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racial/ethnic categories. The median income was \$17,500 (range \$2,500 to \$162,500) and mean education was 13.76 years ( $SD = 2.21$ ). Nearly half of the sample (47.2%) were smokers and the mean body mass index (BMI) was 28.98 ( $\Omega$ ) = 7.08). Here, we report analyses of baseline data obtained prior to any of the parent study-related interventions. All components of the study received IRB approval and subjects were paid \$820 for completing all aspects of the parent study.

Potential study participants were screened by telephone and later interviewed by a physician. Exclusion criteria included pregnancy or current breastfeeding, diagnosis of chronic illness or psychiatric disorder, or history of nasal surgery. Individuals also were excluded from participation if they were regularly taking medication, with the exception of the following medications: birth control, hormone replacement therapy, analgesics, and topical eczema/psoriasis medications.

#### Catecholamines

Urine collection. Urine collection took place under controlled conditions during a 24-hr period of quarantine in a hotel. Participants were provided with 1-liter specimen bottles containing a preservative (sodium metabisulfate), and were instructed to collect all urine voids during the target period, which was divided into two stages. Stage 1 began at 5:00 p.m. and ended at 8:00 a.m. the following morning. Urines collected during Stage 1 comprised the overnight or 15-hr sample. Stage 2 began at 8:00 a.m. and ended at 5:00 p.m. Participants were instructed to save overnight samples and the first morning void in one container, and voids collected during Stage 2 in a second container. All samples were stirred thoroughly, separated into 7 ml aliquots, and stored at -20°C until later analysis. Concentrations of epinephrine (E) and norepinephrine (NE) were derived from the 9-hr and 15-hr samples separately, and these values were combined by multiplying concentration by sample volume, averaging the resulting values, and dividing by the total volume, yielding a weighted average concentration. There were 5 participants who failed to provide Stage 1 (15-hr) urine samples, and 1 participant was missing the Stage 2 (9-hr) sample. Thus, 15-hr data were available for 188 participants, and 24-hr data were available for 187 participants.

Sample preparation. Samples were thawed and centrifuged prior to analysis by solid phase extraction (SPE) and high performance liquid chromatography (HPLC). In preparation for SPE, 3-ml aliquots of urine in duplicate, 3 ml of a standard solution (Bio-Rad) and 3 ml of a normal quantitative urine control (Bio-Rad) each were mixed with 50 µl of internal standard

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### Table 2



Correlations of Urinary Epinephrine and Norepinephrine With Blood Pressure and Salivary Cortisol

Note.  $BP = blood pressure$ .  $AUC = area under the curve$ .

 ${\uparrow}$ p < .10. \*p < .05. \*\*p < .01. \*\*\*p < .001.

are expected to correlate with SNS activity. Table 2 shows that both 15-hr and 24-hr hour urinary E correlated significantly with SBP and salivary cortisol, and both 15-hr and 24-hr urinary NE correlated significantly with cortisol. Comparison with t tests of correlation coefficients associated with 15-hr and 24-hr measures reveals that associations of 15-hr E and NE with BP and cortisol did not differ significantly from correlations of 24–hr E and NE with these physiological measures.

Correlations of urinary catecholamines with BP and cortisol are presented separately by gender in Table 3. Among men, 15-hr but not 24-hr E was correlated significantly with SBP. The t-test comparisons reveal that the difference in size between the two correlations was not statistically significant. Among women, both 15-hr and 24-hr E and NE measures were correlated significantly with salivary cortisol. Correlations of cortisol with 15-hr and 24-hr catecholamine measures, respectively, were not significantly different.

Correlations of urinary catecholamines with BP and cortisol are presented separately for Whites and African Americans in Table 4. Among Whites, both 15-hr and 24-hr E and NE were correlated significantly with SBP, DBP, and salivary cortisol. Only the difference in size between the respective correlations of 15-hr and 24-hr E with cortisol was statistically significant,  $t(98) = 2.49$ ,  $p < .01$ . Among African Americans, none of the urinary catecholamine measures were associated significantly with BP or cortisol. Correlation coefficients associated with 15-hr measures did not differ from those associated with 24-hr measures.

Given that the correlation between 15-hr E and SBP was significant only among the men in the sample—whereas the correlations between all catecholamine



Correlations of Urinary Epinephrine and Norepinephrine With Blood Pressure and Salivary Cortisol:

Table 3

Note BP = blood pressure. AUC = area under the curve.<br>† $p < .10$ . \* $p$ 

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measures and cortisol were significant only among the women—we used z tests to examine whether the size of the correlation coefficients differed between genders. Using similar comparison methods, we examined whether correlations of urinary catecholamines with SBP, DBP, and cortisol differed as a function of race/ethnicity. Results reveal that the correlation coefficients for each of the examined associations were similar across gender and race/ethnicity.

### Discussion

Our data suggest that catecholamine measures obtained via 15-hr overnight urine collection are comparable to those obtained with 24–hr collection among a large, ethnically diverse sample of healthy men and women. Moreover, associations with other physiological markers that are expected to correlate with SNS activity were similarly comparable for 15-hr and 24-hr catecholamine measures.

Although White et al.'s (1995) findings and those reported here both suggest that catecholamine data obtained via overnight collection provide a reasonable substitute for 24-hr measures, the present associations between the two sampling methods are larger than those reported by White et al. There are two features of the present study that likely explain the greater similarity between overnight and 24-hr measures reported here. First, the overnight collection employed by the present study sampled catecholamine excretion over a standardized period of 15 hours, whereas the overnight collection in the previous study sampled urine over a period of approximately 8 hours. A second explanation for the larger correlations reported here is that the present sample was substantially larger than the sample in White et al.'s study. Individual differences that might have resulted in low correlations between overnight and 24-hr measures with their small sample would have been less influential here. Finally, participants in our study were quarantined during the collection period. Although they were allowed to move around the hotel floor (e.g., interact with others, watch television), the possibility of physical exertion was limited. This limitation may have contributed to a greater similarity between daytime and nighttime catecholamine levels than might be observed were measures not taken under controlled conditions.

A few additional limitations of the present study should be noted. The protocol of the parent study from which the present data were taken required participants to stay overnight in a hotel for 1 week. It is possible that individuals who are available to participate in this type of study may not be representative of the general population (e.g., participants may be more likely to be unemployed). Also, all female participants did not provide data during the same phase of the menstrual cycle. Cyclical fluctuations in ovarian hormones have been found to be associated with unchanged BP and heart rate, despite significant variations in plasma NE (Hirshoren et al., 2002). Thus, the lack of association between urinary catecholamines and BP among women may have been accounted for, to some

extent, by menstrual-cycle effects. We explored this possibility by examining the association between urinary catecholamines and where female participants were in their menstrual cycle on the day of urine collection. Results showed no association between menstrual–cycle phase and catecholamines. Relatedly, it should be acknowledged that women taking oral contraceptives (OCs) or hormone replacement therapy (HRT) were not excluded from the present study. Research on the association between these two types of medication and catecholamine levels is limited, but estrogen replacement may be associated with lower levels of NE (Brownley et al., 2004). Thus, it is possible that the presence of women taking OCs or HRT may have influenced the results reported here.

In summary, the present results suggest that 15-hr urinary catecholamine sampling may be an acceptable alternative to 24-hr collection. The large correlations between overnight and 24–hr measures suggest that modest increases in sample size would be necessary to achieve similar power if collecting 15-hr rather than 24–hr samples (32% for E; 18% for NE). Researchers will need to weigh the costs of additional subjects to the benefit of decreased burden when choosing between these two sampling strategies.

### References

- Brownley, K. A., Hinderliter, A. L., West, S. G., Grewen, K. M., Steege, J. F., Girdler, S. S., et al. (2004). Cardiovascular effects of 6 months of hormone replacement therapy versus placebo: Differences associated with years since menopause. American Journal of Obstetrics and Gynecology, 190, 1052-1058.
- Hirshoren, N., Tzoran, I., Makrienko, I., Edoute, Y., Plawner, M. M., Itskovitz-Eldor, J., et al. (2002). Menstrual cycle effects on the neurohumoral and autonomic nervous systems regulating the cardiovascular system. Journal of Clinical Endocrinology and Metabolism, 87, 1569-1575.
- Steptoe, A. (1987). The assessment of sympathetic nervous function in human stress research. Journal of Psychosomatic Research, 31, 141-152.
- Weinkove, C. (1991). Measurement of catecholamines and their metabolites in urine. Journal of Clinical Pathology, 44, 269-275.
- White, I. R., Brunner, E. J., & Barron, J. L. (1995). A comparison of overnight and 24-hour collection to measure urinary catecholamines. Journal of Clinical Epidemiology, 48, 263-267.