ORIGINAL ARTICLES

Endothelial nitric oxide gene haplotype reduces the effect of a single bout of exercise on the vascular reactivity in healthy subjects

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Polymorphisms in the endothelial nitric oxide synthase (eNOS) gene reduce shear stress–induced nitric oxide production. Thus, we investigated the individual and combined impact of 3 variants in the eNOS gene (−786T>C, intron 4b4a, and 894G>T) on vascular reactivity before and after exercise. Sedentary, healthy subjects were studied (105 women/26 men, age 32 ± 1 years (mean ± standard error of the mean)). Genotypes were determined by polymerase chain reaction restriction fragment length polymorphism, and haplotypes were determined by a Bayesian-based algorithm. Vascular reactivity was evaluated by the percentage of change in forearm vascular conductance provoked by 5 minutes of circulatory occlusion before (baseline) and 10, 60, and 120 minutes after a maximal cardiopulmonary exercise test. Vascular reactivity increased 10 minutes after exercise in the entire sample (baseline: 218 ± 11% vs 10 minutes: 284 ± 15%, $P < 0.001$), remained increased at 60 minutes (239 ± 12%, $P = 0.02$ vs baseline), and returned to baseline at 120 minutes (210 ± 10%, $P = 0.83$ vs baseline). Genotype analysis showed that subjects with the 894G>T polymorphism had lower vascular reactivity than wild counterparts (group effect, $P = 0.05$). Furthermore, subjects with haplotype 2 (H2), containing the −786T>C and 894G>T polymorphisms, had lower vascular reactivity than wild counterparts (haplotype 1 (H1)) (group effect, $P = 0.05$), whereas subjects with haplotype 4 (H4), containing only the 894G>T polymorphism, had vascular reactivity similar to that of wild counterparts (H1) (group effect, $P = 0.35$). Altogether, these results indicate that the 894G>T polymorphism reduced exercise-mediated increase in vascular reactivity, particularly when it occurred concomitantly with the −786T>C polymorphism. (Translational Research 2013;161:15–25)

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Abbreviations: ANCOVA = analysis of covariance; ANOVA = analysis of variance; BMI = body mass index; DBP = diastolic blood pressure; eNOS = endothelial nitric oxide synthase; FBF = forearm blood flow; FVC = forearm vascular conductance; H1 = haplotype 1; H2 = haplotype 2; H3 = haplotype 3; H4 = haplotype 4; H5 = haplotype 5; H6 = haplotype 6; H7 = haplotype 7; HDL = high-density lipoprotein; LDL = low-density lipoprotein; MBP = mean blood pressure; NO = nitric oxide; PCR = polymerase chain reaction; SBP = systolic blood pressure; SNP = single nucleotide polymorphism; VO_{2peak} = peak oxygen consumption

AT A GLANCE COMMENTARY

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Background

Recently it was shown that subjects carrying the 894G>T polymorphism in the eNOS gene had blunted vascular reactivity to ischemia after exercise in comparison with wild counterparts. Nevertheless, the impact of other eNOS gene polymorphisms, isolated or combined, on the vascular reactivity after exercise is still unknown.

Translational Significance

The present study showed that only the 894G>T polymorphism reduces the exercise-mediated increase in vascular reactivity, particularly when it occurs concomitantly with the −786T>C polymorphism. Therefore, these findings contribute to translate the impact of eNOS genetic variations on the after-effect of exercise on vascular function.

Dynamic exercise performed with large muscle groups requires complex integrative cardiovascular responses that leads to systemic increase in shear stress.[1] This exercise-mediated increase in shear stress stimulates nitric oxide (NO) production in the whole circulatory system,[2-4] which takes several minutes or hours to return to pre-exercise baseline values.[2-5] Thus, after a single bout of exercise the vascular reactivity is augmented, which is largely dependent on NO[2-5] and has been associated with favorable after-effects of exercise on the cardiovascular system,[6] such as inhibited blood pressure response during sympathoexcitatory maneuvers.[6-8]

The enzyme that catalyzes NO production in response to shear stress over the endothelium is the endothelial nitric oxide synthase (eNOS).[9] The gene that codes this enzyme is located at chromosome 7 (location 7q36) and contains 21 kb. Since the characterization of the eNOS gene in the mid-1990s,[10] many allelic variations were identified. Nevertheless, only some of these have been consistently associated with functional impairments[11-14] and clinical end points.[15] Among these variations are a single nucleotide polymorphism (SNP) in the promoter region (−786T>C, rs2070744), a variable number of tandem repeats polymorphism in the intron 4 (4b4a), and an SNP in the exon 7 (894G>T, rs1799983). The −786T>C polymorphism has been shown to reduce promoter activity of the eNOS gene, which reduces efficiency of eNOS transcription.[16] The 4b4a polymorphism impairs the eNOS mRNA splicing process, which can also reduce efficiency of eNOS transcription.[17] Finally, the 894G>T polymorphism alters the structure of the eNOS enzyme and has been associated with altered eNOS localization at endothelial caveolae,[18] leading to reduced response to shear stress and impaired coordination of the enzyme regulatory cycle.[18] Therefore, it is conceivable that these polymorphisms in the eNOS gene could blunt the enhancement of vascular reactivity that is usually observed after a single bout of exercise.

Our group recently showed that healthy subjects, who carried the 894G>T polymorphism, had blunted vascular reactivity to ischemia[12] and mental stress[13] after a single bout of exercise in comparison with wild counterparts (ie, subjects without the polymorphism). Nevertheless, the impact of other eNOS gene polymorphisms on the vascular reactivity after exercise is still unknown. Most important, the impact of the interaction among eNOS gene polymorphisms on the vascular reactivity after exercise is not known, which is a relevant issue, because the influence of genetic variations on physiologic traits can be more informative when SNPs are analyzed concomitantly as haplotypes (combinations of genetic markers within a chromosome cluster location).[19,20] On the basis of this background, the aim of the present study was to investigate the effect of 3 polymorphisms in the eNOS gene (−786T>C, intron 4b4a, and 894G>T), analyzed individually as genotypes and concomitantly as haplotypes, on the vascular reactivity to an ischemic stimulus performed before and after a single bout of exercise.

MATERIALS AND METHODS

Sample. Subjects were recruited through advertisements at the university and in local newspapers.