The GNAS 393 T>C Polymorphism and the Blood Pressure Response Immediately Following Aerobic Exercise Among Men with Elevated Blood Pressure

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Abstract: A common silent single nucleotide polymorphism (393 T>C) in exon 5 of the guanine nucleotide-binding protein system α subunit (GNAS) gene associates with hypertension, and altered autonomic nervous system function and response to β-blockade. We examined the effects of GNAS 393 T>C on the acute post-exercise BP response among 48 Caucasian men (mean ± SEM, 43.7±1.4 yr) with hypertension (145.1±1.5/85.5±1.1 mmHg). Subjects self disclosed a family history of hypertension. Experiments were non-exercise control and 2 exercise bouts at 40% (LIGHT) and 60% (MODERATE) of peak oxygen uptake. Subjects left the laboratory with an ambulatory BP monitor. Genotypes were detected using polymerase chain reaction and restriction enzyme digestion. Repeated measure ANCOVA tested if BP differed over time among experiments and GNAS genotypes (n=37, TT/TC; n=11, CC). Systolic BP increased 8.0±3.6 mmHg less ($p<0.05$) and diastolic BP tended to decrease 5.1±2.8 mmHg more ($p=0.076$) after LIGHT *vs.* non-exercise control among men with the GNAS T^{393} allele than CC homozygotes. There were no genotype differences in BP after MODERATE *vs.* non-exercise control ($p\geq0.05$). Most men with the GNAS T^{393} allele and a family history of hypertension had lower BP after LIGHT (18/20) *vs.* non-exercise control; whereas 64% of men with the GNAS CC genotype did not have lower BP after LIGHT (7/11), independent of family history of hypertension ($p<0.01$). Men with the GNAS T^{393} allele, a family history of hypertension, and high BP appear to experience the antihypertensive effects of lower intensity, aerobic exercise more so than men with the GNAS CC genotype.

Keywords: G protein, genetics, hypertension, physical activity.

INTRODUCTION

Guanine nucleotide-binding system (G_s) proteins are expressed in all cells and involved in nearly all cellular signal transduction processes [1-4]. G_s proteins are heterotrimers composed of α-, β-, and γ-subunits that determine receptor type and effector specificity. The α-subunit of G_s protein couples β-adrenoceptor activation to the stimulation of cyclic adenosine monophosphate (cAMP). cAMP then stimulates a variety of cAMP dependent protein kinases that modulate short term responses such as changes in potassium and calcium conductance and long term changes such as alterations in gene transcription and cell metabolism [2]. In the cardiovascular system, these actions have important roles in the regulation of cardiac output and peripheral vascular resistance [5, 6]. Abnormalities in the G_s protein-linked pathway are associated with hypertension in rat models [7, 8]. In humans a common silent single nucleotide polymorphism (SNP), 393 T>C (rs7121), of the G_s gene in exon 5 of chromosome 20 (20q13.2) that encodes the α subunit (GNAS) is associated with hypertension [5, 7, 9], and altered autonomic nervous system function [10] and response to β-blockade [6].

Aerobic exercise training decreases blood pressure (BP) 5-7 mmHg [11], and is recommended as lifestyle therapy to prevent, treat and control hypertension [12]. Some if not all of the BP lowering effects of aerobic exercise training programs result from the acute or immediate BP benefits of a single exercise session, termed *post-exercise hypotension* (PEH) [13]. However, PEH does not occur in 25% to 33% of the people with hypertension [14]. When PEH occurs, the greatest BP reductions occur in those with the highest resting BP [11, 14, 15]. The heterogeneity of the individual response to the antihypertensive effects of aerobic exercise is attributed to hypertension being a multifactorial, polygenic disorder [16]; and interactions among genetic, neurohormonal and environmental factors that are not well understood [3, 17].

We have recently found that "risk" alleles in genetic variants of the renin angiotensin system [18, 19] and the α adducin Trp^{460} risk allele [20] associate with the immediate BP lowering effects of a session of cycle exercise performed at 40% of peak oxygen uptake (VO_2peak) but not 60% VO_2peak. GNAS 393 T>C has complex hemodynamic ex-

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pression patterns that are dependent upon interactions among the risk status of the population and the level of sympathetic nervous system stimulation induced by the lifestyle behavior examined [5-7, 9]. Nieminen and colleagues [21, 22] investigated the influence of GNAS 393 T>C on the acute BP response to maximum exercise among young men with normal BP, and found no clinically significant GNAS genotype associations with the post-exercise BP response.

We now extend our search for candidate SNPs that account for some of the variability in the antihypertensive effects of acute aerobic exercise to GNAS 393 T>C. The GNAS T^{393} allele associates with hypertension [5, 7, 9], lower sympathovagal activity in response to postural perturbation [10], and poor response to β-blockade [6]; actions attributed to reduced cAMP vasodilation of the resistance arteries associated with carrying the GNAS T^{393} allele. Our work indicates that risk alleles of genetic variants in BP regulatory pathways associate with the immediate antihypertensive effects of lower intensity, aerobic exercise but not with more vigorous levels of physical exertion [18-20]. For these reasons, we hypothesized that men with the GNAS T^{393} allele would lower BP to a greater degree immediately following lower intensity, aerobic exercise compared to men with the GNAS CC genotype; and consistent with our work [18-20] and the findings of Nieminen *et al.* [6, 21], these GNAS genotype associations would no longer be apparent at more vigorous levels of physical exertion.

METHODS

Subjects

Potential subjects were recruited from the surrounding community and places of work *via* flyer postings and email distribution. As part of the enrollment procedures, volunteers completed a detailed medical health information questionnaire containing questions about caffeine, alcohol, smoking and exercise habits; family history of hypertension; medication use; and presence or absence of various medical diseases and conditions. Volunteers were not enrolled in the study if they had a SBP $\geq$ 160 mmHg and/or DBP $\geq$ 100 mmHg, symptomatic atherosclerotic cardiovascular disease, diabetes mellitus, asthma, thyroid dysfunction, pancreatitis, acute illness, and/or were on medication for depression. Subjects were 48 Caucasian men from 18 to 55 yr with high normal to Stage 1 hypertension [systolic BP (SBP) $\geq$ 130-159 and/or diastolic BP (DBP) $\geq$ 85-99 mmHg]. They were sedentary, had no physical limitations that prevented exercise, and did not smoke. Subjects signed an informed consent approved by the Institutional Review Boards of the University of Connecticut and Hartford Hospital.

Any medications potentially influencing the BP response to exercise including antihypertensives, antilipemics, aspirin, non-steroidal anti-inflammatories, nutritional supplements with the exception of a 1-a-day vitamin, cold medications, and herbal supplements were stopped at least 4 wk before testing occurred. When antihypertensives were withdrawn, study investigators regularly monitored participants' BP during the washout period. Men in who the cessation of antihypertensive medication resulted in resting SBP$\geq$160 and/or DBP$\geq$100 mmHg were excluded and told to resume their medication.

Procedures

The methods of this investigation have been published [15, 18-20]. Briefly, subjects completed an orientation session to acquaint them with the study procedures and determine if their BP met the inclusion criteria. BP was measured in the seating position 3 times, 5 min apart in both arms by auscultation with a mercury sphygmomanometer to the disappearance of the fifth Kortokoff sound and averaged. Waist circumference was measured with a Gulick tape measure (Sammons Preston, Chicago, IL), and height and weight were taken on a standard balance beam scale (Model 339, Detecto, Webb City, MO) and used to calculate body mass index (kg/m^2).

During the orientation session, subjects were asked again to self disclose a family history of hypertension among first degree relatives as a *yes* (n=23) or *no* (n=25). Participants were instructed not to ingest any caffeinated beverage the morning of the testing sessions and to drink caffeinated [$\leq$ 480 ml (2 cups)] and alcoholic ($\leq$2 drinks/d) beverages in moderation throughout study participation. Otherwise, subjects were asked to maintain their usual diet. Weight maintenance was defined as ± 2.25 kg (5.0 lb) of orientation weight and used as an indication that subjects were adhering to their normal dietary patterns throughout the study. Men were weighed prior to the exercise test and the 3 experiments to ensure weight maintenance.

At the end of the orientation session, an ambulatory BP monitor (Accutracker II automatic noninvasive ambulatory BP monitor, Suntech Medical Instruments Inc., Raleigh, NC) was connected to each subject to acquaint them with the apparatus and ensure their BP met the study inclusion criteria. The same investigator attached the ambulatory BP monitor to all subjects. A calibration check was performed with a mercury manometer and a t-tubule (part # 98-0030-00). This arrangement enabled the investigator to hear the Korotkoff sounds determined by the mercury manometer and reference them with the ambulatory BP monitor readings. The calibration check consisted of 3 test runs to ensure that 3 successive ambulatory BP measurements were within 5 mmHg of the auscultatory BP determinations made with the mercury manometer. The intra-investigator coefficient of variation between the ambulatory BP monitor and auscultatory mercury manometer BP measurements was 0.7% for SBP and 1.8% for DBP. The ambulatory BP monitor was programmed to record BP about every 20 min. BP was taken 3 times per hour until 2300 hours. All subjects exited the laboratory with instructions to proceed with their usual activities, not to perform formal exercise for the remainder of the day, to let their arm remain still while the monitor was recording, to record any usual emotional or physical events in a diary, and to return the monitor and diary the next day. The computerized ambulatory BP recordings were considered acceptable if at least 80% of the potential BP readings were obtained. In the rare instance when at least 80% of the potential BP readings were not obtained, experiments were repeated.

Participants then completed a peak graded cardiopulmonary exercise stress test that was used to determine the workload of the 2 experimental exercise sessions. The graded exercise stress test was performed on a cycle ergometer (Monark Ergomedic 818E, Stockholm, Sweden). The proto-

col consisted of 2 min incremental stages of continuous cycling at a constant cadence of 60 rev. min^{-1} with the resistance increased each stage by 0.5 kp (30 W) until volitional exhaustion. VO_2 peak was determined with breath by breath analysis of expired gases (Sensormedics Vmax 29 Metabolic Chart, SensorMedics Corp., Yorba Linda, CA). Heart rate (HR) was recorded continuously with a 12 lead ECG system. The same investigator measured BP by auscultation 30 s prior to the end of each 2 min stage. At the conclusion of the graded cardiopulmonary exercise stress test, subjects were again instrumented with the same ambulatory monitor to further familiarize them with the apparatus.

Subjects completed 3, 40 min experiments in random order on 3 separate days that were at least 48 h apart to avoid acute exercise effects on BP [11, 15]. Each experiment began with a 20 min baseline period of seated rest during which BP and HR were recorded every 2 min. HR was obtained with a HR monitor (Model # 1902750, Polar Electro Inc, Woodbury, NY), and BP by auscultation in the nondominant arm (i.e. hand with which the subject did not write). The same study investigator made all BP determinations during every experiment for all subjects. All BP measurements taken during the baseline period were averaged and denoted as baseline BP. At the conclusion of the 20 min baseline period, volunteers performed either a 40 min non-exercise control session of seated rest; or a 30 min cycle bout on an upright ergometer at light (40% VO_2 peak, LIGHT; equivalent in physical exertion to comfortable walking) or moderate intensity (60% VO_2peak, MODERATE; equivalent in physical exertion to jogging) with a 5 min warm up and cool down of free wheeling (i.e. cycling with no resistance) to total 40 min of exercise. The subjects were blinded to the experiments until the conclusion of the baseline period. Subjects left the laboratory wearing an ambulatory monitor until 2300 hr with a mean attachment time of 12:30 pm.

Blood Sampling and Analysis

Blood samples were taken in advance of the experimental sessions for the purposes of genotyping and fasting blood lipid-lipoprotein, glucose and insulin determinations. Blood samples for catecholamine determinations were obtained during the experiments as follows: at the end of the baseline period, at 30 min of each 40 min experiment, and at the conclusion of the experimental sessions while still in the laboratory but prior to attachment to the ambulatory BP monitor. Samples were drawn into anticoagulated EDTA tubes and centrifuged at 2500g and 23°C for 6 min. Plasma samples were transferred to storage tubes and frozen at -80°C until analysis. Blood lipids-lipoproteins were determined by oxidase assays using colorimetric enzymatic methods (Cobras® Integra™, Roche Diagnostics, Mannheim, Germany). The intra- and inter-assay coefficient of variation for the triglyceride assays were 1.6% and 1.9%, respectively. Low density lipoprotein was calculated with the Friedewald equation [23]. Plasma concentrations of catecholamines were measured in a subsample of 20 men by high performance liquid chromatography (Quest Diagnostics, Wallingford, CT) with low-, normal- and high-quality control values. Epinephrine had an intra-assay coefficient of variation of 0.0%, 9.7% and 8.0%, and an inter-assay coefficient of variation of 0.0%, 10.0% and 8.6% for the three levels of control, respectively. Norepinephrine had an intra-assay coefficient of variation of 0.0%, 6.1% and 4.0%, and an inter-assay coefficient of variation of 0.0%, 8.6% and 5.8%, respectively.

Genotype Analysis

DNA was isolated from anticoagulated EDTA whole blood sample and genotyped for the GNAS 393 T>C SNP (GNAS_000516.2:c.393T>C(p.Ile131Ile). Polymerase chain reaction based restriction fragment polymorphism analysis was performed using primers described by Abe *et al.* [7]. Amplicons were digested with FokI and fragment sizes detected by polyacrylamide gel electrophoresis. Negative and positive controls (known genotypes) and random duplicates were used as quality control. In the event of discordant results, genotyping was repeated.

Statistical Analyses

Ambulatory BP values were averaged at hourly intervals over the course of 9 h after the experiments since this was the timeframe when men were awake and ambulating [15]. Descriptive statistics (mean $\pm$ SEM) were generated on all study variables for the total sample and by GNAS genotype groups. Independent t-tests determined if there were differences in subject characteristics among GNAS genotype groups and the presence or absence of a family history of hypertension. Pearson correlations examined relationships among variables that may affect the post-exercise BP response. These variables included age, body mass index, waist circumference, VO_2peak, and fasting glucose, insulin and blood lipids-lipoproteins. Chi-square was used to examine if GNAS 393 T>C was in Hardy Weinberg Equilibrium; and if there were differences among the number of men that lowered BP after exercise among GNAS genotype groups and those with and without a family history of hypertension. The GNAS 393 T>C genotype was in Hardy Weinberg Equilibrium (Table **1**) (χ^2=0.002, p=0.958) with genotype frequencies of TT 27.1% (n= 13), TC 50.0% (n=24), and CC 22.9% (n=11); and allelic frequencies of T^{393} 52.8% and C^{393} 47.2%.

Three by 3 way repeated measure ANCOVA determined if BP differed over time within and among experimental conditions (non-exercise control, LIGHT and MODERATE) and GNAS 393 T>C genotypes with family history of hypertension as a fixed factor and high density lipoprotein as a covariate. Preliminary statistical analyses revealed that the post-exercise BP response did not differ among carriers of the GNAS T^{393} allele. For this reason and to provide adequate power as determined by our sample size estimates, the GNAS genotypes were combined from three to two [i.e. GNAS TT/TC (n=37) and CC (=11)], and three by two way repeated measures ANCOVA were performed. Significant interaction effects were found between BP and GNAS genotype. Therefore, three way repeated measures ANCOVA then determined if BP differed over time among experimental conditions (non-exercise control, LIGHT, MODERATE) *within* the GNAS genotype groups, and these results are displayed in Table **2**. In order to determine *between* GNAS genotype group differences, two by two way repeated measures ANCOVA determined if BP differed over time among exercise (LIGHT or MODERATE) and non-exercise control

Table 1. Physical Characteristics (mean±SEM) of the Study Sample (n=48) and by GNAS 393 T>C Genotype Groups

Characteristics	Total (n=48)	TT/TC (n=37)	CC (n=11)
Age (yr)	43.7 ± 1.4	43.8 ± 1.5	43.4 ± 3.7
Body Mass Index (kg/m^2)	29.6 ± 0.7	29.9 ± 0.9	28.7 ± 0.8
Waist Circumference (cm)	102.3 ± 2.0	102.6 ± 2.4	101.5 ± 3.6
Orientation Ambulatory Awake SBP (mm Hg)	145.1 ± 1.5	143.7 ± 1.7	150.0 ± 3.0
Orientation Ambulatory Awake DBP (mm Hg)	85.5 ± 1.1	85.1 ± 1.2	86.9 ± 2.5
Relative Maximum VO_2 ($ml.kg^{-1}min^{-1}$)	31.1 ± 0.9	30.8 ± 1.1	32.1 ± 1.5
Total Cholesterol ($mmol{\cdot}L^{-1}$)	4.98 ± 0.05	4.89 ± 0.18	5.27 ± 0.29
Low Density Lipoprotein ($mmol{\cdot}L^{-1}$)	3.10 ± 0.14	3.02 ± 0.16	3.34 ± 0.30
High Density Lipoprotein ($mmol{\cdot}L^{-1}$)	1.10 ± 0.03	1.09 ± 0.04	1.12 ± 0.07
Total Cholesterol / High Density Lipoprotein Ratio (U)	4.6 ± 0.2	4.6 ± 0.2	4.8 ± 0.3
Triglycerides ($mmol{\cdot}L^{-1}$)	1.61 ± 0.14	1.60 ± 0.16	1.65 ± 0.30
Epinephrine ($pmol{\cdot}L^{-1}$)	310.1 ± 12.7 (n=20)	342.2 ± 11.3* (n=16)	279.8 ± 22.7 (n=4)
Norepinephrine ($nmol{\cdot}L^{-1}$)	1.04 ± 0.12 (n=20)	1.34 ± 0.11* (n=16)	0.74 ± 0.22 (n=4)

SBP: systolic blood pressure; DBP: diastolic blood pressure; VO_2: oxygen consumption.
*p<0.05, GNAS TT/TC *vs.* CC.

Table 2. Within-Method (Exercise Intensity) Effect for the Blood Pressure Change (Mean±SEM) from Baseline after Exercise and Non-Exercise Control Over 9 hr Among the Total Sample and GNAS 393 T>C Genotype Groups (95% Confidence Interval)*

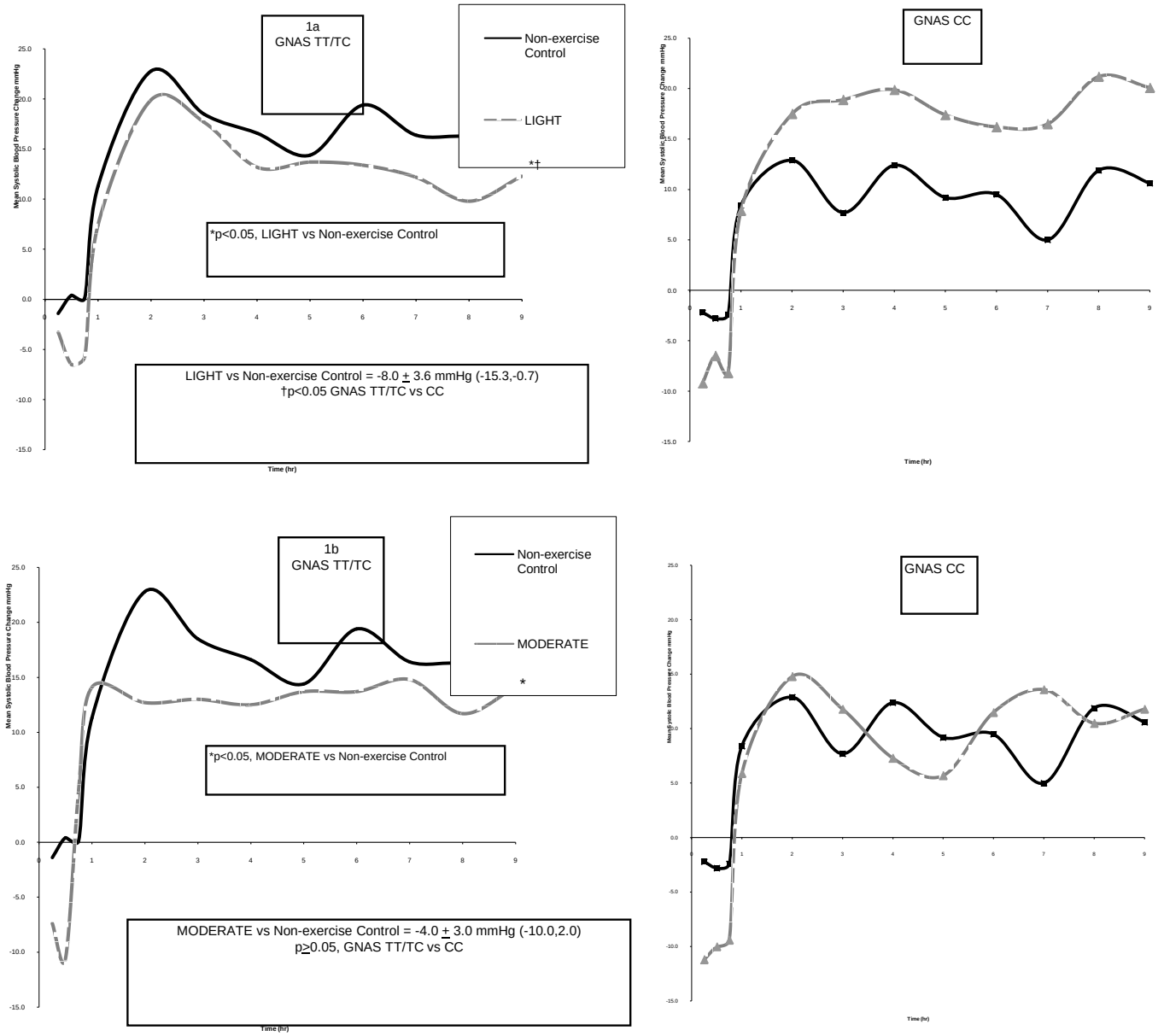
		SBP Response (mmHg)			DBP Response (mmHg)		
		Non-Exercise Control	Light	Moderate	Non-Exercise Control	Light	Moderate
Total Sample (n=48)	Baseline*	125.2 ± 1.6			86.8 ± 1.2		
	Post-Pre Experiment Change	9.5 ± 1.4	9.8 ± 1.5	6.0 ± 1.7†	-2.3 ± 1.1	-1.6 ± 0.9	-3.0 ± 1.0
		(6.7,12.4)	(6.7,12.9)	(2.6,9.5)	(-4.5,0.0)	(-3.6,0.7)	(-5.1,-0.9)
TT/TC (n=37)	Baseline	122.9 ± 1.7†			85.6 ± 1.3‡		
	Post-Pre Experiment Change	12.4 ± 1.2	8.7 ± 1.3*	6.9 ± 1.5†	-1.0 ± 1.0	-2.7 ± 0.9	-2.6 ± 0.9
		(9.9,14.9)	(6.0,11.4)	(3.4,9.9)	(-3.0,1.0)	(-4.6,-0.9)	(-4.5,-0.8)
CC (n=11)	Baseline	132.7 ± 3.4			90.5 ± 2.3		
	Post-Pre Experiment Change	6.7 ± 2.5	11.0 ± 2.8	5.2 ± 3.0	-3.6 ± 2.0	-0.2 ± 1.9	-3.4 ± 1.4
		(1.6,11.7)	(5.4,16.6)	(-0.9,11.3)	(-7.6,0.5)	(-4.0,3.6)	(-7.1,0.4)

* Baseline=Blood pressure average (±SEM) of the pre-experiment 20 min period of seated rest; Post-Pre Experiment Change=Mean of the hourly blood pressure averages over the course of 9 hours after the experiments minus average baseline blood pressure.
†p<0.05, exercise *vs.* non-exercise control; ‡p<0.01 GNAS TT/TC *vs.* CC.
LIGHT: 40% VO_2peak; MODERATE: 60% VO_2peak

among GNAS genotype groups, and these results are displayed in Figs. (**1** and **2**).

In the subsample of subjects (n=20) in which catecholamines were measured, repeated measures ANOVA tested if catecholamines differed over time within and among the experimental conditions and GNAS TT/TC and TT genotype groups. There were no GNAS genotype group differences in the catecholamine response so that data are presented for the entire subsample.

Sample size power calculations were conducted assuming a multivariate approach to analyzing repeated measure BP data [24]. Based upon our previous findings with the α adducin Gly460Trp SNP which had a minor allele frequency of 5% [20], a series of power assessments were fit to estimated BP means and standard deviations for the experimental conditions and GNAS genotype groups. This prior research indicated a moderate effect size could be expected to detect differences in post-exercise BP among the experimental conditions and GNAS genotype groups with BP hourly change correlations of 0.50 across time. Based upon these assumptions, sample sizes of 37 men in GNAS TT/TC and 11 men in the GNAS CC genotype groups were sufficient to provide adequate power for detecting a SBP within method effect (non-exercise control, LIGHT and MODERATE) with a power of 0.827 and a SBP method by GNAS genotype interaction between GNAS genotype group effect with a power of 0.695.



Figs. (1a and b). Mean SBP change from baseline after exercise *vs.* non-exercise control at hourly intervals over 9 hr (**1a**) LIGHT and (**1b**) MODERATE by GNAS TT/TC (n=37) and CC (n=11) genotype groups. LIGHT: 40% VO_2peak; MODERATE: 60% VO_2peak.

All statistical analyses were performed with Statistical Package for the Social Sciences for Windows version 14.0 with $p<0.05$ established as the level of significance. Figures were drawn with Microsoft® Office 2003. BP results (mean±SEM) (95% Confidence Interval) are initially reported for the within method effect (non-exercise control, LIGHT and MODERATE) for the initial assessment of BP change from baseline over time. Results are then presented for the main research question involving the method by genotype interaction effect of the difference in the BP change from baseline over time after exercise *vs.* non-exercise control between GNAS genotype groups.

RESULTS

Subjects

Subjects (n=48) were overweight, Caucasian men with high normal to Stage 1 hypertension, borderline dyslipidemia [25], and below average physical fitness for men of their age [26] (Table **1**). Physical characteristics were not different between GNAS genotype groups (Table **2**) ($p\geq0.05$), with the exception of resting catecholamines that were greater among those with the GNAS T^{393} allele (i.e., TT/TC) than those with the GNAS CC genotype ($p<0.05$).

Total Sample BP Response

Within-Method (Exercise Intensity) Effect

Table 2 presents the BP response from baseline after exercise compared to non-exercise control over the course of 9 h. Among the total sample, SBP increased and DBP decreased from baseline following all experimental conditions over the course of 9 h (Table **2**) ($p<0.001$). However, SBP was increased an average of 3.5±1.5 mmHg less from baseline after MODERATE compared to non-exercise control ($p<0.05$) but not after LIGHT ($p\geq0.05$). The DBP response was not different between exercise and non-exercise control over 9 h ($p\geq0.05$).

GNAS 393 T>C SNP and BP Effects

Within-Method (Exercise Intensity) Effect

Among men with the GNAS T^{393} allele (i.e. TT/TC), SBP increased an average of 3.7±1.6 mmHg less from baseline after LIGHT and 5.5±1.3 mmHg less after MODERATE compared with non-exercise control over 9 h (Table **2**) ($p<0.05$). DBP decreased approximately 1.7±1.2 mm Hg more from baseline after LIGHT and MODERATE compared to non-exercise control over 9 h (Table **2**), but these differences did not achieve statistical significance ($p\geq0.05$). Among men with the GNAS CC genotype, neither the SBP nor DBP change from baseline was different after LIGHT and MODERATE *vs.* non-exercise control over 9 h (Table **2**) ($p\geq0.05$).

Interaction Method (Exercise Intensity) by Genotype Effect

Baseline BP prior to the start of the experiments was lower among men with the GNAS T^{393} allele compared to men with the GNAS CC genotype ($p<0.01$) (Table **2**). Figs (**1** (SBP) and **2** (DBP)) display the mean change in the BP response from baseline after exercise *vs.* non-exercise control at hourly intervals by the GNAS genotype groups. SBP increased 8.0±3.7 mmHg less over 9 h after LIGHT *vs.* non-exercise control among men with the GNAS T^{393} allele (-3.7±1.6 mmHg) compared to those with the GNAS CC genotype (4.3±3.3 mmHg) (Fig. **1a**) ($p<0.05$) but not after MODERATE (Fig. **1b**) ($p\geq0.05$). Similarly, when carriers of the GNAS T^{393} allele (-1.7±1.2 mmHg) were compared to GNAS CC homozygotes (3.4±2.5 mmHg), DBP tended to decrease 5.1±2.8 mmHg more over 9 h after LIGHT than non-exercise control (Fig. **2a**) ($p=0.076$) but not after MODERATE (Fig. **2b**) ($p\geq0.05$).

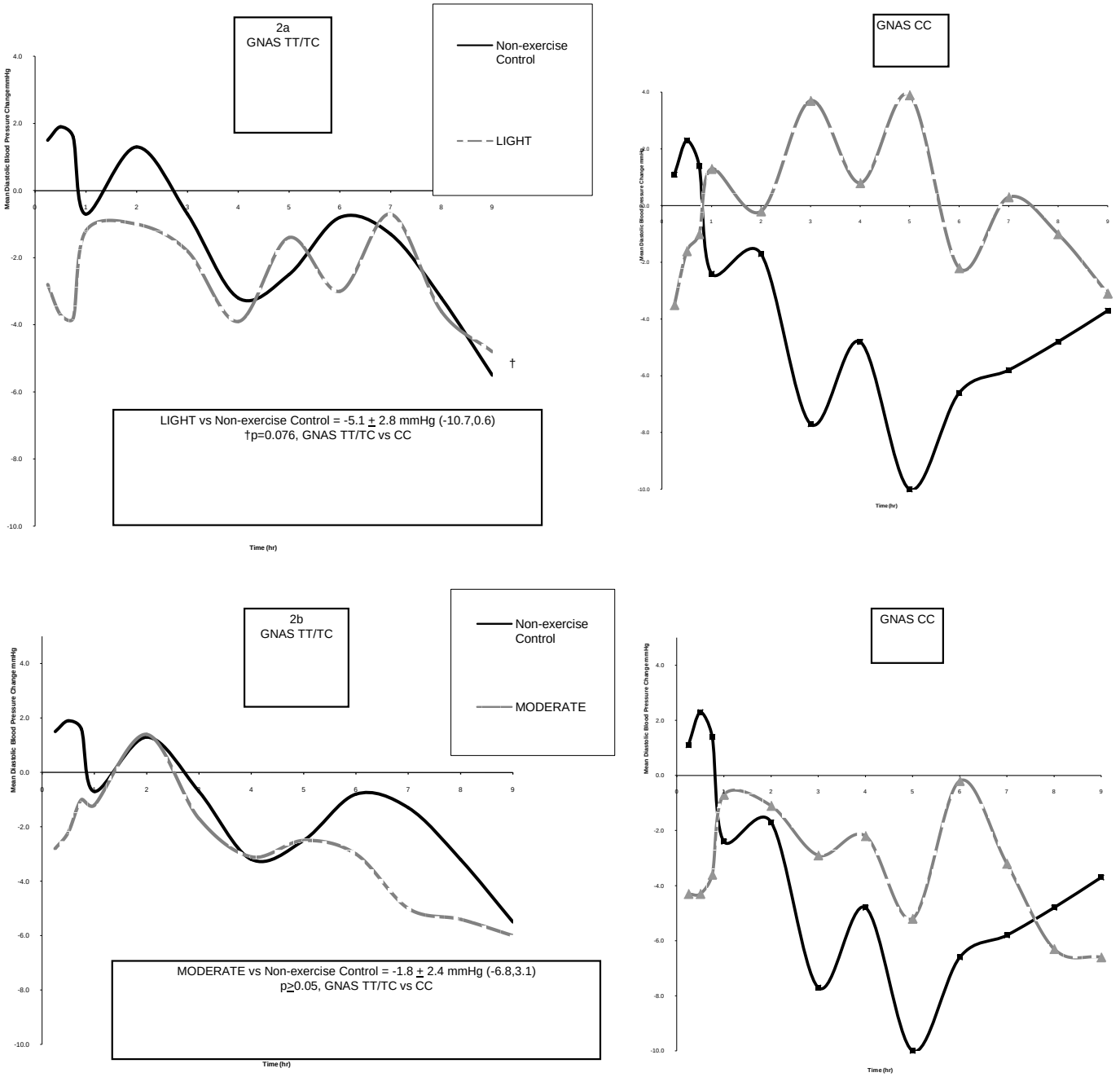
Among GNAS T^{393} allele carriers (n=37), 54.1% reported a family history of hypertension and 45.9% did not; whereas among GNAS CC homozygotes, 27.3% indicated a family history of hypertension and 72.7% did not ($p\geq0.05$). However, 90% of the men with the GNAS T^{393} allele and a family history of hypertension (i.e. 18/20) had lower BP after LIGHT than non-exercise control; whereas 64% of the men with the GNAS CC genotype (i.e. 7/11) did not have lower BP after LIGHT, independent of a family history of hypertension ($p<0.01$).

GNAS 393 T>C SNP and Catecholamines

Epinephrine increased from baseline during and after exercise and non-exercise control ($p<0.001$) (Fig. **3a**). The epinephrine increase from baseline was greater during MODERATE (252.8±88.4 $pmol\cdot L^{-1}$) than non-exercise control (47.2±39.2 $pmol\cdot L^{-1}$) ($p<0.05$) and LIGHT (98.3±33.8 $pmol\cdot L^{-1}$) ($p=0.06$). The epinephrine response did not differ between GNAS genotype groups (GNAS TT/TC n=16; CC n=4) ($p\geq0.05$). Norepinephrine increased from baseline during and after exercise ($p<0.001$) but not non-exercise control ($p\geq0.05$), with the increase during MODERATE (3.15±0.83 $nmol\cdot L^{-1}$) greater than non-exercise control (0.23±0.24 $nmol\cdot L^{-1}$) and LIGHT (1.03±0.28 $nmol\cdot L^{-1}$) ($p<0.05$) (Fig. **3b**). The norepinephrine response did not differ between GNAS genotype groups (GNAS TT/TC n=16; CC n=4) ($p\geq0.05$).

DISCUSSION

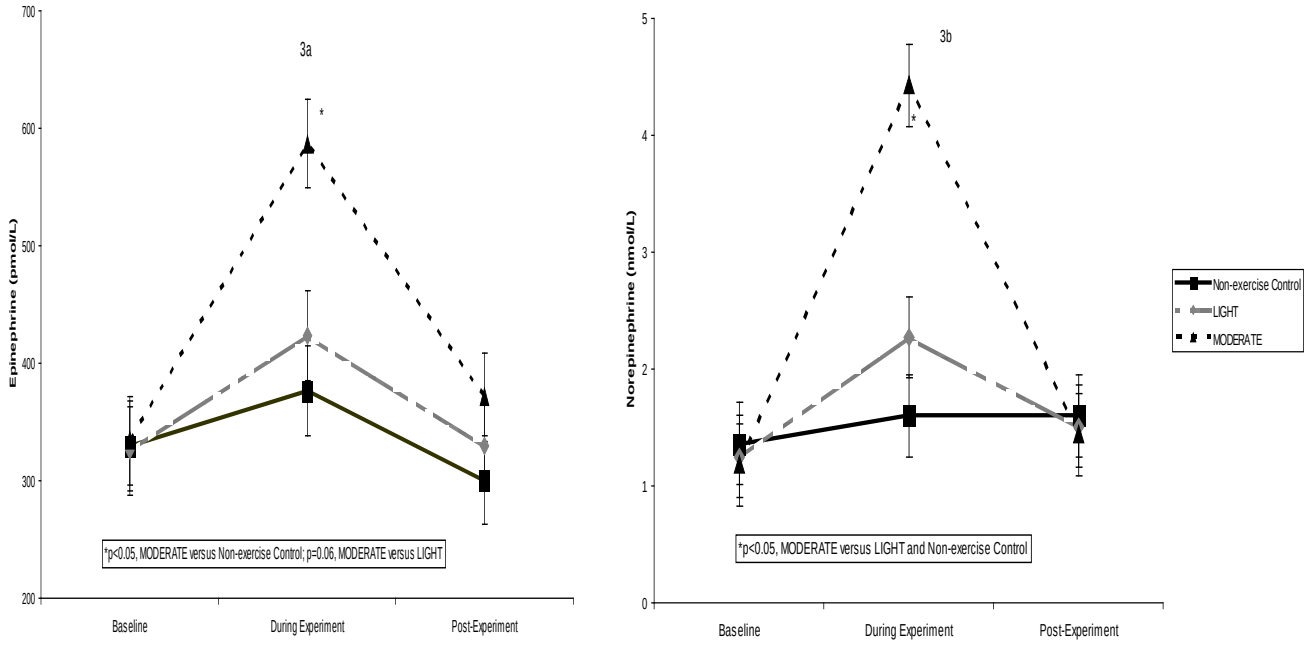
We investigated the influence of GNAS 393 T>C on the BP response immediately following aerobic exercise performed at LIGHT and MODERATE among 48 Caucasian men with high normal to Stage 1 hypertension. SBP increased approximately 8 mmHg less (Fig. **1a**) ($p<0.05$) and DBP tended to decrease 5 mmHg more (Fig. **2a**) ($p=0.076$) after LIGHT *vs.* non-exercise control over 9 h among men with the GNAS T^{393} allele compared to GNAS CC homozygotes. Under these conditions, there were no GNAS genotype differences in the BP response after MODERATE (Figs. **1b** and **2b**) ($p\geq0.05$). Nearly all of the men with the GNAS T^{393} allele and a self-disclosed family history of hypertension had lower BP after LIGHT (i.e., 18/20) compared to non-exercise control; whereas two thirds of men with the GNAS CC genotype did not have lower BP after LIGHT (i.e. 7/11), independent of family history of hypertension ($p<0.01$). Thus, it appears that men with the GNAS T^{393} allele, a family history of hypertension, and high BP appear to experience the antihypertensive effects of lower intensity, aerobic exercise more so compared to men with the GNAS CC genotype.



Figs. (2a and b). Mean DBP change from baseline after exercise *vs.* non-exercise control at hourly intervals over 9 hr (**1a**) LIGHT and (**1b**) MODERATE by GNAS TT/TC (n=37) and CC (n=11) genotype groups. LIGHT: 40% VO_2peak; MODERATE: 60% VO_2peak.

Our results suggest that GNAS 393 T>C SNP may be useful in differentiating between men who do and do not reduce BP after aerobic exercise. These findings are consistent with our previous reports [18-20] and support the notion that risk alleles from genetic variants in major BP regulatory systems (i.e. the renin angiotensin system and now the sympathetic nervous system) are associated with the BP lowering effects of LIGHT. The added value of a family history of hypertension combined with the GNAS T^{393} allele in terms of correctly classifying men who had lower BP after LIGHT than non-exercise control suggests other genetic factors that remain to be identified contribute to the antihypertensive effects of lower intensity, aerobic exercise.

GNAS 393 T>C is a silent SNP resulting in a synonymous codon substitution that does not change the amino acid composition of the protein product. Kimchi-Safaty and colleagues [27] have recently shown that silent mutations have functional consequences due to their influence on protein folding and function that alters substrate specificity. GNAS 393 T>C is associated with complex expression patterns that differ according to the risk status of the population and the level of sympathetic nervous system stimulation associated



Figs. (3a and b). Epinephrine (n=20) and norepinephrine (n=19) response from baseline during and after exercise and non-exercise control. LIGHT: 40% VO_2peak; MODERATE: 60% VO_2 peak.

with the environmental factor examined [5-7, 9]. Jia *et al.* [6] found a higher frequency of the GNAS T^{393} allele among Caucasian middle-aged adults with hypertension compared with those with normal BP; whereas the GNAS C^{393} allele tracked with higher baseline BP among the cohort with hypertension as we found in our study sample (Table **2**).

GNAS 393 T>C associations with BP are reported to be altered by smoking status [7], alcohol consumption [5], glucose tolerance intolerance [9], β blockade [6] and now exercise intensity or the level of physical exertion. We found that men with the GNAS T^{393} allele increased BP less after LIGHT compared to non-exercise control compared to GNAS CC homozygotes; however, these genotype differences were no longer apparent at more vigorous levels of physical exertion. Sympathetic nervous system activation is directly proportional to the intensity of the exercise bout [28] as is shown in Fig. (**3**). The reason for the GNAS genotype BP differences we observed are not readily apparent but may be attributed to a functional impairment of G_s protein β-adrenoceptor coupling among those with the GNAS T^{393} allele resulting in enhanced constriction of the resistance arteries at higher levels of physical exertion [5-7, 9, 10, 29].

Heightened activation of the sympathetic nervous system is implicated in the etiology of hypertension [30]; whereas decreased sensitivity to α-adrenergic receptor stimulation, diminished norepinephrine production, and/or increased norepinephrine uptake following exercise are thought to contribute to post-exercise BP response [11]. Although resting catecholamine levels (GNAS TT/TC > CC) (Table **1**) and baseline BP (GNAS TT/TC < CC) (Table **2**) differed between GNAS genotype groups, there were no differences between GNAS genotype groups and the exercise induced catecholamine response. Thus, the catecholamine results did not offer any explanations for the interactions we found among GNAS genotype and the post-exercise BP response.

The strengths of our study are the stringent criteria by which the ambulatory BP phenotype was assessed and the randomized control study design. Limitations include the small sample size; however, our sample size estimates indicated adequate power to test the primary hypothesis. We purposely restricted the sample to Caucasian men because smaller population subgroups with a more homogenous genetic composition minimize genetic admixture and decrease the likelihood of finding spurious associations with the phenotype of interest [31]. GNAS 393 T>C is located in a recombination hotspot that is in linkage disequilibrium with makers up- and down-stream. Thus, it is possible that the interactions we found between GNAS 393 T>C and the post-exercise BP response are due to other SNPs in the GNAS gene in linkage disequilibrium with GNAS 393 T>C [4]. Overall associations among environmental factors and complex phenotypes such as the post-exercise BP response are often masked by the presence of gene-environment interactions [5, 7, 19, 22]. Our findings demonstrate the importance of conducting intervention studies that examine gene-environment interactions in order to isolate complex genotype-phenotype associations. Admittedly, they need to be validated in a larger, more ethnically diverse sample of men and women.

In summary, men with the GNAS T^{393} allele, a family history of hypertension, and high BP appear to experience the antihypertensive effects of lower intensity, aerobic exercise more so than do men with the GNAS CC genotype. Reasons for our findings need to be more clearly elucidated, but answers may reside in the balance achieved between vasodilator-vasoconstrictor status after stressing the genotype with 2 different levels of physical exertion. A long term goal of personalized medicine is to use genetic and clinical information to more specifically guide lifestyle interventions. Our results indicate that genetic variants in the BP regulatory pathways may eventually be useful in identifying subgroups

of patients likely to respond to lower intensity, aerobic exercise as antihypertensive therapy.

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