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Independent and combined influence of *AGTR1* variants and aerobic exercise on oxidative stress in hypertensives

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Abstract

Angiotensin II (AngII), via the AngII type 1 receptor (AT₁R), contributes to oxidative stress. Aerobic exercise training (AEXT) reduces the risk of cardiovascular (CV) disease, presumably by reducing the grade of oxidative stress. We investigated the independent and combined influence of the *AGTR1* A1166C and –825 T/A polymorphisms on oxidative stress and plasma AngII responses to AEXT in pre- and stage 1 hypertensives. Urinary 8-iso-PGF_{2α} significantly increased with AEXT ($p=0.002$); however, there were no significant changes in superoxide dismutase activity or AngII levels. There was a significant difference in the change in AngII levels with AEXT between A1166C genotype groups ($p=0.04$) resulting in a significant interactive effect of the A1166C polymorphism and AEXT on the change in AngII ($p<0.05$). Only the TT genotype group of the –825 T/A polymorphism had a significant reduction in plasma AngII ($p=0.02$). Risk allele analysis revealed a significant reduction in plasma AngII ($p=0.04$) and a significant increase in urinary 8-iso-PGF_{2α} ($p=0.01$) with AEXT in individuals with two risk alleles only. Our findings suggest that variation in the *AGTR1* gene is associated with differential changes in plasma AngII but not oxidative stress.

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Keywords

AGTR1; angiotensin II; exercise; isoprostanes; oxidative stress

Introduction

Hypertension is a major risk factor for congestive heart failure, coronary artery disease and renal failure. It has a multifactorial etiology with a strong genetic component and is associated with an increase in reactive oxygen species (ROS) and a reduction in endogenous antioxidant defenses, termed oxidative stress (1). Oxidative stress may directly or indirectly contribute to the progression of end-organ injury by promoting hypertension, atherosclerosis or by inducing glomerular damage and renal ischemia.

Angiotensin II (AngII) is the main effector of the renin–angiotensin system (RAS) and is known to cause potent increases in systemic and local blood pressure (BP) via its vasoconstrictive effects (2). Recent *in vitro* evidence raises the possibility that a positive feedback loop may occur during tissue RAS regulation (3–5). Thus, the potential positive feedback loop between the angiotensin II type 1 receptor (AT₁R) and AngII may play an integral role in AngII-related pathophysiological changes in the vasculature and subsequent development of cardiovascular (CV) disease. In addition to the classic actions of AngII, evidence indicates that it may cause CV and end-organ injury independent of its BP elevating effects, through mechanisms that include increases in oxidative stress (6).

Most of the physiological and pathophysiological actions of AngII are mediated by the AT₁R (2). The AT₁R is a member of the seven transmembrane-spanning G-protein coupled receptor family, binds to a heterotrimeric G-protein and typically activates phospholipase C (7). AngII has been shown to increase oxidative stress via the AT₁R in part, because this receptor is linked to activation of NADPH oxidase in vascular walls (8). The AT₁R gene (*AGTR1*) maps to the long arm of chromosome 3, is more than 55 Kb long and contains five exons and four introns with the coding region of the gene located in the fifth exon (9). The *AGTR1* A1166C polymorphism (NCBI ref. SNP ID: rs5186) involves an A→C substitution at position 1166 in the 3'UTR of the *AGTR1* gene. This polymorphism has been shown to be more frequent in hypertensives compared with normotensives (10,11) and has also been associated with oxidative stress in heart failure patients (12). More recently, it has been shown to interrupt the ability of microRNA-155 to attenuate translation, resulting in augmented *AGTR1* expression (13). This suggests a potential functional mechanism by which the 3'UTR A1166C polymorphism can lead to increased oxidative stress and the development of CV disease.

The –825 T/A polymorphism (NCBI ref. SNP ID: rs275651) is located in the promoter region of the *AGTR1* gene and has been found to be in almost complete linkage disequilibrium (LD) with several other single nucleotide polymorphisms (SNPs) in the promoter (9). This polymorphism is reported to destroy a binding site for GATA transcription factors (9) and thus, may alter the expression of the *AGTR1* and the influence of AngII on oxidative stress. In addition, the *AGTR1* –825T/A polymorphism has inconsistently been associated with both increased and reduced risk for CV disease (14,15), though thorough genetic analyses (e.g. accounting for the A1166C polymorphism) have not been performed to clarify a potential mechanistic role.

Physical activity is advocated for the reduction and control of CV disease risk factors including hypertension (16,17). Reports also show that moderate intensity physical activity leads to reductions in markers of oxidative stress and an improved antioxidant capacity (18). Thus, behavioral factors such as aerobic exercise training (AEXT) and genetic factors may result in

modification of oxidative stress, and therefore hypertension, promoting an atheroprotective state in the vasculature.

Based on the evidence provided, the *AGTR1* gene represents a good candidate gene for oxidative stress. Furthermore, we believe that the A1166C and the –825T/A polymorphisms together may explain a portion of the inter-individual variation in the beneficial adaptations that occur in response to AEXT in individuals with CV disease risk factors. Therefore, the purpose of the present study was to investigate the independent and combined influence of the *AGTR1* A1166C and –825 T/A polymorphisms and AEXT on AngII levels and oxidative stress in pre- and stage 1 hypertensives. In addition, in light of the potential influence of the angiotensinogen (*AGT*) M235T polymorphism (NCBI ref. SNP ID: rs699) and the angiotensin converting enzyme insertion/deletion (*ACE I/D*) polymorphism (NCBI ref. SNP ID: rs1799752) on AngII levels and therefore, on oxidative stress and hypertension, we controlled for the effects of the M235T and *ACE I/D* polymorphisms in all statistical analyses.

Subjects and methods

Ethical approval

All subjects signed an informed consent form during their first laboratory visit that was approved by the University of Maryland College Park Institutional Review Board. This study conformed to the latest revision of the Declaration of Helsinki.

Subjects

One hundred pre- and stage 1 hypertensives responding to mailed brochures and to advertisements in newspapers and on the radio were eligible to participate in this study. Participants were 50–75 years of age, sedentary (physical activity <20 min <2 days per week), hypertensive (systolic BP 120–159 mmHg, diastolic BP 80–99 mmHg), non-diabetic, non-smokers, free of CV, renal, liver and lung disease, not on lipid lowering medications, BMI <37 kg/m², and had no medical conditions that would preclude vigorous exercise. Women were postmenopausal (absence of menstrual cycle for >2 years) and agreed to maintain their hormone replacement therapy regime, either on or not on, for the duration of the study.

Screening—Blood was collected after a 12-h overnight fast for blood chemistries and DNA analysis. After the fasting blood draw, participants underwent a 2-h 75-g oral glucose tolerance test to assess diabetes status. Individuals were excluded from the study if they had a hematocrit <35%, evidence of renal (estimated GFR <60 ml/min/1.73 m²) or liver disease, triglyceride (TG) levels >400 mg/dl, fasting blood glucose levels >126mg/dl, or 2-h glucose levels >200mg/dl. Qualified participants underwent a physical examination by a physician to ensure that there was no evidence of CV, pulmonary, or other chronic diseases that would preclude exercise testing or training (19). In addition, a physician-supervised maximal graded treadmill test was performed and the test was terminated when the participant could no longer continue, or CV signs or symptoms occurred (19). Participants who had >2 mV ST-segment depression or CV signs or symptoms were excluded from the study.

Casual BP—Casual BP was measured in all participants on 3 separate days according to the JNC VII guidelines (20). Briefly, participants were fasted for ≥12 h before measurement. They sat quietly in a seated position with feet flat on the floor for 15–20 min and BP was measured at least twice until systolic values were within 4 mmHg and diastolic values were within 4 mmHg. When these criteria were met, the average BP was recorded. The average of the three separately recorded BP values was used as the outcome variable in data analyses.

Dietary stabilization—Qualified participants were stabilized on an American Heart Association (AHA) Step 1 diet for 6 weeks by a registered dietician. This diet consists of ~55% of total daily calories from carbohydrates, <30% from fat and 15% from protein. Weight and BP were recorded at each visit (2 days/week) to the laboratory and participants were required to remain within 5% of their study entry body weight for the entirety of the study. Individuals taking anti-hypertensive medications were tapered off of their medication during dietary stabilization after obtaining written approval from their personal physician. Participants with BP consistently <120/80 or >159/99 mmHg were excluded from the study.

Baseline testing—Percentage of body fat and lean body mass were measured by dual energy X-ray (DEXA) as previously described (21). A second maximal treadmill test, supervised by the study physician, was used to determine participants' CV fitness and to develop individualized exercise prescriptions. The test began at 70% of the peak heart rate achieved during the screening exercise test and the treadmill grade was increased by 2% every 2 min. BP, ECG and oxygen consumption (VO_2) were measured continuously throughout the test. Standard criteria were used to determine if true maximal VO_2 ($\text{VO}_{2\text{max}}$) was achieved (22).

Measurement of plasma Ang II—All blood samples were drawn in the morning after a 12-h overnight fast. After AEXT, blood samples were collected after a 12-h overnight fast and 24–36 h after the last exercise bout. Solid phase extraction of AngII using phenyl cartridges (Phenomenex, Torrance, CA) and an Alltech Vacuum Manifold (Deerfield, IL) were used to isolate AngII peptides. The cartridges were pre-washed with 1 mL of methanol followed by 1 mL of deionized water. The plasma sample (1 mL) was then passed through the cartridge followed once more by 1 mL of deionized water. AngII peptides were eluted with 0.5 mL of methanol, evaporated to dryness using vacuum centrifugation and stored at -20°C until use. The extracted samples were reconstituted with 0.5 mL of EIA buffer (Cayman Chemicals, Ann Arbor, MI) and plasma AngII levels were measured using a commercially available enzyme immunoassay kit according to the manufacturer's instructions (Cayman Chemicals, Ann Arbor, MI). Baseline and final samples from an individual were assayed on the same plate and all samples were assayed in duplicate. The inter-assay and intra-assay coefficient of variation were 5 and 20% respectively.

Measurement of urinary F_2 -isoprostanes—24-h urine was collected to measure urinary 8-iso-PGF $_{2\alpha}$, a major F_2 -isoprostane and a reliable marker of *in vivo* oxidative stress (23). Urinary levels of 8-iso-PGF $_{2\alpha}$ were measured as previously described (24). In brief, an antibody was raised in rabbits by immunization with 8-iso-PGF $_{2\alpha}$ coupled to BSA (*N,O*-bis(trimethylsilyl) acetamide) at the carboxylic acid by 1,1-carbonyldi-imidazole method (24). Urinary samples that were stored at -80°C and later transported to Sweden under dry ice were kept frozen at -70°C until analysis. Un-extracted urine samples were used in the assay. The cross-reactivity of the 8-iso-PGF $_{2\alpha}$ antibody with 15-keto-13,14-dihydro-8-iso-PGF $_{2\alpha}$, 8-iso-PGF $_{2\beta}$, PGF $_{2\alpha}$, 15-keto-PGF $_{2\alpha}$, 15-keto-13,14-dihydro-PGF $_{2\alpha}$, TXB $_2$, 11 β -PGF $_{2\alpha}$, 9 β -PGF $_{2\alpha}$ and 8-iso-PGF $_{3\alpha}$, respectively was 1.7, 9.8, 1.1, 0.01, 0.01, 0.1, 0.03, 1.8 and 0.6%. The intra-assay coefficients of variation were 14.5% at low concentrations and 12.2% at high concentrations (24). The levels of 8-iso-PGF $_{2\alpha}$ were corrected for urinary creatinine values.

Measurement of superoxide dismutase (SOD) activity—Blood samples for plasma SOD analysis were drawn into heparinized tubes, centrifuged at 3000 rpm for 20 min at 4°C , transferred to plastic microtubes (1.5 μL) and stored at -80°C until use. SOD activity was measured using a commercially available assay kit according to the manufacturer's instructions (Cayman Chemicals, Ann Arbor, MI). This assay utilizes a tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit of SOD is defined as the amount of SOD enzyme needed to exhibit 50% dismutation of the superoxide

radical. Baseline and final samples from an individual were assayed on the same plate and all samples were assayed in duplicate. The inter-assay and intra-assay coefficients of variation were 7 and 22%, respectively.

Genotyping—Genomic DNA was extracted from peripheral blood by standard methods. Genotyping was carried out by amplification of target sequences using polymerase chain reaction (PCR) and was performed using gel electrophoresis for three of the four polymorphisms. The primers for those three variants were: *AGTR1* (A1166C) forward primer (FP) 5'-AGA AGC CTG CAC CAT GTT TTG AG-3' and *AGTR1* (A1166C) reverse primer (RP) 5'-CCT GTT GCT CCT CTA ACG ATT TA-3'. *ACE* (I/D) FP 1 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' and *ACE* (I/D) RP 2 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3'; *AGT*(235) FP 5'-GAT GCG CAC AAG GTC CTG T-3' and *AGT*(235) RP 5'-TGC TGT CCA CAC TGG CTC gC-3' (lower case letter identifies a forced cut site for *Bst*UI restriction enzyme). The PCR product was visualized using ultraviolet light as follows: *ACE*, direct visualization of insertion/deletion alleles; *AGTR1*, after digestion with *Dde* I; and *AGT*, after digestion with *Bst*UI. Since the -825 T/A polymorphism does not create or destroy any known enzyme restriction site (9), a genotype assay was developed using the 5'-nuclease allelic discrimination assay (TaqMan Assay, C___8758568_10) which was performed using a TaqMan PCR Core Reagent Kit (Applied Biosystems, Foster City, CA). Fluorescence in each well was measured using an ABI 7300 Real Time PCR system machine (Perkin Elmer, Applied Biosystems Division). Genotypes were determined by the ABI 7300 sequencer detection system software. Sequence-verified control samples were used to validate all assay conditions.

Aerobic exercise training (AEXT)—Participants underwent 6 months of supervised AEXT three times a week. All sessions began and concluded with appropriate warm up and cool down exercises. Heart rate was monitored using a wireless heart rate monitor (Polar Beat, Polar Electro, Inc., Woodbury, NY). Initially, training sessions consisted of 20 min of exercise at 50% $\text{VO}_{2\text{max}}$. Training duration was increased by 5 min per week for the first 5 weeks until 40 min was achieved and then training intensity was increased by 5% per week until 70% of $\text{VO}_{2\text{max}}$ was achieved. Participants added a lower intensity, 45–60 min unsupervised session during weeks 11–24. Only participants who completed >75% of training sessions at the prescribed intensity, frequency and duration were included in the final data analyses. Participants were required to complete food frequency checklists every 2 months to monitor dietary intake and to ensure dietary compliance.

Statistical analysis

Statistical analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC). Data are expressed as means $\pm$ SE. Hardy–Weinberg equilibrium was assessed by χ^2 test. Chi-square tests were also used to compare categorical data. Data distribution was examined using the Shapiro–Wilk test of normality and homogeneity of variances (HOV) was determined using Levene's test. Plasma AngII levels were skewed and therefore were log transformed before analysis. Pearson correlation coefficients were used to examine relationships among outcome variables. In addition, based on current literature, the potential influence of the *AGT* M235T polymorphism and the *ACE* I/D polymorphism on AngII levels and the potential influence of age on oxidative stress levels, supports their use as covariates in the analyses. Analysis of covariance (ANCOVA) was used to compare differences between genotype groups at baseline and in response to AEXT. Baseline values were also used as covariates in the analysis of training-induced changes. Paired *t*-tests were used to determine if there were significant changes in outcome variables with AEXT. Linear regression was used to assess the portion of variation in plasma AngII that was accounted for by the *ACE* I/D and *AGT* M235T polymorphisms. In light of previous reports of associations between the *AGTR1* A1166C polymorphism and hypertension and oxidative stress (10,12), and the suggestion of an

association between the *AGTR1* -825 T/A polymorphism and hypertension (14) and oxidative stress, predicted high-risk and low-risk *AGTR1* genotype combinations were determined and comparisons were made between the risk allele groups. As mentioned earlier, the C allele of the A1166C polymorphism is associated with hypertension, oxidative stress and augmented *AGTR1* expression (10–13,25) and therefore, from a mechanistic perspective it appears appropriate to consider it a risk allele. In addition, the A allele of the -825T/A polymorphism destroys a binding site for GATA transcription factors (9) and thus, may alter *AGTR1* expression. As a result, the A allele may also be considered a risk allele. Because of the low sample size in the group with no risk alleles ($n=3$), participants with 1 risk allele were combined with this group for comparison. Therefore, in a separate analysis, participants were placed into one of three risk groups; group 1 = no risk alleles or one risk allele present (AATA, ACAA, AAAA), group 2 = two risk alleles present (AATT, ACTA, CCAA), and group 3 = three or more risk alleles present (ACTT, CCTA, CCTT). Statistical significance was set at $p \leq 0.05$.

Results

General characteristics of the participants are presented in Table I. Sample sizes shown differ according to variable because of sample viability and the limited sample available to run assays more than once. In addition, not all subjects completed the AEXT program. The rare allele frequency for the *AGTR1* A1166C polymorphism was 21%, which is slightly lower than previously reported (10,26) but is consistent with Hardy–Weinberg equilibrium ($p=0.80$). The rare allele frequency for the *AGTR1* -825 T/A polymorphism was 17%, is similar to previous reports (14,15) and is consistent with Hardy–Weinberg equilibrium ($p=0.96$). In univariate analysis, ethnicity was the only independent predictor of AngII levels ($p=0.02$); however, after accounting for age, gender, A1166C, -825 T/A, M235T and *ACE I/D* polymorphisms, ethnicity was no longer an independent predictor. In multivariate analysis, the *ACE I/D* polymorphism explained ~4% of the observed variance in AngII levels while the *AGT* M235T polymorphism explained <1% of the observed variance in AngII levels.

Baseline genotype differences

There were no significant differences in the oxidative stress variables (urinary 8-iso-PGF_{2α} and plasma SOD activity) or plasma AngII levels at baseline between genotype groups for the *AGTR1* A1166C or -825T/A polymorphisms. Furthermore, baseline characteristics including systolic and diastolic BP were not genotype dependent. There also were no significant differences in baseline characteristics including systolic and diastolic BP, the oxidative stress variables or AngII levels between risk allele groups (Table II).

Aerobic exercise training changes

There was a significant increase in $\text{VO}_{2\text{max}}$ in the entire group after 6 months of AEXT from 24.8 ± 0.5 to 28.8 ± 0.7 ml/kg/min ($p < 0.0001$) indicating that the training stimulus was sufficient to elicit generalized CV adaptations. There were no significant changes in systolic or diastolic BP with AEXT. Urinary 8-iso-PGF_{2α} increased significantly ($p=0.002$) but there were no significant changes in SOD activity or AngII levels in the entire group. The change in urinary 8-iso-PGF_{2α} was positively correlated with the change in SOD activity ($r=0.32$, $p=0.02$).

There was a significant interactive effect between AEXT and the A1166C polymorphism on AngII levels ($p < 0.05$) (Figure 1), resulting in a significant difference in the change in plasma AngII with AEXT between genotype groups. There were no significant differences between genotype groups in changes in either oxidative stress markers or systolic or diastolic BP with AEXT. However, within both genotype groups of the A1166C polymorphism, there was a significant increase in urinary 8-iso-PGF₂ ($p=0.03$, 0.03) (Table III).

For the -825T/A polymorphism, changes with AEXT in oxidative stress markers, and systolic and diastolic BP were not significantly different between genotype groups. However, changes in plasma AngII levels with AEXT were significantly different between genotype groups ($p=0.02$). The TT genotype group had a significant reduction in plasma AngII levels with AEXT ($p=0.03$) while the TA+AA genotype group had a non-significant increase in plasma AngII levels. In the TT genotype group, there also was a significant increase in urinary 8-iso-PGF_{2α} ($p=0.004$).

There were no significant differences between risk allele groups in plasma AngII or oxidative stress changes with AEXT, but there was a significant reduction in plasma AngII in group 2 only ($p=0.04$) (Figure 2). In addition, only group 2 significantly increased urinary 8-iso-PGF_{2α} ($p=0.01$) with AEXT.

Discussion

AngII participates in the pathogenesis of end-organ injury through the regulation of two main receptors: the AT₁R and the AT₂R. However, it is widely shown that the AT₁R mediates the majority of the pathological actions of AngII (27). Furthermore, it has become apparent that an important consequence of AT₁R activation in the CV system is the production and release of ROS (8,28). Genetic variation in the *AGTR1* has been associated with both hypertension and oxidative stress (10,12,29). Risk factors for CV disease are linked and further complicated by the presence of oxidative stress; however, modulation of oxidative stress and the endogenous antioxidant system is regarded as a beneficial approach to reducing the risk of CV morbidity and mortality (30,31).

Studies investigating the effect of long-term AEXT on oxidative stress are lacking. Therefore, given the apparent association between AngII, AT₁R, oxidative stress and hypertension, the present study investigated the independent and combined influence of two *AGTR1* polymorphisms: the A1166C and the -825 T/A variants, and AEXT on AngII levels and oxidative stress in hypertensive subjects. The major findings of the present study were that after 6 months of AEXT: (i) there was a significant increase in oxidative stress in the entire group; (ii) there was a significant interactive effect of the *AGTR1* A1166C polymorphism and AEXT on AngII levels; and (iii) the TT genotype group of the *AGTR1* -825 T/A polymorphism had significantly reduced AngII levels with AEXT compared with A allele carriers.

Our data show that there were no associations at baseline between the *AGTR1* polymorphisms and AngII levels or markers of oxidative stress. Contrary to our findings, Cameron et al. (12) reported that heart failure patients with the CC genotype of the A1166C polymorphism had significantly higher plasma levels of myeloperoxidase and protein carbonyls (markers of oxidative stress) compared with other genotype groups. The A1166C polymorphism is located in the 3'UTR of the *AGTR1* gene and, previously, it was widely speculated that the C allele might be in LD with a functional variant that may alter mRNA stability in response to increasing levels of AngII (10,15). More recently, it has been shown that the C allele may potentially influence the development and progression of CV disease by leading to increased density of the AT₁R (13). The use of different markers of oxidative stress and the substantial difference in populations between the above-mentioned study and the present study could account for the inconsistent findings.

To our knowledge, this is the first study to investigate the influence of the *AGTR1* -825 T/A polymorphism on AngII levels and oxidative stress related to AEXT. We found no differences between genotype groups for the -825 T/A polymorphism at baseline. This promoter polymorphism is in almost complete LD with the *AGTR1* -153 A/G polymorphism (15). The -153 A/G polymorphism was reported to be associated with urinary 8-iso-PGF_{2α} levels, a

reliable systemic marker of oxidative stress (23,32). Given the almost complete LD between these two promoter polymorphisms, we believe that it is reasonable to hypothesize that the -825 T/A polymorphism would also be associated with oxidative stress.

In the present study, risk allele analysis revealed no significant differences at baseline in AngII and urinary 8-iso-PGF_{2α} between risk allele groups. To the best of our knowledge, there is no evidence to suggest a significant LD between the *AGTR1* A1166C and -825 T/A polymorphisms. Using risk allele analysis to determine the interaction between two polymorphisms is reasonable especially when the two polymorphisms are not in LD (9,33). Thus the combination of the two polymorphisms, rather than each alone, may be more informative when determining the genetic contribution to certain phenotypes.

An interesting finding in the present study was that after 6 months of AEXT. There was a significant increase in oxidative stress in apparently healthy, middle-aged to older, pre- and stage 1 hypertensives. Our results are consistent with those of Goto et al. (34) and Bergholm et al. (35) who reported that 12 weeks of AEXT at 70–85% VO_{2max} in previously sedentary healthy young men, resulted in a significant increase in markers of oxidative stress and significantly decreased endothelial function and all circulating antioxidants. The results of our study, which required participants to exercise at 70±5% VO_{2max}, are consistent with these previous reports.

We observed a significant interactive effect of the A1166C polymorphism and AEXT on AngII levels. The differential changes observed in AngII levels among genotype groups with AEXT are interesting and illustrate the complexity of gene–exercise interactions. Recent evidence indicates that the C allele can lead to increased AT₁R density (13) and, though we did not conduct studies to determine the cause of the differential changes in AngII between genotype groups, it is possible that AEXT resulted in an increase in AngII synthesis in C allele carriers of the A1166C polymorphism via non-ACE mechanisms. Although there was not a significant interactive effect of the -825 T/A polymorphism and AEXT on AngII levels, there was a significant difference in the change in AngII between genotype groups, with the TT genotype group having a significant reduction in AngII levels with AEXT.

Despite a reduction in plasma AngII after AEXT in the AA genotype group of the A1166C polymorphism and in the TT genotype group of the -825 T/A polymorphisms, we observed an increase in oxidative stress across all genotype groups. Recent evidence suggests that locally produced AngII may be more important in determining CV risk. It is possible that the measurement of circulating (plasma) levels of AngII may not necessarily represent long-term adaptations that occur in the vasculature in response to AEXT.

To our knowledge, this is the first study to investigate AEXT-induced changes in plasma AngII and urinary 8-iso-PGF_{2α} simultaneously using risk allele analysis. Risk allele analysis revealed that those with two risk alleles exhibited a significant decrease in plasma AngII but this was accompanied by an increase in urinary 8-iso-PGF_{2α}. The results of the risk allele analysis were surprising and opposite of what would be expected and it is not entirely clear why this discrepancy occurred. However, when examining single gene effects, the results were opposite to what was expected (i.e. the C allele [risk allele] of the A1166C polymorphism and the A allele [protective allele] of the -825T/A polymorphism increased AngII levels after training). Therefore, it is not surprising that the combination of the two also revealed unexpected results.

In the present study, we found no genotype main effects for differences in oxidative stress variables at baseline or in response to AEXT. However, our results do suggest that in previously sedentary hypertensive adults the *AGTR1* A1166C and -825 T/A polymorphisms may synergistically interact with an AEXT intensity of 70±5% VO_{2max}, resulting in enhanced oxidative stress in this hypertensive population. Furthermore, our results may have clinical

implications for the influence of AEXT and genetics on oxidative stress involved in the development and progression of CV disease so that when prescribing exercise intensity, consideration should be placed on the patient population and the specific end-point. In addition, it is possible that an exercise intensity of 70% $\text{VO}_{2\text{max}}$ may exceed the threshold for the beneficial effects of AEXT on oxidative stress in this hypertensive population.

Limitations

The lack of measurement of locally produced AngII was a limitation of the present study. An additional limitation of the present study was the inability to measure AT_1R levels directly. However, future studies should determine whether these polymorphisms may have a functional impact on receptor expression *in vivo*.

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Figure 1.

Interaction between aerobic exercise training (AEXT) and the *AGTR1* A1166C polymorphism on Log AngII (angiotensin II). $p < 0.05$ for exercise training $\times$ A1166C interaction.

Figure 2. Changes in plasma angiotensin II (AngII) levels with aerobic exercise training (AEXT) by *AGTR1* risk allele group. *Denotes a significant change within genotype group ($p < 0.05$).

Table 1

Baseline characteristics of study participants by genotype.

	A1166C			-825T/A		
	AA	AC+CC	p-value	TT	TA+AA	p-value
Age (yrs)	58.4±0.8 (62)	58.8±0.9 (38)	0.80	59.2±0.7 (68)	57.2±1.1 (32)	0.14
BMI (kg/m ²)	29.2±0.6 (58)	29.2±0.7 (37)	0.98	29.0±0.6 (65)	29.7±0.9 (30)	0.54
TG (mg/dl)	112.0±8.3 (52)	117.9±9.9 (33)	0.66	109.0±7.4 (60)	127.5±12.0 (25)	0.21
LDL-C (mg/dl)	112.1±4.7 (52)	118.5±5.6 (33)	0.39	109.0±4.1 (60)	129.0±6.6 (25)	0.02*
HDL-C (mg/dl)	53.2±2.7 (52)	48.0±3.3 (33)	0.24	50.0±2.5 (60)	53.4±4.0 (25)	0.48
Systolic BP (mmHg)	132.1±1.8 (53)	133.4±2.2 (32)	0.65	132.0±1.6 (60)	135.0±2.6 (25)	0.32
Diastolic BP (mmHg)	87.2±1.0 (53)	87.0±1.2 (32)	0.81	87.0±1.0 (60)	88.0±1.5 (25)	0.51
GFR (ml/min/1.73 m ²)	78.0±2.1 (51)	79.3±2.6 (31)	0.62	78.0±1.9 (60)	79.0±3.3 (22)	0.81
VO _{2max} (ml/kg/min)	25.0±0.5 (57)	24.4±0.6 (37)	0.55	25.5±0.5 (64)	23.2±0.7 (30)	0.02*
Log AngII (pg/ml)	2.06±0.17 (55)	2.14±0.21 (32)	0.78	2.08±0.17 (58)	2.13±0.24 (29)	0.86
Urinary 8-iso-PGF _{2α} (nmol/mmol creatinine)	0.32±0.02 (55)	0.32±0.02 (32)	0.96	0.31±0.02 (59)	0.33±0.03 (28)	0.64
SOD (U/ml)	1.00±0.09 (58)	1.17±0.11 (33)	0.26	1.09±0.08 (62)	1.00±0.13 (29)	0.55

Values are expressed as means±SE. Parentheses represent sample size.

BMI, Body mass index; TG, Triglycerides; GFR, Glomerular filtration rate; VO_{2max}, Maximal oxygen consumption; LDL-C, Low-density lipoprotein cholesterol; HDL-C, High-density lipoprotein cholesterol; BP, Blood pressure; Log AngII, Angiotensin II; SOD, Superoxide dismutase; 8-iso-PGF_{2α}, 8-iso-prostaglandin F_{2α}.

* Denotes statistically significant difference between genotype groups ($p < 0.05$).

Table IIBaseline characteristics of the *AGTR1* risk allele groups.

	AGTR1 risk allele group			<i>p</i> -value
	Group 1	Group 2	Group 3	
Age (yrs)	57.2±1.4 (20)	58.6±0.8 (53)	59.4±1.2 (27)	0.56
BMI (kg/m ²)	29.6±1.1 (18)	29.2±0.6 (51)	29.1±0.9 (26)	0.94
TG (mg/dl)	125.2±16.7 (14)	111.9±8.4 (48)	113.2±12.7 (23)	0.77
LDL-C (mg/dl)	128.3±9.4 (14)	112.7±4.7 (48)	111.2±7.1 (23)	0.31
HDL-C (mg/dl)	57.0±5.5 (14)	52.3±2.8 (48)	45.5±4.1 (23)	0.28
Systolic BP (mmHg)	130.0±3.3 (16)	135.1±1.8 (45)	130.3±2.5 (24)	0.17
Diastolic BP (mmHg)	87.0±1.9 (16)	88.0±1.1 (45)	86.2±1.5 (24)	0.73
GFR (ml/min/1.73 m ²)	77.0±4.2 (14)	78.0±2.2 (44)	79.5±3.1 (24)	0.88
VO _{2max} (ml/kg/min)	22.8±1.0 (18)	25.5±0.6 (50)	24.7±0.8 (26)	0.06
Log AngII (pg/ml)	2.02±0.30 (19)	2.15±0.18 (46)	2.06±0.27 (22)	0.92
Urinary 8-iso-PGF _{2α} (nmol/mmol creatinine)	0.34±0.03 (18)	0.31±0.02 (46)	0.32±0.03 (23)	0.72
SOD (U/ml)	0.96±0.15 (20)	1.02±0.10 (46)	1.20±0.13 (25)	0.45

Values are expressed as mean±SE. Values in parentheses represent sample size.

BMI, Body mass index; TG, Triglycerides; GFR, Glomerular filtration rate; VO_{2max}, Maximal oxygen consumption; LDL-C, Low-density lipoprotein cholesterol; HDL-C, High-density lipoprotein cholesterol; BP, Blood pressure; Log AngII, Angiotensin II; SOD, Superoxide dismutase; 8-iso-PGF_{2α}, 8-iso-prostaglandin F_{2α}; Group 1, 0–1 risk allele; Group 2, 2 risk alleles; Group 3, ≥3 risk alleles.

Table III

Changes with training by *AGTR1* A1166C and -825T/A genotype groups.

	<i>AGTR1</i> A1166C genotype			<i>AGTR1</i> -825 T/A genotype		
	AA	AC+CC	p-value	TT	TA+AA	p-value
Log AngII (pg/ml)	-0.48±0.18 (30)	0.24±0.24 (16)	0.04*	-0.43±0.16 [†] (32)	0.36±0.26 (14)	0.02*
Urinary 8-iso-PGF _{2α} (nmol/mmol creatinine)	0.07±0.05 [†] (36)	0.15±0.06 [†] (20)	0.38	0.11±0.04 [†] (41)	0.08±0.07 (15)	0.79
SOD (U/ml)	-0.08±0.10 (36)	0.22±0.14 (18)	0.12	0.11±0.09 (38)	-0.17±0.14 (16)	0.11
Systolic BP (mmHg)	-0.05±1.46 (36)	-0.84±1.81 (21)	0.75	-0.48±1.25 (42)	-0.08±2.12 (15)	0.88
Diastolic BP (mmHg)	-0.97±1.03 (36)	0.80±1.28 (21)	0.32	0.66±0.85 (42)	-2.76±1.48 (15)	0.06

Values are expressed as mean±SE.

AngII, Angiotensin II; SOD, Superoxide dismutase; 8-iso-PGF_{2α}, 8-iso-prostaglandin F_{2α}; BP, Blood pressure.[†] Denotes a statistically significant change with training within genotype group ($p<0.05$).* Significant difference between genotype groups ($p<0.05$).