International Scholarly Research Network
ISRN Vascular Medicine
Volume 2012, Article ID 901801, 7 pages
doi:10.5402/2012/901801

Research Article

Endothelial Nitric Oxide Synthase (*NOS3*) +894 G>T Associates with Physical Activity and Muscle Performance among Young Adults

Margaux A. Guidry,[1] Matthew A. Kostek,[1] Theodore J. Angelopoulos,[2] Priscilla M. Clarkson,[3] Paul M. Gordon,[4] Niall M. Moyna,[5] Paul S. Visich,[6] Robert F. Zoeller,[7] Paul D. Thompson,[8] Joseph M. Devaney,[9] Heather Gordish-Dressman,[9] Eric P. Hoffman,[9] and Linda S. Pescatello[1]

[1] *Department of Kinesiology & Human Performance Laboratory, University of Connecticut, Storrs, CT 06269, USA*
[2] *Department of Health Professions, Center for Lifestyle Medicine, University of Central Florida, Orlando, FL 32816, USA*
[3] *Department of Kinesiology, University of Massachusetts, Amherst, MA 01003, USA*
[4] *Department of Physical Medicine and Rehabilitation, University of Michigan, Ann Arbor, MI 48109, USA*
[5] *School of Health and Human Performance, Centre for Preventive Medicine, Dublin City University, Dublin, Dublin 9, Ireland*
[6] *School of Health Sciences, Exercise Science Division, Central Michigan University, Mt. Pleasant, MI 48859, USA*
[7] *Department of Exercise Science and Health Promotion, Florida Atlantic University, Boca Raton, FL 33431, USA*
[8] *Department of Cardiology, Hartford Hospital, Hartford, CT 06106, USA*
[9] *Center for Genetic Medicine Research, Children's National Medical Center, Washington, DC 20010, USA*

Correspondence should be addressed to Margaux A. Guidry, margaux.guidry@gmail.com

Received 4 September 2012; Accepted 5 October 2012

Academic Editors: D. Guidolin, T. Malinski, C. Maziere, and C.-C. Wu

Objective. We examined the influence of missense polymorphism, endothelial nitric oxide synthase (*NOS3*) +894 G>T (rs1799983), on habitual physical activity (PA) and the muscle strength response to resistance training (RT). *Methods*. Men ($n = 354$) and women ($n = 424$; 24.3 ± 8.0 yr) were genotyped. Subjects reported hr/wk in vigorous and light intensity PA and sitting on the Paffenbarger PA questionnaire. One repetition maximum assessed muscle strength. Multivariable and repeated measures ANCOVA tested differences among *NOS3* +894 G>T and PA and RT phenotypes by gender. *Results*. hr/wk in vigorous intensity PA (5.4 ± 1.2 versus 8.3 ± 0.4; $P = 0.019$), more hr/wk in light intensity PA (42.1 ± 2.4 versus 35.8 ± 0.7; $P = 0.011$), and less hr/wk sitting (37.6 ± 2.8 versus 45.8 ± 0.9; $P = 0.006$) than those with the G allele. Women with *NOS3* +894 TT gained more absolute (4.4 ± 0.3 versus 3.7 ± 0.8 kg; $P = 0.013$) and relative (78.3 ± 5.8 versus 61.9 ± 1.8%; $P = 0.007$) strength than those with the G allele. *Conclusions*. *NOS3* +894 G>T associated with PA among men and women and the muscle strength response to RT among women only. Our findings indicate the need for prospective studies examining the influence of *NOS3* variants on PA and the muscle response to RT as well as elucidating underlying mechanistic pathways for the associations observed.

1. Introduction

The endothelial nitric oxide synthase gene (*NOS3*) is located on chromosome 7 (7q36) and encodes NOS3, the rate limiting enzyme for nitric oxide (NO) production [1]. NO is the most potent vasodilator produced by the endothelium, increases in response to physical activity (PA) induced shear stress, and is modulated by genetic predispositions to *NOS3* expression [1]. In addition to being a potent vasodilator, NO is involved in the control of skeletal muscle function [2], skeletal muscle glucose uptake during exercise [2], and mitochondrial ATP production [2], all of which can modulate muscle strength. NO is also produced in the cerebral circulation affecting neuronal activity including the

release of dopamine [3]. Therefore, NO mediated actions can also influence behavior and cognition as well as voluntary movement and motivation [4].

Candidate gene studies report *NOS3* (−786 T>C, rs1800779 and +894 G>T, rs1799983) single nucleotide polymorphisms (SNPs) associate with health/fitness and exercise performance phenotypes. We have shown *NOS3* −786 T>C is associated with resting blood pressure (BP) and the BP response to acute bouts of submaximal [5] and maximal aerobic exercise [6]. Others have reported *NOS3* −786 T>C to be associated with resting forearm blood flow [7] and the parasympathetic modulation response to aerobic exercise training [8] as well as the differentiation of elite power from endurance athletes [9]. *NOS3* +894 G>T has been found to be associated with the BP [10, 11], HR, and stroke volume responses to submaximal and maximal aerobic exercise [12], the nonexercising muscle vasodilation response to isometric handgrip exercise [13], and parasympathetic modulation response to submaximal aerobic exercise [8]. Lastly, *NOS3* is one of the three most studied genes regarding the BP response to exercise training [14].

Collectively, these data indicate *NOS3* SNPs are logical candidates to explore for associations with habitual PA and the muscle strength response to resistance training (RT). Thus, we examined the association of *NOS3* −786 T>C and +894 G>T on habitual PA levels and the muscle strength response to RT among a subsample of healthy, European-derived American adults from the Functional Single Nucleotide Polymorphisms Associated with Human Muscle Size and Strength (FAMuSS) study [15]. We hypothesized that these two *NOS3* SNPs would be associated with habitual PA levels and the muscle strength response to RT.

2. Materials and Methods

The FAMuSS study was conducted by the Exercise and Genetics Collaborative Research Group that was comprised of researchers from 10 university and hospital settings. The experimental design of FAMuSS has been described elsewhere [6, 15–21]. The institutional review boards from the 10 institutions involved with the study approved the study protocol and informed consent was obtained from all individuals prior to enrollment.

Potential study volunteers were recruited and screened at eight RT sites. Individuals who reported use of medications known to affect skeletal muscle function (i.e., corticosteroids, antihypertensive or antilipidemic medications, anabolic steroids, diuretics, arthritis medications, Depo-Provera Contraceptive Injection, Clenbuterol, Rhinocort nasal inhaler, lithium, chronic use of nonsteroidal anti-inflammatory drugs) were excluded from consideration. Subjects were also excluded from participation if they had any previous chronic medical conditions such as diabetes mellitus, and any metal implants in the arms, eyes, head, brain, neck, or heart. Other exclusionary criteria included consuming on average >2 alcoholic drinks daily; use of dietary supplements reported to build muscle size/strength or to cause weight gain such as protein supplements, creatine, or androgenic precursors; and/or gained or lost >2.2 kg within 3 months of study participation.

2.1. Subjects. FAMuSS participants who satisfied the inclusion criteria and elected to participate in the study included 1219 individuals (509 males, 708 females). The Paffenbarger PA Questionnaire [22] was completed to assess the self-reported PA levels of FAMuSS participants. Study investigators obtained measurements of height and weight using a standard balance beam scale. FAMuSS participants who completed the Paffenbarger PA Questionnaire and were genotyped for *NOS3* −786 T>C ($n = 284$, TT/TC $n = 252$, CC $n = 32$) and *NOS3* +894 G>T ($n = 479$, GG/GT $n = 435$, TT $n = 44$) comprising the subsample for this study that totaled 844 men ($n = 347$) and women ($n = 497$).

2.2. Determination of Physical Activity Phenotypes. The Paffenbarger PA Questionnaire was used to estimate weekly PA over the last year prior to study enrollment. The Paffenbarger PA Questionnaire is a validated [23] eight-item instrument used to measure self-reported weekly duration and intensity of PA. Item eight provided data for this study and asked respondents to divide a typical weekday and weekend day into hours spent in five PA categories (vigorous, moderate, and light intensity; sitting; or sleeping) [22]. The PA phenotypes reported in this substudy were those that show statistically significant associations with the *NOS3* genotypes and included hr/wk spent in vigorous and light intensity PA, and sitting.

2.3. One Repetition Maximum Strength Testing (1RM). The dynamic strength of the elbow flexor muscles was assessed in both arms before and after 12 wk of resistance training (RT) by 1RM on a standard preacher curl bench (Yukon International Inc., Cleveland, OH) using Powerblock dumb bells (Powerblocks, Intellbell, Inc., Owatonna, MN) in increments of 1.1 and 2.2 kg. If a weight increase was needed in between the powerblock increments, a 0.6 kg increment was added using Platemates (Benoit Built Inc., Boothbay Harbor, ME). Each subject performed two warm up sets with increasing weight. Subjects were verbally instructed to perform one full range of motion with 100% of the estimated maximum weight extending the elbow to 180° and curling the weight back up to the shoulder. The arm not being tested rested on the lap with the hand in a pronated position. If the lift was successful, a 3 min rest was taken and the weight increased slightly; if the lift was unsuccessful, a 3 min rest was taken and the weight decreased slightly. The procedure was repeated until subjects failed to complete a full range of motion lift. Weights were chosen so that the 1RM could be determined in three to five attempts. Maximum weight lifted was recorded in kg as the greatest amount of weight successfully lifted one time. Study investigators gave verbal encouragement to each subject during each 1RM attempt. The same study investigator administered pre- and post-RT 1RM test for a given subject.

2.4. Resistance Training Program. Subjects underwent 12 wk of gradually progressive, supervised RT of their nondominant arm twice per wk with sessions separated by a minimum

of 48 hr. Each RT session began with a warm up consisting of two sets of 12 repetitions of the biceps preacher curl and overhead triceps extension. After a 3 min rest, subjects performed three sets of 12 repetitions at 65 to 75% of baseline 1RM for each of the following five exercises: biceps preacher curl, biceps concentration curl, standing biceps curl, overhead triceps extension, and triceps kickback. All exercises were performed using dumbbells (Powerblocks, Intellbell, Inc., Owatonna, MN). The speed of each repetition was a total of 4 s, 2 s for the concentric and 2 s for the eccentric phase. A 2 min rest followed each set. At week five, the number of repetitions was decreased to eight and then to six at week 10. Thus, the exercise intensity at weeks five and 10 increased to 75 to 82% 1RM and 83 to 90% 1RM, respectively. Training sessions lasted 45 to 60 min. Muscle strength phenotypes included baseline muscle strength (kg) and the change in absolute (kg) and relative (%) muscle strength.

2.5. DNA Extraction and Genotyping. A sample of whole blood was obtained from each subject, refrigerated, and sent to the Children's National Medical Center Research (Washington, DC), where the DNA was extracted using the Puregene Whole Blood DNA Isolation kit (Gentra Systems, Inc, MN). TaqMan Allelic Discrimination assays were purchased from ABI (Foster City, CA, USA). The two *NOS3* SNP assays were pre-designed by ABI rs1800779 (C_7599687_1_) and rs1799983 (C_3219460_20). The Taqman allelic discrimination assays provided a method to simultaneously detect both alleles during the PCR reaction using allele-specific oligonucleotides each labeled with a different fluorophore (VIC and FAM). The PCR reaction contained 20 ng genomic DNA, 900 nM forward and reverse primers, 200 nM fluorescent allele discrimination probes (FAM and VIC-labeled), and 5 μL TaqMan Genotyping Master Mix (ABI) in a final volume of 10 μL. The PCR profile was 10 min at 95°C (denaturation), and 44 cycles of 15 s at 92°C and 1 min at an annealing temperature of 60°C. The resultant PCR products were analyzed using an ABI 7900HT system and the two alleles are called using SDS 2.3 software and checked manually. *NOS3* SNPs that were genotyped were −786 T>C ($n = 308$, TT $n = 128$, TC $n = 147$, CC $n = 33$) and +894 G>T ($n = 393$, GG $n = 195$, GT $n = 165$, TT $n = 33$).

2.6. Statistical Analyses. Descriptive statistics were calculated on all study variables. Hardy Weinberg Equilibrium (HWE) was determined for each SNP using χ^2. Linkage disequilibrium (LD) was assessed using the r^2 measured between each pair of SNPs. *NOS3* −786 T>C ($P = 0.29$) and +894 G>T ($P = 0.98$) did not deviate from HWE. Multivariable ANCOVA tested associations among *NOS3* genotypes and PA phenotypes. Repeated measures ANCOVA tested associations among *NOS3* genotypes and the muscle strength response phenotypes to RT. These analyses included gender as a between factor and age, body mass index (BMI), and BP as covariates. If significant main effects were found, post hoc analyses were performed with a Bonferroni adjustment for multiple comparisons. The *NOS3* genotype comparisons which emerged as significantly associated with the PA and muscle strength phenotypes were recessive models for *NOS3* −786 TT/TC ($n = 252$) versus CC ($n = 32$) and *NOS3* +894 GG/GT ($n = 479$) and TT ($n = 44$). Alpha level was $P < 0.05$, and analyses were performed using SPSS 14.0 for Windows.

3. Results

3.1. Subject Characteristics. Subjects ($n = 844$) were European-derived American men ($n = 347$) and women ($n = 497$). Age, BMI, and BP (±SEM) did not differ by gender and *NOS3* +894 G>T and −786 T>C genotypes (Table 1) ($P > 0.05$). Subjects were healthy, young, normal weight with normal BP.

3.2. Physical Activity. PA phenotypes and *NOS3* genotype associations did not differ by gender so results are presented in Table 2 for the total sample. Adults with the *NOS3* +894 TT genotype reported less time in vigorous intensity PA ($P = 0.019$), and sitting ($P = 0.006$), but more time in light intensity PA ($P = 0.011$) than adults with the G allele. These PA phenotypes did not differ by *NOS3* −786 T>C genotypes (Table 2) ($P > 0.05$).

3.3. Dynamic Muscle Strength. Among the total sample, absolute ($P = 0.015$) and relative ($P = 0.039$) muscle strength increased pre- to post-RT. However, the muscle strength response was *NOS3* genotype and gender dependent (gender ∗ genotype, $P = 0.006$) so that results are presented separately for men and women in Table 3. Among women, those with the *NOS3* +894 TT genotype had greater absolute ($P = 0.012$) and relative ($P = 0.009$) muscle strength gains pre- to post-RT than those with the G allele. Among men the change in muscle strength pre- to post-RT did not differ between *NOS3* +894 G>T genotypes ($P > 0.05$). The change in absolute and relative muscle strength pre- to post-RT did not differ by *NOS3* −786 T>C genotypes among men and women ($P > 0.05$).

4. Discussion

We investigated the influence of *NOS3* +894 G>T and −786 T>C on habitual PA and the muscle strength response to RT among a large sample of healthy, European-derived American adults from FAMuSS [15]. We found that *NOS3* +894 G>T associated with habitual PA and the muscle strength response to RT. Specifically, adults with the *NOS3* +894 TT genotype spent 7–9 hr/wk more in lower intensity PA, 3-4 hr/wk less in vigorous intensity PA, and 8–10 hr/wk less sitting than adults that were carriers of the *NOS3* +894 G allele. Women with the *NOS3* +894 TT genotype gained ∼20% more dynamic muscle strength than those with the G allele; whereas there were no genotype differences in the muscle strength response to RT among men. In addition, there were no differences among *NOS3* −786 T>C genotypes and PA and muscle performance phenotypes. Thus, it appears *NOS3* +894 G>T may habitually influence the

Table 1: Physical characteristics ($X \pm$ SEM) among the total sample and by *NOS3* +894 G>T and −786 T>C genotypes.

Characteristics	*NOS3* +894 G>T (rs1799983)		*NOS3* −786 T>C (rs1800779)	
	GG/GT	TT	TT/TC	CC
N	548	53	252	32
Age (yr)	23.8 ± 0.3	23.3 ± 0.9	23.8 ± 0.3	23.8 ± 1.1
BMI (kg/m^2)	24.9 ± 0.3	26.6 ± 1.0	24.9 ± 0.3	26.3 ± 1.0
SBP (mmHg)	115.6 ± 0.4	117.0 ± 1.4	115.2 ± 0.7	119.5 ± 2.4
DBP (mmHg)	72.9 ± 0.3	74.1 ± 1.1	72.1 ± 0.6	75.0 ± 1.6

BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure.

Table 2: Time spent ($X \pm$ SEM, hr/wk) in vigorous and light intensity physical activity, and sitting among the total sample and by *NOS3* +894 G>T and −786 T>C genotypes.

Physical activity phenotypes (hr/wk)		*NOS3* +894 G>T (rs1799983)			*NOS3* −786 T>C (rs1800779)	
	Total sample	GG/GT	TT	Total sample	TT/TC	CC
N	479	435	44	284	252	32
Vigorous intensity PA	6.8 ± 0.6	**8.3 ± 0.4**	**5.4 ± 1.2****	7.5 ± 0.7	7.1 ± 0.4	7.9 ± 1.3
Light intensity PA	38.9 ± 1.2	**35.8 ± 0.7**	**42.1 ± 2.4**$^{\#}$	38.8 ± 1.5	36.5 ± 0.8	41.1 ± 2.8
Sitting	41.7 ± 1.5	**45.8 ± 0.9**	**37.6 ± 2.8**$^{\#}$	43.5 ± 1.7	46.3 ± 1.1	40.7 ± 3.2

Covariates included age and body mass index with gender and *NOS3* genotype as between factors.
$^{**}P \leq 0.05$, $^{\#}P \leq 0.01$,*NOS3* +894 TT versus GG/GT (bolded).

preference of light PA, vigorous PA and sitting and the strength gains in women that can result from an RT program.

These findings suggest *NOS3* +894 G>T may be important to consider for a personalized approach to exercise prescription along with a growing number of genetic variants that have been reported to be associated with muscle performance [15, 24–27] and habitual PA [21, 28–32]. For example, when recommending exercise to adults for its overall health benefits, the *NOS3* +894 G>T and PA intensity dependent genotype differences we found could be considered when counseling people to become more physically active due to what appears to be a genetic predisposition to prefer light over vigorous intensity PA among those with the TT genotype. Similarly, women with the TT genotype appeared to make greater muscle strength gains from an RT program and could be counseled to engage in an RT program for this purpose, while women with the G allele could be counseled to participate in an RT program for its overall health benefits. Nonetheless, a personalized approach to exercise prescription based upon genotype such as described with these examples remains a vision of the future rather than a reality of the present [33].

Interestingly, mice without the *NOS3* gene (*NOS3* knockout mice) were found to participate in lower levels of voluntary PA than the control mice with the *NOS3* gene [34]. In turn, the *NOS3* knockout mice displayed a less favorable cardiovascular risk factor profile than their genetic counterparts [34]. These data reinforce the notion that *NOS3* is an important gene to further investigate for its associations with voluntary PA in humans. Whether certain *NOS3* SNPs will confer a higher risk or conversely some protection from being physically inactive is important to enhance our understanding of the true role of exercise in the prevention of common chronic diseases.

NOS3 +894 G>T is a missense mutation in the exon 7. This 894 G/T substitution, which results in an aspartate rather than glutamate at position 298 in the NOS3 protein, is of potential functional relevance as it alters NOS3 localization at the endothelial caveolae [35] that leads to a reduced response to shear stress, impaired enzyme regulation, and reduced NO bioavailability [8, 35]. Tesauro et al. [36] reported the *NOS3* +894 T allele generates protein products with different susceptibility to cleavage, suggesting that this SNP has a functional effect on the NOS3 protein.

NO plays a role as a neurotransmitter in the brain by stimulating soluble guanylyl cyclase to form the second messenger molecule, cyclic guanosine monophosphate (cGMP). cGMP relaxes the blood vessels following exercise, increasing blood flow to muscles post exercise to facilitate glucose uptake [37]. In addition, glutamate-induced dopamine release is also mediated by NO [3]. Glutamate regulates the release of dopamine in several brain regions and has been implicated in the regulation of various behaviors and behavioral disorders [3]. Lightfoot [38] and others [39] have shown that genetic control of PA is centrally mediated, possibly through the actions of dopamine receptors. These combined observations provide insights into biologically plausible peripheral and central explanations for the associations we observed among *NOS3* +894 G>T and PA and the muscle strength response to RT.

NOS3 +894 G>T modulates the hemodynamic response to aerobic [8, 12] and isometric [13] exercise. Hand et al. [12] reported that *NOS3* +894 G>T genotype associations may have resulted from differences in exercise-induced NO

Table 3: Change ($X \pm$ SEM) in dynamic muscle strength pre- to post-RT among the total sample and by gender and *NOS3* +894 G>T and −786 T>C genotypes.

	NOS3 +894 G>T					*NOS3* −786 T>C				
	Total Sample	Men		Women		Total Sample	Men		Women	
		GG/GT	TT	GG/GT	TT		TT/TC	CC	TT/TC	CC
N	601	217	21	331	32	243	95	14	120	14
Pre-RT (kg)	9.0 ± 0.2	12.4 ± 0.2	11.2 ± 0.6	6.4 ± 0.1	5.8 ± 0.3	9.1 ± 0.3	12.3 ± 0.2	12.6 ± 0.7	6.1 ± 0.1	6.5 ± 0.3
Absolute change (kg)	4.1 ± 0.1	4.6 ± 0.1	3.9 ± 0.5	**3.7 ± 0.9**	**4.4 ± 0.3**[#]	3.8 ± 0.2	4.2 ± 0.2	4.5 ± 0.5	3.6 ± 0.1	3.5 ± 0.4
Relative change (%)	54.7 ± 2.2	39.5 ± 1.4	37.4 ± 4.6	**62.4 ± 1.9**	**79.3 ± 6.1**[#]	49.1 ± 2.7	37.0 ± 1.7	37.5 ± 5.0	62.3 ± 2.7	57.4 ± 8.3

Covariates included age and body mass index with gender and *NOS3* genotype as fixed factors.
[#]$P \leq 0.01$, *NOS3* +894 TT versus GG/GT (bolded).

production due to *NOS3* +894 G>T genotype dependent increased or decreased *NOS3* transcription. Silva et al. [8] proposed genetic variations in *NOS3* +894 G>T explain part of the parasympathetic adaption to isometric handgrip training in humans, causing a lower production of NO during and after each exposure to exercise. Lower NO availability could blunt improvements in training (or phenotypes that rely on NO, such as BP) that would normally occur when normal/higher levels of NO are produced [8].

The literature is limited regarding investigating the influence of *NOS3* and control of skeletal muscle or muscle strength from dynamic resistance exercise in humans. NO production during skeletal muscle contraction is essential for the regulation of glucose uptake during exercise [40]. In humans it has been established that NO is involved in the control of skeletal muscle function including force generation [2], and skeletal muscle glucose uptake during exercise [2], actions that could modulate muscle strength. Exercise-induced muscle contraction increases shear stress which releases NO and increases blood flow due to interactions among insulin and l-arginine that enhances glucose skeletal muscle uptake [2]. Pellinger et al. [37] recently reported that the availability of glucose to skeletal muscle is enhanced by postexercise hyperemia, therefore it could be speculated that *NOS3* expression may affect muscle strength due to the increased vasodilation postexercise when greater amounts of NO are present allowing greater availability of glucose to skeletal muscle providing insight into mechanisms for the associations we found between *NOS3* +894 G>T and the dynamic muscle strength response to RT.

Previous studies have suggested that gender differences in NO production could be due to ovarian hormones (i.e., estrogens) [41]. Furthermore, whole-body production of NO is greater in healthy, premenopausal women than in men under ambulatory conditions [41]. Estrogen may influence NO production by activating estrogen receptor mediated genomic pathways and upregulation of *NOS3* [38, 41]. Sex hormones have been postulated to also affect PA levels through the estrogen receptor pathway [38]. This evidence lends insight into possible explanations for the gender dependent effects we found among women but not men regarding *NOS3* +894 T>G associations with the muscle strength response to RT.

Contrary to our findings with *NOS3* +894 G>T, *NOS3* −786 T>C was not associated with self-reported PA levels or the muscle strength response to RT. Consistent with our results, Hand et al. [12] did not find *NOS3* −786 T>C associations with habitual PA level and the hemodynamic response during submaximal or maximal exercise. Nonetheless, it is unclear why we found associations among *NOS3* +894 G>T but not with *NOS3* −786 T>C genotypes and PA and muscle performance phenotypes. *NOS3* +894 G>T is found in the coding region of chromosome 7, while *NOS3* −786 T>C is found in the promoter region [42]. Increased levels of methylation in the promoter region of a gene can reduce transcription of that gene [43]. The expression of *NOS3* is known to be sensitive to epigenetic mechanisms and the methylation of *NOS3* has been found to inversely correlate with its transcriptional activity [44]. Rao et al. [45] and Cooper and Keaney [44] recently reported that the methyl-CpG-binding (MBD2) protein was found to sense DNA methylation and mediate transcriptional repression of *NOS3*, therefore silencing gene transcription and *NOS3* mediated responses. Thus, it can be speculated that due to increased methylation in the promoter region, adults with *NOS3* −786 T>C produced less NO due to transcriptional repression of the *NOS3* gene resulting in the lack of associations we found with self-reported PA levels or the muscle strength response to RT. However, we did not measure NO, so that future studies should be designed to determine whether these suppositions are so.

This study has several limitations. Habitual PA is a complex behavior that is likely influenced by many genes other than *NOS3* genetic variants through multiple pathways. This study was not designed to obtain mechanistic data. Additionally, PA data were collected via questionnaire, thus exposing the possibility of subject recall or social desirability bias. However, the Paffenbarger PA questionnaire has been widely validated in similar populations to the present study and is considered an accurate method of leisure time PA in adults [46].

This investigation is a subset of the FAMuSS study which is the largest study that has investigated candidate genes associated with muscle performance, and the first study to specifically investigate *NOS3* variant associations with PA and muscle strength in humans [15]. Furthermore, FAMuSS

meets the criteria outlined by Hagberg et al. [14, 47] that are necessary for conducting exercise genomics studies including a large sample size, a well-structured exercise intervention with stringent assessment of phenotypes, and quality control of genotyping.

In summary, *NOS3* +894 T>G associated with PA and the muscle response to RT among a young, healthy large sample of European-derived American men and women. Our findings indicate the need for prospective studies examining the influence of *NOS3* variants on PA and the muscle response to RT as well as elucidating underlying mechanistic pathways for the associations observed. Information gathered in future studies will help clinicians better understand genetic predispositions to PA patterns and why some people respond more readily to an RT program than others.

Acknowledgment

This paper was supported by NIH-NINDS R01 NS40606-02 and the University of Connecticut, Center for Health, Intervention, and Prevention.

References

[1] D. J. Green, "Exercise training as vascular medicine: direct impacts on the vasculature in humans," *Exercise and Sport Sciences Reviews*, vol. 37, no. 4, pp. 196–202, 2009.

[2] Y. Goa, "The multiple actions of NO," *Pflugers Archiv European Journal of Physiology*, vol. 459, pp. 829–839, 2010.

[3] J. M. Dominguez, J. W. Muschamp, J. M. Schmich, and E. M. Hull, "Nitric oxide mediates glutamate-evoked dopamine release in the medial preoptic area," *Neuroscience*, vol. 125, no. 1, pp. 203–210, 2004.

[4] C. J. Reaume and M. B. Sokolowski, "CGMP-dependent protein kinase as a modifier of behaviour," *Handbook of Experimental Pharmacology*, vol. 191, pp. 423–443, 2009.

[5] A. L. Augeri, G. J. Tsongalis, J. L. Van Heest, C. M. Maresh, P. D. Thompson, and L. S. Pescatello, "The endothelial nitric oxide synthase -786 T>C polymorphism and the exercise-induced blood pressure and nitric oxide responses among men with elevated blood pressure," *Atherosclerosis*, vol. 204, no. 2, pp. e28–e34, 2009.

[6] K. M. Olson, A. L. Augeri, R. L. Seip, G. J. Tsongalis, P. D. Thompson, and L. S. Pescatello, "Correlates of endothelial function and the peak systolic blood pressure response to a graded maximal exercise test," *Atheroslcerosis*, vol. 222, no. 1, pp. 202–207, 2012.

[7] S. A. Data, M. H. Roltsch, B. Hand, R. E. Ferrell, J. J. Park, and M. D. Brown, "eNOS T-786C genotype, physical activity, and peak forearm blood flow in females," *Medicine and Science in Sports and Exercise*, vol. 35, no. 12, pp. 1991–1997, 2003.

[8] B. M. Silva, F. J. Neves, M. V. Negrao et al., "Endothelial nitric oxide synthase polymorphisms and adaption of parasympathetic modulation to exercise training," *Medicine and Science in Sports and Exercise*, vol. 43, no. 9, pp. 1611–1618, 2011.

[9] F. Gómez-Gallego, J. R. Ruiz, A. Buxens et al., "Are elite endurance athletes genetically predisposed to lower disease risk?" *Physiological Genomics*, vol. 41, no. 1, pp. 82–90, 2010.

[10] T. Rankinen, T. Rice, L. Pérusse et al., "*NOS3* Glu298Asp genotype and blood pressure response to endurance training: the HERITAGE family study," *Hypertension*, vol. 36, no. 5, pp. 885–889, 2000.

[11] J. S. Kim, J. R. Cho, S. Park et al., "Endothelial nitric oxide synthase Glu298Asp gene polymorphism is associated with hypertensive response to exercise in well-controlled hypertensive patients," *Yonsei Medical Journal*, vol. 48, no. 3, pp. 389–395, 2007.

[12] B. D. Hand, S. D. McCole, M. D. Brown et al., "*NOS3* gene polymorphisms and exercise hemodynamics in postmenopausal women," *International Journal of Sports Medicine*, vol. 27, no. 12, pp. 951–958, 2006.

[13] R. G. Dias, M. J. N. N. Alves, A. C. Pereira et al., "Glu298Asp eNOS gene polymorphism causes attenuation in nonexercising muscle vasodilatation," *Physiological Genomics*, vol. 37, no. 2, pp. 99–107, 2009.

[14] J. M. Hagberg, "Do genetic variants alter the effects of exercise training on cardiovascular disease and can we identify the candidate variants now or in the future?" *Journal of Applied Physiology*, vol. 111, pp. 916–928, 2011.

[15] P. D. Thompson, N. Moyna, R. Seip et al., "Functional polymorphisms associated with human muscle size and strength," *Medicine and Science in Sports and Exercise*, vol. 36, no. 7, pp. 1132–1139, 2004.

[16] P. M. Clarkson, J. M. Devaney, H. Gordish-Dressman et al., "ACTN3 genotype is associated with increases in muscle strength in response to resistance training in women," *Journal of Applied Physiology*, vol. 99, no. 1, pp. 154–163, 2005.

[17] M. J. Hubal, H. Gordish-Dressman, P. D. Thompson et al., "Variability in muscle size and strength gain after unilateral resistance training," *Medicine and Science in Sports and Exercise*, vol. 37, no. 6, pp. 964–972, 2005.

[18] M. C. Kostek, Y. W. Chen, D. J. Cuthbertson et al., "Gene expression responses over 24 h to lengthening and shortening contractions in human muscle: major changes in CSRP3, MUSTN1, SIX1, and FBXO32," *Physiological Genomics*, vol. 31, no. 1, pp. 42–52, 2007.

[19] M. A. Kostek, T. J. Angelopoulos, P. M. Clarkson et al., "Myostatin and follistatin polymorphisms interact with muscle phenotypes and ethnicity," *Medicine and Science in Sports and Exercise*, vol. 41, no. 5, pp. 1063–1071, 2009.

[20] L. S. Pescatello, M. A. Kostek, H. Gordish-Dressman et al., "ACE ID genotype and the muscle strength and size response to unilateral resistance training," *Medicine and Science in Sports and Exercise*, vol. 38, no. 6, pp. 1074–1081, 2006.

[21] K. N. Van Deveire, S. K. Scranton, M. A. Kostek et al., "Variants of the ankyrin repeat domain 6 gene (*ANKRD6*) influence muscle and physical activity phenotypes among European derived American adults," *The Journal of Strength & Conditioning Research*, vol. 26, no. 7, pp. 1740–1748, 2012.

[22] R. S. Paffenbarger, R. T. Hyde, A. L. Wing, I. M. Lee, D. L. Jung, and J. B. Kampert, "The association of changes in physical-activity level and other lifestyle characteristics with mortality among men," *New England Journal of Medicine*, vol. 328, no. 8, pp. 538–545, 1993.

[23] J. K. Harris, S. A. French, R. W. Jeffery, P. G. McGovern, and R. R. Wing, "Dietary and physical activity correlates of long-term weight loss," *Obesity Research*, vol. 2, no. 4, pp. 307–313, 1994.

[24] G. De Mars, A. Windelinckx, G. Beunen, C. Delecluse, J. Lefevre, and M. A. I. Thomis, "Polymorphisms in the CNTF and CNTF receptor genes are associated with muscle strength in men and women," *Journal of Applied Physiology*, vol. 102, no. 5, pp. 1824–1831, 2007.

[25] S. M. Roth, M. A. Schrager, R. E. Ferrell et al., "CNTF genotype is associated with muscular strength and quality in humans

across the adult age span," *Journal of Applied Physiology*, vol. 90, no. 4, pp. 1205–1210, 2001.

[26] M. A. I. Thomis, G. P. Beunen, M. Van Leemputte et al., "Inheritance of static and dynamic arm strength and some of its determinants," *Acta Physiologica Scandinavica*, vol. 163, no. 1, pp. 59–71, 1998.

[27] S. Walsh, B. K. Kelsey, T. J. Angelopoulos et al., "CNTF 1357 G>A polymorphism and the muscle strength response to resistance training," *Journal of Applied Physiology*, vol. 107, no. 4, pp. 1235–1240, 2009.

[28] A. M. C. P. Joosen, M. Gielen, R. Vlietinck, and K. R. Westerterp, "Genetic analysis of physical activity in twins," *American Journal of Clinical Nutrition*, vol. 82, no. 6, pp. 1253–1259, 2005.

[29] L. Pérusse, G. Lortie, C. Leblanc, A. Tremblay, G. Thériault, and C. Bouchard, "Genetic and environmental sources of variation in physical fitness," *Annals of Human Biology*, vol. 14, no. 5, pp. 425–434, 1987.

[30] S. Carlsson, T. Andersson, P. Lichtenstein, K. Michaëlsson, and A. Ahlbom, "Genetic effects on physical activity: results from the Swedish Twin Registry," *Medicine and Science in Sports and Exercise*, vol. 38, no. 8, pp. 1396–1401, 2006.

[31] J. H. Stubbe, D. I. Boomsma, J. M. Vink et al., "Genetic influences on exercise participation in 37.051 twin pairs from seven countries," *PLoS ONE*, vol. 1, no. 1, article e22, 2006.

[32] S. Carlsson, T. Andersson, P. Lichtenstein, K. Michaëlsson, and A. Ahlbom, "Genetic effects on physical activity: results from the Swedish Twin Registry," *Medicine and Science in Sports and Exercise*, vol. 38, no. 8, pp. 1396–1401, 2006.

[33] C. E. Garber, B. Blissmer, M. R. Deschenes et al., "Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise," *Medicine and Science in Sports and Exercise*, vol. 43, no. 7, pp. 1334–1359, 2011.

[34] I. Momken, P. Lechêne, R. Ventura-Clapier, and V. Veksler, "Voluntary physical activity alterations in endothelial nitric oxide synthase knockout mice," *American Journal of Physiology*, vol. 287, no. 2, pp. H914–H920, 2004.

[35] M. S. Joshi, C. Mineo, P. W. Shaul, and J. A. Bauer, "Biochemical consequences of the *NOS3* Glu298Asp variation in human endothelium: altered caveolar localization and impaired response to shear," *The FASEB Journal*, vol. 21, no. 11, pp. 2655–2663, 2007.

[36] M. Tesauro, W. C. Thompson, P. Rogliani, L. Qi, P. P. Chaudhary, and J. Moss, "Intracellular processing of endothelial nitric oxide synthase isoforms associated with differences in severity of cardiopulmonary diseases: cleavage of proteins with aspartate vs. glutamate at position 298," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 6, pp. 2832–2835, 2000.

[37] T. K. Pellinger, G. H. Simmons, D. A. Maclean, and J. R. Halliwill, "Local histamine H_1-and H_2-receptor blockade reduces postexercise skeletal muscle interstitial glucose concentrations in humans," *Applied Physiology, Nutrition and Metabolism*, vol. 35, no. 5, pp. 617–626, 2010.

[38] J. T. Lightfoot, "Sex hormones' regulation of rodent physical activity: a review," *International Journal of Biological Sciences*, vol. 4, no. 3, pp. 126–132, 2008.

[39] A. M. Knab, R. S. Bowen, A. T. Hamilton, A. A. Gulledge, and J. T. Lightfoot, "Altered dopaminergic profiles: implications for the regulation of voluntary physical activity," *Behavioural Brain Research*, vol. 204, no. 1, pp. 147–152, 2009.

[40] S. J. Bradley, B. A. Kingwell, and G. K. McConell, "Nitric oxide synthase inhibition reduces leg glucose uptake but not blood flow during dynamic exercise in humans," *Diabetes*, vol. 48, no. 9, pp. 1815–1821, 1999.

[41] P. Forte, B. J. Kneale, E. Milne et al., "Evidence for a difference in nitric oxide biosynthesis between healthy women and men," *Hypertension*, vol. 32, no. 4, pp. 730–734, 1998.

[42] J. Cheng, J. Liu, X. Li et al., "Effect of polymorphisms of endothelial nitric oxide synthase on ischemic stroke: a case-control study in a Chinese population," *Clinica Chimica Acta*, vol. 392, no. 1-2, pp. 46–51, 2008.

[43] "The genomic regulation of physical activity," in *Exercise Genomics*, S. Roth and L. S. Pescatello, Eds., Molecular and Translational Medicine Series, p. 17, Springer, 2011.

[44] M. P. Cooper and J. F. Keaney, "Epigenetic control of angiogenesis via DNA methylation," *Circulation*, vol. 123, no. 25, pp. 2916–2918, 2011.

[45] X. Rao, J. Zhong, S. Zhang et al., "Loss of methyl-CpG-binding domain protein 2 enhances endothelial angiogenesis and protects mice against hind-limb ischemic injury," *Circulation*, vol. 123, no. 25, pp. 2964–2974, 2011.

[46] D. Albanes, J. M. Conway, P. R. Taylor, P. W. Moe, and J. Judd, "Validation and comparison of eight physical activity questionnaires," *Epidemiology*, vol. 1, no. 1, pp. 65–71, 1990.

[47] J. M. Hagberg, T. Rankinen, R. J. F. Loos et al., "Advances in exercise, fitness, and performance genomics in 2010," *Medicine and Science in Sports and Exercise*, vol. 43, no. 5, pp. 743–752, 2011.