Effect of training status on the changes in platelet parameters induced by short-duration exhaustive exercise

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Abstract

It is now well known that hemostasis is directly involved in the benefits induced by physical activity. It has recently been shown that the baseline mean platelet volume (MPV) may be a predictor of endurance performance. We aimed to explore whether platelet parameters are associated with VO₂max as well as running duration and speed in a short-duration exhaustive exercise test. Thirty healthy male subjects (10 sedentary and 20 trained) performed an incremental running test until exhaustion. MPV, platelet distribution width (PDW), platelet (Plt) count, and plateletcrit (Pct) were determined before exercise, immediately after exercise and after 30’ recovery. Training status did not produce any difference in the baseline levels or in the post-exercise increases found in all the parameters tested. VO₂max, test duration, and running speed were not correlated with any baseline parameter. Although MPV was found to be a predictor of endurance performance in long-duration exercise, the results of the present study are consistent with the hypothesis that MPV may not be a significant marker of performance in short-duration exhaustive exercise. Likewise, more research is needed to ascertain whether platelet activation is a reliable performance predictor in other exercise settings.

Introduction

Performance in sports mainly depends on the interrelation of several physiological factors [1, 2]. Accordingly, one of the main goals of training is to improve physiological variables directly involved in athletic performance [3]. In addition, some of these training-induced physiologic adaptations are indeed beneficial to the non-active population, because they are thought to reduce the impact of cardiovascular diseases (CVD) and all-cause mortality [4]. For that reason, biomarkers linked to physiological performance in health and sports fields are very useful to physicians, coaches, and health professionals with respect to controlling the training process and health status, as well as for predicting the performance outcome. To this end, many biomarkers have been evaluated, and subsequently used, to monitor athletes’ adaptations to training, performance status as well as other physiological measures of performance (i.e., VO₂max) [5–7].

Platelets are blood cells that play an important role in the formation of blood clots [8]. By adhering to damaged endothelium and releasing the content of their storage granulocytes, platelets provoke the formation of atheromatos plaques [8]. Platelet hyper-aggregation and hyper-activation are involved in the appearance of atherosclerosis and concomitant CVD, as well as myocardial infarction and stroke [9].

It is now well known that hemostasis is directly involved in the benefits produced by physical activity [10]. Interestingly, it has recently been shown that the baseline platelet size, which is conventionally expressed as mean platelet volume (MPV), may be negatively associated with the time taken to complete a half-marathon race, making it a predictor of endurance performance [11]. MPV and other platelet-related parameters such as platelet distribution width (PDW), platelet (Plt) count, or plateletcrit (Pct) are easily and reliably determined using automated hematological analyzers along with other hematological parameters. Therefore, MPV may represent a simple biomarker of endurance performance, although this phenomenon should be examined in other scenarios such as acute (or short-duration) exhaustive exercise or strenuous physical activity such as ultramarathon running.

To this aim, we studied whether the MPV, PDW, Plt, and Pct are associated with VO₂max as well as running duration and speed in a short-duration exhaustive exercise test, specifically an incremental running test until exhaustion. Moreover, we evaluated the effect of endurance training on these parameters by comparing the platelet-related data between sedentary and endurance-trained subjects.

Methods

Subjects

Thirty healthy male subjects were enrolled in the study. Ten subjects (aged 20–38, body mass index (BMI) 21.1–26.7 kg/m²) were physically inactive (sedentary group, weekly physical activity <1 h) and 20 subjects (aged 21–45, BMI 19.7–24.7 kg/m²) were physically active (trained group, triathletes, and endurance athletes who practiced physical activity weekly >12 h). Exclusion criteria were hematologic, infectious or inflammatory diseases, history of heart disease or hormonal...
impairments (i.e., hypo- or hyperthyroidism), smokers (>1 cigarette/day), hypercholesterolemia (total cholesterol >220 mg/dL or treated for high total cholesterol levels), diabetics (fasting glucose concentration of >126 mg/dL or undergoing pharmacological treatment for diabetes), hypertension (systolic blood pressure >130 mmHg, diastolic blood pressure >90 mmHg or undergoing pharmacological treatment for hypertension), and obesity (BMI >30 kg/m²).

All the participants were informed of the purpose, protocol, and procedures before agreeing to participate in the study, which was approved by the Ethical Committee of the Catholic University of Valencia. This work complies with the principles of the Declaration of Helsinki and was performed in accordance with the Spanish laws regarding research on humans.

Experimental protocol

All the participants reported to the laboratory in fasting conditions between 8 and 9 am. Subjects performed a maximal incremental test on a motorized treadmill until exhaustion. During the warm-up period (5 min), subjects selected the running speed (between 8 and 12 km/h) according to their preferences and habitual training pace. This speed remained fixed during the test, while the slope was increased by 1% every minute. The test ended when the subject was unable to keep running despite verbal encouragement. Ventilatory parameters were recorded during exercise through a respiratory valve and a face mask (Hans Rudolph, Inc., Kansas City, MO) using a gas analyzer (MetaLyzer 3B-R2, Cortex GmbH, Leipzig, Germany). The test was considered maximal when at least two of the following conditions were fulfilled: post-exercise blood lactate >8 mM, respiratory quotient >1.1 and maximal heart rate >maximal heart rate predicted as 220 – age [12]. All trials met at least two of these criteria and were considered maximal.

Blood sampling

Blood samples were taken from the antecubital vein before, just after exercise and after the 30-min recovery period. Blood was collected in standard Vacutainer® tubes (BD Diagnostics, Franklin Lakes, NJ) containing K₃EDTA for hematological measurements. The tubes were kept in ice until performing determinations within 2 h after extraction.

Laboratory methods

MPV, PDW, Plt, Pct, hematocrit (Htc), and hemoglobin (Hb) were assayed in duplicate using a Sysmex XE-2100L (Sysmex, Kobe, Japan) [13]. The intra-assay coefficients of variation were 0.74% for MPV, 2.62% for PDW, 1.90% for Plt, 2.40% for Ptc, 0.36% for Htc, and 0.31% for Hb.

Calculations

Change in plasma volume (ΔPV) after exercise and after recovery was calculated using the Dill and Costill equation [14], as follows:

\[
\Delta PV(\%) = 100 \times \left( \frac{Hb_{pre}}{Hb_{post}} \right) \times \left( 100 - \frac{Htc_{post}}{Htc_{pre}} \right) \times \left( 100 - Htc_{pre} \right)
\]

where Htc is in % and Hb in g/dL.

Statistical analysis

All data variables were analyzed for normality using the Shapiro–Wilk test. When required, data were log-transformed to achieve normality for statistical testing. The effect of exercise and recovery on the platelet parameters was first analyzed using two-way ANOVA for repeated measures (training status: trained, sedentary; sampling time: pre-exercise, post-exercise, after 30-min recovery). Post hoc comparisons were made with

Figure 1. Exercise effects on mean platelet volume (MPV), platelet distribution width (PDW), platelet (Plt) count, and plateletcrit (Pct), in sedentary (void bars) and trained (full bars) subjects. Bonferroni’s post hoc significant comparisons have been indicated.
Bonferroni’s test correction. The baseline and the exercise-induced increases (post-exercise–pre-exercise values) platelet parameters were expressed as absolute or relative (i.e., increase expressed as a percentage of pre-exercise values) and they were compared using a one-way ANOVA for independent measures (training status: trained, sedentary). Pearson’s correlation coefficients were calculated in order to explore the association between continuous variables. The statistical analysis was performed using SPSS, version 21 (IBM Corporation, Armonk, NY). The results were considered statistically significant at \( p < 0.05 \). Data were expressed as mean ± one standard deviation (SD).

Figure 2. Absolute (left) and relative to pre-exercise (right) exercise-induced changes for mean platelet volume (MPV), platelet distribution width (PDW), platelet (Plt) count, and plateletcrit (Pct), in sedentary (○) and trained (●) subjects.
Results

No significant differences in MPV (sedentary 11.02 ± 1.11 fL, trained 11.09 ± 0.88 fL; p = 0.857), PDW (sedentary 13.60 ± 2.34 fL, trained 13.95 ± 2.32 fL; p = 0.695), Plt (sedentary 202 ± 30 x 10^9/L, trained 196 ± 57 x 10^9/L; p = 0.739), or Pct (sedentary 0.22 ± 0.03 %, trained 0.22 ± 0.06; p = 0.739) were found between sedentary and trained subjects at rest.

Exhaustive running caused an increase in platelet parameters (Figure 1). When the effect of exercise was assessed in reference to the subjects’ training status, we did not observe any significant difference in response to exercise (MPV F(2.56) = 0.277, p = 0.759; PDW F(2.56) = 2.725, p = 0.074; Plt F(2.56) = 1.012, p = 0.370; Pct F(2.56) = 1.404, p = 0.254). Moreover, any exercise-induced increases in the tested parameters were different when comparing sedentary and trained subjects (Figure 2). When the Plt values at post-exercise and after recovery were corrected by ΔPV (calculated from changes in Hb and Htc), significant effects of exercise on Plt were found in both sedentary (F(1.213, 10.915) = 18.705, p = 0.001) and trained (F(2,38) = 35.787, p < 0.001) subjects.

VO2max, test duration, and running speed were considered markers of performance in the running incremental test (see Supplementary Table 1). We did not find any significant correlation between baseline levels of platelet parameters and VO2max, test duration, or running speed (Figure 3). When the correlation analysis was performed separately in sedentary and trained subjects, no significant correlation was found either.

Discussion

We failed to find any association between baselines for Plt, MPV, PDW and Pct and VO2max, test duration or running speed in our exercise model (Figure 3). Moreover, no differences in baseline levels of the platelet-related parameters between sedentary and trained subjects were found. These data are seemingly in agreement with those previously reported by Lippi and collaborators, who showed that MPV is a predictor of endurance.
performance in non-exhaustive exercise, such as half-marathons [11], but remains independent of the VO2max. According to this evidence, it seems reasonable to conclude that baseline MPV may not be a predictor of performance in an incremental exhaustive running test. Nevertheless, the sample size in the present study is limited and it would be desirable to confirm these observations in a larger cohort.

The discrepancy with endurance running may be attributable to the metabolic pathways and supporting physiological systems involved in short-term strenuous exercise that are not extensively needed in long-duration exercise (i.e., marathons or ultramarathons). More specifically, platelets may have a role in medium- to long-term exercise by promoting the gradual release of performance-enhancing systemic growth factors [15] and attenuating neuropathic pain and/or fatigue [11], two aspects that are not directly involved in influencing performance in a short incremental running test. On the contrary, increased MPV baseline levels could be attributed to enhanced platelet turnover which could reflect other exercise-induced chronic adaptations without having a direct ergogenic effect.

A temporary increase in MPV is a marker of platelet activation and/or reactivity [16]. Thus, increased MPV after exercise could indicate enhanced platelet activity and risk of thromboembolism. Nevertheless, exercise raised MPV by 2.63%, which is far below the within-subject biological variation reported for this parameter (i.e., 4.3%) [17]. Therefore, the observed MPV increase does not necessarily imply clinical significance. It has been also shown that high- and moderate-intensity exercises do not increase plasma fibrinogen levels [18, 19], whereas acute exercise and training processes have fibrinolytic effects [20, 21]. Therefore, these transient effects of exercise on platelet function do not pose an increased thrombotic risk in healthy subjects. Extensive scientific evidence suggests that engaging in physical activity reduces the incidence of CVD and all-cause mortality, which should encourage non-active subjects to undertake an active lifestyle. In patients suffering from endothelial dysfunction or atherosclerosis, exercise must be prescribed individually and appropriately in order to promote the desired beneficial effects without risks.

Exhaustive incremental running induced an increase in the number of circulating platelets (Figure 1), independent of the post-exercise plasma volume loss [22]. The Pct increase could be attributed to the epinephrine-mediated splenic contraction during exercise that releases thrombocytes into the circulation [23, 24]. In accordance with this, Pct rose after exercise in both sedentary and trained subjects (Figure 1). These data are in agreement with previous studies [25, 26].

MPV and PDW increased after the incremental running test in both sedentary and trained subjects and reverted after the recovery period (Figure 1). Several studies that have investigated the impact of exercise on platelet activation are in accordance with our results [27–30]. Catecholamines play a role in exercise-induced platelet activation through α2-adrenergic receptors in thrombocytes [31]. Endurance-trained subjects show lower α2-adrenergic receptor density in intact platelets along with reduced adrenaline-induced platelet aggregation than non-endurance trained subjects [32]. In this sense, resistance-trained subjects present lower β-TG levels after an acute exhaustive exercise lower exercise test than untrained subjects [33], and 1 h cycling at 70% of VO2max induces higher platelet activation in sedentary subjects than in trained subjects [34]. Moreover, moderate exercise training decreases platelet adhesiveness and aggregability that is reversed back to pretraining states after deconditioning [35].

In conclusion, despite finding MPV to be a predictor of endurance performance in long-duration exercise, the results of the present study are consistent with the hypothesis that MPV may not be a significant marker of performance in short-duration exhaustive exercise. Likewise, more research is needed to ascertain whether platelet activation is a reliable performance predictor in other exercise settings.

Declaration of interest

None of the authors have any conflict of interest.

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References

1. Puthucheary Z, Skipworth JR, Rawal J, Loosemore M, Van Someren K, Montgomery HE. Genetic influences in sport and physical performance. Sports Med 2011;41(10):845–859.
2. Sekulic D, Spasic M, Mirkov D, Cavar M, Satller T. Gender-specific influences of balance, speed, and power on agility performance. J Strength Cond Res 2013;27(3):802–811.
3. Joyner MJ, Coyle EF. Endurance exercise performance: The physiology of champions. J Physiol 2008;586(1):35–44.
4. Schuler G, Adams V, Goto Y. Role of exercise in the prevention of cardiovascular disease: Results, mechanisms, and new perspectives. Eur Heart J 2013;34(24):1790–1799.
5. Banfi G, Colombini A, Lombardi G, Lubkowska A. Metabolic markers in sports medicine. Adv Clin Chem 2012;56(1):1–54.
6. Sanchis-Gomar F, Lippi G. Physical activity – An important preanalytical variable. Biochem Med 2014;24(1):68–79.
7. Romagnoli M, Alis R, Aloe R, Salvagno GL, Basterra J, Pareja-Gameano H, Sanchis-Gomar F, Lippi G. Influence of training and a maximal exercise test in analytival variability of muscular, hepatic, and cardiovascular biochemical variables. Scand J Clin Lab Invest 2014;74(3):192–198.
8. Favaloro EJ, Lippi G, Franchini M. Contemporary platelet function testing. Clin Chem Lab Med 2010;48(5):579–598.
9. Lippi G, Filippozzi L, Salvagno GL, Montagnana M, Franchini M, Guidi GC, Targher G. Increased mean platelet volume in patients with acute coronary syndromes. Arch Pathol Lab Med 2009;133(9):1441–1443.
10. Lippi G, Maffulli N. Biological influence of physical exercise on hemostasis. Semin Thromb Hemost 2009;35(3):269–276.
11. Lippi G, Salvagno GL, Danese E, Skafidas S, Tarperi C, Guidi GC, Schena F. Mean platelet volume (MPV) predicts middle distance running performance. PLoS One 2014;9(11):e112892.
12. Wasserman K, Hansen JE, Sue DY, Stringer WW, Whipp B. 2005. Principles of exercise testing and interpretation. Philadelphia, PA: Lippincott Williams & Wilkins. pp 160–182.
13. England JM, Rowland RM, Bull BS, Coulter WH, Groner W, Jones AR, Koeppke JA, Lewis SM, Shinton NK, Thom R, et al. ICSH recommendations for the analysis of red cell, white cell and platelet size distribution curves. Methods for fitting a single reference distribution and assessing its goodness of fit. Clin Lab Haematol 1990;12(4):417–431.
14. Dill DB, Costill DL. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. J Appl Physiol 1974;37(2):247–248.
15. Pareja-Galeano H, Alis R, Sanchis-Gomar F, Cabo H, Cortell-Ballester I, Gomez-Cabreria MC, Lucia A, Vina J. Methodological considerations to determine the effect of exercise on brain-derived neurotrophic factor levels. Clin Biochem 2015;48(3):162–166.
16. Martin JF, Trowbridge EA, Salmon G, Plumb J. The biological significance of platelet volume: Its relationship to bleeding time, platelet size distribution and assessing its goodness of fit. Clin Lab Haematol 2010;32(4):417–431.
17. Dill DB, Costill DL. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. J Appl Physiol 1974;37(2):247–248.
18. Alis R, Ibanez-Sania S, Basterra J, Sanchis-Gomar F, Romagnoli M. Effects of an acute high-intensity interval training protocol on plasma viscosity. J Sports Med Phys Fitness 2014 (in press).
19. Romagnoli M, Alis R, Martinez-Bello V, Sanchis-Gomar F, Aranda R, Gomez-Cabreria MC. Blood rheology effect of submaximal exercise on young subjects. Clin Hemorheol Microcirc 2014;56(2):111–117.

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20. Szymanski LM, Pate RR. Effects of exercise intensity, duration, and time of day on fibrinolytic activity in physically active men. Med Sci Sports Exerc 1994;26(9):1102–1108.

21. Kupchak BR, Creighton BC, Aristizabal JC, Dunn-Lewis C, Volk BM, Ballard KD, Comstock BA, Maresh CM, Kraemer WJ, Volek JS. Beneficial effects of habitual resistance exercise training on coagulation and fibrinolytic responses. Thromb Res 2013;131(6):e227–e234.

22. Alis R, Sanchis-Gomar F, Primo-Carrau C, Lozano-Calve S, Dipalo M, Aloe R, Blesa J, Romagnoli M, Lippi G. Hemoconcentration induced by exercise. Revisiting the Dill & Costill equation. Scand J Med Sci Sports 2014 (in press).

23. Schaffner A, Augustiny N, Otto RC, Fehr J. The hypersplenic spleen. A contractile reservoir of granulocytes and platelets. Arch Intern Med 1985;145(4):651–654.

24. Chamberlain KG, Tong M, Penington DG. Properties of the exchangeable splenic platelets released into the circulation during exercise-induced thrombocytosis. Am J Hematol 1990;34(3):161–168.

25. Lippi G, Salvagno GL, Danese E, Tarperi C, Guidi GC, Schena F. Variation of red blood cell distribution width and mean platelet volume after moderate endurance exercise. Adv Hematol 2014;2014:192173.

26. Ahmadizad S, El-Sayed MS, MacIaren DP. Responses of platelet activation and function to a single bout of resistance exercise and recovery. Clin Hemorheol Microcirc 2006;35(1–2):159–168.

27. Chaar V, Romana M, Tripetto J, Broquere C, Huissie MG, Hue O, Hardy-Dessources MD, Connes P. Effect of strenuous physical exercise on circulating cell-derived microparticles. Clin Hemorheol Microcirc 2011;47(1):15–25.

28. Maruyama K, Kadono T, Morishita E. Plasma levels of platelet-derived microparticles are increased after anaerobic exercise in healthy subjects. J Atheroscler Thromb 2012;19(6):585–587.

29. Ludlam CA. Evidence for the platelet specificity of beta-thromboglobulin and studies on its plasma concentration in healthy individuals. Br J Haematol 1979;41(2):271–278.

30. Ahmadizad S, El-Sayed MS. The effects of graded resistance exercise on platelet aggregation and activation. Med Sci Sports Exerc 2003;35(6):1026–1032.

31. Wang JS, Cheng LJ. Effect of strenuous, acute exercise on alpha2-adrenergic agonist-potentiated platelet activation. Arterioscler Thromb Vasc Biol 1999;19(6):1559–1565.

32. Lehmann M, Hasler K, Bergdolt E, Keul J. Alpha-2-adrenoreceptor density on intact platelets and adrenaline-induced platelet aggregation in endurance-and nonendurance-trained subjects. Int J Sports Med 1986;7(3):172–176.

33. Creighton BC, Kupchak BR, Aristizabal JC, Flanagan SD, Dunn-Lewis C, Volk BM, Comstock BA, Volek JS, Hooper DR, Szivak TK, et al. Influence of training on markers of platelet activation in response to a bout of heavy resistance exercise. Eur J Appl Physiol 2013;113(9):2203–2209.

34. Singh I, Quinn H, Mok M, Southgate RJ, Turner AH, Li D, Sinclair AJ, Hawley JA. The effect of exercise and training status on platelet activation: Do cocoa polyphenols play a role? Platelets 2006;17(6):361–367.

35. Wang JS, Jen CJ, Chen HL. Effects of exercise training and deconditioning on platelet function in men. Arterioscler Thromb Vasc Biol 1995;15(10):1668–1674.

Supplementary material available online
Supplementary Table 1