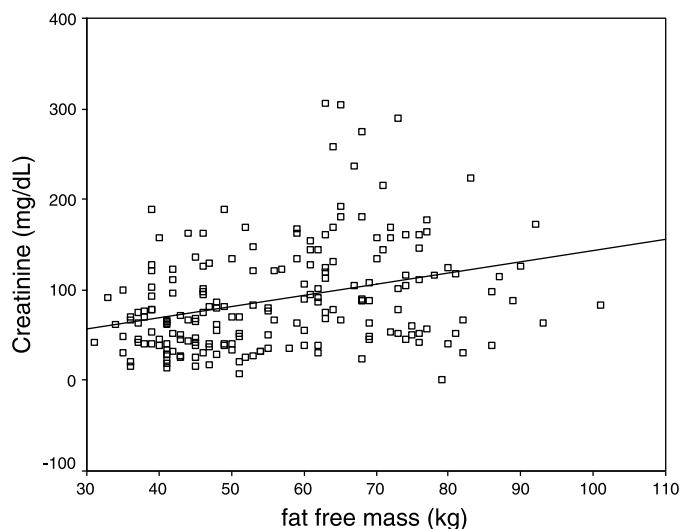


Fig. 1. Fat-free mass and creatinine levels. Regressing urinary creatinine on fat-free mass produces a prediction line that serves as an estimate of the amount of creatinine that is attributable to fat-free mass (i.e., muscle). Note that points (i.e., individuals) vary in their distance from the prediction line. The distance of each point from this line represents a residualized creatinine level or the amount of creatinine that cannot be explained by knowing fat-free mass. To convert mg/dl to mmol/l, multiply by 0.0884.

urinary constituent, the effects of age, education, and income were simultaneously tested in a single-regression model that held constant the influence of gender and ethnicity, factors that have documented effects on creatinine and urinary hormone excretion. For all analyses, degrees of freedom were adjusted for incomplete data.

RESULTS

Response Rate

The response rate among eligible persons was comparable to those of other well-conducted telephone surveys. Among all households called, we were unable to speak to someone (despite repeated attempts) or were unable to determine whether an eligible individual lived in the household in 23%. Among households with eligible individuals, the overall response rate was 45%.

Demographic Differences in Education and Age

Ethnic groups did not differ significantly in household income, but they did differ in highest level of education attained [$\chi^2(8) = 25.505, P < 0.01$]. This difference was attributable to graduate school being completed by a greater proportion of whites than blacks or Hispanics. In addition, the Hispanic group was slightly younger than the white and black groups [$F(2,228) = 8.09, P < 0.001$].

Demographic Differences in Weight, BMI, Fat-Free Mass, and Creatinine Production

Although there was no significant gender difference in BMI, weight and fat-free mass were significantly higher among men compared with women (see Table 3). Compared with whites and Hispanics, blacks had higher weight and BMI. In contrast, the ethnic differences in fat-free mass were not statistically significant. To examine whether urine creatinine concentration differed across gender and ethnicity, a two (gender: men vs.

women) \times three (ethnicity: white, black, Hispanic) analysis of variance was conducted. The analysis yielded a significant main effect for gender [$F(1,218) = 39.20, P < 0.001$], indicating that men have higher levels of urinary creatinine than women. Additionally, there was a main effect for ethnicity [$F(2,218) = 4.22, P = 0.016$], indicating that blacks excrete more creatinine than whites. Creatinine excretion was correlated with fat-free mass [$r(195) = 0.32, P < 0.001$] but not weight [$r(228) = 0.121, P > 0.05$] or BMI [$r(222) = -0.03, P > 0.6$]. The slope of the relationship between creatinine and FFM did not differ by ethnicity or the presence of diabetes. In addition, there was no difference in the ratio of creatinine to fat-free mass by gender or ethnicity.

Creatinine was next regressed on age, education, and income. Ethnicity and gender were entered as covariates to serve as controls. The results revealed that, holding gender and ethnicity constant, age is the only continuous demographic variable related to creatinine production, with creatinine levels decreasing with age ($B = -1.58, SE = 0.86, P = 0.069$; see Table 4).

Demographic Differences in Urinary Catecholamine and Glucocorticoid Excretion

Attention then turned to examining whether there were demographic differences in urinary catecholamine and glucocorticoid excretion. A two (gender) \times three (ethnicity) analysis of variance was conducted for each of the hormones examined by using three types of standardization (volume, creatinine, and residualized change; see Table 3). Regarding volume-standardized hormone levels, the analysis yielded significant main effects for gender, such that men excreted more

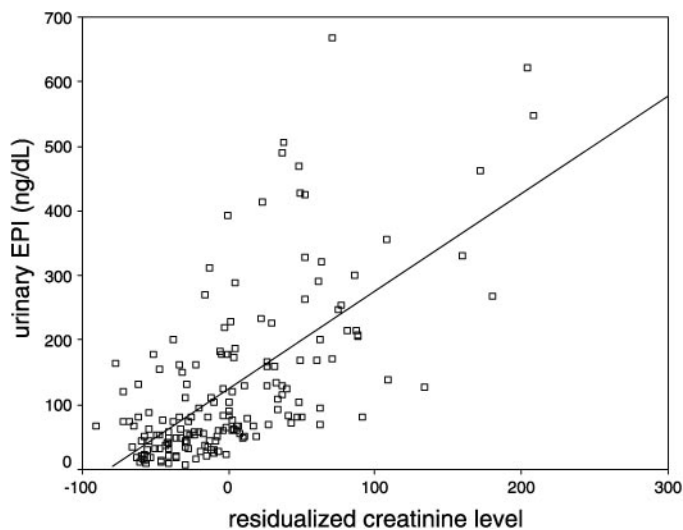


Fig. 2. Residualized creatinine and hormone levels. Regressing urinary hormone [in this case, epinephrine (EPI)] concentration on residualized creatinine level produces a prediction line that serves as the best estimate of the relationship between hormone and residualized creatinine. Points (i.e., individuals) vary in their distance from the prediction line, and the distance of each point from this line represents a residualized hormone level. The residualized hormone level used in analyses, therefore, represents a creatinine-standardized value but one that is standardized relative only to that portion of creatinine that is not related to muscle mass. In other words, this technique does not overcorrect hormone levels for individual differences in creatinine due to muscle mass differences. To convert ng/dl to nmol/l, multiply by 0.0546.

Table 3. Distribution of urinary creatinine and hormones by gender and ethnicity

Variable	All	Men	Women	White	Black	Hispanic
Weight, kg	86.67	92.54 ^b	81.31 ^a	84.63 ^a	92.08 ^b	82.65 ^a
Body mass index, kg/m ²	31.51	31.17 ^a	31.82 ^a	29.93 ^a	33.24 ^b	31.37 ^a
Fat-free mass, kg	56.16	69.83 ^b	44.04 ^a	56.68 ^a	57.22 ^{†‡}	54.21 ^a
Urine volume, ml	546.52	540.57 ^a	552.05 ^a	553.94 ^a	550.12 ^a	532.73 ^a
Creatinine, mg/dl	89.98 (7.95)	114.22 (10.10) ^b	67.23 (5.94) ^a	82.24 (7.27) ^a	103.24 (9.13) ^{b‡}	84.05 (7.43) ^{a,b}
Creatinine/weight	1.07 (0.09)	1.29 (0.11) ^b	0.87 (0.08) ^a	0.98 (0.09) ^a	1.18 (0.10) ^a	1.05 (0.09) ^a
Creatinine/body mass index ratio	2.97 (0.26)	3.8 (0.34) ^b	2.19 (0.19) ^a	2.77 (0.25) ^a	3.35 (0.30) ^a	2.77 (0.25) ^a
Creatinine/fat-free mass ratio	1.6 (0.14)	1.69 (0.15) ^a	1.53 (0.13) ^a	1.44 (0.13) ^a	1.82 (0.16) ^{a‡}	1.56 (0.14) ^a
Epinephrine, ng/dl	127.33 (6.95)	156.66 (8.55) ^b	97.40 (5.32) ^a	107.44 (5.87) ^a	159.57 (8.71) ^{b‡}	113.87 (6.22) ^{a,b}
Epinephrine, ng/mg creatinine	1.43 (0.88)	1.40 (0.91) ^a	1.45 (0.89) ^a	1.32 (0.81) ^a	1.56 (0.97) ^a	1.39 (0.86) ^a
Residualized epinephrine, ng/dl		16.73 (0.91) ^b	-16.55 (-0.90) ^a	-4.62 (-0.25) ^a	10.2 (0.56) ^a	-5.98 (-0.33) ^a
Norepinephrine, ng/dl	2,444.97 (144.50)	2,830.18 (167.18) ^b	2,073.89 (122.57) ^a	2,157.90 (127.54) ^a	2,921.60 (172.67) ^{b‡}	2,261.87 (133.68) ^{a,b}
Norepinephrine, ng/mg creatinine	29.52 (19.74)	26.16 (17.49) ^a	32.74 (21.89) ^b	27.66 (18.49) ^a	31.65 (21.16) ^a	29.32 (19.60) ^a
Residualized norepinephrine, ng/dl		204.33 (12.08) ^b	-187.65 (-11.09) ^a	-28.64 (-1.69) ^a	150.32 (8.89) ^a	-134.41 (-7.94) ^a
Cortisol, ng/dl	1,045.36 (28.84)	1,199.79 (33.10) ^b	904.43 (24.95) ^a	1,101.61 (30.39) ^a	963.09 (26.57) ^a	1,062.74 (29.32) ^a
Cortisol, ng/mg creatinine	14.21 (4.44)	12.80 (4.00) ^a	15.53 (4.85) ^a	15.58 (4.86) ^a	12.19 (3.80) ^a	14.64 (4.57) ^a
Residualized cortisol, ng/dl		101.04 (2.79) ^a	-87.86 (-2.42) ^a	66.66 (1.84) ^a	-132.3 (-3.65) ^a	55.99 (1.54) ^a

Values in parentheses are in SI units (mmol/l for creatinine, nmol/l for epinephrine, norepinephrine, and creatinine, and nmol/mmol of creatinine for the creatinine-adjusted hormone values). Within each demographic comparison (i.e., by gender, by ethnicity), means in the same row that do not share a superscript differ at $P < 0.05$ in the Tukey's honestly significant difference comparison. * $P < 0.1$, men compared with women. † $P < 0.1$, blacks compared with whites. ‡ $P < 0.1$, blacks compared with Hispanics.

overnight epinephrine [$F(1,192) = 12.98, P < 0.001$], norepinephrine [$F(1,208) = 9.53, P = 0.002$], and cortisol [$F(1, 19) = 8.45, P = 0.004$]. Additionally, there was also a main effect for ethnicity, such that blacks excreted more epinephrine and more norepinephrine than whites [$F(2,19) = 12.98, P = 0.021$; $F(2,208) = 3.86, P = 0.023$, respectively]. There was no significant ethnic difference in cortisol excretion.

Regarding creatinine-standardized hormone levels, the results revealed significant main effects for gender, with women excreting higher levels of overnight norepinephrine [$F(1,213) = 11.40, P = 0.001$]. No gender difference in epinephrine production was noted. These results are contrary to the volume-standardized hormones results. Analyses of ethnicity also revealed different results after standardizing by creatinine levels, namely that ethnicity was not

significantly associated with variation in excretion of any of the hormones.

For the residualized hormone levels, consistent with the volume-standardized (but contrary to the creatinine-standardized) analysis, there were significant main effects for gender, with men excreting higher levels of overnight epinephrine and norepinephrine [$F(1,163) = 5.72, P = 0.018$; $F(1,182) = 4.60, P = 0.033$, respectively]. The higher level of cortisol among men compared with women approached statistical significance [$F(1,166) = 3.63, P = 0.058$]. Although ethnicity was not significantly associated with variation in excretion of any of the hormones, the mean levels were consistent with those obtained in the volume-standardized analysis. Analysis of variance testing for interaction effects revealed the gender differences in epinephrine and norepinephrine did not vary by ethnicity. In

Table 4. Demographic differences in creatinine and urinary hormone excretion

Variable	Age	Education	Income
Creatinine, mg/dl	$B = -1.58 (0.86); [B = -0.14 (0.08)]†$	$B = -0.45 (3.31); [B = -0.04 (0.29)]$	$B = -0.11 (1.80); [B = -0.01 (0.16)]$
Epinephrine, ng/dl	$B = -4.89 (2.15); [B = -0.27 (0.12)]*$	$B = 11.16 (8.13); [B = 0.61 (0.45)]$	$B = -5.15 (4.38); [B = -0.28 (0.24)]$
Epinephrine, ng/mg creatinine	$B = -0.01 (0.02); [B = -0.006 (0.01)]$	$B = 0.12 (0.07); [B = 0.07 (0.04)]†$	$B = 0.07 (0.04); [B = -0.04 (0.02)]†$
Residualized epinephrine, ng/dl	$B = -3.52 (1.90); [B = -0.19 (0.10)]†$	$B = 8.77 (6.94); [B = 0.48 (0.38)]$	$B = -7.07 (3.99); [B = -0.39 (0.22)]†$
Norepinephrine, ng/dl	$B = -37.81 (31.73); [B = -2.23 (1.88)]$	$B = -14.41 (120.93); [B = -0.85 (7.15)]$	$B = -33.83 (67.41); [B = -2.00 (3.98)]$
Norepinephrine, ng/mg creatinine	$B = -0.04 (0.23); [B = -0.03 (0.15)]$	$B = 0.96 (0.88); [B = -0.64 (0.59)]$	$B = -1.21 (0.48); [B = -0.81 (0.32)]*$
Residualized norepinephrine, ng/dl	$B = -33.55 (24.57); [B = -1.98 (1.45)]$	$B = 59.82 (90.00); [B = 3.54 (5.32)]$	$B = -98.85 (53.88); [B = -5.84 (3.18)]†$
Cortisol, ng/dl	$B = -25.66 (12.72); [B = -0.71 (0.35)]*$	$B = 5.23 (50.71); [B = 0.14 (1.40)]$	$B = -37.52 (26.60); [B = -1.04 (0.73)]$
Cortisol, ng/mg creatinine	$B = -1.39 (1.01); [B = -0.06 (0.06)]$	$B = -2.66 (3.68); [B = -0.09 (0.25)]$	$B = 1.00 (2.18); [B = -0.09 (0.13)]$
Residualized cortisol, ng/dl	$B = -15.13 (13.53); [B = -0.42 (0.37)]$	$B = -19.36 (51.01); [B = -0.53 (1.41)]$	$B = -27.55 (29.35); [B = -0.76 (0.81)]$

For each urinary constituent, the effects of age, education, and income were simultaneously tested in a single-regression model that held constant the influence of gender and ethnicity. B values are followed by SE in parentheses. B values within brackets are in SI units (mmol/l for creatinine; nmol/l for epinephrine, norepinephrine, and creatinine and nmol/mmol of creatinine for the creatinine-adjusted hormone values). * $P < 0.05$. † $P < 0.10$.

addition, no gender-ethnicity interaction effect was noted in cortisol production.

To examine whether there were age, education, or income-related differences, the urinary hormones were regressed on age, education, and income with gender and ethnicity entered as controls (see Table 4). For volume-standardized hormones, controlling for gender and ethnicity, age was significantly associated with epinephrine excretion. As age increases, there is decreased epinephrine excretion ($B = -4.89$, $SE = 2.15$, $P = 0.024$) and decreased cortisol excretion ($B = -25.66$, $SE = 12.72$, $P = 0.045$). Education and income were not significantly associated with variations in urinary hormone excretion.

For the creatinine-standardized hormones (controlling for gender and ethnicity), contrary to the volume-standardized analyses, age was not significantly associated with variations in excretion for any of the hormones. Level of income was inversely associated with catecholamine excretion. As income increased, epinephrine and norepinephrine excretion decreased ($B = -0.07$, $SE = 0.04$, $P = 0.060$, $B = -1.21$, $SE = 0.48$, $P = 0.012$, respectively).

Regarding the residualized hormones (controlling for gender and ethnicity), consistent with the volume-standardized analyses, education was not significantly associated with variations in urinary hormone excretion. As age increased, epinephrine excretion decreased, although not significantly ($B = -3.52$, $SE = 1.90$, $P = 0.066$). Similarly, as income increased, epinephrine and norepinephrine excretion decreased ($B = -7.07$, $SE = 3.99$, $P = 0.079$, $B = -98.85$, $B = 53.88$, $P = 0.068$, respectively), although not significantly.

DISCUSSION

Among urine tests, multiple 24-h collections from large cohort sizes provide the best opportunity to assess stress hormone production. This approach accounts for day-to-day and circadian differences in hormone production. It also minimizes the effects of hydration status and the timing of sample collection. However, 24-h urine collections are not always practical. As a result, overnight urine sample collections have been used increasingly to assess patterns of hormone production. In this approach, volume-corrected results can be skewed by the hydration status of the study subject and their urine concentration. The most common method of correcting for urine concentration is to correct for urinary creatinine concentration. Placing creatinine concentration in the denominator removes the unit of volume and permits a degree of standardization. Unfortunately, creatinine production varies by gender and ethnicity, and these differences lead to underestimation of hormone production among those with greater muscle mass. This has yielded inconsistencies in the literature with respect to gender and ethnic differences in hormone production.

The results presented here are consistent with prior literature that shows greater creatinine production among men and blacks (7, 10, 14). Men had higher fat-free mass compared with women, but there were no significant gender or ethnic differences in the ratio of creatinine to fat-free mass. This suggests muscle mass largely accounts for differences in creatinine production.

The volume-standardized results from our overnight urine collection are consistent with previous reports showing higher

24-h levels of urinary epinephrine and norepinephrine among men compared with women (6, 17), higher cortisol production among men compared with women (3, 13, 16), and similar cortisol production among whites compared with blacks. However, volume standardization alone is not appropriate for overnight urine samples because it does not correct for urine concentration.

Consistent with previous reports, we found the relationships between gender and urinary stress hormones were significantly affected by creatinine correction (6). Specifically, creatinine standardization resulted in no gender differences in epinephrine or cortisol and higher levels of norepinephrine among women compared with men. We also found no ethnic differences in epinephrine, norepinephrine, or cortisol among blacks compared with whites. The differences between volume-corrected and creatinine-corrected hormone levels appear to be due to the placement of creatinine concentration in the denominator during creatinine standardization. This produces an underestimation of values among those with higher muscle mass.

To account for gender and ethnic differences in creatinine production while at the same time correcting for urine concentration, we calculated residual scores for creatinine and then regressed the urinary hormone levels on these residual creatinine scores. As would be expected, gender and ethnic differences in creatinine levels were substantially reduced in residual creatinine scores.

Analyses of the residual scores for hormones revealed a different pattern of results from that found when using the standard creatinine correction. Importantly, the pattern of results found for the residual scores for hormones was more consistent with values obtained through volume standardization and with previous 24-h urine studies (6, 17). We found the residualized epinephrine, norepinephrine, and cortisol were all higher among men compared with women. Also, although ethnic differences in urinary catecholamine levels were not statistically significant, our results suggest a larger sample size may identify higher epinephrine and norepinephrine among blacks compared with whites. Inferences regarding the relationship between cardiovascular disease and gender-ethnic differences in urinary free hormones should be made with caution, however, because urinary free hormones do not necessarily reflect the activity of plasma glucocorticoid and catecholamine metabolites. Second, we did not assess for variation in length of collection time or nighttime physical activity. Our findings therefore may reflect unmeasured group differences in these variables.

A limitation is the requirement of a measure of fat free mass if the correction procedure developed here is to be implemented. Indexes such as weight and BMI are economical to obtain, but we found weight and BMI to be unsuitable as substitutes for fat-free mass. Although our measure of fat-free mass confirmed that men were characterized by greater muscle mass than women, the BMI scores did not differ between these groups. Moreover, weight and BMI levels were found to be weakly correlated with our measure of fat-free mass and uncorrelated with creatinine levels. Thus, despite the simplicity and economy of weight and BMI measurement, substituting these measures for muscle mass is in effect equivalent to making no correction for the influence of muscle mass on creatinine levels.

Overnight urine samples offer an excellent opportunity for a time-integrated assessment of the stress response system. To date, inconsistencies have been noted between studies that report 24-h and overnight hormone levels (6, 15, 17). We believe these inconsistencies are related to the practice of standardizing urine concentration by dividing by creatinine concentration. Because of gender and ethnic differences in creatinine production, this practice underestimates hormone values among men and blacks. Utilization of residual creatinine scores permits the correction of overnight hormone levels for urine concentration while also eliminating muscle mass differences in creatinine production. Consequently, the correction procedure developed here in a population-based sample that varied across age, gender, and ethnicity should improve the measurement properties of overnight urinary hormone levels and thereby lead to further clarification of the role of the stress response system in gender and ethnic differences in disease. Moreover, given individual differences in muscle mass within gender and ethnicity, this procedure may increase the measurement properties of any urinary hormone assay, whether collected overnight or over a 24-h period, that are to be corrected using creatinine levels.

GRANTS

This research was supported by National Institute on Aging Program Grant P01 AG-18911 (Social Isolation, Loneliness, Health, and the Aging Process).

REFERENCES

1. **Ama PF, Simoneau JA, Boulay MR, Serresse O, Theriault G, and Bouchard C.** Skeletal muscle characteristics in sedentary black and Caucasian males. *J Appl Physiol* 61: 1758–1761, 1986.
2. **Anderson RN.** Deaths: leading causes for 2000. *Natl Vital Stat Rep* 50: 1–86, 2002.
3. **Andrew R, Phillips DIW, and Walker BR.** Obesity and gender influence cortisol secretion and metabolism in man. *J Clin Endocrinol Metab* 83: 1806–1809, 1998.
4. **Contreras N, Shantoshi H, and Tyrell LJ.** Urinary cortisol in the assessment of pituitary-adrenal function: utility of the 24 hour and spot determination. *J Clin Endocrinol Metab* 62: 5–12, 1985.
5. **Gallagher D, Visser M, DeMeersman RE, Sepulveda D, Baumgartner RN, Pierson RN, Harris T, and Heymsfield SB.** Appendicular skeletal muscle mass: effects of age, gender, and ethnicity. *J Appl Physiol* 83: 229–239, 1997.
6. **Gerlo EAM, Schoores DF, and Dupont AG.** Age- and sex-related differences for the urinary excretion of norepinephrine, epinephrine, and dopamine in adults. *Clin Chem* 37: 875–878, 1991.
7. **Goldwasser P, Aboul-Magd A, and Maru M.** Race and creatinine excretion in chronic renal insufficiency. *Am J Kidney Dis* 30: 16–22, 1997.
8. **Greenberg GN and Levine RJ.** Urinary creatinine excretion is not stable: a new method for assessing urinary toxic substance concentration. *J Occup Med* 31: 832–838, 1989.
9. **Hansen AM, Garde AH, Christensen JM, Eller NH, and Netterstrom B.** Reference intervals and variation for urinary epinephrine, norepinephrine and cortisol in healthy men and women in Denmark. *Clin Chem Lab Med* 39: 842–849, 2001.
10. **James GD, Sealey JE, Alderman M, Ljungman S, Mueller FB, Pecker MS, and Laragh JH.** A longitudinal study of urinary creatinine and creatinine clearance in normal subjects: race, sex, and age differences. *Am J Hypertens* 1: 124–131, 1988.
11. **Janssen I, Heymsfield SB, Wang Z, and Ross R.** Skeletal muscle mass and distribution in 468 men and women aged 18–88 yr. *J Appl Physiol* 89: 81–88, 2000.
12. **Jenner DA, Harrison GA, Prior IAM, Leonetti DL, and Fujimoto WY.** 24-h catecholamine excretion: relationships with age and weight. *Clin Chim Acta* 164: 17–25, 1987.
13. **Litchfield WR, Hunt SC, Jeunemaitre X, Fisher NDL, Hopkins PN, Williams RR, Corvol P, and Williams GH.** Increased urinary free cortisol: a potential intermediate phenotype of essential hypertension. *Hypertension* 31: 569–574, 1998.
14. **Mattix HJ, Hsu C, Shaykevich S, and Curhan G.** Use of the albumin/creatinine ratio to detect microalbuminuria: implications of sex and race. *J Am Soc Nephrol* 13: 1034–1039, 2002.
15. **Reuben IB, Talvi SLA, Rowe JW, and Seeman TE.** High urinary catecholamine excretion predicts mortality and functional decline in high-functioning, community-dwelling older persons: MacArthur Studies of Successful Aging. *J Gerontol A Biol Sci Med Sci* 55: M618–M624, 2000.
16. **Shamim W, Yousufuddin M, Bakhai A, Coats AJ, and Honour JW.** Gender differences in urinary excretion rates of cortisol and androgen metabolites. *Ann Clin Biochem* 37: 770–774, 2000.
17. **Souza MCP, Rumlper WV, Douglass LW, and Howe JC.** Urinary catecholamine excretion in men and women: between- and within subject variation. *J Nutr Biochem* 9: 396–401, 1988.
18. **Tran JG, Kovacs SJ, McIntosh TS, Davis HM, and Martin DE.** Morning spot and 24-hour urinary 6 β -hydroxycortisol to cortisol ratios: intraindividual variability and correlation under basal conditions and conditions of CYP 3A4 induction. *J Clin Pharmacol* 39: 487–494, 1999.
19. **White IR, Brunner EJ, and Barron JL.** A comparison of overnight and 24 hour urine collection to measure urinary catecholamines. *J Clin Epidemiol* 48: 263–267, 1995.