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Genetic Variation in the Presynaptic Norepinephrine Transporter is Associated with Blood Pressure Responses to Exercise in Healthy Humans

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Abstract

Background—The presynaptic norepinephrine (NE) transporter (NET) mediates synaptic clearance and recycling of NE. NET-deficient transgenic mice have elevated blood pressure, heart rate, and catecholamine concentrations. However, the *in vivo* effects of common *NET* variants on cardiovascular regulation at rest and during exercise are unknown.

Methods—We studied cardiovascular responses and plasma catecholamine concentrations at rest and during bicycle exercise at increasing workloads (25, 50 and 75 W) in 145 healthy subjects. We used multiple linear regressions to analyze the effect of common, purportedly functional polymorphisms in *NET* (rs2242446 and rs28386840) on cardiovascular measures.

Results—44% and 58.9% of subjects carried at least one variant allele for *NET* T-182C and A-3081T, respectively. Systolic blood pressure (SBP) during exercise and SBP area-under-the-curve were higher in carriers of variant *NET* alleles ($P=0.003$ and 0.009 for T-182C and A-3081T, respectively) and *NET* haplotype -182C/-081T compared to -82T/-3081A (all $P<0.01$). Diastolic blood pressure (DBP) during exercise was also higher at lower, but not at higher exercise stages in carriers of *NET* -182C ($P<0.01$) and -081T ($P<0.05$). *NET* genotypes were not associated with catecholamine concentrations or heart rate.

Conclusion—Common genetic *NET* variants (-182C and -081T) are associated with greater blood pressure response to exercise in humans.

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Keywords

orepinephrine transporter; Blood pressure; Exercise; Polymorphism

Introduction

Genetic factors contribute substantially to interindividual variability in cardiovascular responses to exercise, stress, and drugs [1]. Variants in genes encoding adrenergic receptors and their signaling mechanisms [2–4] account for some of this variability, but other genetic determinants are unknown. The timing and extent of synaptic norepinephrine (NE) removal is determined by active clearance from the synapse by the presynaptic NE transporter (NET, *SLC6A2*). Genetic variations that affect this reuptake process could have profound effects on autonomic responses. Indeed, mice with deletion of *NET* have provided valuable insights into the potential cardiovascular consequences of altered transporter activity [5]. In these animals, NET-deficiency results in higher resting blood pressure, heart rate, and plasma NE and epinephrine (EPI) concentrations; these differences are further accentuated by activity [5].

Although little is known about the effect of genetic variation in *NET* on regulation of cardiovascular function in humans, a rare mutation (Ala457Pro), identified and studied in a single pedigree, results in a transporter with greatly reduced activity (residual activity approximately 2%) [6]. The same mutation also exhibits dominant-negative effects on the expression of wildtype NET protein in transfected cells [7]. Consistent with these findings, carriers of this mutation exhibit traits of orthostatic intolerance and also have higher systolic and diastolic blood pressure, heart rate, and NE concentrations than controls [6]. Several other variants in human norepinephrine transporter gene (*NET*, *SLC6A2*), such as the promoter region polymorphisms (*NET* [*SLC6A2*], rs28386840, NET, A-3081T) and (*NET* [*SLC6A2*], rs2242446, NET, T-182C), have been identified [8,9]. The T allele of the A-3081T polymorphism drives decreased transcriptional expression of reporter constructs and is selectively transmitted to subjects with attention deficit hyperactivity disorder (ADHD) [8]. However, the effects of these variants on *in vivo* catecholamines and catecholamine mediated responses have not been studied.

Physical exercise is characterized by incremental sympathetic activation and concomitant parasympathetic withdrawal. Therefore, exercise is likely to accentuate functional effects of genetic variations affecting sympathetic signal transduction [10]. Accordingly, we examined whether common polymorphisms in *NET* (rs28386840 [A-3081T] and rs2242446 [T-182C]) alter cardiovascular responses in healthy humans at rest and during exercise. More specifically, we examined the hypothesis that polymorphisms associated with reduced NET expression result in higher heart rate, blood pressure, and plasma catecholamine concentrations at rest and during exercise.

Methods

Subjects

This study was approved by the Institutional Review Board of the Vanderbilt University Medical Centre, Nashville, TN, and all subjects gave written informed consent. We studied 145 healthy subjects aged 19–50 years, all of whom also contributed data to a previous study [10]. All subjects were non-smokers without a history of major medical illness, without active intermittent disease or any medications or dietary supplements for at least 1 week, and received a controlled alcohol-free and caffeine-free diet (providing 150 mmol of sodium, 70 mmol of potassium, and 600 mmol of calcium daily) for 5 days before the study. Each

subject reported the intensity, duration, and frequency of his/her physical activity over the last 4 weeks, and this was converted into Metabolic Equivalents (METs) using standard charts [11]. The exercise score for each subject was defined as the number of METs/week. The subjects also reported their race and that of their parents and grandparents using check-boxes to choose among “Caucasian”, “African-American”, “Hispanic”, “Chinese”, “Japanese”, and “other” (the latter to be specified). Multiple choices were permitted. A subject was assigned to a racial group when both parents and at least 3 out of 4 grandparents were of the same race. The details of subject recruitment are described elsewhere [10].

Protocol

The exercise protocol was well standardized and previously described in detail [10,12,13]. On the morning (8:00–10:00 am) of the study day, subjects were admitted to a temperature-controlled room (22–23°C) in the Vanderbilt University Clinical Research Center after an overnight fast. A 20 G intravenous cannula was inserted into an antecubital arm vein for blood sampling. Digitized ECG signals were continuously acquired on a personal computer at 500 MHz using the Windaq software (v. 2.20, Dataq Instruments Inc., Akron, Ohio). After 30 minutes of supine rest, subjects slid to an adjacent electronically braked supine bicycle ergometer. The exercise protocol consisted of cycling at a constant rate (60 revolutions/min) at sequentially increasing workloads of 25, 50 and 75 W, for 2 minutes each. Average heart rate was determined from digitized ECG signals at rest and during the second minute of each of the 3 exercise stages. Blood pressure was measured manually at the right brachial artery and by a semiautomated oscillometric device (Dinamap MPS; GE Medical Systems) at the left brachial artery at rest and during the second minute of each of the 3 exercise stages using a cuff size appropriate for the arm circumference [14]. The mean of the manual and automated blood pressure readings was calculated at rest and each of the 3 exercise stages for analysis. Blood samples for plasma catecholamines (EPI and NE) were collected twice, immediately before the start of exercise and then at the termination of exercise.

Genotyping

Subjects were genotyped for *NET* polymorphisms rs28386840 (A-3081T, n=141) and rs2242446 (T-182C, n=140) using allelic discrimination (TaqMan®) assays, performed in the Vanderbilt Center for Human Genetics Research DNA Resources Core. For each SNP, four nanograms of whole genome amplified DNA was used as template in reactions containing 900 nM forward and reverse amplification primers, 200 nM VIC® and FAM® dye labeled probes, and 1X TaqMan® Universal PCR Master Mix. Thermal cycling (95°C for 10 min, followed by 50 cycles of 92°C for 15 sec and 60°C for 1 min) and product detection were accomplished using an ABI 7900HT Real-Time PCR System (ABI). For quality assurance, we repeated genotyping for 20% of the samples, yielding concordant results.

Plasma catecholamine determination

Blood was collected into cooled heparin-coated tubes which were immediately placed on ice until centrifuged at 4°C for 10 minutes at 3000rpm. Plasma was harvested and stored in tubes containing 40µL of reduced glutathione (6%) at –20°C until assayed. NE and EPI concentration were measured using high-performance liquid chromatography (HPLC) with electrochemical detection. Dihydroxybenzylamine was used as an internal standard [15].

Data analysis, statistics and haplotype inference

Normally distributed data are expressed as mean ± standard deviation (SD) or 95% confidence interval. The effect of *NET* polymorphisms on cardiovascular outcomes (heart

rate, systolic and diastolic blood pressure) and plasma NE and EPI concentrations was analyzed using multiple linear regression models at rest and during 3 exercise stages (25, 50 and 75W); all models included adjustment for potential confounders (age, sex, race, exercise score, and in addition, pre-exercise heart rate, systolic, and diastolic blood pressure for models that analyzed these outcomes during exercise). Increasing exercise intensity is characterized by progressive sympathetic activation and parasympathetic withdrawal; heart rate and blood pressure at exercise are therefore determined by sympathetic activity to a greater degree than at rest. Thus, functional effects of genetic *NET* variants are expected to be increasingly pronounced with escalating exercise, and we therefore analyzed the phenotype at rest and during 3 incremental exercise intensities. In addition, we also modeled multiple measurements on the same patient using a measure that summarizes responses [16]. For this purpose, we used the area under the curve (AUC) of the graph plotting the parameter of interest over exercise intensity as a single summary response, reflecting data from rest and all 3 exercise stages. Such a summary variable is commonly used to incorporate both baseline and post-intervention measures into data analysis while avoiding statistical problems associated with multiple comparisons for repeated measures [16–18]. In these analyses, the response feature was the area under the cardiovascular outcome-exercise intensity curve (AUC) for heart rate, systolic, and diastolic blood pressure, calculated as a summary measure of responses during exercise for each subject.

We tested for Hardy-Weinberg equilibrium using χ^2 test with 1 degree of freedom. For the analysis of outcomes, we combined subjects homozygous for the variant *NET* alleles with the heterozygous subject into one group (“variant allele carriers”). All tests were two-tailed, and $P < 0.05$ was considered significant. Analyses were performed using the statistical software STATA v.10.0 (StataCorp, College Station, TX) and R (www.r-project.org). The R package “haplo.stats” was used to carry out haplotype inference using the expectation maximization algorithm.

Sample size calculation

The sample size of the cohort ($n=145$) was previously determined by the sample size calculation of an earlier study [10]. Systolic blood pressure at maximal exercise (75W) in that analysis was 150.5 ± 16.4 mmHg [10]. In a mixed North American population, 50% of subjects carry at least one T allele of the *NET* A-3081 T polymorphism [8]; therefore, we estimated that our sample size ($n=145$) would provide 85% power to detect a difference of 8.2 mmHg (corresponding to 50% of the standard deviation) in systolic blood pressure at maximal exercise between carriers and non-carriers of *NET* A-3081T ($\alpha=0.05$).

Results

Subject characteristics, genotypes, and haplotypes

We studied 145 subjects (83 European Americans and 62 African Americans; 82 females). Subject characteristics and genotyping results for *NET* polymorphisms are summarized in Table 1. The mean exercise score for our subjects was 14.8 ± 21.6 METS/week and did not differ between *NET* genotype groups (all $P > 0.25$). All genotypes conformed to Hardy-Weinberg equilibrium in each racial group (all $P > 0.13$) except for the *NET* A-3081T variant in African Americans ($\chi^2=4.46$; $P=0.03$), a finding possibly attributable to genetic admixture. Minor allele frequencies were in the expected range [8]. The two *NET* variants were in strong linkage disequilibrium (LD) in European Americans ($r^2 = 0.94$) but not in African Americans ($r^2=0.19$). *NET* haplotypes -3081A/-82T (haplotype 1), -081T/-182C (haplotype 2), and -081T/-82T (haplotype 3) had frequencies of 61.1%, 22.6 %, and 15.9 %, respectively. Haplotype frequencies differed among European Americans (73.4%, 25.3%,

and 0.6%) and African Americans (43.7%, 19.3%, and 36.9%). *NET* haplotype -3081A/-182C (haplotype 4) was rare (0.4%) and was not used in analysis.

Cardiovascular responses

Systolic blood pressure—There were no significant differences in resting systolic or diastolic blood pressure among genotypes for the *NET* variants (Table 2). However, *NET* variants were significantly associated with systolic blood pressure response during exercise: carriers of the variant T allele of A-3081T and C allele of T-182C had higher systolic blood pressure than wild type homozygotes at all exercise stages (Table 3 and 4, Figure 1). The summary measure, systolic blood pressure AUC, was also significantly higher in carriers of the variant alleles than in wild type homozygotes for both *NET* T-182C and A-3081T ($P=0.003$ and 0.009 , respectively; Table 4 and 5.).

We also analyzed the two racial groups separately. Both *NET* variants were significantly associated with systolic blood pressure response at all exercise intensities in European Americans ($n=83$, all $P<0.01$; Figure 2). In African Americans, with a smaller sample size ($n=62$) similar findings were only observed for the *NET* T-182C variant with significant differences in systolic blood pressure response at 50 W ($P=0.02$) and a trend in the same direction at 25 and 75W of exercise intensity ($P=0.08$ and 0.11 , respectively; Figure 2).

NET -081T/-182C haplotype (haplotype 2) was associated with significantly higher SBP than *NET* -3081A/-82T haplotype (haplotype 1) at all exercise intensities. SBP-AUC was also higher in -081T/-182C than -3081A/-82T haplotype carriers (mean adjusted difference, 466.4 mmHg · Watt; 95% CI, 159.6 to 773.1; $P=0.003$). When comparing SBP between haplotype 2 and 1 separately in European Americans and African Americans, the differences were significant only in the former ($P=0.03$, 0.004 and 0.008 at exercise intensities of 25, 50 and 75 W, respectively, and $P=0.02$ for SBP-AUC).

Diastolic blood pressure—Carriers of the variant *NET* -182C allele had higher diastolic blood pressure than wild type homozygotes at exercise intensities of 25 W (mean adjusted difference, 3.9 mmHg; 95% CI, 1.2 to 6.6 mmHg; $P=0.004$) and 50 W (3.8 mmHg; 95% CI, 1.0 to 6.6 mmHg; $P=0.008$; Table 3.). Similarly, carriers of the variant *NET* -081T allele had higher diastolic blood pressure than wild type homozygotes at exercise intensities of 25 W (mean adjusted difference, 2.9 mmHg; 95% CI, 0.2 to 5.7 mmHg; $P=0.04$) and 50 W (3.0 mmHg; 95% CI, 0.2 to 5.9 mmHg; $P=0.04$; Table 3). Diastolic blood pressure AUC was not significantly associated with either of these *NET* polymorphisms (all $P>0.17$; Table 5.). *NET* haplotypes were not associated with DBP at rest (all $P>0.44$), or during exercise (all $P>0.10$), nor with DBP-AUC (all $P>0.26$). Race-stratified haplotype analysis yielded similar negative results.

Heart rate—Resting heart rate, heart rate during exercise, and heart rate AUC were not significantly related to *NET* polymorphisms (Tables 2, 3, and 5) or *NET* haplotypes (all $P>0.39$). Race-stratified haplotype analysis yielded similar negative results.

Catecholamine concentrations—Mean plasma NE concentrations at rest were approximately 18% lower in carriers of the variant *NET* -182C allele than in wild type homozygotes (mean difference, 42.4 pg/ml; 95% CI, 9.0 to 75.8 pg/mL; $P=0.01$; Table 2). Similarly, *NET* -081T/-182C haplotype (haplotype 2) was associated with significantly lower resting plasma NE concentrations than the -3081A/-82T haplotype (haplotype 1) (mean difference, 39.5 pg/ml; 95% CI, 9.4 to 69.7 pg/ml; $P=0.01$). In contrast, NE concentrations after exercise and plasma EPI concentrations at rest or after exercise were not

associated with any of the genetic variants (Tables 2 and 3.). Race-stratified haplotype analysis did not yield any significant results.

Discussion

This is the first study to assess the physiological effects of common *NET* variants on cardiovascular function at rest and during exercise. In a highly controlled setting, we found that two *NET* promoter polymorphisms, A-3081T and T-182C, were associated with higher systolic and diastolic blood pressure during exercise in healthy humans. Furthermore, haplotype analysis revealed that -081T/-182C haplotype (haplotype 2) was associated with significantly higher exercise systolic blood pressure than -3081A/-82T haplotype (haplotype 1). The *NET* T-182C variant affected blood pressure response to exercise in African Americans and European Americans, whereas the A-3081T variant was significant only in European Americans, in whom the two variants are in strong LD. This finding suggests that the *NET* T-182C variant may account for the observed physiological effects, or that the variants differ in their association with blood pressure responses in African Americans and European Americans.

The T allele of *NET* A-3081T polymorphism results in reduced promoter function and consequently reduced *NET* expression compared to the wild-type A allele [19]. The functional effects of the *NET* T-182C variant on gene transcription have not been assessed. At the neuropsychiatric level, the A-3081T variant has been associated with ADHD and depression [8,19], whereas the T-182C variant has been associated with depression [19,20] and response to antidepressant medication [21]. Reduced *NET* expression is expected to result in delayed clearance of norepinephrine from the noradrenergic synapses [6,22] and, thus, increased synaptic noradrenergic activity. The enhancement of noradrenergic effects would be most significant in phases of increased sympathetic activation, such as during exercise.

In addition to delaying the clearance of synaptic NE, deficient *NET* activity leads to an increase in sensitivity of post-synaptic adrenergic receptors. This is demonstrated by a marked increase in locomotor responses to sympathomimetics such as amphetamines and cocaine in *NET* deficient mice and in vascular responses to sympathomimetic drugs such as α_1 -agonist phenylephrine and β -agonist isoproterenol in humans with pharmacological blockade of the *NET* [22,23]. Therefore, in addition to delayed clearance of NE, enhanced post-synaptic adrenergic receptor sensitivity may contribute to exaggerated blood pressure response to exercise in the *NET* variant allele carriers.

Our findings of increased blood pressure in the carriers of variant alleles, one of which codes for a reduced-function *NET*, are therefore concordant with the *in vitro* data and with *in vivo* findings in subjects with a rare genetic loss-of function *NET* variant (Ala457Pro) as well as with the pharmacological inhibition of *NET* [6,24]. These findings are of potential clinical relevance, as exaggerated blood pressure response during exercise is associated with future adverse cardiovascular outcomes including hypertension [25–28].

The association of *NET* variants with DBP during exercise was less marked than that of SBP. During exercise, the increase in SBP is much more pronounced than that of DBP (in our study, Δ BP were 39.0 ± 13.5 mmHg and 14.5 ± 9.4 mmHg for SBP and DBP, respectively), reflecting a decrease in local peripheral resistance secondary to local tissue regulation in well-perfused muscle tissues [29]. Thus, SBP during exercise is a better marker of central sympathetic tone than DBP. This fact may have contributed to the weaker association between *NET* variants and DBP compared to SBP.

Heart rate at rest and during exercise was not associated with the *NET* variants. Increase in blood pressure activates a baroreceptor-mediated vagal response that decreases heart rate [30]. It is therefore possible that differences in heart rate responses in carriers of *NET* variant alleles are countered by the baroreceptor response to a greater increase in blood pressure in these subjects. These findings suggest that exaggerated blood pressure response in the *NET* variant allele carriers was driven by a greater increment in stroke volume or peripheral resistance, rather than heart rate.

Although *NET* mediates rapid uptake of NE at the synaptic cleft and thus directly decreases synaptic NE concentrations [31,32], the transporter may not have a large role in dictating resting plasma NE or EPI concentrations [33–35]. Therefore, although reduction in *NET* function is expected to increase the synaptic norepinephrine concentration, changes in plasma NE concentrations are likely to occur only with severe reductions in *NET* function that allow significant spillover of synaptic NE. Indeed, loss of *NET* function in *NET* gene targeted mice [5], or severely decreased *NET* function due to a rare genetic loss-of-function polymorphism (Ala457Pro) [6] or pharmacologic blockade [24], result in increased plasma NE concentrations. However, in our study, the C allele carriers of the *NET* T-182C polymorphism had significantly lower resting plasma NE concentrations. Post-exercise plasma NE and resting and post-exercise plasma EPI concentrations were not associated with this variant.

Plasma NE concentration reflects the amount of NE that is released into the synapse, the amount that spills over from the synapse, and the amount that is cleared, and therefore, is an inaccurate estimate of synaptic NE concentration [33,34]. Thus, more specific measures of synaptic NE release such as radioisotope-based spillover studies will be required to specifically address the role of these two reduced-function *NET* variants in regulating synaptic NE kinetics [33].

Plasma EPI is mainly secreted from adrenal medulla in response to exercise and acts systemically, therefore EPI concentrations are less likely affected by synaptic transporters such as *NET* [36,37]. Moreover, *NET* is 9-fold less effective in clearing synaptic EPI than NE [38] and is therefore unlikely to make a major contribution to plasma EPI concentrations.

Our study had some limitations. It is possible that these variants could potentially affect plasma catecholamine concentrations in presence of other genetic or environmental factors, particularly diseases associated with an increase in sympathetic activity. However we studied healthy subjects under highly controlled conditions to minimize the influence of these confounders, and thus cannot extrapolate our findings to disease states. Additionally, the effects of *NET* polymorphisms are known to be dependent on the degree of adrenergic stimulation and physical activity [5]. Since we studied healthy subjects who did not exercise to exhaustion, we might not have detected the full magnitude of functional effects of *NET* variants. Certainly, it will be important to study the role of these polymorphisms in other populations such as the elderly and patients with heart failure, where adrenergic activation is often more chronic and predicts poor outcome.

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Figure 1. The effect of *NET* polymorphisms on systolic blood pressure at rest and during exercise (all subjects, n=145)

Panel A (left): *NET* rs2242446 (T-182C), Panel B (right): *NET* rs28386840 (A-3081T). * adjusted $P < 0.01$; ** adjusted $P < 0.001$.

Figure 2. The effect of *NET* polymorphisms on systolic blood pressure at rest and during exercise in European Americans (n=83) and African Americans (n=62)

Panel A (top left): *NET* rs28386840 (A-3081T) in European Americans, Panel B (top right): *NET* rs2242446 (T-182C) in European Americans, Panel C (bottom left): *NET* rs28386840 (A-3081T) in African Americans, Panel D (bottom right): *NET* rs2242446 (T-182C) in African Americans.

Table 1

Subject characteristics and resting and post-exercise plasma catecholamine concentrations

Parameter (n)	Mean $\pm$ SD
Age (yrs)	26.5 $\pm$ 6.3
Weight (kg)	74.7 $\pm$ 16.8
Height (cm)	171.2 $\pm$ 9.9
BMI (kg/m ²)	25.4 $\pm$ 5.2
Sex (Male/Female)	63/82
Race (European American/African American)	83/62
Exercise score (METS/week)	14.8 $\pm$ 21.6
Epinephrine (pg/mL)	
Rest	21.5 $\pm$ 16.6
Post-exercise	49.0 $\pm$ 39.2
Norepinephrine (pg/mL)	
Rest	220.8 $\pm$ 100.8
Post-exercise	472.6 $\pm$ 243.7

Table 2
Subject characteristics, baseline cardiovascular measures, and catecholamine concentrations by *NET* genotypes

Genotype	Race European American/ African American	Sex (male/female)	Baseline heart rate (bpm)	Baseline systolic BP (mmHg)	Baseline diastolic BP (mmHg)	Baseline epinephrine (pg/mL)	Baseline norepinephrine (pg/mL)
All Subjects	83/62	63/82	67.7 ± 7.8	111.4 ± 12.0	65.2 ± 7.4	21.45 ± 16.6	220.8 ± 100.8
<i>NET</i> rs2242446 T-182C							
Wild type (n=79)	39/40	36/43	66.8 ± 7.7	111.4 ± 11.6	65.8 ± 8.0	22.4 ± 19.3	237.3 ± 114.1
Variant (n=61) [†]	39/22	25/36	68.6 ± 7.9	111.5 ± 12.8	64.6 ± 6.5	20.3 ± 13.3	194.9 ± 74.3 *
<i>NET</i> rs28386840 A-3081T							
Wild type (n=58)	43/15	30/28	67.9 ± 7.1	110.4 ± 11.7	64.6 ± 7.4	21.2 ± 18.0	232.4 ± 101.1
Variant (n=83) [‡]	39/44	32/51	67.7 ± 8.2	111.7 ± 11.9	65.5 ± 6.6	22.0 ± 15.9	210.1 ± 97.4

* P = 0.014 (adjusted for age, sex, race and exercise score) for variant vs. the wild type

[†] Variant includes subjects that were hetero- or homozygous for the variant allele

Table 3

NET variants and cardiovascular responses and catecholamine concentrations during exercise

Genotype	Systolic BP (mmHg)			Diastolic BP (mmHg)			Heart Rate (bpm)			Post-exercise catecholamines	
	25W	50W	75W	25W	50W	75W	25W	50W	75W	Epi-nephrine (pg/mL)	Norepi-nephrine (pg/mL)
NET rs2242446 (T-182C)											
Wild type	122.1 ± 14.5	132.9 ± 16.5	147.6 ± 16.9	70.3 ± 9.6	74.0 ± 10.4	79.1 ± 10.7	93.4 ± 9.6	105.5 ± 11.7	118.2 ± 15.2	47.8 ± 41.2	438.7 ± 191.7
Variant	126.7 ± 14.4[†]	139.6 ± 15.7[†]	153.7 ± 15.8[†]	72.6 ± 8.8[‡]	75.9 ± 8.7[‡]	79.6 ± 9.9	93.7 ± 11.7	106.8 ± 12.8	119.5 ± 14.5	51.5 ± 37.3	436.9 ± 204.1
NET rs28386840 (A-3081T)											
Wild type	120.1 ± 13.6	130.2 ± 15.5	145.0 ± 16.8	68.8 ± 8.5	72.0 ± 9.3	77.9 ± 10.0	93.3 ± 9.4	104.2 ± 11.5	116.9 ± 16.0	48.7 ± 41.6	441.7 ± 188.7
Variant	126.4 ± 14.8[‡]	139.1 ± 15.9[‡]	153.3 ± 14.9[‡]	73.1 ± 9.5[*]	76.4 ± 9.1[*]	80.4 ± 10.2	93.8 ± 11.7	107.6 ± 13.1	120.7 ± 14.9	55.5 ± 37.9	442.5 ± 207.0

* P < 0.05;

[‡] P ≤ 0.01;

[†] P ≤ 0.001 for variant vs. wild type; all P values adjusted for age, sex, race, exercise score, fitness, and the baseline value of the outcome measure; HR = Heart rate, BP = Blood pressure, Variant includes subjects that were hetero- or homozygous for the variant allele

Table 4

Effect of *NET* genotypes, race, and covariates on SBP (at rest and at maximal exercise) and on SBP-AUC

Parameter	Rest (mmHg)		75 W (mmHg)		SBP-AUC (mmHg · Watt)	
	β (95% CI)	P value	β (95% CI)	P value	β (95% CI)	P value
<i>NET T-182C</i>						
Age	0.05 (−0.26, 0.35)	0.76	0.6 (0.2, 1.0)	0.003	22.3 (−6.6, 51.2)	0.15
Sex	11.5 (7.7, 15.3)	<0.001	0.3 (−4.9, 5.4)	0.91	791.9 (438.4, 1145.5)	<0.001
Race	6.8 (3.0, 10.6)	0.001	5.3 (0.4, 10.2)	0.03	789.2 (423.9, 1154.5)	<0.001
Fitness	−0.02 (−0.12, 0.07)	0.58	0.05 (−0.07, 0.16)	0.43	2.9 (−6.0, 11.8)	0.52
Resting SBP	N/A	-	0.8 (0.6, 1.0)	<0.001	N/A	-
<i>NET T-182C</i>	1.5 (2.2, 5.1)	0.44	7.4 (2.9, 11.9)	0.001	531.1 (181.9, 880.3)	0.003
<i>NET A-3081T</i>						
Age	0.02 (−0.3, 0.3)	0.9	0.6 (0.2, 1.0)	0.002	19.8 (−8.7, 48.3)	0.17
Sex	12.2 (8.5, 15.8)	<0.001	0.4 (−4.9, 5.6)	0.89	783.7 (435.4, 1132.1)	<0.001
Race	5.3 (1.5, 9.1)	0.006	2.9 (−2.0, 7.8)	0.24	530.8 (165.8, 895.8)	0.005
Fitness	−0.04 (−0.12, 0.05)	0.40	0.04 (−0.06, 0.14)	0.42	0.43 (−7.5, 8.3)	0.91
Resting SBP	N/A	-	0.8 (0.5, 1.0)	<0.001	N/A	-
<i>NET A-3081T</i>	−1.3 (2.4, 5.0)	0.48	6.8 (2.2, 11.5)	0.004	470.3 (7405.1, 9176.7)	0.009

Regression coefficients for *NET T-182C* and *A-3081T* genotypes and other covariates, with their respective 95% confidence intervals (CI) and P values. Genotypes were coded 0 or 1 representing absence or presence of at least one *NET -182 C* and *-3081 T* allele; sex and race were coded 0/1 for female/male and European American/African American, respectively, so that regression coefficients reflect sex and racial differences. SBP-AUC = Systolic blood pressure area under the curve, SBP = Systolic blood pressure, N/A = not applicable. Systolic blood is reported in mmHg.

For each change of 1 unit in the covariate, the outcome (SBP or SBP-AUC) changes by the amount indicated by the respective β -coefficient. Thus, for example, for each allele of *NET T-182C*, resting SBP increase by 1.5 mmHg (95% CI, 2.2 to 5.1; $P=0.44$), SBP at maximal exercise increases by 7.4 mmHg (95% CI, 2.9 to 11.9 mmHg; $P=0.001$), and SBP-AUC increases by 531.1 mmHg · Watt (95% CI, 181.9 to 880.3 mmHg · Watt; $P=0.003$).

Table 5

NET genotypes and area-under-the curve analysis for systolic and diastolic blood pressure and heart rate

	SBP-AUC (mmHg.Watt)	DBP-AUC (mmHg.Watt)	HR-AUC (mmHg.Watt)
<i>NET</i> rs2242446 (T-182C)			
Wild type	9540.1 ± 1080.2	5397.8 ± 669.5	7278.0 ± 759.8
Variant	9896.3 ± 1074.5[‡]	5508.7 ± 544.7	7363.4 ± 833.2
<i>NET</i> rs28386840 (A-3081T)			
Wild type	9366.1 ± 1045.3	5273.9 ± 620.5	7246.6 ± 754.0
Variant	9875.9 ± 1043.1[‡]	5556.4 ± 572.7	7386.7 ± 854.5

[‡]P<0.01 for variant vs. the wild type; all P values adjusted for age, sex, race, fitness and the baseline value of the outcome measure; HR = Heart rate, BP =Blood pressure, AUC=Area under the curve. Variant includes subjects that were hetero- or homozygous for the variant allele