Platelet Activation in Essential Hypertension During Exercise: Pre- and Post-Treatment Changes With an Angiotensin II Receptor Blocker

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BACKGROUND
Acute exercise may exert deleterious effects on the cardiovascular system through a variety of pathophysiological mechanisms, including increased platelet activation. However, the degree of exercise-induced platelet activation in untreated hypertensive (UH) individuals as compared with normotensive (NT) individuals has yet to be established. Furthermore, the effect of antihypertensive treatment on exercise-induced platelet activation in essential hypertension (EH) remains unknown.

METHODS
Study 1 consisted of 30 UH and 15 NT subjects. UH subjects who received treatment were included in study 2 and were followed-up after a 3-month treatment period with an angiotensin II receptor blocker (ARB; valsartan). Circulating monocyte–platelet aggregates (MPA) and platelet P-selectin were measured as platelet activation markers at baseline, immediately after a treadmill exercise test, and 10, 30, and 90 minutes later.

RESULTS
Maximal platelet activation was observed at 10 minutes after peak exercise in both groups. In UH subjects, MPA levels remained increased at 30 minutes after peak exercise, despite BP fall to baseline levels. MPA levels were significantly higher in UH subjects than NT subjects at maximal exercise and at 10 and 30 minutes of recovery. Post-treatment MPA levels increased significantly only at 10 minutes into recovery and were similar to those of NT subjects.

CONCLUSIONS
Acute high-intensity exercise exaggerates platelet activation in untreated patients with EH compared with NT individuals. Angiotensin II receptor blockade with adequate BP control greatly improves exercise-induced platelet activation in EH. Further studies are needed to clarify whether this phenomenon depends purely on BP lowering or benefits also from the pleiotropic effects of ARBs.

Keywords: blood pressure; essential hypertension; exercise; hypertension; monocyte-platelet aggregates; P-selectin; platelet activation.

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Regular physical activity of at least 30 minutes most days a week exerts beneficial cardiovascular effects and is recommended by the European Society of Hypertension7 and American Society of Hypertension,8 with the elderly included in these recommendations.9 Nevertheless, acute physical activity is a known trigger of acute cardiac events.10 This phenomenon may partially be attributed to the fact that acute and dynamic exercise induces platelet activation, as shown by increased mean platelet volume,11 β-thromboglobulin and thromboxane B2 levels,12,13 platelet aggregation,14,15 expression of platelet P-selectin,16,17 and platelet–leukocyte aggregates,18,19 leading to increased thrombotic events. Whether platelet activation in response to exercise is exaggerated in untreated

Blood pressure (BP) levels are directly associated with cardiovascular and overall mortality,1 making essential hypertension (EH) a major public health problem that leads to >7 million deaths annually worldwide.2 Increased platelet activity has been documented in EH and represents an important factor contributing to the prothrombotic state characterizing the disease.3 Among the established methods that estimate platelet activation, the measurement of monocyte–platelet aggregates (MPA) represents the most sensitive and robust marker in several clinical settings.4,5 including EH.6 We have recently shown that MPA levels are higher in untreated subjects with EH and correlate independently with the levels of both systolic BP (SBP) and diastolic BP (DBP), but especially with SBP.5

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patients with EH, compared with normotensive people, is currently unknown.

Thus, the objectives of our study were, first, to estimate the degree of platelet activation before, during, and after high-intensity exercise and its relationship to the BP response in subjects with untreated EH as compared with normotensive individuals and, second, to investigate whether antihypertensive treatment reduces exercise-induced platelet activation in EH.

METHODS

Study population

Study 1. Our study population consisted of consecutive patients attending the Hypertension Unit of the 2nd Propedeutic Department of Internal Medicine, Thessaloniki, Greece, during a 6-month period (January 2011–June 2011). Healthy individuals were recruited from the community during the same period. Based on initial estimations, total sample size required was calculated at 40 subjects.

The study’s inclusion and exclusion criteria were the following:

1. All subjects were aged 18–65 years.
2. All subjects were clinically healthy, with no evidence from medical history and physical examination of cardiovascular disease (other than EH) or other significant co-morbidity.
3. Hypertensive individuals were recently diagnosed with essential hypertension. Secondary hypertension was excluded, when indicated, by measuring plasma renin activity, serum aldosterone, and urinary catecholamine levels. Patients with masked or white coat hypertension were excluded with the use of ambulatory BP measurement.
4. Participants had never been treated with antihypertensive agents and were not on regular treatment with lipid-lowering therapy, aspirin, nonsteroidal anti-inflammatory drugs, or other antiplatelet medication.
5. All subjects gave written informed consent, and the procedures followed were in accordance with institutional guidelines. The study was approved by the Hippokrateion Hospital Ethics Committee and was in accordance with the principles of the Helsinki Declaration.

Study 2. After completion of the study procedures, all hypertensive individuals were subsequently referred to the hypertension specialists of our clinic. When antihypertensive therapy was indicated, treatment was initiated with an angiotensin II receptor blocker (valsartan 160 mg). Patients did not receive any other medication. All patients who received antihypertensive treatment were reevaluated at a follow-up visit after a 3-month period where the same protocol was repeated.

BP measurements

Office BP was measured in the dominant arm using a validated oscillometric device (Omron 705IT, Omron Healthcare Europe BV, Hoofddorp; The Netherlands) after 15 minutes of rest in a quiet, temperature-controlled room (23 °C) in the seated position. Office BP of each subject was determined as the mean of the second and third values of 3 consecutive BP readings taken at 2-minute intervals. Ambulatory BP measurement was subsequently performed in the left upper arm using a SpaceLabs (Spacelabs Healthcare, Issaquah, WA) 90207 device according to a standard protocol: BP and heart rate were measured at 15-minute intervals during a standard working day and at 30-minute intervals during the night. Daytime and nighttime were defined according to the individual’s awake and sleeping pattern during that particular day. A minimum of 70% successful readings was required for inclusion in the study. A dipping pattern was defined as a difference in mean BP >10% between the daytime and nighttime hours. EH was defined as office BP >140/90 mm Hg and an average daytime BP on ambulatory BP measurement >135/85 mm Hg according to the European Society of Hypertension and American Society of Hypertension.

Estimation of atherosclerotic burden

Additionally, to estimate atherosclerotic burden in these subjects, measurement of carotid intima-media thickness was performed in all patients and healthy control subjects with an ultrasound imaging system (Prosound a7; Aloka Medical, Switzerland) using a 7.5-MHz linear vascular probe. Measurements were performed 1 cm below the bifurcation in the anterior and posterior wall of both left and right common carotid arteries.

Laboratory measurements

All subjects underwent a treadmill exercise test, according to the Bruce protocol. During the test, continuous electrocardiographic recording was used, and BP was measured manually every 3 minutes. Target heart rate was defined as 85% of maximal predicted heart rate for age and sex. The test was prematurely terminated if the participant was unable or refused to continue or developed significant arrhythmias, hypotension, electrocardiographic changes, or angina or where there were technical difficulties. All tests were performed at 9 AM after an overnight fast and the subject having abstained from caffeine and smoking for at least 8 hours.

With the subject in the supine position, an intravenous indwelling cannula (18 G) was inserted and isotonic saline (0.9% sodium chloride) was infused at a rate of 1 ml/min during the test. To avoid artificial platelet activation, the first 5 ml of blood were discarded, and the following 2 ml were used for all measurement. The first sample was drawn with the patient in the supine position just before the treadmill exercise (45 minutes after placement of the intravenous cannula), the second at peak exercise, and the third, fourth, and fifth at 10, 30, and 90 minutes, respectively, during the recovery period, with the subject in the supine position. These time points were selected on the basis of preliminary experiments, which clarified the time course over which circulating MPA decreased to pre-exercise test levels.

At each time point, platelet activation was assessed by measuring the expression of P-selectin on the platelet
surface and the level of MPA using whole blood double immunofluorescence flow cytometry (FC 500; Beckman Coulter, Switzerland), as previously described. Briefly, to access the monocyte population, forward and side light scatter characteristics were gated, and the population positive for both CD14 and CD42b was taken to represent MPA, which were quantified as a percentage of the total monocyte population (Figure 1). The population double-positive for CD42b and CD62P was taken to represent the platelets expressing surface P-selectin. Preliminary experiments showed a satisfactory coefficient of variation. Mean intra- and interassay coefficients of variation were determined at 16.0% and 12.0%, respectively.

**Statistical analysis**

Analysis was performed using the Statistical Package for Social Sciences 15.0 for Windows (SPSS, Chicago, IL). Results are presented for continuous variables as mean ± SD (or for non-normal variables as median ± interquartile range) and for qualitative variables as frequencies. Statistical analyses were carried out using the independent samples Student t test or the Mann–Whitney U test to compare differences between mean values between the 2 groups and repeated measures analysis of variance (with Bonferroni post hoc test) or Friedman test to compare differences within the same group at different time points. Logarithmic

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**Figure 1.** Quantification of monocyte-platelet aggregates by double immunofluorescence flow cytometry at different time points in a hypertensive subject pre- and post-treatment (untreated hypertensive (UH) and treated, respectively) and a normotensive (NT) individual. Abbreviations: 10R, 10 minutes into the recovery period; 90R, 90 minutes into the recovery period.
transformation was performed when appropriate, where data were non-normally distributed. Multivariable analysis (binary logistic regression) was used when appropriate. A probability value of $P < 0.05$ was considered statistically significant.

**RESULTS**

**Demographics**

Overall, 30 patients with untreated EH (UH) and 15 age- and sex-matched normotensive (NT) subjects were eligible for and agreed to participate in the study. No significant differences were observed between the 2 groups in regard to smoking status, body mass index, lipid profile (total, high- and low-density lipoprotein cholesterol, and triglycerides) and glucose levels, as shown in Table 1. All of the study participants reported an alcohol intake of <5 units/week (1 unit = 12 mg of alcohol). As expected, UH subjects had significantly higher SBP and DBP (both office and ambulatory) compared with NT subjects.

Carotid intima-media thickness in UH subjects was similar to carotid intima-media thickness in NT subjects ($0.62 \pm 0.15$ mm vs. $0.51 \pm 0.10$ mm and $0.47 \pm 0.09$ mm for left and right common carotid arteries, respectively; $P = 0.09$ for each), and in all cases was <0.9 mm, indicating that these subjects had no significant atherosclerotic burden, at least at the level detectable by carotid intima-media thickness.

**Baseline exercise test**

All study participants achieved target heart rate, with no reasons for premature termination of the exercise test. Maximum metabolic equivalents were no different between the 2 groups ($9.68 \pm 1.82$ in UH subjects vs. $10.2 \pm 1.67$ in NT subjects; $P = 0.33$), indicating that all participants had successfully performed high-intensity exercise.

SBP increased significantly at maximal exercise both in UH and NT subjects, whereas DBP remained unchanged in both groups. SBP had returned to baseline levels at 10 and 30 minutes after exercise in both groups. At all time-points, SBP and DBP levels were significantly higher in UH subjects compared with NT subjects.

Maximal platelet activation was observed at 10 minutes after peak exercise in both groups. In UH subjects, MPA levels increased significantly at maximal exercise compared with baseline and remained increased at both the 10-minute and 30-minute time points during recovery, even though SBP levels were back to baseline levels at these times. By contrast, NT subjects exhibited significantly increased MPA levels at the 10-minute but not at the 30-minute time point during recovery. Between groups, MPA levels were significantly higher in UH subjects than NT subjects at maximal

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Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NT, normotensive subject; SBP, systolic blood pressure; UH, untreated hypertensive subject.
exercise, and at 10 and 30 minutes of recovery, as shown in Figure 2.

Platelet P-selectin expression also increased during exercise, both in UH and NT subjects. However, at no time point were significant differences found between UH and NT subjects.

Three-month follow-up exercise test

Seventeen subjects received antihypertensive treatment with an angiotensin receptor blocker (valsartan 160 mg) and were followed up after a 3-month period. Subjects who received treatment did not present any significant differences in baseline characteristics as compared with those who did not receive treatment. There were no treatment side-effects. All treated subjects achieved adequate BP control (office SBP/DBP: 123.7 ± 9.8/82.7 ± 11.3 mm Hg; 24-hour SBP/DBP: 125.7 ± 6.5/78.7 ± 8.2 mm Hg; daytime SBP/DBP: 129.8 ± 7.4/83.7 ± 6.7 mm Hg; dipping 11.3% ± 7.8%).

During exercise at the follow-up visit, SBP levels at maximal exercise and 10 minutes into recovery were significantly increased compared with baseline levels, whereas DBP levels remained unchanged. However, BP levels at all time points were significantly decreased compared with pretreatment levels. In regard to platelet activation, MPA levels increased significantly only at 10 minutes into recovery, compared with baseline levels, indicating the absence of prolonged platelet activation in treated hypertensives. Compared with pretreatment levels (Figure 3), a significant decrease of MPA levels was observed at maximal exercise and 10 and 30 minutes of recovery. Interestingly, the post-treatment MPA response during the exercise test was similar to the MPA response of normotensive subjects. More specifically, when MPA values in hypertensive subjects post-treatment were compared with those of NT subjects, the pattern of activation was similar, with significantly increased MPA levels only at 10 minutes after exercise as compared with baseline in both groups.

Platelet P-selectin levels increased significantly in hypertensive subjects after treatment at peak exercise and at 10 and 30 minutes of recovery. Nevertheless, the values at these time points were significantly decreased compared with pretreatment levels, as shown in Figure 4. Of note, as with

Figure 2. Circulating monocyte–platelet aggregate (MPA) levels in untreated hypertensive (UH) and normotensive (NT) subjects in response to high-intensity exercise. \*P < 0.05 vs. NT. Abbreviations: 10R, 10 minutes into the recovery period; 30R, 30 minutes into the recovery period; 90R, 90 minutes into the recovery period.

Figure 3. Circulating monocyte–platelet aggregate levels in hypertensive patients pre- and post-treatment in response to high-intensity exercise. \*P < 0.05 vs. post-treatment. Abbreviations: 10R, 10 minutes into the recovery period; 30R, 30 minutes into the recovery period; 90R, 90 minutes into the recovery period; UH, untreated hypertensive subject.
circulating MPA levels, no difference was observed between expression levels of platelet P-selectin in hypertensive subjects after treatment and normotensive subjects.

**DISCUSSION**

This study was conducted in patients with a recent diagnosis of EH who were on no medical treatment and had no clinically detectable atherosclerosis. We found that high-intensity acute exercise induces platelet activation both in UH patients and in healthy individuals. However, UH patients experience a prolonged and significantly higher increase of MPA levels, suggesting exaggerated platelet reactivity in response to exercise as compared with NT control subjects. Furthermore, antihypertensive treatment with renin-angiotensin system (RAS) antagonists reduced MPA to similar levels to those found in NT individuals, indicating that high BP is an important factor contributing to this phenomenon.

High-intensity acute exercise may exert deleterious effects on the cardiovascular system through a variety of pathophysiological mechanisms that involve sympathetic nervous system (SNS) activation and changes in coagulation and fibrinolysis and platelet activation. In particular, acute exercise is a well-established trigger of increased SNS activity, which in turn leads to an increase in catecholamine levels, which has also been documented in patients with untreated EH. Plasma adrenaline and noradrenaline levels decreased after exercise, falling to almost pre-exercise levels at the end of the recovery period. In addition, several studies in healthy individuals and in patients with cardiovascular disease indicate that high-intensity acute exercise induces platelet activation, as shown by increased mean platelet volume, p-selectin, β-thromboglobulin and thrombomodulin B2 levels, platelet aggregation, expression of platelet P-selectin, and platelet–leukocyte aggregates.

High-intensity acute exercise may increase platelet reactivity in UH patients through a number of pathophysiological mechanisms. Studies have documented that even an acute rise in BP leads to platelet activation mainly through P-selectin expression on the platelet surface and β-thromboglobulin secretion, possibly through increased shear forces on the platelet surface. Additionally, enhanced SNS activity and the resultant increase in catecholamine levels may directly induce platelet activation through binding of noradrenaline to platelet α1-adrenergic receptors. Enhanced SNS activity is known to occur in the early stages of EH and is also intensified during acute exercise. Indeed, in a previous study conducted in our department applying the same methodology in newly diagnosed untreated hypertensive patients, we showed a 10-fold increase of plasma noradrenaline and noradrenaline levels at exercise peak, which then returned to baseline levels at the end of the recovery period (10 minutes after maximal exercise). Post-treatment changes of plasma catecholamine levels during exercise followed the same pattern but were significantly decreased compared with pretreatment values. The hypothesis that catecholamine secretion may be implicated in exercise-induced platelet activation is also supported by a study from Li et al., who documented exercise-induced platelet activation in healthy normotensive individuals by measuring circulating platelet–platelet microaggregates and platelet–leukocyte aggregates, a phenomenon which was independent of thrombin because thrombin inhibition by argatroban or enoxaparin failed to counteract it. Therefore, the authors concluded that other pathophysiological mechanisms, such as enhanced SNS activity, may at least partially mediate this phenomenon.

Our observation that platelet reactivity was found to persist despite the rapid BP fall to pre-exercise levels is in accordance with this hypothesis. This may be attributed to the longer-standing effects on platelet activity of both high BP and circulating catecholamines levels. Upon platelet activation, primary aggregation occurs through the binding of fibrinogen to its platelet receptor, glycoprotein IIb-IIIa, followed by secondary irreversible aggregation due to the platelet release reaction from dense and α-granules, leading to stable thrombus formation. The molecular mechanisms...
responsible for the progression of aggregation involve so-called “inside-out signaling” due to the stimulation of platelet receptors, which is an ongoing self-limited process. Although the half-life of catecholamines is very short (1–2 minutes), the duration of platelet activation caused by their release is likely to be longer.

This study demonstrates for the first time a significant reduction of exercise-induced platelet activation in patients with adequate BP control after treatment with an angiotensin II receptor blocker (valsartan 160 mg). Apart from the hemodynamic effect of BP lowering, inhibition of the RAS has also been shown to decrease SNS discharge. Underlying mechanisms involve reduced noradrenaline release from adrenergic nerve terminals and increased responsiveness of adrenergic receptors. It is likely that the above multiple actions of RAS inhibitors, including angiotensin II receptor blockers, may account for the beneficial effects observed on platelet activity. Interestingly, however, a recent study in a small heterogeneous group of previously treated hypertensive subjects failed to show a significant reduction of exercise-induced platelet activity (measured only by platelet P-selectin and platelet–platelet aggregates levels) after a 2-month treatment with RAS inhibitors.

Platelet activation is a well-described phenomenon in patients with essential hypertension. Circulating MPA levels represent a reliable marker of platelet activation in the clinical setting, as confirmed by a recent study by Burdess et al. This study compared the reproducibility and correlation indices of several markers of platelet activation (MPA, platelet P-selectin, platelet CD40L, platelet-derived microparticles, monocyte CD40, and monocyte 11b) in patients with peripheral vascular disease. MPA levels proved to be the marker of choice in the clinical setting, characterized by good reproducibility and correlation with all the other markers of platelet and monocyte activation. Our study bears out this finding, as MPA levels were more sensitive than platelet P-selectin expression in detecting differences between UH and NT subjects. Although platelet P-selectin levels were numerically higher in UH subjects than NT subjects during exercise, these differences did not reach significance.

Our study has some limitations. First, the patients included in the study were relatively young (mean age = 42.7 ± 6.2 years), untreated hypertensive patients with a recent diagnosis of EH and no comorbidities. Thus, our findings are relevant only to the first stages of EH, and care should be taken in extrapolating these findings to the whole hypertensive population. Second, given that all individuals studied were relatively sedentary, the effects of acute high-intensity exercise in individuals exercising regularly remain unknown. Third, the effects of different RAS blockers or antihypertensive agents of different classes on exercise-induced platelet activation remain to be further investigated. Finally, it should be noted that flow cytometry is limited in availability and relatively high in cost, thereby limiting its use in everyday clinical practice as a marker of platelet activation.

To our knowledge, this is the first study to document that acute high-intensity exercise exaggerates platelet activation in untreated patients with EH compared with NT individuals. This phenomenon may, at least in part, explain the deleterious effects of acute intense exercise in the cardiovascular system—namely, the high incidence of thrombotic cardiac events in both apparently previously healthy individuals and patients with cardiovascular disease. Our study also indicates that antihypertensive treatment with adequate BP control greatly improves exercise-induced platelet activation in hypertensive patients. Whether this is purely dependent on BP lowering or on the specific drug used remains to be investigated. It is possible that the pleiotropic effects of angiotensin II receptor blockers, as used here, may be more beneficial than other antihypertensive drug types. However, this is a hypothesis which needs to be investigated in future studies.

ACKNOWLEDGMENT
E. Gkaliagkousi and E. Gavriliaki contributed equally to this study.

DISCLOSURE
The authors declared no conflict of interest.

REFERENCES


