Urinary norepinephrine is a metabolic determinant of 24-h energy expenditure and sleeping metabolic rate in adult humans

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Funding: This research was supported by the Intramural Research Program of the National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health.

Conflict of interest Statement: The authors have nothing to disclose.

Precis: A higher urinary norepinephrine excretion rate is associated with higher 24-h energy expenditure and sleeping metabolic rate during non-stressed, energy balance conditions in adult humans

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Abbreviations: 24EE, 24-h energy expenditure; AFT, awake-fed thermogenesis; AUC, area under the curve; BMI, body mass index; CI, 95% confidence interval; DIT, diet-induced thermogenesis; EE, energy expenditure; EE_0, energy expenditure in the inactive, awake state; FFM, fat-free mass; FM, fat mass; OGTT, oral glucose tolerance test; PFAT, percentage fat; 24RQ, 24-h respiratory quotient; SMR, sleeping metabolic rate; SNS, sympathetic nervous system; SPA, spontaneous physical activity.

Abstract

BACKGROUND:

Inter-individual variability in 24-hour energy expenditure (24EE) during energy balance conditions is mainly determined by differences in body composition and demographic factors. Previous studies suggested that 24EE might also be influenced by sympathetic nervous system activity via catecholamine (norepinephrine, epinephrine) secretion. Therefore, we analyzed the association between catecholamines and energy expenditure in 202 subjects from a heterogeneous population of mixed ethnicities.

METHODS:

Participants (n=202, 33% female, 14% Black, 32% Caucasian, 41% Native Americans, 11% Hispanic, age: 36.9±10.3 years (mean±SD), percentage body fat: 30.3±9.4) resided in a whole-room calorimeter over 24-h during carefully controlled energy balance conditions to measure 24EE and its components: sleeping metabolic rate (SMR), awake-fed thermogenesis (AFT), and spontaneous physical activity (SPA). Urine samples were collected, and 24-h urinary epinephrine and norepinephrine excretion rates were assessed by high-performance liquid chromatography.

RESULTS:

Both catecholamines were associated with 24EE and SMR (norepinephrine: +27 and +19 kcal/day per 10 µg/24h; epinephrine: +18 and +10 kcal/day per 1 µg/24h) in separate analyses after *adjustment* for age, sex, ethnicity, fat mass, fat-free mass, calorimeter room and temperature, and physical activity. In a multivariable model including both norepinephrine and epinephrine, only norepinephrine was independently associated with both 24EE and SMR (both $p<0.008$) while epinephrine became insignificant. Neither epinephrine nor norepinephrine were associated with *adjusted* AFT (both $p=0.37$) but epinephrine was associated with *adjusted* SPA (+0.5% per 1 µg/24h).

CONCLUSIONS:

Our data provide compelling evidence that sympathetic nervous system activity, mediated via norepinephrine, is a determinant of human energy expenditure during non-stressed, eucaloric conditions.

Keywords: obesity, energy expenditure, catecholamines, sympathetic nervous system, epinephrine, norepinephrine

Introduction

Inter-individual differences in 24-hour energy expenditure (24EE) are mainly explained by differences in body composition, age, sex, and ethnicity(1-4). Although these measures account for up to ~85% of the variance in 24EE among individuals, the remaining ~15% are yet unexplained. Previous studies suggested that the extent of activity of the sympathetic nervous system (SNS) might also affect energy expenditure (EE)(5-7).

The SNS plays an important role in modulating heart rate, blood pressure, lipolysis, brown adipose tissue activity, and digestion(8, 9). These effects are mediated by the tyrosine-derived catecholamines (dopamine, norepinephrine, and epinephrine), which can act as sympathetic neurotransmitters and/or hormones that bind to α- and β- adrenergic receptors in target tissues(10). The SNS can be divided in two branches(11): I) The "central sympathetic" branch releases norepinephrine (the metabolite derived from dopamine and the main sympathetic neurotransmitter) from postganglionic sympathetic nerve endings directly into target tissues. Due to synaptic spillover and, to lesser extent, adrenal secretion, norepinephrine also accumulates in the blood stream(12). II) The "sympathoadrenal" branch is represented by the adrenal medulla whose chromaffin cells almost exclusively secrete epinephrine (the metabolite derived from norepinephrine) into circulation(10, 12). Both branches can be specifically, and somewhat independently, activated by different stressors, i.e., cold exposure or overeating generally lead to a greater central sympathetic activation while emotional stressors largely activate the sympathoadrenal branch(11, 13).

Seminal studies by Young, Landsberg, and others have shown that diet- and cold-induced increases in catecholamines are associated with concomitant increases in thermogenesis in rodents and humans(13-18). Further, treatment with catecholamines and catecholamine reuptake inhibitors increases thermogenesis in humans(14, 19-21). In line with these findings, two studies reported a positive association between norepinephrine – but not epinephrine – and the unexplained variance of 24EE measures during non-stressed, eucaloric conditions(2, 22). However, in the first study, catecholamines were analyzed in plasma and not in urine(2) – which could constitute a limitation as catecholamines may vary according to exposure and time of day(23) – while in the second study, only males of Caucasian and Native American descent were analyzed(22).

Thus, in this cross-sectional study, we measured 24EE, the components of 24EE, and urinary catecholamine concentrations in a larger and more heterogeneous population (n=202 subjects) of mixed ethnicities during 24 hours of carefully controlled energy balance conditions to assess the associations between urinary catecholamines and accurate measure of energy metabolism in healthy adults.

Material and Methods

Subjects

For this cross-sectional analysis, we used data from four different studies which were conducted from 2009 to 2017 on our research unit in Phoenix, AZ, and which had similar baseline procedures and logistics. In total, 202 individuals were included in the analysis (**Table 1, Supplemental Figure 1)** (supplemental material can be found at (24)). Subjects who participated in multiple studies were only included once (at their first study participation). Inclusion criteria for the current analysis were valid measurements of 1) 24EE during energy balance conditions (percentage deviation from exact energy balance (defined as 24-h energy intake minus 24EE) between –20% and 20%), 2) urinary catecholamines from 24-h urine collection, and 3) body composition. All individuals were 18 years or older and living in and nearby Phoenix, Arizona. All participants were weight stable (variation <2.3 kg within past 6 months) prior to admission by self-report and were confirmed to be healthy by history, physical examination, and fasting blood tests. Drug tests were performed prior to admission to exclude recent use of alcohol, cigarettes, and other drugs that might alter SNS activity. All participants provided written informed consent prior to any study procedures. The Institutional Review Board of the NIDDK approved all four studies.

Upon admission to the clinical research unit, participants were placed on a standard weight-maintaining diet (WMD; 50% carbohydrate, 30% fat, and 20% protein), using unit-specific equations based on weight and sex(25). Daily fasting body weight measured by calibrated scale was maintained within 1% of the admission weight during the inpatient period by *adjusting*, if necessary, daily energy intake of the WMD(26). Participants were instructed to refrain from vigorous exercise and to restrict their activities to those available on the research unit during their stay. Diabetes was excluded in all 202 participants based on oral glucose tolerance test performed after three days on the WMD as part of the standard procedures of the study protocols(27). Plasma glucose concentrations were measured using the Analox GM9 glucose oxidase method (Analox Inst. USA Inc., Lunenburg, MA, USA). Insulin concentrations were measured by an automated immunoenzymometric assay (Tosoh Bioscience Inc., Tessenderlo, Belgium). Body fat mass (FM), fat-free mass (FFM) and percentage fat (PFAT) were estimated by total-body dual-energy X-ray absorptiometry (DXA) (DPX-1 and DPX-L; Lunar Radiation, Madison, WI). To account for the usage of different DXA machines over time, previously validated regression equations were used to make DXA data comparable across different DXA machines(28).

Energy expenditure measurements

Twenty-four-hour EE and substrate oxidation were assessed in a large, open-circuit indirect whole-room calorimeter (respiratory chamber), as previously described(29). Before entering the calorimeter, subjects received breakfast at 7AM. During the 24 hours inside the respiratory chamber, total energy intake was equal to approximately 80% of the WMD to account for the limited physical activity inside

the calorimeter. Three meals were provided to volunteers via an airlock at 11AM, 4PM, and 7PM. All unconsumed food was returned to the metabolic kitchen for weighing for accurate calculation of intake. The 24-hour energy balance was calculated as the difference between actual energy intake minus 24EE. Carbohydrate and fat oxidation rates were calculated from the 24-hour respiratory quotient (24RQ), accounting for protein oxidation calculated from the measurement of 24-h urinary nitrogen excretion(30).

We also calculated the components of 24EE, namely, sleeping metabolic rate (SMR), the awake-fed thermogenesis (AFT, a surrogate for diet-induced thermogenesis [DIT] including cost of arousal(31)), the EE in the inactive, awake state (EE_0, representing daytime EE at zero physical activity(32)), and the energy cost of spontaneous physical activity (SPA). The EE_0 was calculated as the intercept of the regression line between EE and SPA 1-min data points between 10:00AM and 1:00AM and then extrapolated to 15 hours, as previously described(31). Participants' SPA was measured by radar sensors and expressed as the percentage of time when activity was detected(33). SMR was calculated as the average EE between 11:30PM and 5:00AM overnight when subject movement was less than 1.5% (<0.9 sec/min) and extrapolated to a 24-hour period, as previously described(29). AFT was calculated as the difference between EE_0 minus SMR and extrapolated to 15 hours, when the subject was awake and fed(31). Chamber ambient temperature averaged 23.8±2.1°C. Participants were asked to remain sedentary and not to exercise while residing in the respiratory chamber.

Catecholamine measurements

A constant fraction of the circulating concentrations of plasma epinephrine and norepinephrine is excreted into the urine(34, 35) which permits assessment of urinary catecholamine excretion over a 24-h period instead of a single time point of the blood draw. Studies comparing urinary levels of catecholamines with corresponding determinations of both hormones in plasma indicate a significant positive association between the concentrations obtained from these measurements(34, 36, 37). This was confirmed in the present study by comparative analysis of plasma concentration and 24-hour urinary catecholamine excretion in n=14 participants with both these measurements (**Supplemental Figure 2**)(24). Additionally, we assessed urinary normetanephrine and metanephrine which are the respective O-methylated metabolites of norepinephrine and epinephrine(38).

Urine was collected in a refrigerator inside the calorimeter during each 24EE assessment, then stored at −70°C until measured for the 24-hour excretion rate of norepinephrine, epinephrine, normetanephrine, and metanephrine to evaluate SNS activity. The diet given inside the respiratory chamber did not contain any caffeinated and alcoholic beverages, as well as catecholamine-rich foods that could alter physiologic urinary catecholamine levels.

Urinary catecholamines and metanephrines were measured by Mayo Clinic Laboratories (Rochester, MN, USA) using High-Performance Liquid Chromatography(39). The catecholamine and metanephrine excretion rate over 24 hours was obtained by multiplying catecholamine/metanephrine concentration (μg/L) by urinary volume (L) and extrapolating urine collection time to 24 hours in cases where it was

different than 24 hours. The mean±SD urinary collection time was 23.4±2.0 hours. Throughout the manuscript, the terms “norepinephrine”, “epinephrine”, and “catecholamines” refer to urinary excretion rates. Epinephrine and norepinephrine separated by study group are shown in **Supplemental Figure 3** . We detected a storage time effect on norepinephrine (r=0.15, p=0.03, **Supplemental Figure 4A**) but not on epinephrine (p=0.63, **Supplemental Figure 4B**) and on both metanephrines (both p>0.16).

According to the Mayo Clinic laboratory reference ranges for catecholamines (https://neurology.testcatalog.org/show/CATU), all epinephrine levels were within normal range (<21 µg/24 hours), whereas norepinephrine levels of 17 participants (8%) were outside the normal range (15–80 µg/24 hours). Therefore, we performed a sensitivity analysis by excluding these participants with above-normal norepinephrine levels which led to similar results (data not shown).

Statistical analysis

Data analyses were performed using SAS software (SAS 9.3, Enterprise guide version 5.1; SAS Institute, Cary, NC). Data are expressed as mean±SD, except for skewed data, which are expressed as median with interquartile range (IQR). A p-value <0.05 was considered statistically significant.

Epinephrine and norepinephrine were $\log_{10}$-transformed to meet the assumptions of parametric tests (i.e., homoscedasticity and Gaussian distribution of data). The Pearson’s correlation coefficient was used to quantify the associations between catecholamines and EE, body composition, or demographic parameters. Differences in catecholamine levels among ethnicities were assessed with analysis of variance (ANOVA) with post-hoc *adjustments* (Tukey method). The total area under the curve (AUC) during the OGTT was calculated using the trapezoidal rule.

Multivariate regression analysis was used to *adjust* values of 24EE and its components by including age, sex, ethnicity, FM, FFM, calorimeter ambient temperature, SPA levels (only 24EE analyses), and calorimeter suite (Room 1 or Room 2) in the regression models as covariates (**Supplemental Table 1**) as previously done(31, 40). Both calorimeter rooms are identical, and participants were randomly assigned to the rooms based on calorimeter availability. Sex, ethnicity, and “study group” showed no interaction effect in all models (data not shown); therefore, we did not perform separate analyses for subgroups of these covariates. We evaluated the independent determinants of norepinephrine and epinephrine by including age, sex, ethnicity, percentage body fat, fasting glucose, fasting insulin, and storage time in the regression models as covariates (**Table 2**).

The residual values (observed minus predicted values) of 24EE and its components obtained from these regression models were considered as the unexplained variability in EE measures after adjustment for known EE determinants(40). For each EE variable, *adjusted* values were then derived by adding the average value calculated in the whole cohort to the residual values obtained by regression analysis. Norepinephrine and epinephrine were then tested as potential determinants of *adjusted* EE values in univariate (each catecholamine included as the only predictor in the regression model) and multivariate (both catecholamines included as predictors in the same regression model) analyses, where *β*

coefficients were expressed both as absolute values (µg/24h) and as fold-change values with their 95% confidence intervals (CI). Sensitivity analyses were also performed by including both norepinephrine and epinephrine in the multivariable regression models of unadjusted EE measures including the aforementioned covariates and similar results were obtained (data not shown). A sample size of 202 individuals had >0.80 power (α=0.05) to detect an increase of 0.01 in R^2 attributed to two independent variables (norepinephrine and epinephrine) in a linear regression model for 24EE including covariates with a combined total R^2 =0.80.

Results

Characteristics of the 202 participants (136 males, 66 females) are shown in **Table 1**. The average unadjusted 24EE during energy balance was 2150±373 kcal/day and the average 24RQ (=0.87) was equal to the expected food quotient of the diet(30). The average deviation from 24-h energy balance inside the calorimeter was 3.7% and there were no differences between both calorimeter rooms (p=0.10). Epinephrine and norepinephrine correlated with each other (r=0.31, p<0.0001; **Supplemental Figure 5**), also in a partial correlation after *adjustment* for BMI (partial r=0.39, p<0.0001). Both catecholamines were not associated with age (both p>0.30, **Supplemental Figure 6**).

Determinants of urinary catecholamine excretion rates

Norepinephrine

Norepinephrine positively correlated with BMI (r=0.36, p<0.0001, **Figure 1A**), PFAT (r=0.17, p=0.01, **Figure 1B**), fasting insulin (r=0.38, p<0.0001, **Supplemental Figure 7A**), and the total AUC of insulin during the OGTT (r=0.32, p<0.0001, **Supplemental Figure 7B**). After *adjustment* of norepinephrine by BMI, the association with fasting insulin was still present (r=0.17, p=0.02) but there was no longer an association with the total AUC of insulin during the OGTT (p=0.13). To assess the impact of all determinants of norepinephrine concentration in a single model, a multivariate regression analysis was performed. Here, only fasting insulin was an independent determinant of norepinephrine (partial r=0.28, p=0.0001, **Table 2**).

Epinephrine

Epinephrine was negatively correlated with BMI (r=–0.16, p=0.02, **Figure 1C**) and PFAT (r=–0.31, p<0.0001, **Figure 1D**) while there were no correlations with fasting insulin and the total AUC of insulin during the OGTT (both p≥0.13, **Supplemental Figures 7C-D**). Results were similar after *adjustment* of epinephrine by BMI. To assess the impact of all determinants of epinephrine concentration in a single model, a multivariate regression analysis was performed. Here, only sex was an independent predictor of epinephrine (partial r=0.16, p=0.03, **Table 2**).

Association between urinary catecholamines and energy expenditure measurements

24-hour energy expenditure measures

Both norepinephrine (partial r=0.27, p=0.0002, **Figure 2A**) and epinephrine (partial r=0.21, p=0.003, **Figure 2B)** were positively associated with 24EE after *adjustment* for age, sex, ethnicity, FM, FFM, calorimeter ambient temperature, SPA levels, and calorimeter suite in separate, univariate analyses. Similar results were obtained when *adjusting* for BMI instead of FM and FFM (norepinephrine: partial r=0.23, p=0.001; epinephrine: partial r=0.22, p=0.003). On average, a 2-fold greater norepinephrine excretion was associated with a greater *adjusted* 24EE by 201 kcal/day (CI: 98−305 kcal/day) while a 2-fold greater epinephrine excretion was associated with a greater *adjusted* 24EE by 165 kcal/day (CI: 52−269 kcal/day).

To determine whether the associations between both catecholamines and 24EE were due to a common upstream signal or due to an independent effect of one specific catecholamine, we included both hormones in the multivariate regression analysis of *adjusted* 24EE along with covariates. We found that only norepinephrine (partial r=0.21, p=0.004), but not epinephrine (p=0.08), was an independent determinant of *adjusted* 24EE. This result for norepinephrine was further confirmed when considering the epinephrine-to-norepinephrine ratio (reflecting the conversion of norepinephrine to epinephrine), which was not a significant determinant of *adjusted* 24EE (p=0.44). We also assessed the direct metabolites of norepinephrine and epinephrine, i.e. the 24-h urinary excretion rate of normetanephrine and metanephrine and found similar associations with regard to *adjusted* 24EE (**Supplemental Figures 8A-B**).

Norepinephrine and epinephrine were not associated with 24RQ and lipid oxidation rate during energy balance, either in unadjusted analyses or after *adjustment* for their known determinants (all p>0.31). Norepinephrine was positively associated with unadjusted (r=0.27, p=0.0001) but not with *adjusted* carbohydrate oxidation rate (p=0.14) while no associations were found for epinephrine (all p>0.13). Both norepinephrine and epinephrine were positively associated with unadjusted protein oxidation rate (r=0.39, p<0.0001; and r=0.19, p=0.006, respectively); however, after *adjustment* for covariates, only norepinephrine (partial r=0.33, p<0.0001, **Supplemental Figure 9A**), but not epinephrine (p=0.06, **Supplemental Figure 9B**), was associated with *adjusted* protein oxidation rate. The association between norepinephrine and protein oxidation rate remained significant when *adjusting* for BMI instead of FM and FFM (partial r=0.35, p<0.0001).

Sleeping metabolic rate

We further analyzed the components of 24EE to elucidate which components may explain the positive associations between catecholamines and 24EE. We first analyzed the association between catecholamines and SMR. We found that norepinephrine (partial r=0.24, p=0.0008, **Figure 3A**) and epinephrine (partial r=0.15, p=0.04, **Figure 3B**) were associated with *adjusted* SMR in separate, univariate analyses. Similar results were obtained when *adjusting* for BMI instead of FM and FFM (norepinephrine: partial r=0.19, p=0.01; epinephrine: partial r=0.17, p=0.02). On average, a 2-fold

greater norepinephrine excretion was associated with a greater *adjusted* SMR by 137 kcal/day (CI: 57−216 kcal/day) while a 2-fold greater epinephrine excretion was associated with a greater *adjusted* SMR by 90 kcal/day (CI: 6−174 kcal/day). In the multivariate analysis testing both catecholamines as independent determinants of *adjusted* SMR, only norepinephrine (β: +16 kcal/day per 10 µg/24h, partial r=0.19, p=0.007), but not epinephrine (p=0.25), was an independent determinant of *adjusted* SMR. This was further confirmed when considering the epinephrine-to-norepinephrine ratio which was not associated with *adjusted* SMR (p=0.32).

Energy expenditure in the inactive state

The EE_0 represents the energy expended in the awake, inactive state and includes components of SMR and AFT. We found that norepinephrine (partial r=0.24, p=0.0007, **Figure 3C**) and epinephrine (partial r=0.19, p=0.007, **Figure 3D**) were associated with *adjusted* EE_0 in separate, univariate analyses. On average, a 2-fold greater norepinephrine excretion was associated with a greater *adjusted* EE_0 by 132 kcal/day (CI: 56−207 kcal/day) while a 2-fold greater epinephrine excretion was associated with a greater *adjusted* EE_0 by 109 kcal/day (CI: 31−188 kcal/day). In the multivariate analysis testing both catecholamines as independent determinants of *adjusted* EE_0, only norepinephrine (β: +15 kcal/day per 10 µg/24h, partial r=0.20, p=0.01), but not epinephrine (p=0.09), was an independent determinant of *adjusted* EE_0. This was further confirmed when considering the epinephrine-to-norepinephrine ratio which was not associated with *adjusted* EE_0 (p=0.56).

"Awake and fed" thermogenesis

We also analyzed the association between catecholamines and AFT (a surrogate for diet-induced thermogenesis [DIT] that includes the energy cost of arousal). However, neither catecholamine measure was associated with AFT (both p=0.37, **Figure 3E-F**, respectively). The ratio of epinephrine to norepinephrine was also not correlated with *adjusted* AFT (p=0.58).

Spontaneous physical activity

We also analyzed the associations between catecholamines and *adjusted* SPA inside the respiratory chamber and found that only epinephrine was associated with *adjusted* SPA (r=0.19, p=0.008, **Supplemental Figure 10B**) while norepinephrine showed no association (p=0.96, **Supplemental Figure 10A**). On average, a greater epinephrine excretion by 2–fold or 1 µg/24h was associated with a greater *adjusted* SPA by 4.0% (CI: 1.1−7.0%) or 0.5% (CI: 0.2−0.8%), respectively. In line with these results, we found that only epinephrine (p=0.004), but not norepinephrine (p=0.30), was an independent predictor of *adjusted* SPA. This was further confirmed when considering the ratio of epinephrine to norepinephrine which was now a significant determinant of *adjusted* SPA (r=0.17, p=0.03).

Discussion

In a cohort including 202 healthy participants, we demonstrated that epinephrine and norepinephrine were determinants of 24EE, non-activity energy expenditure (EE_0) and sleeping metabolic rate (SMR)

while only epinephrine was associated with spontaneous physical activity (SPA) during carefully controlled conditions of energy balance and after *adjustment* for the known determinants of energy expenditure. In statistical models in which both catecholamines were included, only norepinephrine was an independent determinant of 24EE, EE_0, and SMR. Both catecholamines were not associated with "awake and fed" thermogenesis (AFT, a surrogate for diet-induced thermogenesis).

Norepinephrine was higher in participants with greater adiposity, while epinephrine was lower. This "dissociation" of central sympathetic and sympathoadrenal activity in obesity was previously reported(41-43). However, after further *adjustment* for age, sex, ethnicity, fasting glucose and insulin, only insulin was still associated with greater norepinephrine. This is in line with the hypothesis from *Landsberg* who proposed that insulin increases in adiposity as a compensatory mechanism to limit weight gain by increasing sympathetic activity and consequently metabolic rate(44).

Urinary norepinephrine is a metabolic determinant of 24-h, resting, and sleeping metabolic rate

Our results show that, after accounting for body composition, age, sex, ethnicity, and calorimeter parameters, both epinephrine and norepinephrine were determinants of 24EE, SMR, and EE_0 when analyzed separately. However, only norepinephrine was an independent determinant of these EE measures as it remained a significant determinant of EE when both catecholamines were introduced into the multivariable regression models. In addition, the epinephrine-to-norepinephrine ratio showed no significant association with all three EE measures, suggesting that the results for epinephrine are driven by its association with norepinephrine (**Supplemental Figure 5**).

The effect of norepinephrine on 24EE arises from its association with SMR, a surrogate for resting metabolic rate (RMR) which is defined as the resting, post-absorptive state. On average, RMR accounts for 70–80% of the daily calories spent, and thus, is a major contributor to 24EE(45). Previous studies already found in various settings that norepinephrine (but not epinephrine) is a metabolic determinant of RMR(2, 22, 46). Our study extends these findings to a larger and more heterogeneous, ethnically diverse population.

A 10 µg/24h greater urinary norepinephrine excretion rate was associated with a greater 24EE by 27 kcal/day and a greater SMR by 19 kcal/day. Norepinephrine explained 1.1% of the additional variance which is admittedly a small effect but similar to what Toubro *et al.* previously reported(2). These authors argued that the weak association could be due to the measurement of plasma catecholamines at only one time point (after exiting the calorimeter) and advocated the measurement of catecholamines over 24h to obtain a better estimate of SNS activity during 24 hours of energy balance. This was done by Saad. *et al*. who found a positive association between urinary norepinephrine (but not epinephrine) excretion rate and 24EE measures in a cohort of Caucasian men whereas this association was not found in Native American men(22). In this present study, we confirm and extend the associations between catecholamine excretion rate and EE measures to women and across different ethnicities including Native Americans. Yet, the effect of catecholamines, although significant, contributes to only a small

part of the inter-individual variance in 24EE, at least in healthy, unstressed participants during energy balance conditions.

The physiological mechanisms by which norepinephrine modulates EE is likely by its effects on α_1- and β_1-receptors throughout the body by increasing blood pressure, heart rate, and cardiac output(47). Likewise, norepinephrine may modulate EE via its binding to β_3-adrenergic receptors in the membrane of brown adipocytes where it increases fatty acid β-oxidation and heat production(48). We also found that norepinephrine was an independent determinant of protein oxidation rate which indicates that it might increase EE via protein catabolism resulting in enhanced gluconeogenesis(49). However, norepinephrine is also associated with anabolic actions on skeletal muscle protein metabolism(50).

Urinary catecholamines are not associated with diet-induced thermogenesis

Neither urinary norepinephrine nor epinephrine were associated with *adjusted* AFT (a surrogate measure of DIT). However, studies in rodents suggest that greater sympathetic activity does increase DIT(13, 14, 51). In humans, the results are mixed with some studies (including ours) failing to show a correlation between catecholamines and DIT(5, 52-54) while others did demonstrate an association(54-56). These divergent findings might be due to different measurement techniques (catecholamine appearance rate vs 24h urinary catecholamine excretion), assessment of DIT (e.g., AFT is the sum of DIT plus the cost of being awake), or to provided energy intake (most studies measured DIT after one single meal while we measured DIT over 24 hours which comprised 4 separate meals). Furthermore, it might be that only "nutrient stress" like overfeeding leads to sufficient SNS activation to significantly increase metabolic rate and DIT(14, 56).

Urinary epinephrine is associated with greater spontaneous physical activity

A greater 24-hour urinary epinephrine excretion was associated with greater *adjusted* SPA. This is supported by other studies which show that higher SNS activity was associated with increased physical activity in Native American and Caucasian men(37). Interestingly, higher SPA measured while residing in the respiratory chamber also predicted less future weight gain(57). We speculate that the higher epinephrine levels during energy balance are a consequence rather than a cause of higher SPA in our present cohort as increased physical activity was shown to be causal for the increase in epinephrine secretion(58, 59).

Limitations

Our study has limitations. This is a cross-sectional analysis, thus, causality of the associations between catecholamines and EE measures could not be determined, although treatment with catecholamines was previously shown to increase metabolic rate(14). Also, subjects of Native American descent are overrepresented in our cohort (41%) which may have biased our findings as SNS activity appears to be a stronger determinant of energy expenditure in Caucasians than in Native Americans(22). However, we *adjusted* all EE measures by ethnicity to account for this overrepresentation. Further, the measurement

of urinary catecholamines is not a complete indicator of SNS activity as significant amounts of monoamines filtered at the glomerulus do not reach the final urine(60) and as the kidneys contain the enzymes necessary to synthesize most neurotransmitters which may distort the results(61). Lastly, urinary catecholamines are spillover measures and may not represent the "real" end-organ effects of the SNS.

Conclusion

Both catecholamines were determinants of 24-h energy expenditure, resting energy expenditure, and sleeping metabolic rate. However, these associations were driven by norepinephrine rather than epinephrine. Although the effects of catecholamines on energy expenditure were admittedly small, they were measured in non-stressed subjects during energy balance conditions. Stressful situations like cold exposure or overeating might increase catecholamine levels to a greater extent and ultimately have a greater impact on energy expenditure during these conditions, thus representing an important metabolic factor for the susceptibility to weight gain.

Acknowledgements

The authors thank the dietary, nursing, and technical staff of the National Institutes of Health Clinical Unit in Phoenix, AZ, for their assistance. Most of all, the authors thank the volunteers for their participation in the study.

Funding: This research was supported by the Intramural Research Program of The National Institutes of Health, The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). P.P. was supported by the program "Rita Levi Montalcini for young researchers" from the Italian Minister of Education and Research.

Authors contributions: Dr. Hollstein carried out the initial analyses, interpreted the results, wrote the manuscript, and approved the final manuscript as submitted. **Dr. Ando** carried out the initial analyses, interpreted the results and approved the final manuscript as submitted. **Dr. Basolo** carried out the initial analyses, interpreted the results and approved the final manuscript as submitted. **Dr. Votruba** interpreted the results and approved the final manuscript as submitted. **Dr. Krakoff** interpreted the results and approved the final manuscript as submitted. **Dr. Piaggi** designed the study, interpreted the results, edited the text and approved the final manuscript as submitted. Drs. Hollstein and Piaggi are the guarantors of this work, and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Disclosure summary: The authors have nothing to disclose.

Competing interests: There are no competing financial interests in relation to the work described.

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Tables

Table 1. Baseline characteristics of the study cohort (n = 202).

Demographic characteristics	
Male (%)	136 (67.3)
Ethnicity, n (%)	
Native American	83 (41.1)
Caucasian	65 (32.2)
Black	28 (13.9)
Hispanic	23 (11.4)
Other[$]	3 (1.5)
Age (years)	36.9 ± 10.3 (18, 66)
Body composition measurements	
Height (cm)	170.3 ± 8.8 (150.5, 195.0)
Body weight (kg)	89.2 ± 21.9 (47.5, 171.0)
BMI (kg/m^2)	30.8 ± 7.7 (17.7, 60.6)
Body fat (%)	30.3 ± 9.4 (7.6, 51.8)
FM (kg)	28.1 ± 13.7 (5.3, 83.3)
FFM (kg)	61.1 ± 12.1 (35.5, 100.6)
Energy expenditure measurements during 24-h energy balance	
24EE (kcal/day)	2150 ± 373 (1350, 3378)
Food intake (kcal/day)	2218 ± 353 (1461, 3353)
Energy balance (kcal/day)	69 ± 147 (–348, 422)
Energy balance (%)	3.7 ± 6.8 (–14.1, 20.0)
SMR (kcal/day)	1662 ± 282 (1060, 2559)
24RQ (ratio)	0.86 ± 0.04 (0.70, 1.02)
EE_0 (kcal/15h)	1422 ± 245 (916, 2192)
AFT (kcal/15h)	384 ± 112 (145, 710)
Lipid oxidation (kcal/day)	707 ± 352 (–385, 1925)

Carbohydrate oxidation (kcal/day)	1031 ± 336 (–194, 2095)
Protein oxidation (kcal/day)	385 ± 104 (149, 1094)
SPA (%)	7.1 ± 4.5 (0.4, 27.5)
Urinary catecholamines during energy balance	
Norepinephrine (µg/24h)	34.3 ± 16.9 (6.4, 114.6); 30.5, 23.2 – 42.7*
Epinephrine (µg/24h)	4.1 ± 2.0 (1.0, 13.2); 3.6, 2.6 – 5.0*
Epinephrine-to-norepinephrine ratio	0.14 ± 0.08 (0.02, 0.53); 0.12, 0.08 – 0.17*
Urinary metanephrines during energy balance	
Normetanephrine (µg/24h)	276.5 ± 126.7 (65.5, 792.0); 251.1, 191.4 – 324.2*
Metanephrine (µg/24h)	118.2 ± 47.7 (23.8, 305.1); 113.9, 87.8 – 141.8*
Measurements of glucose metabolism	
Fasting glucose (mg/dL)	94.1 ± 7.7 (77, 118)
2-h glucose during OGTT (mg/dL)	121.1 ± 29.0 (53, 194)
Total glucose AUC during OGTT (µIU/mL×180min)	22,752 ± 3,908 (13,853, 34,800)
Fasting insulin (µIU/mL)	13.9 ± 15.7 (1.2, 147.5); 9.0, 6.0 – 16.5*
2-h insulin during OGTT (µIU/mL)	121 ± 29.0 (4.0, 1100.0); 60.0, 32.0 – 121.5*
Total insulin AUC during OGTT (µIU/mL×180min)	16,881 ± 15,221 (2,895, 129,885); 12,255, 7,673 – 20,085*

Values are presented as mean ± SD for continuous variables or number (frequency) for categorical variables with minimum and maximum in parentheses. Skewed values are also expressed as medians with interquartile ranges (*). BMI, body mass index; FM, fat mass; FFM, fat-free mass; 24EE, 24-h energy expenditure; 24RQ, 24-h respiratory quotient, EE_0, energy expenditure in the inactive, awake state; AFT, awake-fed thermogenesis; SPA, spontaneous physical activity, SMR, sleeping metabolic rate; OGTT , oral glucose tolerance test.

$Other ethnicities comprise one subject of Asian and two subjects of unknown ethnicity.

Table 2. Multivariable regression models for the determinants of 24-h urinary norepinephrine and epinephrine excretion rates during isocaloric conditions.

	Urinary norepinephrine (µg/24h) log_{10}-transformed	**Urinary epinephrine (µg/24h) log_{10}-transformed**
Explained variance (global P value)	**R^2=0.20** **P<0.0001**	**R^2=0.16** **P=0.0004**
Age (years)	0.002±0.002 (–0.001, 0.005) Partial r: 0.105 P value: 0.16	–0.001±0.002 (–0.004, 0.002) Partial r: –0.032 P value: 0.67
Sex (male)	0.06±0.05 (–0.03, 0.15) Partial r: 0.09 P value: 0.21	**0.1±0.05** **(0.01, 0.19)** **Partial r: 0.16** **P value: 0.03**
Ethnicity (Caucasian)	–0.1±0.12 (–0.34, 0.13) Partial r: –0.07 P value: 0.38	–0.11±0.12 (–0.35, 0.12) Partial r: –0.07 P value: 0.33
Ethnicity (Native American)	–0.05±0.12 (–0.28, 0.18) Partial r: –0.03 P value: 0.65	–0.15±0.12 (–0.39, 0.08) Partial r: –0.1 P value: 0.2
Ethnicity (Black)	–0.08±0.12 (–0.32, 0.16) Partial r: –0.05 P value: 0.5	–0.03±0.12 (–0.27, 0.21) Partial r: –0.02 P value: 0.81
Ethnicity (Hispanic)	–0.05±0.12 (–0.29, 0.19) Partial r: –0.03 P value: 0.7	–0.03±0.12 (–0.27, 0.22) Partial r: –0.02 P value: 0.83
Percentage body fat (%)	0.001±0.003 (–0.005, 0.006) Partial r: 0.021 P value: 0.78	–0.003±0.003 (–0.008, 0.003) Partial r: –0.073 P value: 0.33
Fasting Glucose	0.002±0.002 (–0.003, 0.006) Partial r: 0.052 P value: 0.49	–0.0014±0.0022 (–0.0058, 0.003) Partial r: –0.0465 P value: 0.53
Fasting Insulin (log_{10}-transformed)	**0.22±0.06** **(0.11, 0.34)** **Partial r: 0.28** **P value: 0.0001**	0.03±0.06 (–0.08, 0.14) Partial r: 0.04 P value: 0.59

Storage time (months)	0.00003±0.00002 (–0.00001, 0.00006) Partial r: 0.10592 P value: 0.16	0.000004±0.000019 (–0.000034, 0.000041) Partial r: 0.01405 P value: 0.85
Intercept	0.52±0.46 (–0.4, 1.44) P value: 0.26	0.73±0.47 (–0.19, 1.66) P value: 0.12

The β coefficient estimate in each cell is reported with ± SE and the 95% confidence interval in parentheses, along with partial correlations and P value. Significant results are highlighted in bold.

Similar results were obtained when introducing the 3-h total area under the curve (AUC) of the insulin/glucose response during the oral glucose tolerance test (model with norepinephrine: AUC insulin: partial r=0.21, p=0.005) into the linear model in place of fasting insulin/glucose, when introducing body mass index in the linear model in place of percentage body fat, and after exclusion of subjects with impaired glucose tolerance (n=65).

Figure legends

Figure 1. Associations between adiposity measures and 24-h urinary norepinephrine and epinephrine excretion rates during isocaloric conditions.

Association between urinary norepinephrine excretion and BMI (panel A) and percentage body fat by DXA (panel B). Association between urinary epinephrine excretion and BMI (panel C) and percentage body fat by DXA (panel D). Black circles denote male, white circles denote female participants. Y-axes are formatted on a logarithmic scale (log_{10}) to account for the skewed data distribution of raw catecholamine values.

BMI, body mass index.

Figure 2. Greater 24-h urinary norepinephrine (A) and epinephrine (B) excretion rates are associated with relatively higher *adjusted* 24EE during energy balance and weight stability.

On average, a greater urinary norepinephrine excretion rate by 10 µg/24h (panel A) and a greater urinary epinephrine excretion rate by 1 µg/24h (panel B) were associated with a greater *adjusted* 24EE by 27 kcal/day (CI: 13−40 kcal/day) and 18 kcal/day (CI: 7−30 kcal/day), respectively.

The *adjusted* 24EE values were calculated via linear regression analysis including fat-free mass, fat mass, age, sex, ethnicity, calorimeter temperature, spontaneous physical activity, and calorimeter room as covariates after adding the average 24EE to the residual values obtained from the regression model. X-axes are formatted on a logarithmic scale (log_{10}) to account for the skewed data distribution of raw catecholamine values. Black circles denote male, white circles denote female participants.

24EE, 24-h energy expenditure; CI, 95% confidence interval.

Figure 3. Associations between 24-h urinary norepinephrine and epinephrine excretion rates and components of 24EE during energy balance.

Associations between urinary norepinephrine (panel A) and epinephrine (panel B) excretion rates and *adjusted* sleeping metabolic rate (SMR). On average, a greater urinary norepinephrine excretion rate by 10 µg/24h and a greater urinary epinephrine excretion rate by 1 µg/24h were associated with a greater *adjusted* SMR by 19 kcal/day (CI: 9−29 kcal/day) and 10 kcal/day (CI: 2−19 kcal/day), respectively.

Associations between urinary norepinephrine (panel C) and epinephrine (panel D) excretion rates and *adjusted* EE_0. On average, a greater urinary norepinephrine excretion rate by 10 µg/24h and a greater urinary epinephrine excretion rate by 1 µg/24h were associated with a greater *adjusted* EE_0 by 18 kcal/day (CI: 8−27 kcal/day) and 11 kcal/day (CI: 3−20 kcal/day), respectively.
Lack of associations between urinary norepinephrine (panel E) and epinephrine (panel F) excretion rates and *adjusted* AFT.

All *adjusted* EE measures (SMR, EE_0, and AFT) were calculated via linear regression analysis including fat-free mass, fat mass, age, sex, ethnicity, calorimeter temperature, and calorimeter room as covariates after adding the respective average value calculated in the whole cohort to the residual values obtained from the respective regression model. X-axes are formatted on a logarithmic scale (log_{10}) to account for the skewed data distribution of raw catecholamine values. Black circles denote male, white circles denote female participants.

24EE, 24-h energy expenditure; AFT, awake-fed thermogenesis; CI, confidence interval; EE_0, energy expenditure in the inaktive, awake state; FFM, fat-free mass; FM, fat mass; SMR, sleeping metabolic rate.

Figure 1

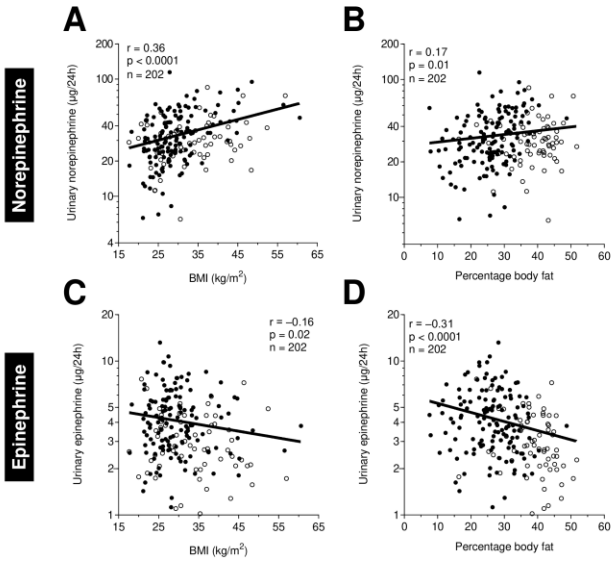

Figure 2

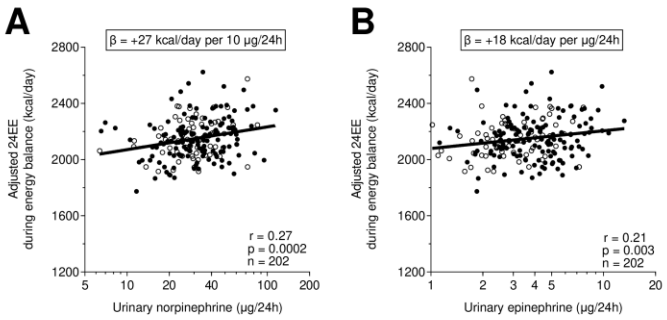

Figure 3