

## Relation of Obesity and Diet to Sympathetic Nervous System Activity

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The hypothesis that dietary intake and obesity stimulate the sympathetic nervous system was investigated in a cross-sectional study of 572 men aged 43–85 years from the Normative Aging Study. Habitus was represented by body mass index, as a measure of overall adiposity, and by the ratio of abdomen-to-hip circumference (abdomen/hip ratio), as a measure of centripetal fat distribution. Sympathetic activity was assessed by measurement of 24-hour urinary norepinephrine excretion. Increased body mass index and total caloric intake were independently associated with increased 24-hour urinary norepinephrine excretion ( $p=0.0001$  and  $p=0.0055$ , respectively). In addition, mean urinary norepinephrine excretion was higher in subjects classified as either hyperglycemic (serum fasting glucose  $\geq 113$  mg/dl) and hyperinsulinemic (serum fasting insulin  $\geq 19$   $\mu$ IU/ml) ( $p=0.0023$ ) or in subjects classified as either hyperglycemic or hyperinsulinemic ( $p=0.0063$ ) than the mean urinary norepinephrine excretion in normal subjects. These relations were demonstrated to be independent of age, smoking status, and physical activity. Our results are consistent with the hypothesis that insulin mediates sympathetic stimulation in response to dietary intake and increases sympathetic nervous system activity in the obese. (*Hypertension* 1991;17:669–677)

Although hyperinsulinemia has emerged as a significant risk factor for the development of hypertension,<sup>1–3</sup> the role played by insulin in the pathogenesis of increased blood pressure remains unclear. Dietary intake has been shown to alter sympathetic nervous system activity, with caloric restriction suppressing<sup>4</sup> and overfeeding stimulating<sup>5</sup> sympathetic output in both animal<sup>4,5</sup> and clinical<sup>6–11</sup> studies. Insulin has been identified as an important mediator in this relation.<sup>12–14</sup> The prevalence of hyperinsulinemia observed in the obese has raised the possibility that sympathetic nervous system activity may also be stimulated.<sup>15</sup> Clinical studies of the relation between the level of sympathetic nervous system activity and the level of obesity, however, have produced varying results,<sup>16–27</sup> with some studies showing a positive relation,<sup>16–20</sup> some showing an inverse

relation,<sup>21,22</sup> and others showing no effect.<sup>23–27</sup> The existence of subtypes of obesity and differences in other characteristics among samples may explain, in part, the incongruity of these findings.

The purpose of this investigation was to cross-sectionally assess the relation of overall obesity, centripetal fat distribution, dietary intake, and insulin and glucose levels to sympathetic nervous system activity. This study adds to previous studies by addressing these relations in the Normative Aging Study (NAS), a large, population-based study of men.

### Methods

The NAS is an ongoing longitudinal, multidisciplinary study established by the Veterans Administration in 1961. Details of the study protocol have been presented elsewhere.<sup>28</sup> Volunteers were initially screened on the basis of clinical, laboratory, radiological, and electrocardiographic criteria to identify an initially healthy population. History or presence of coronary heart disease, diabetes, cancer, peptic ulcer, gout, recurrent asthma, or bronchitis were criteria for initial exclusion from the study. Subjects were also excluded if their systolic blood pressure exceeded 140 mm Hg or their diastolic blood pressure exceeded 90 mm Hg. Body composition and hyperlipidemia were not used as screening criteria.

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The protocol for this substudy was approved by the Human Studies Subcommittee of the Research and Development Committee, Department of Veterans Affairs, Veterans Administration outpatient clinic; written informed consent was obtained from all subjects.

Subjects reported for a physical examination, an anthropometric examination, and blood and urine tests every 3–5 years. Subjects fasted overnight and abstained from smoking after 8:00 PM the night before their examination. An oral glucose tolerance test (100 g) was administered to the subjects on arrival. Serum glucose was measured in duplicate on an autoanalyzer by the hexokinase method.<sup>29</sup> Serum insulin determination was performed with use of a solid-phase iodine-125 radioimmunoassay (Coat-A-Count Insulin 1987, Diagnostic Products Corp., Los Angeles).

A series of anthropometric measurements were taken with each participant standing erect with his feet together, wearing undershorts and socks only. Weight was measured on a balance beam scale (Continental Scientific Corporation, Chicago) to the nearest 0.5 lb and converted to kilograms. Stature was measured against a wall chart to the nearest 0.1 in. and converted to meters. Body mass index was calculated as weight (kg) divided by stature (m) squared. Abdomen circumference was measured to the nearest 0.1 cm at the level of the umbilicus perpendicular to the axis of the body; hip circumference was measured at the greatest protrusion of the gluteal muscles to the nearest 0.1 cm, and the ratio of abdomen circumference-to-hip circumference (abdomen/hip ratio) was calculated.

Information on smoking status was collected by interview. The participants were categorized as never smokers, current smokers, or former smokers according to their status on the day of their examination. In the statistical analyses, comparisons were made with never-smokers defined as the reference group.

Dietary data were derived from a semiquantitative food frequency questionnaire (SFFQ)<sup>30,31</sup> mailed to subjects and completed before their visit to the study. The SFFQ lists food items (with serving sizes) and elicits information on frequency of intake. Macronutrient scores were computed by multiplying the frequency of intake by the macronutrient content of the food item.

The SFFQ also provides information on physical activity. Responses to questions about the number of flights of stairs climbed per day, walking pace, and the frequency of various physical activities were used to derive a continuous physical activity variable that assessed total kilocalories used per week based on the scale devised by Paffenbarger et al.<sup>32</sup>

Twenty-four-hour urine samples were collected at home by participants and brought in at the time of their examination. In addition, a questionnaire eliciting information on urine collection times, missed collections, spilled urine, and medication use was completed by the subject. Values for norepinephrine

and epinephrine were obtained by high-performance liquid chromatography with electrochemical detection according to the method of Smedes et al<sup>33</sup> as modified by Macdonald and Lake.<sup>34</sup> The intra-assay coefficient of variation for urine samples (corrected for recovery) was 4–6% for norepinephrine and epinephrine; the interassay coefficient of variation was 6–7%.

Blood pressure was measured with a standard mercury sphygmomanometer and a 14-mm cuff (W.A. Baum Co., Inc., Copiague, N.Y.). Systolic and fifth phase diastolic blood pressure were measured to the nearest 2 mm Hg in each arm and averaged to provide one systolic and one diastolic measurement. Subjects were considered to be hypertensive if they received any medication for high blood pressure.

#### Study Subjects

Data from examinations conducted between February 1987 and May 1989 were evaluated. A 24-hour urine sample was provided by 717 (81%) of the subjects. A total of 43 urine samples were excluded from the analyses for the following reasons: eight samples had a volume under 500 ml; nine samples had a collection time of less than 15 hours; five samples were from subjects reporting an incomplete collection; five samples did not have accompanying questionnaires; and 16 samples were from subjects taking L-dopa, methyldopa, or thiorazine on the day of their urine collection. Five additional samples were excluded from the analyses because the total daily caloric intake from the food frequency questionnaire was not within the range set a priori (600–4,600 kcal/day), and they were considered to represent either underreporting or overreporting. Sixty-seven observations were excluded because of missing values for one or more of the study variables. Finally, 30 subjects taking insulin were excluded. After all the exclusions were made, data from 572 subjects were available for analysis.

Table 1 presents the descriptive statistics for the study sample and the subjects not included in the analyses (except those subjects excluded because they were taking insulin). An analysis of subjects not included in the present study showed a significant difference in stature and a difference of borderline significance in body mass index. The excluded subjects, on average, were shorter than the study subjects (mean stature  $\pm$ SD, 1.74  $\pm$  0.07 m versus 1.75  $\pm$  0.06 m,  $p=0.04$ ) and had a slightly higher body mass index (mean body mass index  $\pm$ SD, 27.0  $\pm$  3.8 versus 26.5  $\pm$  3.5,  $p=0.07$ ). There were no other significant differences in the study variables between the included and excluded subjects. Mean age of subjects in the study sample was 62 years and ranged from 43 to 85 years. The majority of subjects in the study sample were former smokers ( $n=321$ , 56%), followed by never smokers ( $n=198$ , 35%), and current smokers ( $n=53$ , 9%).

TABLE 1. Characteristics of the Study Sample and the Exclusions

Variable	Mean value of variable $\pm$ SD		Exclusions (n)	p value
	Study sample	Exclusions		
Age (yr)	62.2 $\pm$ 7.9	61.9 $\pm$ 8.2	285	0.68
Weight (kg)	82.0 $\pm$ 12.5	82.2 $\pm$ 13.0	281	0.84
Height (m)	1.75 $\pm$ 0.064	1.74 $\pm$ 0.077	257	0.04
BMI (wt [kg]/ht [m] <sup>2</sup> )	26.5 $\pm$ 3.5	27.0 $\pm$ 3.8	257	0.07
Abdomen circumference (cm)	100.4 $\pm$ 9.3	100.9 $\pm$ 9.6	256	0.54
Hip circumference (cm)	102.6 $\pm$ 7.1	103.0 $\pm$ 7.8	254	0.46
Abdomen/hip ratio	0.9782 $\pm$ 0.047	0.9789 $\pm$ 0.046	251	0.85
Urinary catecholamines ( $\mu$ g/24 hr)				
Norepinephrine	48.0 $\pm$ 20.1	44.7 $\pm$ 19.3	86	0.15
Epinephrine	6.8 $\pm$ 3.8	6.8 $\pm$ 4.3	86	0.95
Serum insulin ( $\mu$ IU/ml)				
Basal	11.4 $\pm$ 7.9	12.0 $\pm$ 8.4	220	0.37
2 hours PC	66.4 $\pm$ 66.8	68.6 $\pm$ 60.2	184	0.69
Serum glucose (mg/dl)				
Basal	101.2 $\pm$ 17.7	102.3 $\pm$ 15.2	251	0.40
2 hours PC	116.9 $\pm$ 49.3	113.8 $\pm$ 41.1	208	0.38
Caloric intake (kcal/day)	2,003.0 $\pm$ 639.0	1,941.0 $\pm$ 644.2	258	0.19
Physical activity (kcal/wk)	1,743.0 $\pm$ 1,749.5	1,675.2 $\pm$ 1,756.1	257	0.60

Study sample  $n=572$ . The sample size for exclusions based on missing values varies depending on the variable. BMI, body mass index; PC, postcarbohydrate.

### Statistical Analyses

Subjects were defined as hyperglycemic and hyperinsulinemic based on their fasting serum glucose and insulin levels. Hyperglycemia was defined as a fasting serum glucose level greater than or equal to the top 10% of the sample distribution for serum glucose (113 mg/dl). Hyperinsulinemia was defined as a fasting serum insulin level greater than or equal to the top 10% of the sample distribution for serum insulin (19  $\mu$ IU/ml). To evaluate differences in sympathetic nervous system activity among normoglycemic and hyperglycemic and normoinsulinemic and hyperinsulinemic subjects, we classified subjects into three insulin/glucose groups: 1) subjects who were normoglycemic and normoinsulinemic (insulin/glucose group 1;  $n=444$ ); 2) subjects who were hyperglycemic or hyperinsulinemic but not both (insulin/glucose group 2;  $n=103$ ); and 3) subjects who were both hyperglycemic and hyperinsulinemic (insulin/glucose group 3;  $n=25$ ). For the regression analyses, two indicator variables were formed. The first variable (insulin/glucose III) represents the comparison of subjects who were hyperglycemic and hyperinsulinemic with subjects who were normoglycemic and normoinsulinemic. The second variable (insulin/glucose II) represents the comparison of subjects who were either hyperglycemic or hyperinsulinemic with subjects who were normoglycemic and normoinsulinemic.

Variables representing energy-adjusted macronutrient intakes were computed. Residuals from a linear regression model, with absolute macronutrient intake as the dependent variable and total caloric intake as the independent variable, were added to

the expected value for each macronutrient (fat, carbohydrate, protein) for the mean total caloric intake of the study sample.<sup>35</sup> These macronutrient variables, independent of total caloric intake, were created so that total caloric intake and macronutrient intake could be independently assessed in the same regression model.

Pearson product-moment correlations were calculated between age, the habitus variables, the dietary variables, insulin and glucose levels, physical activity, and both of the catecholamines. Analysis of covariance was used to compare catecholamine values by tertile of body mass index, abdomen/hip ratio, and total caloric intake, and by insulin/glucose category, after adjustment for age, smoking, and physical activity. Multiple linear regression analysis was used to assess the independent relations of body mass index, abdomen/hip ratio, dietary intake, and the insulin/glucose variables to norepinephrine and epinephrine while adjusting for age, cigarette smoking, and physical activity. Interaction terms were formed between abdomen/hip ratio and body mass index, abdomen/hip ratio and total caloric intake, abdomen/hip ratio and age, body mass index and total caloric intake, and body mass index and age. In addition to the main effect terms, age, smoking, and physical activity, the interaction terms were included in separate regression models to test whether they were significant predictors of urinary norepinephrine and epinephrine excretion.

The natural logarithm (ln) for urinary norepinephrine and epinephrine and physical activity were used to improve the linearity assumption for the linear regression models. The exponents of the adjusted

TABLE 2. Pearson Product-Moment Correlations

Variable	ln norepinephrine	p value	ln epinephrine	p value
Age	-0.07	0.0798	-0.18	0.0001
Body mass index	0.25	0.0001	-0.05	0.2197
Abdomen circumference	0.27	0.0001	-0.11	0.0074
Abdomen/hip ratio	0.16	0.0002	-0.10	0.0228
Total caloric intake	0.11	0.0098	0.09	0.0275
ln serum insulin				
Fasting	0.09	0.0223	-0.10	0.0186
2 hours PC	0.08	0.0623	-0.13	0.0014
ln serum glucose				
Fasting	0.16	0.0001	-0.01	0.8117
2 hours PC	0.10	0.0220	-0.12	0.0028
ln physical activity	-0.07	0.0783	0.01	0.7460

Study sample  $n=572$ . PC, postcarbohydrate.

least-squares means and confidence limits for norepinephrine and epinephrine from the analyses of variance were tabulated to facilitate the interpretation of the comparisons. The ln for basal and 2-hour postcarbohydrate insulin levels were used in the correlations. Residuals were generated from the final multiple linear regression models to assess goodness-of-fit. All statistical analyses were performed with use of Statistical Analysis System AOS/VS version 5.18.<sup>36</sup>

### Results

The pattern of univariate correlation of urinary amine excretion with body habitus, insulin, glucose, and physical activity differed for norepinephrine and epinephrine (Table 2). Significant positive correlations were demonstrated between urinary norepinephrine excretion and the habitus variables: body mass index, abdomen circumference, and abdomen/hip ratio. In contrast, urinary epinephrine excretion was negatively correlated with abdomen circumference and abdomen/hip ratio and was not significantly

correlated with body mass index. Urinary norepinephrine and serum basal insulin were positively correlated, whereas urinary epinephrine was negatively correlated with basal insulin. Correlations between urinary norepinephrine excretion and the measures of serum glucose were slightly stronger than those observed for norepinephrine and insulin. Norepinephrine was positively correlated with both basal glucose and 2-hour postcarbohydrate glucose. Urinary epinephrine was negatively correlated with serum 2-hour postcarbohydrate glucose levels. Values for both norepinephrine and epinephrine increased with total caloric intake; urinary epinephrine decreased with age, whereas urinary norepinephrine was unrelated to age.

Table 3 illustrates the degree to which urinary norepinephrine and epinephrine excretion were associated with body mass index, abdomen/hip ratio, total caloric intake, and insulin/glucose category after adjusting for age, smoking, and ln physical activity. As in the unadjusted correlations, urinary norepi-

TABLE 3. Mean Catecholamine Values by Tertile of Study Variables and by Insulin/Glucose Group

Variable	Tertile or group		
BMI (wt [kg]/ht [m] <sup>2</sup> )	I (<25.0)	II (25.0-27.6)	III (>27.6)
Norepinephrine	41.41 (39.19, 43.75)	42.99 (40.73, 45.39)	49.38 (46.75, 52.16)
Epinephrine	6.20 (5.74, 6.70)	6.04 (5.60, 6.52)	5.51 (5.11, 5.96)
Abdomen/hip ratio	I (<0.96)	II (0.96-1.0)	III (>1.0)
Norepinephrine	41.65 (39.42, 44.00)	44.24 (41.89, 46.72)	47.72 (45.15, 50.43)
Epinephrine	6.31 (5.85, 6.82)	6.00 (5.56, 6.48)	5.45 (5.04, 5.89)
Total caloric intake (kcal/day)	I (<1,700)	II (1,700-2,180)	III (>2,180)
Norepinephrine	42.47 (40.18, 44.90)	45.10 (42.68, 47.66)	45.90 (43.44, 48.49)
Epinephrine	5.63 (5.22, 6.09)	5.85 (5.42, 6.32)	6.26 (5.80, 6.76)
Insulin/glucose groups	1 ( $n=444$ )	2 ( $n=103$ )	3 ( $n=25$ )
Norepinephrine	43.07 (41.56, 44.64)	48.52 (45.03, 52.27)	54.72 (46.98, 63.73)
Epinephrine	5.99 (5.69, 6.29)	5.77 (5.20, 6.41)	5.22 (4.21, 6.46)

Values are mean (confidence limits) in  $\mu\text{g}/24$  hr, adjusted for age, smoking, and ln physical activity. Study sample  $n=572$ . Insulin/glucose group 1, subjects normoinsulinemic and normoglycemic (defined as basal insulin  $<19$   $\mu\text{IU}/\text{ml}$  and basal glucose  $<113$   $\text{mg}/\text{dl}$ , respectively); insulin/glucose group 2, subjects either hyperinsulinemic and normoglycemic or hyperglycemic and normoinsulinemic; insulin/glucose group 3, subjects both hyperinsulinemic and hyperglycemic.

TABLE 4. Regression Analyses With Norepinephrine and Epinephrine as Dependent Variables

Independent variables	ln norepinephrine			ln epinephrine		
	$\beta$	SEE( $\beta$ )	<i>p</i> value	$\beta$	SEE( $\beta$ )	<i>p</i> value
Age (yr)	-0.0017	0.002	0.4142	-0.0130	0.002	0.0001
Smoking status						
Current vs. never	0.0275	0.059	0.6430	0.1061	0.084	0.2094
Former vs. never	0.0030	0.034	0.9291	-0.0259	0.049	0.5980
ln physical activity (kcal/wk)	-0.0141	0.016	0.3926	-0.0175	0.023	0.4557
Caloric intake (1,000 kcal/day)	0.0692	0.024	0.0055	0.0816	0.035	0.0211
BMI (wt [kg]/ht [m] <sup>2</sup> )	0.0238	0.005	0.0001	-0.0081	0.007	0.2636
Abdomen/hip ratio	0.4267	0.373	0.2544	-0.8717	0.531	0.1015

Study sample  $n=572$ . SEE, standard error of the estimate; BMI, body mass index.

nephrine excretion was positively associated and urinary epinephrine excretion was negatively associated with the habitus variables. Mean norepinephrine levels adjusted for age, smoking, and ln physical activity increased by 19% from the lowest to the highest tertile of body mass index and increased by 14.5% from the lowest to the highest tertile of abdomen/hip ratio. In contrast to norepinephrine, mean urinary epinephrine levels decreased by 11% from the lowest to the highest tertile of body mass index and decreased by 14% from the lowest to the highest tertile of abdomen/hip ratio. Mean values for urinary excretion of both catecholamines increased with increases in caloric intake. From the lowest to the highest tertile of total caloric intake, urinary norepinephrine increased by 8% and urinary epinephrine increased by 11%. Urinary norepinephrine and epinephrine had opposite associations with the insulin/glucose category. The hyperinsulinemic and hyperglycemic subjects (group 3) had the highest mean value for urinary norepinephrine, whereas group 2 (subject either hyperinsulinemic or hyperglycemic) had the next highest values, and subjects with normal values for insulin and glucose (group 1) had the lowest mean urinary norepinephrine values. In contrast, the mean value for urinary epinephrine excretion was highest in insulin/glucose group 1, intermediate in insulin/glucose group 2, and lowest in insulin/glucose group 3. Mean urinary norepinephrine values (confidence limits) were the highest in the insulin-requiring diabetics not included in the above analyses: 57.25 (95% confidence interval=49.10, 66.76).

Regression analyses were performed to determine whether the measures of habitus were related to ln urinary norepinephrine and ln epinephrine independent of total caloric intake (data not tabulated). Body mass index was positively associated with ln norepinephrine controlling for age, smoking, ln physical activity, and total caloric intake (regression coefficient [ $\beta$ ] $\pm$ SEE=0.0263 $\pm$ 0.004,  $p=0.0001$ ). Using the same covariates with ln epinephrine rather than ln norepinephrine as the outcome variable, the regression coefficient for body mass index was negative ( $\beta$  $\pm$ SEE=-0.0132 $\pm$ 0.006,  $p=0.0426$ ). In the above two regression models substituting abdomen/hip ratio

for body mass index, abdomen/hip ratio was positively associated with ln norepinephrine ( $\beta$  $\pm$ SEE=1.1872 $\pm$ 0.342,  $p=0.0006$ ) and negatively associated with ln epinephrine ( $\beta$  $\pm$ SEE=-1.1305 $\pm$ 0.478,  $p=0.0185$ ). Total caloric intake was positively related to both ln norepinephrine and ln epinephrine in all four regression models.

In an attempt to determine whether body fat distribution was associated with ln urinary norepinephrine independent of total body adiposity, body mass index and abdomen/hip ratio were included together in two more regression analyses controlling for age, smoking, ln physical activity, and total caloric intake (Table 4). With ln urinary norepinephrine as the dependent variable, body mass index remained statistically significant but abdomen/hip ratio did not. In a similar regression model with ln urinary epinephrine as the outcome variable, neither body mass index nor abdomen/hip ratio were statistically significant predictors of ln epinephrine. Total caloric intake was positively related to both ln norepinephrine and ln epinephrine. To ensure that the effect of total calories on urinary norepinephrine activity was not due to dietary sodium intake, the relation of urinary sodium, as a measure of dietary sodium, to ln urinary norepinephrine was examined (data not shown). In a subgroup of subjects with data available for urinary sodium ( $n=472$ ) as well as the other covariates, we determined the correlation between ln urinary norepinephrine and urinary sodium levels ( $r=0.09$ ,  $p=0.04$ ). When urinary sodium was added to the regression models the relations between body mass index and ln urinary norepinephrine and total caloric intake and ln urinary norepinephrine were unchanged.

To test whether the interactions of certain of the study variables (body mass index and age, body mass index and total caloric intake, body mass index and abdomen/hip ratio, abdomen/hip ratio and age, and abdomen/hip ratio and total caloric intake) were significant predictors of the catecholamines, regressions that included age, smoking, ln physical activity, total caloric intake, an interaction term, and the main effect terms (of the interaction) were modeled. None of the interaction terms were significant (results not shown).

TABLE 5. Regression Analysis With Norepinephrine and Epinephrine as Dependent Variables

Independent variables	ln norepinephrine			ln epinephrine		
	$\beta$	SEE( $\beta$ )	<i>p</i> value	$\beta$	SEE( $\beta$ )	<i>p</i> value
Age (yr)	-0.0036	0.002	0.0753	-0.0123	0.002	0.0001
Smoking status						
Current vs. never	0.0221	0.060	0.7143	0.1054	0.084	0.2146
Former vs. never	0.0097	0.035	0.7810	-0.0336	0.049	0.4945
ln physical activity (kcal/wk)	-0.0241	0.016	0.1479	-0.0088	0.023	0.7045
Caloric intake (1,000 kcal/day)	0.0711	0.025	0.0050	0.0810	0.035	0.0226
Insulin/glucose III	0.2438	0.079	0.0023	-0.1317	0.112	0.2399
Insulin/glucose II	0.1155	0.042	0.0063	-0.0407	0.059	0.4918

Study sample  $n=572$ . SEE, standard error of the estimate. Insulin/glucose III is defined as a comparison of the hyperinsulinemic and hyperglycemic group ( $>19 \mu\text{IU/ml}$  and  $<113 \text{ mg/dl}$ , respectively, variable=1) vs. the normoinsulinemic and normoglycemic group (variable=0). Insulin/glucose II is defined as a comparison of the hyperinsulinemic and normoglycemic group and the normoinsulinemic and hyperglycemic group (variable=1) vs. the normoinsulinemic and normoglycemic group (variable=0).

The variable representing subjects with a combination of hyperinsulinemia and hyperglycemia (insulin/glucose III) was positively associated with ln urinary norepinephrine after adjusting for age, smoking, ln physical activity, and total caloric intake (Table 5). The variable that represented subjects that were considered either hyperinsulinemic or hyperglycemic but not both (insulin/glucose II) was also positively related to ln urinary norepinephrine in the same model (Table 5). When body mass index and abdomen/hip ratio were added to the regression model with ln norepinephrine as the outcome variable, the effects of the two insulin/glucose variables (III and II) on ln norepinephrine were attenuated but remained of borderline statistical significance. In the same model, the effect of body mass index on ln norepinephrine was also attenuated; the regression coefficient for body mass index decreased by 14% when insulin/glucose was included in the model but remained statistically significant. In a similar regression model with ln urinary epinephrine as the outcome variable, neither insulin/glucose variable was a statistically significant predictor of ln epinephrine (results not shown).

The mean systolic blood pressure was  $128.6 \pm 16.4$  mm Hg. The mean diastolic blood pressure was  $78.8 \pm 8.7$  mm Hg. Urinary norepinephrine was not associated with systolic blood pressure ( $r=0.05$ ,  $p=0.23$ ) but was borderline associated with diastolic blood pressure ( $r=0.08$ ,  $p=0.068$ ). In contrast to norepinephrine, urinary epinephrine was negatively associated with systolic blood pressure ( $r=-0.09$ ,  $p=0.033$ ) and diastolic blood pressure ( $r=-0.079$ ,  $p=0.060$ ). Urinary norepinephrine was also higher in hypertensive subjects (mean=3.83) compared with nonhypertensive subjects (mean=3.77,  $p=0.066$ ). Urinary epinephrine was actually higher in nonhypertensive subjects (mean=1.84) relative to hypertensive subjects (mean=1.69,  $p=0.093$ ).

#### Discussion

The relations of dietary intake, habitus, and insulin and glucose status to sympathetic nervous system

activity, as measured by 24-hour urinary norepinephrine excretion, were examined in 572 male participants from the NAS. Increased body mass index and total caloric intake were independently associated with increased urinary norepinephrine. In addition, mean urinary norepinephrine excretion was higher in subjects who were defined as hyperglycemic and hyperinsulinemic than in normal subjects. These associations were demonstrated to be independent of the effects of age, smoking status, caloric intake, and physical activity on urinary norepinephrine excretion.

The level of sympathetic nervous system activity in human obesity has been controversial. Our finding of a positive relation between overall obesity, as measured by body mass index, and 24-hour urinary norepinephrine excretion in a large, "free-living" population supports the presence of increased sympathetic nervous system activity in the obese. Our results are consistent with other studies that have shown that plasma norepinephrine concentration,<sup>16-18</sup> appearance rate,<sup>19</sup> and urinary norepinephrine excretion<sup>20</sup> are positively associated with obesity. Other investigators have found either no relation between obesity and sympathetic nervous system activity<sup>23-27</sup> or an inverse association of sympathetic nervous system activity and obesity.<sup>21,22</sup> Lack of a conclusive relation between sympathetic nervous system activity and obesity suggests that obesity and its pathogenesis may be heterogeneous.<sup>37</sup> Difficulties in measurement of sympathetic nervous system activity and the inclusion or exclusion of different numbers of various subtypes of obesity (e.g., familial, adult onset, experimental, upper body, or centripetal) might help to explain these discrepant results.

The extent to which the sympathetic nervous system is involved in diet-induced thermogenesis has aroused considerable interest. The effect of dietary intake on sympathetic nervous system activity also has been demonstrated previously in humans.<sup>6-11</sup> In the present population-based study, a positive relation was demonstrated between total caloric intake and 24-hour urinary norepinephrine independent of body mass index. Our results are consistent with

clinical studies that have shown a decrease in norepinephrine appearance rate in circulation,<sup>6</sup> a decrease in urinary norepinephrine excretion<sup>7</sup> in response to fasting, and an increase in norepinephrine appearance rate<sup>6,8</sup> and standing plasma norepinephrine levels in response to overfeeding.<sup>9</sup>

An enhanced effect of glucose intake over protein intake and fat intake on norepinephrine levels also has been confirmed in humans<sup>10</sup> even when caloric intake was restricted.<sup>11</sup> Correlations between macronutrient intake and total caloric intake necessitate an adjustment for total caloric intake in the regression analyses when examining the effect of a specific macronutrient.<sup>35</sup> Independent of total caloric intake, carbohydrate intake, fat intake, and protein intake were not positively related to norepinephrine excretion. Variability in the estimation of the nutrient values in addition to strong correlations between total caloric intake and carbohydrate intake ( $r=0.88$ ) and fat intake ( $r=0.86$ ) may have inhibited the demonstration of independent relations of the macronutrients to sympathetic nervous system activity. Unexplained variability in the estimation of norepinephrine excretion due to the nature of the 24-hour urine collection may also have influenced these results. Sample collection was performed at home by participants rather than under study conditions. Factors that affect sympathetic nervous system activity, such as dietary intake and physical activity, were not controlled during the collection but estimated as a yearly average. Increased statistical power may be necessary to detect an effect of carbohydrate or fat intake over and above the effect of total caloric intake on sympathetic nervous system activity.

Measurements of plasma and urinary norepinephrine as indexes of sympathetic nervous system activity lack sensitivity. Norepinephrine is not a circulating hormone but is released locally from sympathetic nerve endings; moderate changes in sympathetic activity, therefore, may not be reflected in plasma levels.<sup>38</sup> Furthermore, in certain cases, norepinephrine may be released into circulation from the adrenal medulla, thereby obscuring the measurement of norepinephrine from sympathetic nerve endings.<sup>39</sup> Plasma norepinephrine measurements, moreover, are impractical in epidemiological studies because of the need to acquire the blood specimen under carefully controlled circumstances.

Twenty-four-hour urinary norepinephrine excretion may be a more integrated measure of sympathetic nervous system activity, although discriminating norepinephrine of sympathetic nervous system origin from norepinephrine of adrenal medullary origin continues to exist as a problem. In this study, the dissociation of the sympathetic nervous system and adrenal medulla responses may be indicated by the opposite relations of norepinephrine and epinephrine to the measures of body habitus, glucose, and insulin.<sup>7</sup> These opposite relations suggest that urinary norepinephrine may have provided a measure of sympathetic nervous system activity rather

than adrenal medullary activity. However, further studies should address the relation of the sympatho-adrenal system to insulin secretion and habitus.

Dietary intake may affect sympathetic nervous system activity through insulin-mediated glucose metabolism.<sup>12</sup> Insulin infusion with glucose maintenance has been associated with increased norepinephrine levels in normal human subjects.<sup>13,14</sup> In contrast, plasma glucose and epinephrine concentrations have been shown to be inversely related.<sup>40</sup> Results from the present study appear to support the hypothesis that insulin and glucose levels are directly correlated with sympathetic nervous system activity and inversely correlated with adrenal medullary activity. Urinary epinephrine was negatively correlated with insulin and glucose levels and was lower in subjects classified as hyperglycemic or hyperinsulinemic than in normal subjects. Conversely, mean urinary norepinephrine values were significantly higher in subjects classified as hyperglycemic and hyperinsulinemic than in subjects with normal values. It is of interest that the highest mean value for urinary norepinephrine was found in the insulin-requiring diabetics who were excluded from the analysis.

Our findings showed independent positive effects of both caloric intake and insulin/glucose group on urinary norepinephrine excretion. Insulin-mediated glucose metabolism is more proximate to sympathetic nervous system activity in the causal pathway than dietary intake. If the effect of caloric intake were entirely mediated through insulin levels, the expected result, when both diet and insulin were included in the regression model, might be a stronger relation of the insulin/glucose group to norepinephrine and a weaker relation between dietary intake and norepinephrine. Dietary intake, in this study, was measured as a yearly average of total calories, whereas serum insulin and serum glucose were measured temporarily more proximate to the 24-hour urine collection. A chronic effect of caloric intake and a more immediate effect of insulin-mediated glucose metabolism on sympathetic nervous system activity may be suggested by our results.

Body mass index is known to be associated with increased insulin levels and insulin resistance.<sup>41</sup> Thus, it is not surprising that the inclusion of body mass index with the two insulin/glucose variables in the regression model to predict urinary norepinephrine decreased the effect of the latter two variables on norepinephrine. These results are consistent with our hypothesis that the mechanism by which obesity increases sympathetic nervous system activity is an increase in insulin/glucose levels. Analogous to the effect of dietary intake on sympathetic nervous system activity, the effect of body mass index (and centripetal adiposity) on sympathetic nervous system activity may involve insulin-mediated glucose metabolism. Our data are consistent with independent effects of diet and obesity on increases in insulin levels and sympathetic nervous system activity.

When the NAS began in 1961, all subjects were normotensive. Over the 25 years of follow-up, roughly 40% of the population developed hypertension (defined as taking antihypertensive medications or having a systolic blood pressure of 150 mm Hg or more or a diastolic blood pressure of 95 mm Hg or more). The relation of the various factors described in this manuscript (e.g., insulin, urine catecholamines, and diet to current blood pressure levels) have not yet been analyzed but remain an important goal of this ongoing investigation.

There are a few limitations of this investigation that should be mentioned. Some misclassification in the estimates of dietary intake and sympathetic nervous system activity may have resulted from the 24-hour urine self-collection and the semiquantitative food frequency questionnaire. Increased sensitivity in the estimates of norepinephrine, epinephrine, and dietary intake would have been possible had this study been conducted in a clinical research setting. The lack of variance in dietary intake, however, that would have resulted from controlled conditions would have made impossible the assessment of the effect of diet on sympathetic nervous system activity. Moreover, assuming that such misclassification is nonselective, the finding of a positive relation between total caloric intake and urinary norepinephrine suggests that the true relation may be even stronger. Although our results were independent of age, smoking status, and physical activity, sympathetic nervous system activity is determined by multiple factors, and it is possible that an unmeasured antecedent variable may explain the apparent relations of total caloric intake and level of obesity to sympathetic nervous system activity.

In conclusion, our results are consistent with insulin-mediated sympathetic stimulation in response to increased dietary intake and may represent an adaptive mechanism used in the obese to dissipate extra calories and limit weight gain. This hypothesis may also explain the association of obesity and hypertension. Hyperinsulinemia and increased sympathetic nervous system activity in the obese may contribute to increases in blood pressure through effects on renal sodium reabsorption and sympathetic stimulation of the vasculature. Further exploration of the relation of obesity, hyperinsulinemia, and sympathetic nervous system activity to blood pressure is warranted.

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