Published in final edited form as:

Psychophysiology. 2009 March ; 46(2): 276–284. doi:10.1111/j.1469-8986.2008.00779.x.

Prolonged Platelet Activation in Individuals with Elevated Blood Pressure in response to a Moderate Exercise Challenge

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Abstract

We examined the magnitude of 20-min moderate exercise-induced platelet activation in 50 volunteers with normal (n= 31) or elevated BP (EBP; n=19). Blood was drawn before, immediately after and 25-min after exercise. Antibody-staining for platelet activation markers P-selectin and fibrinogen receptors was done with and without adenosine diphosphate (ADP) stimulation in whole blood for flow cytometric analyses. Exercise led to increases in % aggregated platelets and % platelets expressing P-selectin or PAC-1 binding (p 's ≤ 0.001). This increase in % platelets expressing P-selectin continued even after 25-min rest only in the EBP group ($p \leq 0.01$) accompanied by an increase in % aggregated platelets ($p \leq 0.05$). Although ADP stimulation led to increased platelet activation at rest, it was attenuated following exercise, even among EBP individuals. A moderate exercise challenge induced prolonged platelet activation in individuals with EBP but attenuation in activation to further stimulation by an agonist. Findings suggest that a recovery period after physical stress appear critical in individuals with high BP regarding platelet activation and aggregation, which can lead to an acute coronary syndrome in vulnerable individuals.

Keywords

high blood pressure; PAC-1; physical stress; platelets; P-selectin; sympathetic nervous system

Introduction

Epidemiological studies indicate that an episode of vigorous exercise can trigger an acute coronary syndrome in susceptible individuals (Siscovick, Weiss, Fletcher & Lasky, 1984). On the other hand, regular exercise reduces cardiovascular risk, including thrombotic risk (Arraiz, G. A., Wagle, D. T., & Mao, 1992; Morris, Everitt, Pollard, Chave, & Semmence, 1980; Paffenbarger, Hale, & Hyde, 1975; Yamamoto, Lizumi, Hirota, Shimonaka, Nagamatsu, Horie, & Morita, 1989). Platelet activation leads to their aggregation and coagulation and thus, is a critical step resulting in thrombus formation and possible subsequent cardiovascular insult. Many studies demonstrate that acute strenuous exercise leads to an increase in platelet activation, adhesiveness and aggregability: bicycle or treadmill exercise until exhaustion or anaerobic threshold was associated with platelet activation indicated by increased platelet P-selectin expression after incubation with platelet

agonist TRAP-6 (Hilberg, Gla, Schmidt, Losche, Franke, Schneider & Gabriel, 2003). Acute exercise also led to enhanced platelet intracellular calcium concentration and aggregation in response to an agonist collagen (Kasuya, Kishi, Sakita, Numano, & Mitsuaki, 2002), decreased sensitivity to nitric oxide (Sakita, Kishi, & Numano, 1997), and increased adhesiveness to fibrinogen and sensitivity to agonists adenosine diphosphate (ADP) and clonidine (Wang & Cheng, 1999).

Studies of exercise and platelet activation help delineate pathways of thrombosis that may underlie the epidemiological findings of increased risk of a cardiovascular event during strenuous physical exertion in some individuals, including those with hypertension. Markers of platelet aggregation/aggregability at rest (Winther, Gleeup & Hedner, 1991) and in response to ADP (Vlachakis & Aledort, 1979), handgrip exercise (Vlachakis & Aledort, 1980), or bicycle ergometer exercise (Lemne, Vesterqvist, Egberg, Green, Jogestrand & de Faire, 1992) are greater in individuals with hypertension, and these can be reduced by antihypertensive medications (Winther et al., 1991), although there remain conflicting findings (Jimenez, Tofler, Chen, Stubbs, Solomon & Muller, 1993).

This effect of physical exertion on platelet activation is, in part, mediated by sympathetic nervous system (SNS) activation and increased levels of circulating catecholamines (Ikarugi, Taka, Nakajima, Noguchi, Watanabe, Sasaki, Haga, Ueda, Seki & Yamamoto, 1999; Kawano, Nobuo, Homori, Kawano, Maki, Kimura, Yanagisawa, Ohsaki, Takahashi, Shiohara, Ishikawa & Yoshino, 2000; von Kanel & Dimsdale, 2000). Acute exercise, even moderate, leads to a significant increase in circulating levels of plasma catecholamines (e.g., Dimsdale & Moss, 1980). Epinephrine (epi) is a weak platelet agonist, ineffective alone, but significantly potentiates the effects of other agonists such as ADP and thrombin (e.g., Keraly, Kinlough-Rathbone, Packham, Suzuki & Mustard, 1986). Both exercise and epinephrine infusion enhanced platelet fibrinogen binding in response to ADP and thrombin (Wallen, Goodall, Nailin & Hjemdahl, 1999). Platelets express α_2 -adrenoreceptors, and *in vitro* activation of platelets are mediated by α_2 receptors (Grant & Scrutton, 1979). Epi-induced increase in platelet aggregation is abolished by non-selective β blockade (von Kanel & Dimsdale, 2000), and metoprolol, a selective β_1 -blocker, abolishes exercise-induced platelet activation, indicating that β -adrenoceptors are also involved (Kawano et al., 2000). Studies also suggest the role of increased sheer stress in exercise-induced platelet activation but with mixed findings (Ersöz, Zergeroglu, Fiçicilar, Ozcan, Oztekin, Aytay, & Yavuzer, 2002; Paszkowiak & Dardik, 2003).

Platelet activation can be detected by assessing expression of surface markers. One of cell adhesion molecules P-selectin is detected on the outer membrane of platelets upon stimulation, which results in an increased rate of fibrin formation and continued platelet activation (Larsen, Celi, Gilbert, Furie, Erban, Bonfanti, Wagner & Furie, 1989). Fibrinogen facilitates the formation of platelet-platelet aggregates (Shattil, Kashiwagi & Pampori, 1998) therefore, an increased platelets with activated fibrinogen binding sites may help promote the hypercoagulable state following exercise (El-Sayed, 1996). Monoclonal antibody PAC-1, binds to the fibrinogen binding site on the GP IIb-IIIa complex (surface receptor for adhesion) of the activated platelet surface (Shattil, Cunningham, & Hoxie, 1987). Flow cytometry is a sensitive and reliable method to directly measure expression of these activation-dependent surface markers on platelets in whole blood with minimal handling, greatly reducing the chances of *in vitro* platelet activation (Kestin, Ellis, Barnard, Errichetti, Rosner & Michelson, 1993; Schmitz, Rothe, Ruf, Barlage, Tschope, Clemetson, Goodall, Michelson, Nurden & Chankey, 1998).

We used flow cytometry to examine the effect of 20-minute moderate exercise on platelet activation in individuals with normal vs. elevated BP by assessing surface markers P-

selectin and PAC-1 binding and platelet aggregation. We also examined platelet activation 25-minutes after exercise since few studies have examined possibly persistent platelet activation following moderate exercise in individuals with elevated BP (EBP). We hypothesized that 1) overall, platelet activation will increase after a 20-min of moderate exercise bout and 2) elevated BP group will show a greater platelet activation responses after exercise as compared to those in the normal BP (NBP) group, which may be mediated in part by circulating catecholamine levels.

Methods

Participants

Fifty-seven healthy men and women aged 20–55 ($M \pm SD = 39 \pm 10.3$) with “normal” (screening Systolic BP (SBP) ≤ 129 and/or Diastolic BP (DBP) ≤ 84 mmHg; $n = 35$) or “elevated” (screening SBP ≥ 130 and/or DBP ≥ 85 mmHg; $n = 22$) BP¹ were recruited from the local community via various advertisements (e.g., newspaper, flyers, brochures, and websites) and word of mouth referrals for a larger study of sympathetic nervous system regulation of cellular adhesion of immune cells. Platelet activation data were not collected for six participants because of technical difficulties (i.e., problems with blood draws for five and antibody staining error for flow cytometry for one), and one participant with elevated BP did not complete the exercise protocol due to a complaint of over-exertion. Thus, four and two individuals were in the normal and elevated BP groups, respectively among the six participants who were excluded from the data analyses. Thus, data from 50 individuals were included in this investigation. The protocol was approved by the University of California, San Diego (UCSD) Human Research Protections Program, and participants gave written informed consent. Screening BP was measured using a Dinamap Compact BP[®] monitor (Critikon, Tempa, FL) to confirm normal or elevated BP and defined as the average of three seated blood pressure measures on two separate days taken in the right arm after having rested for 5 minutes.

Inclusion criteria were: 80–150% of ideal body weight, and being healthy (no medical symptoms or conditions other than high BP). A history and physical examination was performed by a licensed physician, a normal electrocardiogram was confirmed, and a blood test for metabolic, liver, and thyroid panels was done. Exclusion criteria were: a history or signs of heart disease, liver or renal disease, diabetes, psychosis, severe asthma, pregnancy, morbid obesity, and current use of prescription medication except for anti-hypertensive medication. Human chronic gonadotropin screening was also done for women of childbearing age to confirm absence of pregnancy. Participants were instructed to abstain from any medications for a week minimum before the exercise test. For six participants who were taking anti-hypertension medications, they were carefully tapered off followed by a three-week wash-out period before the protocol began. Approval had been obtained from each patient’s physician for stopping anti-hypertension medications, and their BP was monitored on a daily basis throughout the duration of the study to ensure safety.

Exercise Testing

In order to determine each individual’s cardiovascular fitness, peak oxygen consumption (VO_{2peak}) was assessed by having participants exercise on a treadmill (TMX 425C;

¹We categorized “normal” as BP ≤ 129 and/or 84 mmHg and “elevated” as BP ≥ 130 and/or 85 mmHg according to the 2003 Guidelines for Management of Hypertension by the European Society of Hypertension (ESH) (Guidelines Committee, 2003 European Society of Hypertension – European Society of Cardiology guidelines for the management of arterial hypertension, 2003). The “elevated BP” group included those with elevated SBP and/or DBP and mild hypertension (subjects’ SBP/DBP range: 130–166/66–100 mmHg; designated as “high normal” and “mild hypertension” by the ESH Guidelines). The “normal BP” group included individuals who would be categorized as “optimal” and “normal” according to the ESH Guidelines.

Trackmaster Fitness, Eastlake, OH) until full exertion (voluntary cessation) using the standard Bruce protocol where the speed and grade of the treadmill increased gradually from 1.7mph and 10% every three minutes. Treadmill exercise was selected for its familiar motion (walking) to all participants regardless of their fitness level. Subjects' expired gas was analyzed by Sensormedics metabolic cart (Sensormedics, Yorba Linda, CA) equipped with Vmax software (version 6-2A), and ECG was recorded using Marquette CardioSoft V. 3 (GE medical systems, Milwaukee, WI). Oxyhemoglobin saturation (SpO₂) was monitored using pulse oximetry (Ohmeda, Datex, Louisville, CO), and perceived effort during the exercise was recorded using Borg's 6-20 scale ratings of perceived exertion (RPE; Borg, 1970).

About a week later, participants returned between 8:00 and 8:30 am and exercised on the treadmill for 20 minutes at 65–70% of their VO_{2peak}. Blood for platelet assays and catecholamines was collected before and 0 minute and 25 minutes after exercise (after the 2-minute cool-down of walking at 1.5 mph on the treadmill) in supine through an intravenous (i.v.) catheter inserted into an antecubital vein using minimal tourniquet. Participants refrained from consuming nicotine, caffeine, alcohol, and vigorous exercise 24-hours prior to the exercise test.

Hemostasis Measures

Blood used to determine platelet count (PLT), platelet size as per mean platelet volume (MPV), and hematocrit (Hct) was drawn in EDTA. The complete blood count with differential was analyzed at the UCSD Medical Center Hematology Laboratory.

Platelet Activation by Flow Cytometry

Blood was drawn in tubes with acid citrate dextrose (ACD) to determine expression of P-selectin and PAC-1 binding on the platelet surface. In order to minimize artificial activation of platelets, i.v. lines were flushed with normal saline only, and the first two cc's of blood drawn were discarded. Blood was kept at room temperature and the antibody-staining process began within 10 minutes of collection at each time point to minimize effects of time (between blood draw and sample processing). A whole blood method was employed, eliminating erythrocyte lysis, centrifugation, and washing, further avoiding artificial platelet activation from handling. Whole blood (5 µl) was incubated with fluorochrome-conjugated antibodies (Becton Dickinson Immunocytometry Systems, San Jose, CA) at predetermined saturating concentrations (30 µl) in polystyrene tubes for 15 minutes at room temperature in the dark. The specific antibody-fluorochrome conjugates used were PAC-1-FITC, CD62P-PE, and CD61-PerCP (an activation-independent platelet marker). IgG1-PE isotype mAb and RGDS (a peptide blocker of the fibrinogen binding site on the GP IIb-IIIa complex) were used to control for non-specific binding. A portion of blood samples at each time point was also stimulated with ADP (4 µM, final concentration) prior to immunostaining.

Samples were fixed with 1 ml of 1% formaldehyde in PBS containing 0.1% NaN₃ to avoid further activation or aggregation of platelets and analyzed on a Coulter EPICS Elite flow cytometer within 24 hours. Platelets were identified by and distinguished from red blood cells by the presence of the CD61 antigen. Single vs. aggregate platelets were then discriminated by size on forward and side scatter plots (smaller vs. larger groups identified as single vs. aggregate platelets, respectively) and separately gated in order to examine surface activation marker levels (fibrinogen binding sites and P-selectin) of single vs. aggregate platelets (Figure 1)². This size-dependent discrimination of platelets has been used in previous studies (Ault, Rinder, Mitchell, Rinder, Lanbren & Hillman,; Frojmovic, Wong, & van de Ven, 1991). PAC-1 binding and P-selectin expression were then, calculated as % of total single or aggregate platelet numbers. In addition, average per platelet levels of

PAC-1 binding and P-selectin were estimated by mean fluorescent intensity (MFI). PAC-1 and P-selectin expression and MFI and % aggregate platelets are all indicative of platelet activation. Ten thousand gated (platelets identified by CD61 positive staining) events were collected per tube.

Catecholamines

Blood for catecholamines was preserved in EDTA on ice before and after the task and after a 25-min quiet rest. After centrifugation in a refrigerated centrifuge, plasma was stored at -80°C until the assays were done. Epinephrine (Epi) and norepinephrine (NE) were determined by radioenzymatic assay as described previously (Kennedy & Ziegler, 1990). The intra- and inter-assay coefficients of variation for the assay were 6.5% and 11% respectively. Catecholamine data were log-transformed for normalization prior to statistical analyses.

Statistical Analyses

All analyses were performed using SPSS 14.0 (SPSS, Inc., Chicago, IL). The main analysis was done using two separate ANOVA. First, data were analyzed using repeated measures ANOVA to examine overall Time effects for pre, post, and 25-min post exercise measurements to test the first hypothesis (*'platelet activation will increase after 20-min of moderate exercise regardless of the BP levels'*). Secondly, 3 Time (pre, post, and 25-min post exercise)-by-2 Group (NBP vs. EBP) repeated measures ANCOVA, controlling for age, body mass index (BMI), and cardiovascular fitness ($\text{VO}_{2\text{peak}}$) was performed to examine Time-by-Group interaction for the second hypothesis (*'the EBP group will show a greater platelet activation responses after exercise as compared to the NBP group'*). F and P values were based on linear or quadratic function as appropriate based on the polynomial expansion since the platelet activation in response to a stressor mostly followed the quadratic function (i.e., increase and decrease or plateau). The data were transformed to achieve normal distribution as needed. As a secondary analysis, a series of multiple regression was performed to further examine the effects of BP groups (block 3) on post-exercise platelet activation after controlling for demographic variables (block 1: age, BMI, and fitness) and pre-exercise values (e.g., block 2: % aggregates pre exercise).

In addition, Epi and NE responses to exercise were also analyzed by 3 Time-by-2 Group repeated measures ANCOVA, controlling for age, BMI, and fitness. Multiple regression analyses were performed to further examine the effects of catecholamines (block 4) on platelet activation in response to exercise after controlling for demographic characteristics (block 1), pre-exercise platelet activity values (block 2), and BP groups (block 3).

Results

Demographic Characteristics & Exercise Responses

Twenty-nine men and 21 women completed the protocol. Among them, 31 individuals had normal BP, and 19 individuals had elevated BP. Ethnic composition was following: White (58%), Black (18%), non-white Hispanic (4%), Asian (12%), and other (8%). Table 1 presents demographic characteristics and metabolic responses to the exercise challenge in the NBP vs. EBP individuals. Compared to the NBP group, the individuals with EBP were 12 years older on average ($p \leq 0.001$) and exhibited significantly higher resting SBP and

²We have sorted platelets based on this gating of size discrimination using FacStar Plus flow cytometer equipped with Win-Fcm software (BD Immunocytometry Systems, San Jose, CA). Two platelet populations were collected in PBS with 10% fetal calf serum and examined under a Olympus BX50 fluorescent microscope and two populations were visually verified as single vs. aggregate platelets.

DBP as compared to the normal BP group, by definition ($p \leq 0.01$). The EBP group also showed higher BMI ($p \leq 0.01$) and lower cardiovascular fitness ($p \leq 0.05$) assessed by a peak oxygen consumption test as compared to the NBP group.

During the moderate exercise challenge (65–70% $\text{VO}_{2\text{peak}}$) both SBP and DBP were higher in the EBP group. Although HR during exercise was higher in the NBP individuals, respiratory exchange ratio (R), or perceived exertion rating (RPE) during exercise were not different between the groups, indicating that all individuals exercised at a relatively same objective (i.e., close to their anaerobic threshold) and subjective exercise intensity regardless of the BP groups or fitness, as intended. Participants rated their average exercise intensity using the Borg's perceived exertion scale (Borg, 1970) as 13.6 ("somewhat hard") on average.

Overall Platelet Activation in response to Exercise

Unstimulated—PLT, MPV, and Hct all increased significantly pre to post 20 minutes of moderate aerobic exercise (p 's ≤ 0.001) with no BP-group differences. Repeated measures ANOVA showed significant overall Time effect (pre, post, 25-min post exercise) for % single platelets, % aggregates, % PAC-1 expressing (PAC-1⁺) platelets, and % P-selectin⁺ platelets; exercise led to a decrease in % single platelets [$F_{(1, 46)} = 7.73$, $P \leq 0.01$] and an increase in % aggregates [$F_{(1, 47)} = 11.86$, $P \leq 0.001$] (Figure 1). And, % single and aggregate platelets continued to decrease and increase, respectively, even 25 minutes after exercise. Percent PAC-1⁺ [$F_{(1, 45)} = 6.87$, $P \leq 0.05$] or % P-selectin⁺ [$F_{(1, 45)} = 126.02$, $P \leq 0.001$] single platelets increased in response to exercise and remained elevated at 25-min post exercise. Percent P-selectin⁺ [$F_{(1, 47)} = 54.94$, $P \leq 0.001$] aggregates also significantly increased and remained elevated at 25-min post exercise and its levels of expression (by MFI) on both single [$F_{(1, 45)} = 74.59$, $P \leq 0.001$] and aggregates [$F_{(1, 47)} = 104.78$, $P \leq 0.001$] also significantly increased after exercise and remained elevated. Percent PAC-1⁺ [$F_{(1, 47)} = 54.94$, $P \leq 0.001$] aggregates significantly increased and remained elevated at 25-min post exercise [$F_{(1, 47)} = 7.92$, $P \leq 0.01$]. Average levels of PAC-1 expression on single [$F_{(1, 45)} = 3.93$, $P = 0.054$] and aggregate [$F_{(1, 47)} = 4.02$, $P = 0.051$] platelets increased after exercise and remained elevated 25-min post exercise. There were one to three missing data points depending on the platelet variables as indicated by the df due to technical difficulties/errors during the sample acquisition by flow cytometry.

ADP stimulated—As described in the methods section, 4 μM ADP was added to the blood samples before antibody staining to examine the effect of exercise on further activation of platelets by an agonist (added *in vitro*). The ADP stimulatory effects on platelets were clearly shown at rest, but ADP-induced platelet activation was blunted after exercise. This exercise inhibitory effect on ADP-induced platelet activation disappeared 25-min post exercise; % aggregates only slightly increased after exercise and *in vitro* ADP, but decreased below baseline values after 25-min [$F_{(1, 46)} = 4.41$, $P \leq 0.05$] (Figure 1). Percent P-selectin⁺ single or aggregates did not change significantly after exercise and ADP stimulation, although P-selectin expression levels on aggregates increased after exercise and ADP and returned to baseline values 25-min post exercise ($p \leq 0.05$). PAC-1 expression levels decreased on both single platelets and aggregates after exercise and ADP stimulation (p 's ≤ 0.05).

Platelet Activation after Exercise in Normal vs. Elevated BP Individuals

Unstimulated—The Time-by-Group interaction was significant for % aggregate platelets [$F_{(1, 44)} = 4.55$, $P \leq 0.05$] (Figure 1) and for % P-selectin⁺ single [$F_{(1, 42)} = 13.77$, $P \leq 0.001$] and aggregate platelets [$F_{(1, 44)} = 12.68$, $P \leq 0.001$]; % P-selectin⁺ platelets slightly increased after exercise and plateaued after a 25-min recovery in the NBP group, but they continued to

increase even after 25-min recovery in the EBP group (Figure 3). Post-hoc analyses of simple Time effect for each BP group confirmed that the increases in % P-selectin⁺ platelets from post-exercise to 25-min recovery was significant only in the EBP group. In addition, the group difference at each time point on % P-selectin⁺ single or aggregate platelets was significant only at the 25-min recovery time point. A change in % single platelets pre to post exercise was not significantly different between the NBP and HBP groups, although the EBP group exhibited a continued decrease, whereas the NBP group showed little changes from post-exercise to 25-min after exercise (Figure 1). There was no significant interaction found in % PAC-1 expressing platelets, although the EBP group showed no signs of recovery at 25-min post exercise, whereas the NBP group did (Figure 2).

As a secondary analysis, 3-step multiple regression was performed (block 1: age, BMI, fitness; block 2: baseline platelet activation levels; block 3: BP status) for each platelet activation variable post-exercise and 25-min recovery. These confirmed that BP status significantly predicted platelet activation at 25-min recovery, even after controlling for demographic variables and baseline activation levels. BMI, baseline activation levels, and BP status were significant predictors in the final model for both % P-selectin⁺ single [model: $R = 0.74$, $\Delta R^2 = 0.17$, $F = 10.04$, $P \leq 0.001$; coefficients for BP group: $p \leq 0.001$] and aggregates [model: $R = 0.66$, $\Delta R^2 = 0.18$, $F = 6.66$, $P \leq 0.001$; coefficients for BP group: $p \leq 0.001$].

ADP stimulated—When further stimulated by ADP *in vitro*, the Time-by-Group interaction was significant for % PAC-1⁺ single platelets such that inhibitory effect of exercise on ADP-induced platelet activation that was shown in NBP individuals did not appear in the EBP group [$F_{(1, 39)} = 5.90$, $P \leq 0.05$] (Figure 2). Simple effects of Time for each BP group revealed, though, that these changes were not significant in either group when examined separately. For % P-selectin⁺ single platelets, the NBP group showed little changes in its %, whereas the EBP group exhibited increases after exercise and *in vitro* ADP stimulation, although the Time-by-Group interaction was not significant (Figure 2). However, multiple regression showed that, BP status significantly predicted % P-selectin⁺ single platelets [model: $R = 0.79$, $\Delta R^2 = 0.06$, $F = 12.84$, $P \leq 0.001$; coefficient for BP status: $p \leq 0.05$] and aggregates [model: $R = 0.70$, $\Delta R^2 = 0.05$, $F = 7.72$, $P \leq 0.001$; coefficient for BP status: $p = 0.06$] in response to exercise and ADP stimulation.

Catecholamines

Time-by-Group repeated measures ANCOVA revealed that there was no significant difference between the BP groups in NE or Epi responses to exercise. Levels of NE (pre: 253 ± 87.81 , post exercise: 599 ± 470 , 25-min post: 292 ± 121 pg/ml) and Epi (pre: 41 ± 31 , post-exercise: 80 ± 87 , 25-min post: 42 ± 25 pg/ml) increased significantly post exercise and returned near the baseline values after 25-min recovery in both groups. Multiple regression analysis showed that neither baseline NE or Epi nor post-exercise NE or Epi predicted platelet activation in response to exercise.

Discussion

Strenuous exercise/physical exertion has been previously shown to activate platelets (Hilberg et al., 2003; Wang & Cheng, 1999), and this process may be implicated in acute cardiovascular events in vulnerable individuals (Siscovick et al., 1984). However, the literature remains limited in acute moderate exercise-induced activation of platelets in hypertensive individuals. We compared the effects of a bout of moderate (standardized to each individual's fitness levels) treadmill exercise on platelet activation in individuals with normal and elevated BP. We found that 20-min moderate exercise led to an increase in

platelet activation that continued even after 25-min recovery; increases in % aggregates and % platelets that were expressing P-selectin or activated fibrinogen binding sites after exercise clearly indicated exercise-induced platelet activation.

More importantly, this platelet activation and increases in aggregation seen in response to moderate exercise was significantly greater in individuals with elevated BP as compared to individuals with normal BP. Our findings are in agreement with previous reports showing greater platelet aggregation in response to acute exercise regardless of the exercise type in hypertensive individuals (Lemne et al., 1992; Vlachakis & Aledort, 1980). This difference in platelet responses to a moderate exercise bout between our BP groups was most pronounced after 25-min recovery than immediately post-exercise such that individuals with EBP exhibited further activation after termination of the exercise at least for 25 minutes, whereas NBP individuals showed a clear sign of “recovery” (no further activation). This suggests that individuals with EBP experience prolonged platelet activation or delayed recovery from the hypercoagulable state induced by physical exertion. Thus, delayed recovery may be a critical factor to be considered in exercise-induced platelet activation in association with increased risk of acute cardiovascular events in high BP individuals even more so than their immediate responses to physical exertion, especially to moderate exercise. This finding emphasizes the importance of “recovery” from moderate physical exertion in hypertension and extends on the existing literature that reports mainly on immediate platelet responses to exercise in this population. Whether this finding applies to more or less intense exercise challenges in hypertensive individuals and to what degree merit further investigation.

On the contrary to platelet activation induced by a vigorous bout of exercise, studies show that exercise training led to attenuated exercise-induced increases in platelet adhesiveness and aggregability, which was reversed after 8–12 weeks of no training (Wang, Li, Chen, & Chen, 2005; Wang, Jen & Chen, 1995; Wang, Jen & Chen, 1997). Also, physically active individuals did not show vigorous exercise-induced platelet activation, whereas sedentary individuals did (Kestin et al., 1993). The mechanisms of regular exercise effects on platelet activation remain unclear. Lekakis and colleagues recently reported increased fibrinolysis activity (thus, decreased clot formation) during moderate exercise, which may be one of the mechanisms of reduced platelet aggregation after regular exercise (Lekakis, Triantafyllidi, Galea, Koutroumbi, Theodoridis, Komporozos, Ikonomidis, Christopoulou-Cokkinou, Kremastinos, 2008).

Possible underlying mechanisms of prolonged platelet P-selectin expression and aggregate formation in response to exercise in individuals with EBP remains unclear in our investigation. Plasma catecholamine levels during exercise did not appear to directly mediate exercise-induced platelet activation in our sample of individuals; neither baseline NE or Epi nor post-exercise NE or Epi levels predicted platelet activation in response to exercise, even though significant SNS activation was evident during the exercise challenge. Circulating NE or Epi responses to the exercise were not different between the two BP groups. In addition, in our group of individuals with EBP, platelet activation responses were profoundly different from that of NBP individuals 25-min after exercise. This is dissimilar to circulating NE or Epi responses to exercise where the increases in the levels shown immediately after exercise disappeared 25-min post-exercise in both groups. However, the mediating role of SNS should not be disregarded solely based on our findings of circulating levels of catecholamines. Although it is speculative since platelet adrenoceptors were not assessed in our study, greater platelet activation levels after moderate exercise in our group of individuals with EBP is possibly in part mediated by elevated adrenergic receptor responses (e.g., higher density or sensitivity) that continued during the recovery period. Alpha 2-adrenoceptor density on platelets is significantly higher in hypertensive individuals compared to normotensives (Brodde, Daul, O'Hara & Bock, 1984; Portaluppi, Manfredini,

Varani, Gessi, Caiazza & Borea, 2000; Varani, Gessi, Caiazza, Rastelli, Portaluppi & Borea, 1998), and their responses to epinephrine are greater in hypertensive individuals (Brodde et al., 1984). As previously mentioned, β blockers Epi- or exercise-induced platelet aggregation (Kawano et al., 2000; von Kanel & Dimsdale, 2000) indicating the involvement of β receptors in their activation. Different duration or intensity of exercise also leads to differential responses of platelet α_2 -adrenoreceptors (Kempen, Saris, Senden, Menheere, Blaak & Van Baak, 1994; Wang & Cheng, 1999). In addition, possible abnormal shear stress during exercise and slow recovery may also partly contribute to greater platelet activation in individuals with EBP, given that shear stress is implicated in platelet activation (Paszowski & Dardik, 2003) and that decreases in BP in hypertensive individuals after antihypertensive medication treatments is accompanied by reduced platelet aggregation or activation (Dietrich, Heesen, Boldt & Hempelmann, 1996; Labios, Martinez, Gabriel, Guiral & Aznar, 2006). A future study is needed to identify the mechanisms of hypertension-related platelet activation in response to moderate exercise.

In general, 20 minutes of moderate treadmill exercise may have led to transient desensitization of platelets to further stimulation by an agonist as seen by the reduction in % platelets expressing P-selectin or PAC-1 after stimulation with 4 μ M ADP. This potentially inhibitory effect of acute exercise on further agonist-induced platelet activation was more clearly shown in NBP individuals immediately after exercise. Interestingly, blunted platelet activation upon ADP stimulation or complete recovery appeared 25-min post exercise among EBP individuals. These findings suggest that although acute exercise leads to prolonged effects on platelet activation in individuals with elevated BP, it may have an inhibitory effect against exaggerated/additional platelet reactivity to an agonist. This finding is in agreement with Wang and colleagues (Wang et al., 1994), who first demonstrated that healthy men who performed 30-min bicycle ergometry exercise at 50–55% of their VO_{2max} exhibited less ADP-induced platelet aggregation after exercise than at rest. In contrast, strenuous exercise (bicycle ergometry until exhaustion) led to significant increases from rest in ADP-induced aggregation. Arm ergometry exercise at 75% VO_{2peak} did not change ADP-induced platelet aggregation, but produced a significant increase in collagen-induced aggregation (Ersoz et al., 2003). These findings imply that the effects of exercise on agonist-induced platelet activation may be exercise intensity-dependent and agonist-specific. Nevertheless, a decrease in platelet responsivity may underlie some of the cardioprotective effects of long-term regular moderate exercise, given that regular moderate exercise results in attenuated platelet activation both at rest and in response to a maximal exercise challenge (Wallen et al., 1999).

The findings of the differences in platelet activation after acute exercise in high BP individuals from this study need to be interpreted with caution because of the following reasons. First, although the demographic and basic physiological characteristics (age, BMI, and fitness) were statistically controlled for, we did not match participants on those variables, resulting in a significant difference in them between the groups. Therefore, possible physiological effects of those variables on the group differences in platelet activation cannot be completely disregarded. Additionally, HR during exercise were significantly higher in the NBP group, which may suggest a difference in SNS activation between the groups. It should be noted, though, that our plasma catecholamine data show no difference in their responses to the exercise challenge. Also, the difference in HR also may have been largely driven by the age difference, as it is known that age is closely related to HR during exercise. Average exercise HRs for the normal and elevated BP groups were 88 and 84% of their maximal HRs reached during the VO_{2peak} test, indicating that two groups exhibited similar exercise HRs relative to their maximal HRs. Thirdly, participants in the EBP group in this study exhibited a relatively mild BP level (average BP of 145/87 mm Hg). Further study is needed to examine if the findings from this study apply to the individuals

with more severe hypertension who may exhibit larger and more immediate platelet activation after acute exercise. Lastly, we observed higher than anticipated % aggregated platelets in our baseline samples, although whole blood staining of platelets for flow cytometry is a reliable method to measure platelet activation with minimal handling, greatly reducing the chances of *in vitro* platelet activation. This elevated aggregation could have occurred due to possible anticipatory anxiety before the exercise challenge and/or occurred during 15 minutes of antibody staining before fixation. Future studies may benefit from fixation before antibody staining and by largely diluting blood samples.

In summary, a moderate exercise (65–70% $\text{VO}_{2\text{peak}}$) challenge induced an increase in platelet activation that was longer-lasting for individuals with EBP. Meanwhile, a moderate exercise bout led to inhibition of platelet activation by *in vitro* ADP stimulation 25-min post exercise. This exercise-induced (prolonged) platelet activation vs. attenuated platelet activation upon further agonist stimulation may have clinical implications. Prolonged coagulatory responses in hypertensive individuals after physical stress may suggest more caution needed in evaluating post-exercise responses in these individuals. Simultaneously, exercise intensity-dependent platelet responses need further investigation, as moderate exercise may provide protection against further activation from other agonists. An acute episode of maximal strenuous exercise in the literature appears to cause platelet activation and potentiates the response to any additional agonists, which may lead to the increased risk of thrombosis and sudden death from an acute coronary syndrome in vulnerable individuals. Acute moderate exercise, on the other hand, may protect an individual from thrombosis risk in part because of desensitization of platelets to further stimulation, based on our findings. Future studies to compare different exercise types and intensity and to observe for a longer recovery period will help better delineate the effects of different physical exertions on platelet activation in association with BP. In addition, underlying mechanisms of possible moderate-exercise induced desensitization of platelets for further activation warrants investigation.

Acknowledgments

This work was supported by grants RO1 HL-57265 and MO1-RR00827 from the National Institutes of Health.

The authors would like to express their gratitude to Elaine Dillon, R.N., the UCSD Clinical Trials Center, and the VA Flow Cytometry Research Core Facility for their invaluable assistance in data collection.

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Figure 1.

Flow cytometric histogram and scatter plots to identify platelets ($CD61^{+}$) and single vs. aggregated platelets that were unstimulated and ADP stimulated. Single and aggregated platelets were identified and separately gated based on the size discrimination using Forward Scatter (FS) and Side Scatter (SS) on a log scale. Gating of single and aggregate platelets were further verified by using sorting and microscopic examination. An increase in aggregate platelets is shown in the ADP stimulated sample.

Figure 2.

Percent single (top) and % aggregate (bottom) platelets before, immediately after, and 25-min after exercise (unstimulated in thin lines; left vs. ADP stimulated in thick lines; right) in normal (NBP in dotted lines) vs. elevated BP (EBP) individuals (mean $\pm$ SEM). Values are % of total (CD61⁺) platelets. * denotes $p \leq 0.05$ for Time-by-Group interaction. For main effects for Time see the text in Results.

Figure 3. Percent PAC-1 (left) and % P-selectin (right) expressing single platelets before, immediately after, and 25-min after exercise (unstimulated in thin lines vs. ADP stimulated in thick lines) in normal (NBP in dotted lines) vs. elevated BP (EBP) individuals (mean $\pm$ SEM). * and *** denote $p \leq 0.05$ and $p \leq 0.001$ for Time-by-Group interaction. For main effects for Time see the text in Results.

Figure 4.

Percent P-selectin expressing aggregate platelets before, immediately after, and 25-min after exercise (unstimulated in thin lines vs. ADP stimulated in thick lines) in normal (NBP in dotted lines) vs. elevated BP (EBP) individuals (mean $\pm$ SEM). ** denotes $p \leq 0.01$ for Time-by-Group interaction. For main effects for Time see the text in Results.

Table 1

Demographic characteristics and metabolic and cardiorespiratory responses during a 20-min exercise at 65–70% $\dot{V}O_{2peak}$ of individuals with normal and elevated blood pressure (BP)

<i>Variables</i>	Normal BP (N=31)	Elevated BP (N=19)	t/χ^2
Age (years)	35.2 (9.4)	46.8 (7.5)	−4.81 ***
Gender (male/female) [^]	17/14	12/7	0.30
Systolic blood pressure (mmHg) at rest	114.41 (8.71)	144.91 (9.87)	−11.43 ***
Diastolic blood pressure (mmHg) at rest	73.17(8.91)	87.11 (8.14)	−5.55 ***
Body mass index (kg/m ²)	24.20 (4.07)	28.54 (4.09)	−3.66 **
$\dot{V}O_{2peak}$ (ml/kg/min)	37.84 (8.66)	31.04 (10.55)	2.48 *
Systolic blood pressure (mmHg) during 20-min exercise	149.34 (21.49)	185.37 (20.84)	−5.75 ***
Diastolic blood pressure (mmHg) during 20-min exercise	70.21 (9.09)	87.79 (9.45)	−6.45 ***
Heart rate (bpm) during 20-min exercise	155.32 (19.99)	142.16 (14.99)	2.44 *
Respiratory exchange ratio (R) during 20-min exercise	0.93 (0.07)	0.93 (0.07)	0.63
Ratings of perceived exertion (RPE) during 20-min exercise	13.93 (2.07)	13.08 (2.41)	1.32

*, **, and *** denote $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.

[^] denotes Chi-square tests