Original Research

The Influence of Tissue Plasminogen Activator I/D Polymorphism on the tPA Response to Exercise

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ABSTRACT

International Journal of Exercise Science 11(3): 1136-1144, 2018. The purpose was to determine if the Alu-insertion (I)/deletion (D) polymorphism of the tissue plasminogen activator (tPA) gene influences the tPA response to maximal exercise. Fifty male subjects (age = 23.6 ± 4.7 yrs) completed a maximal treadmill exercise test. Blood samples were drawn before and immediately after exercise for determination of plasma tPA antigen and activity. Isolated DNA was amplified via polymerase chain reaction, electrophoresed, and visually amplified to determine tPA genotype. Subjects were classified as possessing the D allele (D) (n = 28) or being homozygous for the I allele (I) (n = 22). Differences in tPA antigen and activity were assessed using a two-factor (genotype and time) repeated measures analysis of variance. There were significant main effects for time for tPA antigen and activity ($p < 0.05$), but no main effect for genotype. Furthermore, there was no genotype x time interaction due to a similar increase in tPA antigen in the D group (pre-exercise = 5.83 + 2.39 ng/ml, post-exercise = 21.88 + 7.38 ng/ml) and the I group (pre-exercise = 5.61 + 2.82 ng/ml, post-exercise = 19.05 + 7.67 ng/ml) and a similar increase in tPA activity in the D group (pre-exercise = 0.39 ± 0.19 IU/ml, post-exercise = 9.73 ± 4.22 IU/ml) and I group (pre-exercise = 0.45 ± 0.29 IU/ml, post-exercise = 9.76 ± 5.50 IU/ml). The I/D polymorphism of the tPA gene does not influence the tPA antigen nor tPA activity responses to maximal exercise in healthy, young, sedentary males.

KEY WORDS: Genetics, fibrinolysis, hemostasis, exercise

INTRODUCTION

Cardiovascular disease (CVD) can ultimately manifest itself as one of several acute events, including stroke and myocardial infarction (MI). Typically, hemorrhage or ulceration of

atherosclerotic plaque initiates an acute ischemic event. This result of a clot formation occurs inside the ulcerated plaque, occluding the blood vessel. However, plaque ruptures have been observed on autopsy in individuals without history of ischemic events (4, 16). This suggests that altered hemostasis, in addition to plaque rupture, is necessary for occlusive clot formation. In fact, decreased fibrinolysis, the ability to lyse inappropriate or excessive clots, is a risk factor for cardiovascular events (e.g. ischemic events, mortality, and presence of CVD) (3, 6, 10, 13, 17, 21, 24).

Although regular physical activity is an important part of CVD prevention and rehabilitation, acute exercise can transiently increase the risk of triggering an acute event (14, 20, 22, 25). Mittleman et al. (14) analyzed responses from 1228 patients who were interviewed an average of four days following their MI. Heavy exertion (defined as ≥6 METS) within an hour of their MI were reported in 4.4 percent of the patients. Additionally, of the 48.5 percent of 849 patients in a Multicenter Investigation of Limitation of Infarct Size study where MI triggers could be identified, 14.1 percent were due to moderate physical activity (25). Further evidence (22) from 133 men following cardiac arrest indicate the risk of cardiac arrest being highest during exercise, particularly in those who are sedentary in nature. Ragosta et al. (20) concluded that individuals who died during or immediately after exercise was mainly due to atherosclerosis. It was also concluded that death during exercise in asymptomatic subjects is rare.

This increased risk of a cardiac event may be related to an altered coagulative and/or fibrinolytic response to acute exercise. Evidence illustrates that MIs resulting from physical stress are more likely to occur due to an occlusive thrombus compared to infarctions triggered by other causes (5). While the fibrinolytic response to physical exercise is clinically important, the mechanism(s) that determine the magnitude of this response is(are) not well established. Considering the direct role plasminogen has in lysing arterial thromboses, investigating factors that affect plasminogen in warranted.

The main enzyme of the fibrinolytic system is tissue plasminogen activator (tPA), which catalyzes the conversion of plasminogen to plasmin and subsequently dissolves fibrin into fibrin dimer proteins. It is possible there is considerable genetic influence on the tPA response to exercise, as the release of endothelial tPA is regulated at the level of transcription once the initial endothelial stores are exhausted (11). For example, an Alu-insertion (I)/deletion (D) polymorphism of the tPA gene is associated with variability in the release rate of tPA from the vascular endothelial cells in the forearm both at rest (9, 12) and in response to a mental stress test (9). These reported differences in tPA release did not result in different plasma tPA activity or antigen across genotypes. It is therefore reasonable to believe that the clearance of tPA is large enough under the conditions reported (9, 12) to prohibit differences in plasma tPA activity or antigen.

Exercise causes a dramatic increase in tPA release, resulting in a ~5-fold increase in tPA antigen and a ~380-fold increase in tPA activity (2). It is then worth considering that tPA genotype influences the tPA antigen and activity response to acute, maximal exercise. This, even despite

the lack of influence on resting tPA antigen or activity. Therefore, the purpose of this study was to evaluate the Alu-insertion/deletion polymorphism of the tPA gene to determine if an association exists between this polymorphism and the tPA response to acute, maximal exercise.

METHODS

Participants
Fifty non-smoking, sedentary apparently healthy males participated in this study. Forty-seven subjects were Caucasian, two were Hispanic, and one was African American. To reduce the potential for confounding by factors that impact fibrinolysis, subjects were less than 40 years of age, not taking any medications, and apparently free from known cardiovascular and/or metabolic disease, including diabetes. Written informed consent was obtained from all subjects prior to testing. This research was approved by the University Committee on Research Involving Human Subjects (UCRIHS).

Table 1. Participant characteristics

	Height (cm)	Weight (kg)	Age (years)
D allele (n=28)	181.4 ± 7.7	82.8 ± 11.7	23.7 ± 4.6
I/I (n=22)	179.9 ± 6.7	86.2 ± 16.0	23.5 ± 4.8

Demographic values are expressed as mean ± standard deviation for tPA D allele (containing the D allele as either DD or ID), and I/I (homozygous for the I allele). cm = centimeters, kg = kilograms

Protocol
Treadmill test: all testing was conducted in the morning between 0700 and 1000 hours to minimize diurnal variation in fibrinolysis (1). To minimize postural changes in tPA (29), subjects assumed a semi-recumbent position prior to exercise for 30 minutes followed by collection of a venous blood sample. Subjects then completed a maximal, incremental treadmill exercise test, which began at a 0.0% incline and a speed of 2.5 mph and increased at a rate of 0.5 mph each minute until 6 mph was achieved. At this point, elevation increased at a rate of 3% per minute until subjects reached volitional exhaustion. Heart rate (HR) was measured every minute using telemetry (Polar Vantage; Gays Mills, WI). Oxygen uptake (VO_2) was measured continuously using a Sensormedics 2900 metabolic measurement cart (Sensomedics Inc; Yorba Linda, CA). The highest minute average of VO_2 achieved throughout the test was defined as the subject's maximal oxygen uptake (VO_{2max}). To ensure that a maximal effort had been given, subjects were required to achieve at least two of the following criteria: 1) achievement of 90% age-predicted maximal heart rate (HR_{max}), 2) maximal respiratory exchange ratio (RER) ≥ 1.15, and 3) maximal blood lactate concentration ($[La^-]$) ≥ 8.00 mmol/L. Blood samples for the determination of blood lactate (YSI Inc, Yellow Springs, OH), hematocrit, and tPA were collected immediately before the treadmill exercise and immediately post-exercise (2). A single blood sample was used for the determination of blood lactate.

Blood sampling: venous samples were obtained with the subjects in a semi-recumbent position. At each time point (pre-exercise, post-exercise), 5 ml of venous blood was drawn into an EDTA tube (Vacutainer; Franklin Lakes, NJ) and 5 ml of blood was drawn into a tube containing an

acidified citrate solution (Biopool International; Ventura, CA). Five hundred μl of whole blood from the EDTA tube was used for DNA extraction and determination of hematocrits. Blood collected in acidified citrate was immediately spun at 10,000 rpm for 20 min at 4°C to obtain platelet-poor plasma, which was stored at -80°C until assayed. Cooper et al. (2) demonstrated that tPA antigen and activity increases following a maximal treadmill test remained elevated only two minutes post-test. Within the fourth minute, post-test values decreased significantly from immediately post-test values. Therefore, a single sample was collected on our participants immediately post-test for analysis.

Blood assays: both tPA antigen and activity were measured to represent total tPA, by tPA antigen, and free tPA represented by tPA activity. An enzyme-linked immunosorbancy assay (ELISA) (American Diagnostica Inc.; Greenwich, CT) was used to measure tPA antigen. An amidolytic activity assay (Biopool International; Ventura, CA) was used to measure tPA activity. All samples were assayed in duplicate. Intra-assay coefficients of variation were 4.0% and 5.0% for tPA antigen and tPA activity, respectively. Inter-assay coefficients of variation for tPA antigen and tPA activity were 5.7%and 5.8%, respectively. Post-exercise tPA activities and antigens were corrected for plasma volume changes, which were calculated using the difference between pre and post-exercise hematocrit (26).

Determination of genotypes: DNA was isolated from whole blood using a QIAamp® DNA Blood Mini Kit (Qiagen, Valencia, CA). DNA was amplified by polymerase chain reaction (PCR) using oligonucleotide forward and reverse primers of the polymorphic region for the tPA polymorphism 5'-TCCGTAACAGGACAGCTCA-3' and 5'-ACCGTGGCTTCAGTCATGGA-3', respectively. To determine validity of classification of homozygous D subjects, we performed a second PCR to determine the presence, or absence, of an insertion allele. This was done using an insertion specific primer (5`-GATCACGAGGTCAGGAGAT-3`) for amplification. The PCR was performed in a final volume of 50 μl containing the following: 300 ng of genomic DNA; 12 pmol of each primer; 1.5 mmol/L $MgCl_2$; 200 μM dATP, dGTP, dCTP, dTTP; 1x reaction buffer; and 0.25 U Taq polymerase. The thermocycling procedure (Gene Amp®, PCR System 2700, Applied Biosystems, Foster City, CA) consisted of 10 minutes at 94°C, 32 cycles of 1 minute at 93°C, 1 minute at 64°C, and 100 seconds at 72°C. The final step was extended for seven minutes. The insertion specific PCR used the same reverse primer with the addition of the insertion specific primer and a lower annealing temperature of 60°C. Amplified DNA was electrophoresed in a 1% agarose gel stained with ethidium bromide. The products were detected with UV light to determine genotype for tPA. Because of the relatively low frequency of the homozygous D genotype (9, 23), and because previous data suggest that having the D allele can result in decreased resting tPA release (9), the heterozygous subjects and subjects homozygous for the D allele were combined into one group (D allele). Subjects classified as homozygous for the I allele were classified into another group (I/I).

Statistical Analysis
Differences in tPA antigen, tPA activity, and blood lactate concentrations were assessed using repeated-measures analysis of variance with genotype (D allele, I/I) as the between-subjects

factor, and exercise time (pre-exercise, post-exercise) as the within-subjects factor. Differences in height, weight, age, VO_{2max}, HR_{max}, respiratory exchange ratio (RER), and blood lactate between groups were assessed using analysis of variance. Post-hoc tests were performed using the Tukey HSD test (JMP version 3, Cary, NC). A priori significance level was set at $p < 0.05$ for all statistical tests.

RESULTS

Descriptive data for both genotype groups are listed in Table 1. There were no statistical differences between groups for height, weight, or age. Analysis for group differences for maximal heart rate, VO_2 max, RER, and Peak Blood [La$^-$] showed no significant differences between groups for these variables (Table 2).

Table 2. Results from the treadmill tests

	HRmax (bpm)	VO₂max (ml/kg/min)	RER	Blood [La⁻] (mmol/L)
Group D (n=28)	195 ± 9	47.10 ± 7.10	1.21 ± 0.05	9.447 ± 2.140
Group I (n=22)	194 ± 12	48.60 ± 7.67	1.18 ± 0.08	8.834 ± 2.033

Values are expressed as mean ± standard deviation for tPA Group D (containing the D allele as either DD or ID), and Group I (homozygous for the I allele). bpm = beats per minute, ml = milliliters, kg = kilograms, min = minute, RER = respiratory exchange ratio, La⁻ = lactate, mmol/L = millimoles per Liter

Pre-exercise and post-exercise tPA antigen and tPA activity for both the D allele group and the I/I group are displayed in Figure 1. There were main effects for time for tPA antigen and activity ($p < 0.05$). There was no significant main effect for genotype for tPA antigen or tPA activity. Furthermore, there was no genotype x time interaction due to a similar increase in tPA antigen in the D group (pre-exercise = 5.83 ± 2.39 ng/ml, post-exercise = 21.88 ± 7.38 ng/ml) and the I group (pre-exercise = 5.61 ± 2.82 ng/ml, post-exercise = 19.05 ± 7.67 ng/ml) and a similar increase in tPA activity in the D group (pre-exercise = 0.39 ± 0.19 IU/ml, post-exercise = 9.73 ± 4.22 IU/ml) and I group (pre-exercise = 0.45 ± 0.29 IU/ml, post-exercise = 9.76 ± 5.50 IU/ml).
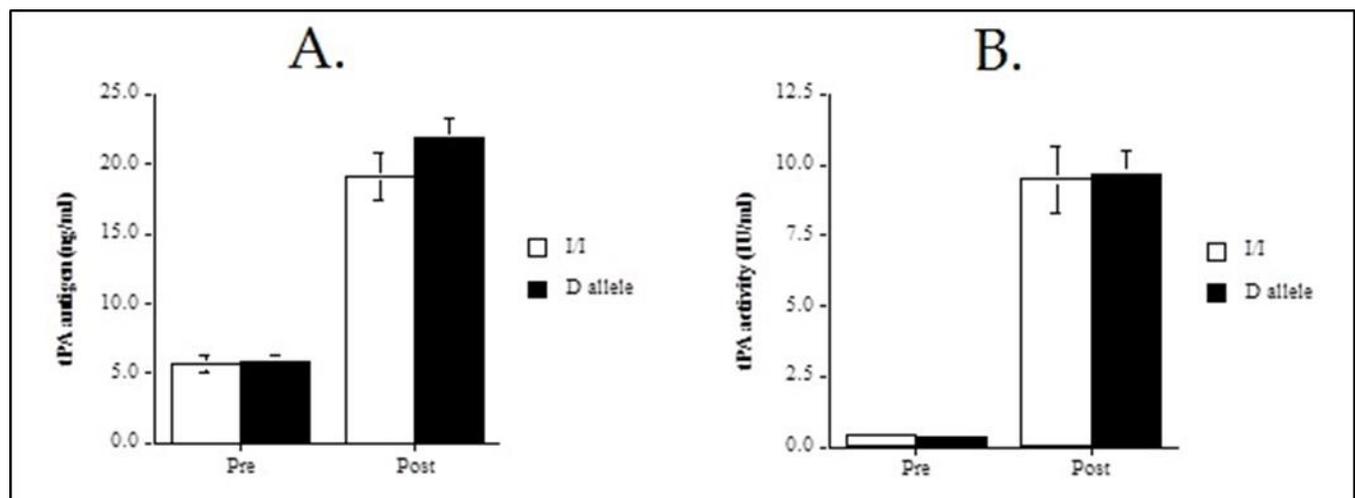

Figure 1. Pre and post-exercise values for A.) tPA antigen (ng/ml), and B.) tPA activity (IU/ml). D allele (containing the D allele as either DD or ID), and I/I (homozygous for the I allele).

DISCUSSION

The major finding of the present study is that individuals possessing at least one D allele of the tPA gene do not differ in tPA antigen or tPA activity response to maximal exercise from individuals that are homozygous I. Previous data showed no association between I/D polymorphism and resting plasma tPA antigen (8, 27) nor resting plasma tPA activity (27). Similarly, resting tPA antigen and activity were similar across groups in this study. Although previous data suggests a higher resting tPA release rate in individuals with the I/I genotype (9, 12), tPA release did not translate into significant differences in tPA antigen or activity. This lack of tPA difference is presumably due to the release of tPA being balanced by inactivation and increased clearance. However, exercise results in rather large increases in tPA release, which was hypothesized to result in a higher post-exercise plasma tPA in individuals homozygous for the I allele. However, these data suggest an identical exercise response for individuals homozygous for the I allele and individuals possessing the D allele.

Our results suggest the increased release of tPA occurring in subjects homozygous for the I allele is not biologically significant since we found no difference between genotype groups in plasma tPA. Another possibility is increased tPA release during exercise occurs from other sources, and the I/D polymorphism is only associated with differences in endothelial tPA release. Wang et al. (28) observed that tPA is also synthesized and released from sympathetic nerve terminals in the rat. Furthermore, skeletal muscle has been identified as a source of tPA synthesis (7). Therefore, the D allele of the tPA I/D polymorphism may be associated with a decreased endothelial cell release of tPA at rest, but this difference may be offset by tPA release from other sources. This possibility is speculative and future research should ascertain the role of this polymorphism on tPA release from different sites in response to acute exercise.

It is also possible the tPA I/D polymorphism did not influence tPA release in our subjects. However, Jern et al. (9) reported greater release of tPA in homozygous I subjects at rest in 51 apparently healthy, non-obese, nonsmoking, male subjects. Considering subjects in the present study were very similar to those studied by Jern et al. (9), it is likely that the subjects in the current study in the I group also had a higher resting release rate of tPA. However, unlike the Jern et al. (9) article this cannot be proven with the present data, as we did not measure release of tPA. Even so, Jern et al. (9) found that there was no correlation between the net tPA release rate and the arterial plasma level of either tPA antigen or tPA activity. Data from the present study suggest that, even if the release of tPA during exercise is higher in subjects homozygous for the I allele, it is not associated with plasma tPA levels.

If the regulation of tPA levels is not explained through the I/D polymorphism, other mechanisms must be explored. Moore et al. (15) found that plasma tPA levels are influenced by the angiotensin-converting enzyme (ACE) I/D and plasminogen activator inhibitor-1 (PAI-1) 4G/5G polymorphism. Once tPA is bound to PAI-1 it has a slower clearance rate, which can elevate tPA antigen levels, and increase tPA release. Pretorius et al. (19) demonstrated that ACE inhibition increases constitutive endothelial tPA release through endogenous bradykinin. It is

also possible that acute tPA responses by mechanisms affecting the release of stored tPA and regulation of tPA may only affect restocking of tPA stores and not the release and/or circulating levels (19).

Giri et al. (5) observed that myocardial infarctions resulting from physical exertion were more frequently due to occlusive thrombus formation than infarctions resulting from other triggers. Specifically, all 64 out of the 64 of the patients with an exertion-related acute MI were reported to having an intracoronary thrombus ≥2 mm. This compared to only 35 out of 576 patinets that experienced a non-exertion-related acute MI, a reported p-value of 0.001. Thus, coagulation and/or fibrinolytic responses to physical stress may have important clinical significance. van der Bom et al. (27) noted an increased risk MI in individuals with the I/I genotype. This finding is in contrast to the study by Jern et al. (9) who found no association between genotype and risk of MI. Findings by Hooper et al. (8) showed a nonsignificant positive association between the D allele and MI in African American adults. Regardless, results from the present study suggest that any association between the tPA I/D polymorphism and risk of MI is not likely due to a suppressed tPA response to exercise in subjects with the D allele. The subjects in the present study were all sedentary males and predominantly Caucasian. Therefore, the results cannot be generalized across genders, physical training status, or race. Maximal aerobic exercise was utilized in the current study to elicit a tPA antigen and tPA activity response, a known effect on increasing plasma tPA antigen and activity (2, 18). While acute, maximally intense exercise did not elicit a difference in the change in plasma levels of tPA antigen nor tPA antigen between groups, future research should determine the effects of varying levels of duration of physical activity on tPA response between the tPA I/D genotypes.

In conclusion, the I/D polymorphism of the tPA gene does not influence the tPA antigen nor the tPA activity responses to maximal exercise in healthy, young, sedentary males. Future studies should evaluate mechanisms of tPA release, including the influence of other polymorphisms on the tPA response to exercise.

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