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Exercise Training, NADPH Oxidase p22phox Gene Polymorphisms, and Hypertension

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Abstract

Introduction—Oxidative stress that is mediated through NADPH oxidase activity plays a role in the pathology of hypertension, and aerobic exercise training reduces NADPH oxidase activity. The involvement of genetic variation in the p22phox (*CYBA*) subunit genes in individual oxidative stress responses to aerobic exercise training has yet to be examined in Pre and Stage 1 hypertensives.

Methods—Ninety-four sedentary Pre and Stage 1 hypertensive adults underwent 6 months of aerobic exercise training at a level of 70% $\dot{V}O_{2\max}$ to determine whether the *CYBA* polymorphisms, C242T and A640G, were associated with changes in urinary 8-*iso*-prostaglandin $F_{2\alpha}$ (8-*iso*-PGF_{2 α}), urinary nitric oxide metabolites (NO_x), and plasma total antioxidant capacity (TAC).

Results—Demographic and subject characteristics were similar among genotype groups for both polymorphisms. At baseline, a significant ($P = 0.03$) difference among the C242T genotype groups in 8-*iso*-PGF_{2 α} levels was detected, with the TT homozygotes having the lowest levels and the CC homozygotes having the highest levels. However, no differences were found at baseline between the A640G genotype groups. After 6 months of aerobic exercise training, there was a significant increase in $\dot{V}O_{2\max}$ ($P < 0.0001$) in the entire study population. In addition, there were significant increases in both urinary 8-*iso*-PGF_{2 α} ($P = 0.002$) and plasma TAC ($P = 0.03$) levels and a significant decrease in endogenous urinary NO_x ($P < 0.0001$). Overall, aerobic exercise training elicited no significant differences among genotype groups in either *CYBA* variant for any of the oxidative stress variables.

Conclusions—We found that compared with *CYBA* polymorphisms C242T and A640G, it was aerobic exercise training that had the greatest influence on the selected biomarkers; furthermore, our

results suggest that the C242T *CYBA* variant influences baseline levels of urinary 8-*iso*-PGF_{2α} but not the aerobic exercise-induced responses.

Keywords

OXIDATIVE STRESS; AEROBIC EXERCISE; *CYBA* GENE; NITRIC OXIDE; ISOPROSTANES

It has been suggested that oxidative stress contributes to the pathogenesis of hypertension, cardiovascular disease (CVD), and atherosclerosis (18,23,39). It has also been suggested that oxidative stress can be modulated by both acute and chronic exercises (16,36). This is just one of the many reasons why regular physical activity continues to be recommended as a nonpharmacological lifestyle modification for the prevention and management of hypertension (21). Oxidative stress is associated with either an increase in the formation of reactive oxygen species (ROS), primarily superoxide (O₂⁻), or a decrease in antioxidant reserves (14). The NADPH oxidase enzyme complex is the principal source of vascular O₂⁻ in humans (8), and aerobic exercise training has been shown to reduce the expression of NADPH oxidase (1). Gene expression studies in endothelial cells have shown that laminar shear stress, which simulates the increased vascular blood flow during aerobic exercise training, alters the expression of oxidative stress genes (9,30). In particular, laminar shear stress has been found to downregulate NADPH oxidase subunit expression, including p22phox, in endothelial cells (13), but the mechanisms explaining how exercise can externally regulate these NADPH genes in human subjects is not well understood. Also, the involvement of genetic variation of the p22phox (*CYBA*) subunit genes in individual oxidative stress responses to aerobic exercise training has yet to be determined in Pre and Stage 1 hypertension. Therefore, to our knowledge, the present study is the first to specifically examine the influence of two *CYBA* polymorphisms, C242T and A640G, and their interaction with aerobic exercise training, on urinary 8-*iso*-prostaglandin F_{2α} (8-*iso*-PGF_{2α}), endogenous urinary NO metabolite (NO_x), and plasma total antioxidant capacity (TAC) in a population of Pre and Stage 1 hypertensive adults.

The NADPH oxidase enzyme complex is composed of a membrane-bound cytochrome, which includes subunits gp91phox and p22phox, and a cytosolic component composed of subunits p47phox, p40phox, p67phox, and a G protein, Rac (25). The p22phox subunit is essential to this enzyme's activity, and activation of NADPH through this membrane-bound subunit protein has been shown in vascular cells (35). Furthermore, many of the stimuli found to activate NADPH oxidase increase expression of the p22phox subunit (35,40). The *CYBA* gene is located on the long arm of chromosome 16 (at q24), encodes the alpha subunit of the membrane-bound component, spans 8.5 kb, and contains five introns and six exons (12). Several *CYBA* gene variants have been associated with CVD and hypertension (19,26). Two polymorphisms are of particular interest, A640G and C242T. In a previous study, we examined the effects of 6 months of aerobic exercise training on systemic oxidative stress responses in middle-aged to older whites with at least one lipid abnormality. In that study, we reported that the A allele of the A640G (rs1049255) variant, which is found in the 3' untranslated region, was associated with a training-induced reduction in plasma thiobarbituric acid-reactive substances levels, a common indicator of lipid peroxidation (31). Similarly, Gardemann et al. reported that the AA genotype was found more frequently in individuals with coronary artery disease. Through both of these studies, a potential role for this polymorphism in NADPH oxidase activity and oxidative stress can be hypothesized.

Along with the A640G variant, the C242T (rs4673) *CYBA* polymorphism has also been previously found to influence NADPH oxidase gene expression. This *CYBA* C242T gene variant is in exon 4 and causes a structural modification in the protein from the histidine-to-tyrosine substitution in a heme binding site (12). Protein structural changes that result from a genetic code substitution can lead to functional changes in the protein. Thus, the resulting

structural change in p22phox from this C242T polymorphism has been related with CVD, hypertension, and endothelial function (7,15,19,22,26). In particular, studies have reported that the T allele is associated with reduced NADPH oxidase activity in blood vessels of patients with coronary artery disease (19) and in blood vessels of healthy individuals (38). These findings have led to the hypothesis of a protective effect for T allele carriers in both diseased and healthy states (19,22). Many clinical studies have examined the relationships between these two specific *CYBA* polymorphisms and vascular disease, but results are conflicting and the mechanisms are undefined. Moreover, research that examines their influence in individual oxidative stress responses to aerobic exercise training has yet to be determined in Pre and Stage 1 hypertension.

Because the F₂-isoprostanes are currently the best available biomarker for whole-body lipid production, we measured urinary excretion of 8-*iso*-PGF_{2α} (33). In addition, to determine the cumulative levels of systemic antioxidants in the plasma and body fluids, we measured plasma TAC, which is regarded as one of the best quantitative *in vivo* laboratory measures (32). Finally, because a hallmark of early hypertension is impaired vasodilatory abilities, and nitric oxide (NO) has long been known to induce vascular smooth muscle relaxation (17), we measured urinary NO_x levels before and after aerobic exercise training. Thus, the purpose of this study was to investigate the association of the *CYBA* C242T and A640G polymorphisms with oxidative stress responses to 6 months of aerobic exercise training in Pre and Stage 1 hypertensive adults.

MATERIALS AND METHODS

Recruitment and screening

Ninety-four apparently healthy sedentary men ($n = 44$) and women ($n = 50$) volunteered to participate in this study. Subjects responded to media advertisements and underwent a telephone interview to assess their initial eligibility. The study was approved by the Institutional Review Board of the University of Maryland, College Park, and all subjects provided their written informed consent during their first laboratory visit.

It is understood that any contribution made by gene polymorphisms to complex oxidative stress phenotypes is likely to be small. Therefore, in gene association studies, it becomes necessary to control for as many confounding factors as possible and to do so before any baseline testing begins. Along with the physical examination and exercise test, screening for impaired glucose tolerance or type 2 diabetes was important. Also controlling for heterogeneity in diet among subjects was essential. Subjects' medical histories were reviewed on their first laboratory visit to ensure they met the study inclusion criteria listed above. A 12-h overnight fasting blood sample was drawn for blood chemistries and DNA typing. Each subject had their fasting plasma glucose level determined and underwent a 2-h, 75-g oral glucose tolerance test. Those with fasting plasma glucose >126 mg·dL⁻¹ or a 2-h glucose level >200 mg·dL⁻¹ were excluded from the study.

All subjects had Pre or Stage 1 Hypertension (systolic BP = 120–159 mm Hg, diastolic BP = 80–99 mm Hg). Subjects were also sedentary (aerobic exercise less than two times per week, <20 min per session, sedentary job), 50–75 yr of age, nondiabetic, not on lipid-lowering medications, had no diagnosed CVD, except for being Pre or Stage 1 Hypertensive, and had a body mass index (BMI) <37 kg·m⁻². Qualified subjects could not have any medical conditions precluding their ability to perform vigorous exercise. As part of the screening process, subjects meeting these inclusion criteria underwent a physical examination and a physician-supervised maximal treadmill exercise test to screen for cardiovascular, pulmonary, or other chronic diseases that would preclude them from aerobic exercise training. Also, before baseline testing began, hypertensive subjects using one antihypertensive medication were tapered off their

medication under close supervision of the study physician. They were expected to remain off their medication for the duration of the study. Subjects on more than one antihypertensive medication were excluded. All women were more than 2 yr postmenopausal and maintained their hormone replacement therapy regimen, either on or not on, for the duration of the study. Previous research from our laboratory has shown that hormone replacement therapy does not alter the exercise training-induced oxidative stress responses to aerobic exercise (2,31). All plasma and urine samples were collected after a 12-h overnight fast. All samples collected after 6 months of aerobic exercise training were collected 24–36 h after the last exercise training session to avoid the acute effects of prior exercise on outcome variables.

Dietary stabilization

Before any baseline testing began, all qualified subjects underwent 6 wk of dietary instruction (2 d·wk⁻¹, for 1 h each session) with a registered dietician on how to maintain the American Heart Association low-fat (<30% total calorie intake) and low-sodium (<3 g·d⁻¹) diet. Subjects were then required to follow this prescribed diet and be weight-stable for at least 3 wk before undergoing baseline testing. In addition, subjects had to maintain the diet and continue to be weight-stable throughout the entire study. To ensure dietary compliance, subjects completed 7-d food records at the beginning and end of aerobic exercise training and food frequency questionnaires every 2 months. To eliminate the potential effect of dietary nitrate intake on urinary NO_x levels, participants were required to follow a low-nitrate diet for the 2 d preceding and on the day of urine collection.

Baseline testing

At the completion of the 6-wk dietary stabilization period and before beginning aerobic exercise training, subjects completed baseline testing that consisted of measurement of plasma lipoprotein lipids, collection of blood and urine samples for laboratory assay, 24-h urine and blood pressure monitoring, and maximal oxygen consumption ($\dot{V}O_{2\max}$). $\dot{V}O_{2\max}$ was determined by indirect calorimetry during a graded exercise test to exhaustion as described previously (37). $\dot{V}O_{2\max}$ was then used to derive valid exercise prescriptions for the aerobic exercise training intervention.

Exercise training

The 6 months of supervised aerobic exercise training consisted of three sessions per week. All training sessions began and concluded with appropriate warm-up, stretching, and cool-down exercises. HR monitors were used to determine exercise rate and to ensure that subjects trained at an HR corresponding to the prescribed intensity. The training program began at 20 min of exercise at 50% $\dot{V}O_{2\max}$ followed by graded increases during the next 10 wk until 40 min at a level of 70% $\dot{V}O_{2\max}$ was reached, where it remained for the remainder of the 6 months. Subjects added a single lower-intensity 45- to 60-min exercise session during weeks 12 to 24. Only subjects who completed >75% of training sessions at the prescribed intensity, duration, and frequency were included in the final data analysis.

NADPH p22phox, A640G and C242T, genotyping

Genomic DNA was extracted from peripheral blood by standard methods. The *RsaI* restriction site gain created by the C242T substitution and the *DraIII* site gain created by the A640G substitution were genotyped by standard restriction fragment length polymorphism techniques as described (22).

Total antioxidant capacity

TAC was measured using a commercially available kit (Cayman Chemical, Ann Arbor, MI) that measured the combined antioxidant activities of all aqueous and lipid soluble antioxidants

in plasma, including vitamins, proteins, lipids, and enzymes. This assay relies on the ability of antioxidants in plasma to inhibit the oxidation of ABTS® (2,2'-azino-di-[3-ethylbenzthiazoline sulphonate]) to ABTS® + by metmyoglobin). The capacity of the antioxidants in plasma to prevent ABTS® oxidation is compared with that of Trolox, a water-soluble vitamin E analog, and is quantified as millimolar Trolox equivalents. The amount of ABTS® + produced was measured by reading the absorbance at 750 nm. Baseline and final samples were assayed on the same plate, and all samples were assayed in duplicate. The intra-assay and interassay CV were 10.5% and 4.4%, respectively.

Measurement of urinary nitrates/nitrites (NO_x)

Urinary NO_x levels were measured using a modified Griess assay. Before performing the assay, urine samples were centrifuged at 3000 rpm for 15 min at 4°C. The assay was performed as previously described (6,37). Briefly, the assay involved the reduction of nitrate to nitrite using nitrate reductase (*Aspergillus* species) and measurement of nitrite via the magenta-colored azo dye formed when nitrite reacts with the Griess reagents; 0.1% *N*-[1-naphthylethylenediamine dihydrochloride] and 1% sulfanilamide in 5% phosphoric acid. The concentration of urinary NO_x represents the total amount of urinary NO end products (nitrate and nitrite), and absorbance was read at 541 nm and determined by using an Emax Maxline Microplate Reader (Sunnyvale, CA). Baseline and final samples were assayed on the same plate, and all samples were assayed in duplicate. The interassay and intra-assay CV were 4% and 13%, respectively.

Measurement of urinary 8-iso-PGF_{2α}

The 24-h urine collection was used to measure urinary 8-iso-PGF_{2α}. Urinary levels of 8-iso-PGF_{2α} were measured in the laboratories of Dr. Basu in Uppsala, Sweden. In brief, an antibody was raised in rabbits by immunization with 8-iso-PGF_{2α} coupled to bovine serum albumin (BSA) at the carboxylic acid by 1,1,-carbonyldiimidazole method (4). Urinary samples were stored at -80°C and later transported to Sweden under dry ice (-70°C) for analysis. Unextracted urine samples were used in the assay. The cross-reactivity values of the 8-iso-PGF_{2α} antibody with 15-keto-13,14-dihydro-8-iso-PGF_{2α}, 8-iso-PGF_{2α}, PGF_{2α}, 15-keto-PGF_{2α}, 15-keto-13,14-dihydro-PGF_{2α}, TXB₂, 11β-PGF_{2α}, 9β-PGF_{2α}, and 8-iso-PGF_{3α}, respectively, were 1.7%, 9.8%, 1.1%, 0.01%, 0.01%, 0.1%, 0.03%, 1.8%, and 0.6%. The levels of 8-iso-PGF_{2α} were corrected for urinary creatinine values. The detection limit of the assay was 8 pg·mL⁻¹.

Statistical analysis

Data distribution was examined using the Shapiro–Wilk test of normality, and homogeneity of variances was determined using Levene's test. All data were found to be normally distributed. The Hardy–Weinberg equilibrium was assessed by χ^2 test. ANCOVA was used to compare differences between genotype groups at baseline and in response to aerobic exercise training. There were no gender by genotype interactions, therefore these groups were combined for all analyses. Paired *t*-tests were used to determine whether there were significant changes in outcome variables with aerobic exercise training. Pearson correlation was used to determine whether there were relationships between outcome variables. Statistical analyses were performed using SAS version 9.1 (SAS Institute, Inc, Cary, NC). Data are expressed as means ± SE, and significance was set at *P* < 0.05.

RESULTS

Baseline characteristics

Subject characteristics and frequencies of *CYBA* C242T and A640G genotypes are presented in Table 1. No significant differences existed between genotype groups for any of the subject

characteristics, and these results remained after adjusting for gender, which is similar to our previous reports on adults with at least one lipid abnormality (31).

At baseline, there was a significant difference in urinary 8-*iso*-PGF_{2α} ($P = 0.03$) among C242T genotype groups (Fig. 1) with the CC homozygotes having the highest levels and the TT homozygotes having the lowest levels. In addition, *post hoc* analysis revealed that there was a significant difference in urinary 8-*iso*-PGF_{2α} levels between the CC and CT genotype groups ($P = 0.01$; Table 2). Although there were no significant differences among genotype groups for plasma TAC or urinary NO_x levels, the TT homozygotes tended to have higher plasma TAC levels compared with the CT and CC genotype groups ($P = 0.08$; Table 2). There were no significant baseline differences among A640G genotype groups for any of the outcome variables.

Exercise training-induced changes

In the total study population, there was a significant increase in $\dot{V}O_{2\max}$ from 24.8 ± 0.5 to 28.8 ± 0.7 mL·kg⁻¹·min⁻¹ ($P < 0.0001$) after 6 months of aerobic exercise training indicating that the training protocol was sufficient to elicit generalized cardiovascular adaptations. Also, in the total study population, there was a significant increase in urinary 8-*iso*-PGF_{2α} levels from 0.32 ± 0.01 to 0.42 ± 0.03 nmol·mmol⁻¹ creatinine ($P = 0.002$) and in plasma TAC from 2.76 ± 0.01 to 3.00 ± 0.13 mmol·L⁻¹ ($P = 0.03$). Finally, we report a significant decrease in urinary NO_x from 3.0 ± 1.0 to 0.60 ± 0.04 μmol·L⁻¹ × 10⁻³ ($P < 0.0001$) for the entire study population.

There were no significant differences in changes in any of the outcome variables among the C242T genotype groups with aerobic exercise training, but within-genotype group analysis revealed that the CC homozygotes significantly decreased ($P = 0.04$) systolic blood pressure and tended to decrease ($P = 0.07$) diastolic blood pressure (Table 2). Within-group analysis also revealed that the CT genotype group showed a significant increase ($P = 0.01$) in urinary 8-*iso*-PGF_{2α} levels, the CC homozygotes showed significant increase in TAC ($P = 0.04$), and all of the genotype groups showed a significant decrease in urinary NO_x levels with aerobic exercise training (Table 2).

Among the A640G genotype groups, there were no significant differences in changes in any of the outcome variables with 6 months of aerobic exercise training. However, within-genotype group analysis revealed that there was a significant increase in urinary 8-*iso*-PGF_{2α} levels in the AA genotype group ($P = 0.04$) and GG genotype group ($P = 0.02$) but not the AG genotype group (Table 2). Also, similar to the C242T polymorphism, within-genotype group analysis for the A640G variant revealed that all of the genotype groups showed a significant decrease in urinary NO_x levels with aerobic exercise training (Table 2).

DISCUSSION

To our knowledge, this is the first study to specifically examine the influence of two *CYBA* polymorphisms, C242T and A640G, and 6 months of aerobic exercise training on urinary 8-*iso*-prostaglandin F_{2α} (8-*iso*-PGF_{2α}), urinary NO_x, and plasma TAC in a population of Pre and Stage 1 hypertensive adults. Aerobic exercise training creates an increase in unidirectional blood flow and therefore causes a shear stress along the blood vessel walls from repeated bouts of the shear frictional force. Research shows that aerobic exercise training alters the balance between vascular oxidants and the endogenous antioxidants by decreasing NADPH oxidase enzyme activity and improving endothelial function (31). Research also shows that genetic variation in the p22phox subunit plays a role in oxidative stress responses (7) and in hypertension (26). Furthermore, compelling evidence suggests that early stages of

hypertension, similar to that of our population, may be associated with vascular oxidative stress, an increased O_2^- production, and an increase in NO production (5,24,28).

Reactive oxygen species, particularly O_2^- , are involved in several diffusion limited enzymatic reactions, and the O_2^- radical also interacts with arachidonic acid, an omega-6 polyunsaturated fatty acid, forming a group of compounds called isoprostanes (11,27). Steady-state excretion of 8-*iso*-PGF_{2α} is an established and easily measured urinary marker of oxidative stress (3, 33). In our subjects at baseline, we found a significant difference in urinary 8-*iso*-PGF_{2α} levels for the C242T polymorphism. Specifically, the TT homozygotes had the lowest levels among the genotype groups, and this is consistent with previous reports of the T allele's protective effect (19,22). A recent nonexercise training study examined the functional effects of the *CYBA* C242T variant on NADPH oxidase activity in blood vessels from healthy adults and reported that the TT homozygotes of the C242T polymorphism had reduced respiratory burst (38). This lower oxidative state in the TT homozygotes is consistent with our results at baseline where the TT genotype group had the lowest levels of urinary 8-*iso*-PGF_{2α}. However, in our subjects after 6 months of aerobic exercise training at 70% $\dot{V}O_{2max}$, within-group analyses showed that specific genotype groups had significant increases in urinary 8-*iso*-PGF_{2α} levels; the CT and CC genotype groups of the C242T polymorphism, and the AA and GG genotype groups of the A640G polymorphism. However, among the genotype groups, we found no significant differences in the changes in 8-*iso*-PGF_{2α} levels after aerobic exercise training. This suggests a potential association of the C242T polymorphism with NADPH oxidase enzyme activity at baseline but not with aerobic exercise training. Finally, because the difference that existed at baseline was abolished with aerobic exercise training, our data suggest that the effects of aerobic exercise training were greater than the effects of the *CYBA* polymorphisms on the 8-*iso*-PGF_{2α} responses in this population. This is another example of the often greater influence that environmental factors have over the small gene effects on phenotype characteristics.

In addition, for the entire group, we found that, after 6 months of aerobic exercise training, there was a significant overall increase in the oxidative state, as evidenced by increased urinary 8-*iso*-PGF_{2α} and decreased urinary NO_x levels. These findings are consistent with those reported by Goto et al. In their study, different exercise training intensities were compared, and they reported that 12 wk of aerobic exercise training at 75% $\dot{V}O_{2max}$ caused sedentary healthy young men to have a significant increase in plasma oxidative stress levels. In the study by Goto et al., mild- (25% $\dot{V}O_{2max}$) and moderate- (50% $\dot{V}O_{2max}$) intensity exercise training did not elicit an increase in plasma 8-OHd6 levels. Plasma 8-OHd6 levels for these exercise groups had no significant change from rest to exercise to recovery (16). In the present study, an exercise training intensity of 70% $\dot{V}O_{2max}$ was used, which is similar to the high-intensity group from the study of Goto et al. Also, our subjects were Pre and Stage 1 hypertensive adults, and prehypertension status has been shown associated with higher oxidative stress levels (10). Taken together, the training level of 70% $\dot{V}O_{2max}$ and the known association between hypertension and higher oxidative stress levels could have contributed to the increased plasma 8-*iso*-PGF_{2α} levels we report.

As mentioned, for our entire group, 6 months of aerobic exercise training elicited a significantly higher oxidative state. Along with significantly increased plasma 8-*iso*-PGF_{2α} levels, in the group, we found significant training-induced decreases in urinary NO_x levels, although other training studies have reported increases in endogenous NO_x with training (29). Typically, a higher oxidative state through increased levels of ROS leads to an imbalance in endothelial cell function and thus altered NO release (28). Ultimately, this has clinical implications because NO, as the primary vascular vasodilative factor, is critical to endothelial function (20). Furthermore, data suggest that increases in blood pressure are associated with impaired endothelial-dependent vasodilation (5). Therefore, in our subjects, the complex etiology of hypertension may have contributed to the training-induced decrease in NO_x levels seen. Also,

as discussed, the higher-intensity aerobic exercise training in the current study could have affected the subjects' oxidative response and consequently reduced their urinary NO_x levels. Further clinical exercise studies are needed in this population to fully elucidate exercise training intensity levels that optimize the oxidative stress reduction in Pre and Stage 1 hypertensive adults. Also, further studies are needed to fully understand the differences in oxidative stress response associated with changes in fitness level in this population because it is possible that a larger change in fitness level could contribute to a more robust effect on some of the oxidative stress variables than what we report.

Interestingly, in addition to the increased oxidative stress we found in the present study, we report that, in our entire study population, there was a concurrent increase in antioxidant capacity after aerobic exercise training. TAC has been found to increase with acute exercise (34); however, there is little information on changes in TAC levels with long-term aerobic exercise training. We found a significant increase in plasma TAC for the entire group with 6 months of aerobic exercise training, and the only specific *CYBA* genotype-dependent response to aerobic exercise training observed was within the CC homozygote group. This significant increase in plasma TAC for the entire group indicates that the aerobic exercise training program elicited an antioxidant response in our population. Within the C242T genotype groups at baseline, there was a tendency for higher plasma TAC levels in TT homozygotes, but this tendency was abolished with aerobic exercise training, again demonstrating that the aerobic exercise training had a greater influence on oxidant and antioxidant responses than the *CYBA* polymorphisms did. Recently, Chrysohoou et al. (10) confirmed an association between prehypertension and TAC levels. The authors reported that, in general, prehypertensive subjects had 7% lower TAC levels than normotensive subjects. In our study, the subjects were either Pre or Stage 1 hypertensive, so the fact that TAC levels increased across the entire group demonstrates either a beneficial clinical effect from long-term aerobic exercise training on antioxidant capacity or an increased antioxidant state in response to the increased oxidant state we report.

It should be noted that several limitations exist in the present study. First, a functional measurement of overall endothelial function would have added additional information about the reduced NO_x levels reported. A reduction in urinary NO_x levels with aerobic exercise training indicates a decrease in total body systemic NO levels but does not give a quantitative measure of endothelial function. Another thing that could be included in the future are measurements of nitrotyrosine production to show the peroxynitrite-mediated protein nitration associated with reactive nitrogen intermediates. Nitrotyrosine measures provide an index of peroxynitrite production, and these data may have also supplied further insight into the reduction in NO_x observed in the present study. In addition, we only measured one marker of oxidative stress, which may not reflect the total oxidative stress status. There are many other markers of oxidative stress, but 8-*iso*-PGF_{2α} continues to be regarded as one of the most stable and reliable biomarkers for lipid peroxidation. Finally, it is understood that measuring TAC only reflects the ability to scavenge hydrogen peroxide and not specifically O_2^- , which is the oxidant produced by NADPH oxidase. However, it must be noted that a large source of hydrogen peroxide in the cell results from the spontaneous dismutation of O_2^- by action of superoxide dismutase (SOD) enzyme. Therefore, TAC measures in a laboratory do inherently reflect O_2^- scavenging to some extent. Future studies should include measurement of SOD activity to actually quantify O_2^- scavenging.

CONCLUSIONS

In conclusion, the most important finding from our study was a significant difference among the C242T genotype groups in baseline urinary 8-*iso*-PGF_{2α} levels, which was not evident after aerobic exercise training at 70% $\dot{V}\text{O}_{2\text{max}}$. This finding suggests that the C242T polymorphism

may influence the baseline levels but not the aerobic exercise training-induced changes in 8-*iso*-PGF_{2α} levels in Pre and Stage 1 hypertensive adults. Our finding has relevance because the C242T polymorphism causes a nonconservative histidine-to-tyrosine substitution, which leads to a structural change in the p22phox protein. However, because this genotype-dependent relationship with baseline urinary 8-*iso*-PGF_{2α} levels was abolished with 6 months of aerobic exercise training, we believe that compared with the *CYBA* polymorphisms that we studied, the aerobic exercise training program had a larger impact on the oxidative stress responses in this population.

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FIGURE 1.
Baseline 8-iso-PGF_{2α} levels in *CYBA* C242T genotype groups.

Table 1
Baseline subject characteristics and polymorphic frequencies for the genotype and allele groups.

	C242T Genotype				A640G Genotype			
	CC (n = 36)	CT (n = 48)	TT (n = 10)	AA (n = 19)	AG (n = 53)	GG (n = 22)		
Age (yr)	58.4 ± 0.7	59.0 ± 1.0	58.0 ± 1.8	57.1 ± 1.6	59.0 ± 0.7	59.1 ± 1.0		
Gender (male/female)	15:21	25:23	4:6	8:11	26:27	10:12		
BMI (kg·m ⁻²)	30.0 ± 0.7	29.0 ± 0.6	28.0 ± 1.4	29.0 ± 1.0	29.3 ± 0.6	28.2 ± 0.7		
TC (mg·dL ⁻¹)	192.2 ± 6.1	193.7 ± 5.5	181.4 ± 10.2	185.0 ± 5.0	189.0 ± 6.0	205.0 ± 6.8		
HDL-C (mg·dL ⁻¹)	52.0 ± 3.2	50.6 ± 3.3	45.5 ± 3.3	45.4 ± 4.1	52.0 ± 3.0	51.3 ± 4.7		
LDL-C (mg·dL ⁻¹)	114.6 ± 5.5	117.0 ± 4.4	109.0 ± 9.0	109.0 ± 5.2	113.0 ± 4.5	126.0 ± 7.0		
Triglycerides (mg·dL ⁻¹)	122.5 ± 10.8	119.5 ± 9.4	115.4 ± 13.0	127.0 ± 15.1	113.0 ± 8.0	133.0 ± 15.0		
VO _{2max} (mL·kg ⁻¹ ·min ⁻¹)	25.2 ± 0.8	25.0 ± 0.7	25.1 ± 1.0	25.9 ± 0.9	24.3 ± 1.0	25.6 ± 0.8		
Genotype frequency (%)	38.3	51.1	10.6	20.2	56.4	23.4		
Allele frequency	C: 0.64		T: 0.36		A: 0.48		G: 0.52	

Values are mean ± SE except for frequency data.

BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; VO_{2max}, maximal oxygen consumption.

Table 2
Oxidative stress and blood pressure changes with aerobic exercise training by *CYBA* genotype groups.

	C242T Genotype			A640G Genotype		
	CC	CT	TT	AA	AG	GG
8- <i>iso</i> -PGF _{2α} (nmol·mmol ⁻¹ creatinine)						
Baseline	0.36 ± 0.02 ^{*†}	0.29 ± 0.02 ^{*†}	0.28 ± 0.04 [*]	0.29 ± 0.03	0.32 ± 0.02	0.35 ± 0.03
Changes with training	0.16 ± 0.06	0.10 ± 0.04 [‡]	-0.01 ± 0.10	0.12 ± 0.07 [‡]	0.06 ± 0.04	0.20 ± 0.07 [‡]
TAC (mmol·L ⁻¹)						
Baseline	2.8 ± 0.1	2.7 ± 0.1	3.4 ± 0.3	2.8 ± 0.2	2.7 ± 0.1	3.0 ± 0.2
Changes with training	0.3 ± 0.2 [‡]	0.1 ± 0.15	0.6 ± 0.3	0.3 ± 0.2	0.1 ± 0.1	0.6 ± 0.2
NO _x (μmol·L ⁻¹ × 10 ⁻³)						
Baseline	2.7 ± 0.2	2.9 ± 0.2	2.6 ± 0.4	2.9 ± 0.3	2.8 ± 0.2	2.5 ± 0.3
Changes with training	-0.19 ± 0.06 [‡]	-0.20 ± 0.05 [‡]	-0.18 ± 0.10 [‡]	-2.19 ± 0.09 [‡]	-1.94 ± 0.05 [‡]	-2.01 ± 0.08 [‡]
SBP (mm Hg)						
Baseline	133.0 ± 2.0	133.0 ± 1.9	132.0 ± 4.2	131.9 ± 3.3	134.3 ± 1.6	130.2 ± 2.4
Changes with training	-2.8 ± 1.5 [‡]	1.4 ± 1.3	-3.4 ± 2.5	3.2 ± 2.1	-1.8 ± 1.2	-1.6 ± 2.1
DBP (mm Hg)						
Baseline	86.7 ± 1.2	87.3 ± 1.1	88.2 ± 2.6	88.1 ± 1.8	87.2 ± 1.0	86.1 ± 2.0
Changes with training	-2.0 ± 1.1	0.7 ± 0.95	-3.0 ± 1.8	-0.10 ± 1.6	-0.64 ± 0.89	-1.8 ± 1.6

Values are mean ± SE.

^{*} Significant difference ($P < 0.05$) among the three genotype groups.

[†] Significant difference ($P < 0.05$) between any two of the genotype groups.

[‡] Significant change ($P < 0.05$) within genotype group with aerobic exercise training.

8-*iso*-PGF_{2α}, 8-*iso*-prostaglandin F_{2α}; DBP, diastolic blood pressure; NO_x, urinary nitrates/nitrites; SBP, systolic blood pressure; TAC, total antioxidant capacity.