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The *KCNMB1* E65K Variant is Associated with Reduced Central Pulse Pressure in the Community-Based Framingham Offspring Cohort

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Abstract

Objectives—Genetic variants that influence large conductance calcium-activated potassium channel (BKCa) function may alter arterial function and contribute to the known heritability of arterial stiffness and blood pressure. The $\beta 1$ -subunit (*KCNMB1*) of the BKCa channel includes two coding region polymorphisms. *E65K*, a gain-of-function polymorphism, is predicted to enhance BKCa channel opening and vasorelaxation, whereas *V110L* has no known effect. We and others have reported that *E65K* carriers have reduced blood pressure.

Methods—To test our hypothesis that *E65K* has a favorable effect on arterial function, we related arterial tonometry and brachial artery phenotypes to genotypes in 1,100 Framingham Offspring Study participants with available genotypes and phenotypes (53% women; mean age 61.5±9.4 years).

Results—The minor allele frequency was 0.10 for *E65K* and 0.09 for *V110L*; both were in Hardy-Weinberg equilibrium (χ^2 $p > 0.05$), and haplotype analysis found $R^2=0.01$. *E65K* was associated with lower augmented pressure (7.4±3.3 vs. 9.0±3.8 mm Hg, $p=0.01$) and central pulse pressure (47.1±7.3 vs. 50.7±7.8 mm Hg, $p=0.01$) in multivariable analyses. No association was noted between

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Conflicts of Interest: Dr. Mitchell is owner of Cardiovascular Engineering, Inc, a company that designs and manufactures devices that measure vascular stiffness. The company uses these devices in clinical trials that evaluate the effects of diseases and interventions on vascular stiffness. Dr Mitchell has reported receiving consulting and speaking fees from OMRON Healthcare, Inc. The remaining authors report no conflicts.

E65K and mean arterial pressure, carotid-femoral pulse wave velocity or brachial artery diameter, flow velocity or volume flow. *V110L* was not associated with tonometry or brachial measures.

Conclusions—A diminished augmented pressure in *K*-carriers suggests a reduced or delayed wave reflection and supports the hypothesis that *E65K* reduces arterial impedance mismatch in the arterial tree. Our findings in a middle-aged community-based cohort, if replicated, would support that *E65K* has a favorable effect on arterial function and pulsatile hemodynamic load.

Keywords

KCNMB1; Single Nucleotide Polymorphism; Genetics; Vascular Tonometry

Introduction

Large-conductance calcium-activated potassium (BKCa) channels are expressed in vascular smooth muscle cells and regulate arterial tone by altering resting membrane potential [1]. BKCa channels in blood vessels are composed of α -pore-forming and β_1 -regulatory subunits. The β_1 -subunit, which is expressed specifically in vascular smooth muscle cells, is required for calcium-dependent BKCa channel opening, a principal mechanism by which the BKCa is activated [2]. Blood vessels from mice deficient in the β_1 -subunit are resistant to calcium-triggered BKCa channel opening and show increased arterial tone [3]. Consistent with a direct vascular effect on blood pressure, β_1 -subunit deficient mice are hypertensive [2].

The human gene encoding the β_1 -subunit, *KCNMB1*, contains two known non-synonymous polymorphisms: *E65K* and *V110L*, both of which have been associated with cardiovascular phenotypes. The *E65K* polymorphism has been associated with lower diastolic pressure [4,5], particularly in the setting of anti-hypertensive therapy [6,7]. *V110L*, which has not been related to altered blood pressure, was found to be associated with reduced cardiovascular mortality in a clinical trial sub-study [7]. Whereas, there is mounting evidence that *E65K* guards against hypertension, the mechanism of the protective effect has not been fully established.

Arterial stiffness has been associated with cardiovascular morbidity through increases in systolic and pulse pressure and end organ damage [8–11]. Arterial tonometry measures, which characterize components of arterial stiffness and wave reflection, are heritable [12], suggesting a genetic contribution to pressure waveform morphology and pressure pulsatility. We hypothesized that the effect of *KCNMB1* polymorphisms on blood pressure and cardiovascular outcomes may be mediated in part through direct effects on arterial stiffness or wave reflection. To test this hypothesis we compared tonometry phenotypes in the Framingham Offspring Cohort.

Methods

Study Participants

The design of the Framingham Offspring study, involving a community-based sample of predominantly white Americans, has been described previously in detail [13]. Medical history, medication use, laboratory tests, physical examinations, and brachial artery and tonometry measures were assessed at examination cycle seven (1998–2001), when 2,271 unrelated participants underwent tonometry assessment. Separately, during the sixth examination (1995–1998), 1,809 unrelated individuals provided blood samples for DNA extraction [14]. These unrelated individuals were selected for DNA collection without regard to phenotypic features. Participants with arterial tonometry measures were eligible for inclusion in this analysis if they had DNA available. This resulted in 1,265 participants eligible for genotype-tonometry analysis, of which 1,100 (1025 for carotid-femoral PWV) had complete covariate information.

Additional assessment of brachial artery function, as a secondary analysis, was available in a subset of 1,084 participants who also had DNA, tonometry and full covariate measures available. All participants provided written informed consent; and the Boston University Medical Center Institutional Review Board approved the study.

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using standard methods. The *KCNMB1* polymorphisms *rs11739136* (*E65K*) and *rs2301149* (*V110L*) were genotyped using Taqman Assay (Applied Biosystems 7900HT). Genotyping rates were 99.7% for *E65K* and 99.8% for *V110L*. Linkage disequilibrium and haplotype block analysis were constructed using *Haploview* software [15].

Tonometry Measures

Arterial tonometry of the brachial, radial, femoral and carotid arteries, with simultaneous ECG monitoring, was performed as previously described [16]. Mean arterial pressure was determined by integrating the calibrated brachial pressure waveform obtained by tonometry. Carotid-femoral pulse wave velocity (PWV) was calculated from the carotid and femoral waveforms and body surface measurements of transit distance as previously described [16]. Central pulse pressure was assessed as peak minus trough of the calibrated carotid tonometry waveform. The forward wave amplitude was defined as the difference between pressure at the foot and the first systolic inflection point or peak. The augmented pressure was defined as the difference between central pulse pressure and the forward wave. Augmentation index was computed as previously described [17].

Brachial Artery Measures

Brachial artery diameter at baseline and 1-minute after reactive hyperemia, induced by 5-minute forearm cuff occlusion, were assessed as previously described [18], by using a Toshiba SSH-140A ultrasound system and commercially available software (Brachial Tools, version 3.2.3). Mean Doppler flow velocity was analyzed using a semi-automated signal averaging approach (Cardiovascular Engineering, Inc.) and multiplied by brachial artery cross-sectional areas to obtain volume flows. Forearm vascular resistance was calculated by dividing mean arterial pressure by mean volume flow at baseline and during hyperemia.

Statistical Analysis

Given the low minor allele frequency of both polymorphisms, a dominant mode of inheritance was used in all analyses (*E*=wild type, *K*=substitution for *E65K*; *V*=wild type, *L*=substitution for *V110L*). We assessed whether either genotype was associated with adjusted-mean forward wave, augmented pressure, central pulse pressure, augmentation index, mean arterial pressure and carotid-femoral PWV using analysis of covariance. Models included known and suspected correlates of arterial function, including: age, age-squared, sex, heart rate, height, weight, total/high-density lipoprotein cholesterol ratio, fasting glucose, current smoking, presence of diabetes, presence of cardiovascular disease, antihypertensive medication use, lipid-lowering medication, aspirin use and use of hormone replacement therapy. Based on prior publications [5–7,19], we also assessed interactions between *E65K* and anti-hypertensive medications, heart rate, age and sex by the addition of a multiplicative term to the full multivariable model. We also compared mean heart rate by *E65K* and *V110L* genotypes using a T-test.

As secondary analyses, additional analyses were performed to test for association of *E65K* genotype with measures of brachial artery diameter, flow velocity, flow volume and forearm vascular resistance using the above listed covariates. Values of forearm vascular resistance were highly skewed and were natural log-transformed to normalize their distribution.

Additional subgroup multivariate analyses of tonometry and brachial artery measures were performed on subjects not receiving anti-hypertensive therapy. All analyses were performed using SAS software [20]. A two-sided $p < 0.05$ was considered statistically significant for all primary and secondary analyses. No adjustment for multiple testing was performed.

Results

Associations of *KCNMB1 E65K* and *V110L* with tonometry measures were examined in 1,100 participants (53% women; mean age 61.5 ± 9.4 years) in whom full covariate data was available. The clinical characteristics of this cohort are shown in Table 1. The minor allele frequency was 0.10 for *E65K* and 0.09 for *V110L*; both polymorphisms conformed to Hardy-Weinberg equilibrium testing (χ^2 test $p > 0.05$). Haplotype analysis of *E65K* and *V110L* found the rare alleles within the haplotype to be out-of-phase ($R^2 = 0.01$), with the frequency of the *K-L* haplotype exceedingly low ($< 1\%$).

The *E65K*-substitution was associated with an 18% lower augmented pressure ($p = 0.01$) and 7% lower central pulse pressure ($p = 0.01$) compared to wildtype, with a trend toward lower augmentation index ($p = 0.07$; Table 2). No association was noted between *E65K* and forward wave amplitude, carotid-femoral PWV or mean arterial pressure (Table 2). Associations between *V110L* and forward wave, augmented pressure, augmentation index, central pulse pressure, mean arterial pressure, and carotid-femoral PWV were assessed and no association was identified ($p = 0.74, 0.60, 0.47, 0.97, 0.89$ and 0.54 , respectively, data not shown).

Given that age, heart rate, sex and anti-hypertensive therapy have been shown previously to modify the association between *E65K* and blood pressure, we tested for these interactions in the augmented pressure and central pulse pressure models. No statistical interactions were observed ($p \geq 0.15$). Since augmented pressure may be altered in the presence of increased peripheral resistance or various disease states, we compared the distribution of *E65K* genotypes by the presence of diabetes, hypertension or cardiovascular disease using χ^2 analysis. No differences were noted ($p \geq 0.65$). A prior publication, that included *V110L*, found that *KCNMB1* polymorphisms were associated with heart rate variability and baroreflex sensitivity [19]. To explore potential relations between genotype and heart rate, we tested for associations between *V110L* and *E65K* with heart rate and no association was identified ($p > 0.51$).

As part of secondary analyses to determine whether differences in muscular artery or distal microvascular function contributed to the association between *E65K* and augmented pressure, we examined the association between *E65K* and measures of brachial artery diameter, flow velocity, flow volume and vascular resistance at rest and during hyperemia. No associations between brachial artery measures and *E65K* were identified (Table 3).

When the analysis was restricted to subjects not receiving anti-hypertensive treatment ($n = 713$) the *E65K*-substitution was associated with lower augmented pressure ($p = 0.003$) and a lower augmentation index ($p = 0.004$; Table 4). Examination of brachial artery measures in subjects not receiving antihypertensive therapy ($n = 707$) found *E65K*-carriers to exhibit higher flow-mediated dilation ($p = 0.03$) and hyperemic flow-volume ($p = 0.03$) and lower hyperemic forearm vascular resistance ($p = 0.03$, Table 5).

Discussion

In this analysis we have examined relations between two non-synonymous *KCNMB1* polymorphisms, which were previously shown to be associated with reductions in blood pressure and cardiovascular outcomes, and components of the arterial waveform. *E65K* was associated with lower augmented pressure and central pulse pressure, without effects on aortic PWV or muscular artery diameter or flow. The association was not affected by age, gender,

and anti-hypertensive therapy. We found no association between tonometry measures and *V110L*.

KCNMB1-E65K was first reported to have a protective effect on diastolic hypertension in a Spanish cohort (n=3876) [4]. Subsequently, the INVEST trial found that *K*-carriers achieved blood pressure control more rapidly and were less likely to require multiple medications compared to non-carriers [7]. Most recently we have shown that *E65K*-carriers have lower systolic and diastolic pressure in the setting of anti-hypertensive therapy in two independent cohorts [6]. Physiology studies in β 1-deficient mice, which are hypertensive, demonstrate profound abnormalities of blood vessel relaxation. While altered potassium handling with acute volume expansion has also been described in these mice, the mean arterial blood pressure in β 1-deficient mice is not salt-sensitive [21], suggesting that abnormalities of systemic arterial relaxation rather than salt handling have the greatest effect on blood pressure in these animals. Consistent with a direct effect on arterial stiffness in humans we now report associations with lower augmented pressure and central pulse pressure in *E65K*-carriers. These associations were not explained by age, sex, anti-hypertensive therapy or atherosclerotic disease risk, suggesting that the genetic effect may be related directly to alterations in vascular structure or function. By comparison, the *V110L* polymorphism, which has not been shown to affect BKCa-channel function, was not associated with tonometry measures in our cohort. While our studies support an important role for *E65K* and arterial stiffness, the potential for altered salt and mineralocorticoid effects mediated by *E65K* warrants further study.

Our findings add to a growing body of literature suggesting that *E65K* has protective effects on the vasculature. Fernandez-Fernandez, et al.[5], demonstrated that the *E65K* gain-of-function mutation may result in membrane hyperpolarization, which they predicted would cause vascular smooth muscle cell relaxation and blood vessel dilation. To our knowledge our study is the first to test for this predicted effect using direct measures of vascular function. It was difficult to predict whether *E65K* would cause a generalized effect on all blood vessels because the BKCa channel is expressed in a range of arteries, including the aorta, large muscular arteries and small resistance arterioles [22–24]. In this analysis, *K*-allele carriers had a reduction in augmented pressure, suggesting a reduction in reflected wave amplitude. The reflected pressure wave is formed as forward traveling pressure waves reflect from sites of impedance mismatch. Mismatch occurs when larger, more compliant (low impedance) arterial segments connect with smaller, stiffer (high impedance) arteries and resistance vessels [16]. Diminished augmented pressure in *K*-carriers suggest that *E65K* reduces arterial impedance mismatch somewhere in the arterial tree, perhaps by altering muscular artery or resistance vessel diameter or elastance. The latter physical attributes are the principal determinants of local vascular impedance of an arterial segment. Therefore, our results support a role for *E65K* in the modulation of wave reflection in humans.

The amplitude of the reflected wave is determined by both the size of the initial forward traveling wave and the proportion of that wave that is reflected, which can be estimated by using augmentation index. Since neither an index of relative wave reflection (the augmentation index) nor forward wave amplitude had clear association with *E65K*, it is difficult to determine whether lower augmented pressure and central pulse pressure was attributable to reduced relative wave reflection or combined reductions in relative wave reflection and forward wave amplitude. However, our subgroup analysis in subjects not receiving antihypertensive therapy found reduced augmentation index in *K*-carriers, suggesting an effect via the reflected wave. Additional studies that measure both pressure and flow, so that true forward and reflected wave amplitude can be evaluated, may clarify this remaining question.

We found no difference in carotid-femoral PWV, suggesting that the mutant channel does not affect aortic wall stiffness, which is closely related to carotid-femoral PWV. Because there

were no significant differences in brachial artery diameters at rest in *K*-carriers, we conclude that the mutant BKCa channels did not have a general effect on the diameter of the brachial artery and presumably other medium-sized muscular arteries. We did not observe an association with mean arterial pressure, suggesting that if there was a reduction in global peripheral resistance, it was offset by an increase in cardiac output, resulting in no change in mean arterial pressure. Further studies that include measures of cardiac output and total peripheral resistance will be required to clarify the relation between *E65K* and global resistance vessel function.

Measures of brachial artery diameter and flow were potentially confounded by the use of anti-hypertensive agents from a variety of medication classes in our sample. In a subgroup analysis of subjects not receiving anti-hypertensive agents, we found a greater increase in brachial artery size and flow volume in the setting of hyperemia in *K*-carriers, suggesting that *E65K* may have an effect on muscular arteries. The effect of *E65K* on brachial artery measures was identified only in the setting of hyperemia, suggesting that *K*-carriers may have increased sensitivity to endothelium-dependent or other mediators of smooth muscle cell relaxation. Indeed, nitric oxide has been shown to positively regulate smooth muscle cell calcium “sparks” that trigger *KCNMB1*-dependent BK channel opening in pressurized rat cerebral arteries[25]. Our finding that flow-mediated brachial artery dilation was greater in *K*-carriers, if confirmed, would support a mechanistic link between *E65K* and nitric oxide.

Because our results are consistent with a prior linkage study [12] and findings in the Spanish cohort and the INVEST trial [4,5,7], we consider a false positive association to be less likely. We sought to reduce the effects of multiple testing by restricting the initial analyses to the linear analysis of several measures of arterial stiffness with two non-synonymous polymorphisms, after taking into account known covariates. We consider our exploration for modifiers of *E65K* as secondary analyses and restricted these analyses to pre-specified interaction terms in order to minimize the risk of false-positive associations.

Strengths and limitations

The routine ascertainment of tonometric measures, genotypes, and covariate data in a community-based sample are strengths of the study. Our estimates of forward and reflected wave amplitude were based on assessment of pressure only. In order to ascertain the true forward and reflected wave amplitude, both pressure and flow are required. We acknowledge that we did not account for multiple testing and that our findings will need to be replicated in an external cohort. In addition, the generalizability of our findings to individuals who are younger, or are not white or of European descent will need to be tested. Because the genotyping was conducted in the standard Framingham unrelated DNA plate set, many of the individuals who were phenotyped were not genotyped.

Conclusion

The regulation of vascular tone is complex and influenced by both genetic and environmental factors. The *KCNMB1-E65K* polymorphism is associated with reductions in augmented pressure and central pressure pulsatility, consistent with a protective effect of this polymorphism. This trend toward protection may be mediated by reducing end organ damage due to excessive pressure pulsatility, which can occur with advancing age and in the presence of various risk factors and disease states. The finding of reduced pressure pulsatility in *K*-carriers of the *KCNMB1-E65K* polymorphism supports the hypothesis that prior findings of lower blood pressure and a greater response to medical therapy were mediated in part through a direct vascular effect of the polymorphism.

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Abbreviations and Symbols

BKCa

Large-conductance Calcium-activated Potassium Channels

PWV

Pulse Wave Velocity

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Table 1
Characteristics of the Sample

Variable	Total Cohort [†]
Basic Characteristics: (n=1100)	
Age (years)	61.5 (9.4)
Males (%)	47.0
Body mass index (kg/m ²)	27.8 (5.1)
Height (cm)	66.1 (3.7)
Weight (kg)	173.6 (38.0)
Heart Rate (bpm)	64 (10)
Systolic Blood Pressure (mm Hg)	128 (19)
Diastolic Blood Pressure (mm Hg)	74 (10)
Total/HDL cholesterol ratio	4 (1)
Glucose (mg/dL)	104 (25)
Hypertension (%)	47.2
Cardiovascular Disease (%)	13.6
Diabetes (%)	11.8
Current Smoking (%)	12.6
Anti-Hypertensive Medication Use (%)	35.2
Aspirin Use (%)	37.6
Lipid-lowering Medication Use	21.6
Hormone Replacement Therapy (% Females)	35.5
Arterial Tonometry Measures: (n=1100)	
Carotid-Femoral Pulse Wave Velocity (m/s) (n=1025)	10.3 (3.7)
Forward Wave Pressure (mm Hg)	41.3 (13.4)
Reflected Wave Pressure (mm Hg)	8.7 (7.6)
Augmentation Index (%)	16.3 (11.3)
Central Pulse Pressure (mm Hg)	50.1 (16.4)
Mean Arterial Pressure (mm Hg)	101.1 (12.3)
Brachial Artery Measures: (n=1084)	
Baseline Diameter (mm)	4.3 (0.9)
Baseline Flow Velocity (cm/s)	7.9 (4.6)
Baseline Volume Flow (ml/s)	1.2 (0.9)
Baseline FVR, ln (dyne/s/cm ⁻⁵)	11.9 (0.7)
Flow-Mediated Dilation (%)	2.9 (2.9)
Hyperemic Flow Velocity (cm/s)	50.2 (21.5)
Hyperemic Volume Flow (ml/s)	7.0 (3.6)
Hyperemic FVR, ln (dyne·s·cm ⁻⁵)	10.0 (0.6)

[†] Mean (SD) reported, unless otherwise noted.

Table 2Multivariable-Adjusted[†] Tonometry Measures by *KCNMB1* E65K Polymorphism

Phenotype	Genotype	n	Least-Square Means	P value
Carotid-Femoral Pulse	E	828	10.3 (2.5)	0.88
Wave Velocity (m/s)	K	197	10.2 (2.5)	
Forward Pressure (mm Hg)	E	893	41.7 (6.0)	0.11
	K	207	39.7 (6.2)	
Augmented Pressure (mm Hg)	E	893	9.0 (3.8)	0.01
	K	207	7.4 (3.4)	
Augmentation Index (%)	E	893	16.7 (6.0)	0.07
	K	207	15.1 (5.7)	
Central Pulse Pressure (mm Hg)	E	893	50.7 (7.8)	0.01
	K	207	47.1 (7.3)	
Mean Arterial Pressure (mm Hg)	E	893	101.3 (4.8)	0.32
	K	207	100.3 (4.9)	

[†] Adjusted for: age, age-squared, sex, heart rate, height, weight, total/high-density lipoprotein cholesterol ratio, fasting glucose, current smoking, presence of diabetes, presence of cardiovascular disease, hormone replacement therapy, antihypertensive medication use, lipid-lowering medication, and aspirin use.

Table 3Multivariable-Adjusted[†] Brachial Artery Measures by *KCNMB1* E65K Polymorphism

Phenotype	Genotype	n	[‡] Least-Square Means	[‡] P value
Baseline Brachial Artery	E	879	4.2 (0.7)	0.51
Diameter (mm)	K	205	4.3 (0.7)	
Baseline Flow	E	879	8.0 (2.0)	0.24
Velocity (cm/s)	K	205	8.3 (1.9)	
Baseline Flow Volume (ml/s)	E	879	1.2 (0.5)	0.11
	K	205	1.3 (0.5)	
Baseline FVR, ln (dyne·s·cm ⁻⁵)	E	879	11.9 (0.4)	0.39
	K	205	11.8 (0.4)	
Flow-Mediated	E	879	2.8 (1.2)	0.12
Dilation (%)	K	205	3.1 (1.1)	
Hyperemic Flow	E	879	50.1 (11.2)	0.72
Velocity (cm/s)	K	205	50.9 (11.1)	
Hyperemic Flow Volume (ml/s)	E	879	7.0 (2.0)	0.26
	K	205	7.4 (2.0)	
Hyperemic FVR, ln (dyne·s·cm ⁻⁵)	E	879	10.0 (0.3)	0.24
	K	205	9.9 (0.3)	

[†] Adjusted for: age, age-squared, gender, heart rate, height, weight, total/high-density lipoprotein cholesterol ratio, fasting glucose, current smoking, presence of diabetes, presence of cardiovascular disease, hormone replacement therapy, antihypertensive medication use, lipid-lowering medication, and aspirin use.

Table 4

Multivariable-Adjusted[†] Tonometry Measures by *KCNMB1 E65K* Polymorphism in Individuals not Receiving Antihypertensive Agents[‡]

Phenotype	Genotype	n	Least-Square Means	P value
[‡] Carotid-Femoral Pulse	E	540	9.4 (2.1)	0.65
Wave Velocity (m/s)	K	133	9.5 (2.2)	
Forward Pressure (mm Hg)	E	577	39.3 (4.9)	0.91
	K	136	39.0 (5.3)	
Augmented Pressure (mm Hg)	E	577	8.2 (3.1)	0.003
	K	136	6.6 (2.4)	
Augmentation Index (%)	E	577	16.4 (5.6)	0.004
	K	136	14.0 (4.6)	
Central Pulse Pressure (mm Hg)	E	577	47.5 (6.0)	0.22
	K	136	45.6 (5.9)	
Mean Arterial Pressure (mm Hg)	E	577	99.1 (4.8)	0.29
	K	136	97.8 (4.4)	

[†] Adjusted for: age, age-squared, sex, heart rate, height, weight, total/high-density lipoprotein cholesterol ratio, fasting glucose, current smoking, presence of diabetes, presence of cardiovascular disease, hormone replacement therapy, lipid-lowering medication, and aspirin use.

[‡] n=713; Carotid-Femoral Pulse Wave Velocity (m/s), n=673.

Table 5

Multivariable-Adjusted[†] Brachial Artery Measures by *KCNMB1 E65K* Polymorphism in Individuals not receiving Antihypertensive Agents[‡]

Phenotype	Genotype	n	[‡] Least-Square Means	[‡] P value
Baseline Brachial Artery	E	572	4.1 (0.7)	0.14
Diameter (mm)	K	135	4.2 (0.6)	
Baseline Flow	E	572	7.9 (2.0)	0.24
Velocity (cm/s)	K	135	8.3 (1.9)	
Baseline Flow Volume (ml/s)	E	572	1.1 (0.5)	0.09
	K	135	1.2 (0.5)	
Baseline FVR, ln (dyne·s·cm ⁻⁵)	E	572	11.9 (0.4)	0.18
	K	135	11.9 (0.5)	
Flow-Mediated	E	572	3.1 (1.2)	0.03
Dilation (%)	K	135	3.7 (1.2)	
Hyperemic Flow	E	572	53.2 (10.4)	0.26
Velocity (cm/s)	K	135	55.3 (10.7)	
Hyperemic Flow Volume (ml/s)	E	572	7.1 (2.1)	0.03
	K	135	7.8 (2.0)	
Hyperemic FVR, ln (dyne·s·cm ⁻⁵)	E	572	10.0 (0.3)	0.03
	K	135	9.9 (0.3)	

[†] Adjusted for: age, age-squared, gender, heart rate, height, weight, total/high-density lipoprotein cholesterol ratio, fasting glucose, current smoking, presence of diabetes, presence of cardiovascular disease, hormone replacement therapy, lipid-lowering medication, and aspirin use.

[‡] n=707