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Original Research

High Intensity Interval Exercise Increases Platelet and Transforming Growth Factor-β Yield in Platelet-Rich Plasma

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Abstract

Background: Platelet-rich plasma (PRP) is an emerging orthobiologic treatment for musculoskeletal conditions like osteoarthritis. Two studies have demonstrated the influence of longer duration exercise on PRP composition, but no study has ever explored the impact of high intensity interval exercise (HIIE) on PRP content.
Objective: To quantify cellular and growth factor content changes in PRP after 4 minutes of HIIE.
Design: Controlled laboratory pilot study.
Setting: Academic sports medicine center.
Participants: Ten healthy volunteers (5 male, 5 female).
Intervention: Volunteers had PRP prepared from 15 mL of whole blood using a single spin, plasma-based system (autologous conditioned plasma [ACP]) immediately before and after 4 minutes of HIIE on a stationary exercise bike (Tabata protocol).
Main Outcome Measure: The PRP was sent for complete blood counts and enzyme-linked immunosorbent assay (ELISA) to quantify transforming growth factor (TGF)-β, platelet-derived growth factor (PDGF), insulin-like growth factor (IGF)-1, and vascular endothelial growth factor (VEGF).
Results: Mean platelet count in PRP increased from 367.4 ± 57.5 k/μL to 497.7 ± 93.3 k/μL after 4 minutes of HIIE ($P < .001$). TGF-β also increased from 8237.2 ± 7676.5 pg/mL to 21 535.7 ± 4062.6 pg/mL postexercise ($P = .004$). The other cellular components (leukocytes, red blood cells, and mean platelet volume) and growth factors (PDGF, IGF-1, and VEGF) were not significantly changed.
Conclusions: A short 4-minute bout of HIIE significantly increased the total platelet count and TGF-β concentration in PRP.

Introduction

Platelet-rich plasma (PRP) is an autologous solution of concentrated platelets derived from whole blood.[1] The primary goal of concentrating platelets over baseline is to increase the yield of anabolic growth factors (GFs) found in platelet' alpha-granules, such as platelet-derived growth factor (PDGF), transforming growth factor (TGF)-β, and fibroblast growth factor (FGF).[2,3] In vitro data have demonstrated that PRP has a protective and anabolic effect on chondrocytes and tenocytes.[4-7] PRP injections are used to treat osteoarthritis (OA) and tendinosis, although high-quality studies supporting these indications are limited.[8-14]

Cellular and GF contents of PRP are highly variable due to several processing and physiologic variables. Different concentration methods greatly alter the composition of PRP.[15]

Plasma-based PRP systems use a low-velocity, short-duration centrifugation to yield lower platelet concentrations with reduced leukocytes, whereas buffy-coat systems use two higher velocity centrifugation cycles to yield more densely concentrated platelets and leukocytes.[3,16] Due to

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their lower cellular content, plasma-based systems have lower GF concentrations but also have fewer inflammatory and catabolic mediators such as interleukin (IL)-1 and matrix metalloproteases (MMP).[3,17] Patient-specific variables such as age, gender, medical comorbidities, and medication use all impact the GF content of PRP.[18-23]

Exercise status also affects the cellular content of whole blood and PRP, which is important as exercise state can be easily modified. The physiologic mechanism underlying this response is referred to as exercise-induced thrombocytosis and has been well detailed in hematology literature.[24] Up to one third of platelets are stored in the spleen, which are released during acute stress, such as exercise.[25] One study demonstrated a 24% increase in whole blood platelet count and a 3.7% increase in mean platelet volume (MPV), suggesting that not only are more platelets released, but those that are released are also larger.[26] Anz et al was the first to demonstrate that additional platelets could be captured in PRP if the blood was drawn immediately after 20 minutes of continuous exercise. However, it may be difficult to implement 20 minutes of exercise prior to a procedure in clinical practice. Therefore, a shorter bout of high-intensity interval exercise (HIIE) may be simpler to implement routinely. HIIE increases platelet count in peripheral blood, but the impact of HIIE on PRP cellular and growth factor content has not been explored.[27]

Therefore, the purpose of the present study was to quantify changes in PRP content induced by a short bout of HIIE. This study specifically analyzed changes in cellular and GF yield caused by a 4-minute HIIE protocol. We hypothesized that HIIE would lead to an immediate increase in platelet and GF yield.

Methods

This study was approved by our institutional review board. Ten healthy volunteers were recruited and provided voluntary, written informed consent prior to the study. Inclusion criteria included age > 18 years, no musculoskeletal conditions that would interfere with exercise, no systemic inflammatory conditions (such as rheumatoid arthritis, systemic lupus erythematous etc), and no cardiovascular risk factors.

The interval exercise used in this study was based on the original protocol described by Tabata et al.[28] Briefly, using a Schwinn Airdyne Evolution Comp exercise bike (Nautilus Inc., Vancouver, WA), participants completed a 5-minute warm-up (at a self-selected pace) and then performed 8 intervals. Each interval consisted of 20 seconds of work followed by 10 seconds of rest. The 10 participants were nonrandomly assigned to one of two intensity groups: (1) strenuous intensity (defined as 15 out of 20 on the Borg Rate of Perceived Exertion [RPE] scale and (2) maximum intensity (defined as 20 out of 20 on the Borg RPE scale).[29] Individuals were assigned to a group at the time of recruitment based on baseline fitness activity, with those participants already engaged in HIIE assigned to maximum intensity. Five participants were recruited into each group. Participants were encouraged to perform the protocol 3-4 times prior to the testing day to allow for appropriate conditioning. Participants were also instructed to avoid nonsteroidal anti-inflammatory drugs and aspirin for 1 week prior to testing and to avoid exercise on the testing day.

On the testing day, prior to any exercise, participants had preexercise PRP prepared using a plasma-based system (autologous conditioned plasma [ACP]; Arthrex, Naples, FL). Each participant underwent standard venipuncture by a single phlebotomist under sterile conditions for a total blood draw of 13 mL, which was mixed with 2 mL of Anticoagulant Citrate Dextrose Solution, Solution A (ACD-A) [Citra Labs, LLC., Braintree, MA] per manufacturer's recommendation. This underwent a single centrifugation at 1500 rpm for 5 minutes. Resultant PRP was labeled and stored in a 4°C refrigerator.

The participant then performed the HIIE protocol described previously. The entire exercise protocol and recovery period was monitored by a staff physician and certified athletic trainer for safety and adverse events. Once the exercise protocol was completed, participants were allowed a recovery period (including rest, walking if desired, and fluid intake) before undergoing the second blood draw. Maximum recovery time allowed was 5 minutes, but if participants recovered sooner, they were permitted to undergo the second blood draw at their discretion. The second blood draw and PRP processing was performed identically to the first.

Once all PRP processing was complete, samples were sent to the comparative orthopedics laboratory and divided into 2 aliquots, 1 for cell counts, 1 for GF analysis. Complete blood counts (including platelet, leukocyte, red blood cells [RBC], MPV) were determined using an automated cell counter within 6 hours. For GF analysis, a single freeze-thaw cycle was used to induce platelet activation and release of GFs as described by Zimmerman et al and Schnabel et al.[30,31] Samples were individually separated into aliquots before being stored in a −80°C refrigerator. Thirty minutes later, the samples were thawed at room temperature for another 30 minutes and thereafter frozen for a second time at −80°C.

Growth Factor Quantification

Cryopreserved samples were thawed at room temperature prior to analysis using enzyme-linked immunosorbent assay (ELISA) kits specific for each GF (R&D Systems, Minneapolis, MN) according to manufacturer's recommendations. Active PDGF-BB, TGF-β, vascular endothelial growth factor (VEGF), and insulin-like growth factor-1 (IGF-1) concentrations were determined in duplicate aliquots of all samples.

Statistics

Paired sample *t*-tests were used to determine mean differences between cell counts and growth factor concentration before and after HIIE exercise. An a priori alpha level of 0.05 was used to determine significance. A post-hoc Bonferroni correction was applied to correct for family-wise error rate, changing the level deemed statistically significant to $P \leq .006$. All data were analyzed using IBM SPSS Statistics version 25 (IBM, Armonk, NY).

Results

Ten healthy participants were recruited for participation. Their demographics are presented in Table 1. There were 5 males and 5 females, with an average age of 39.6 ± 9.8 years with a range of 28 to 60. Three participants (2 male, 1 female) completed the maximal exertion protocol (20 RPE) and 7 participants (3 male, 4 female) completed the strenuous protocol (15 RPE). The average time to postexercise blood draw completion was 4 minutes, 36 seconds with a SD of 21.5 seconds.

Table 1
Demographics for 10 healthy, volunteer participants.

	Participant Demographics
Gender	5 male 5 female
Age (y), mean ± SD	39.6 ± 9.8
Intensity (Rate of Perceived Exertion scale)	7 strenuous (15) 3 maximal (20)

Table 2
Cellular and growth factor changes after 4 minutes of high intensity interval exercise

	Pre Exercise	Post Exercise	Significance
Platelets (x10^3/μL)	367.4 ± 57.5	497.7 ± 93.3	$P < .001$*
Mean platelet volume (fL)	7.9 ± 0.6	8.3 ± 1.0	$P = .013$
Red blood cells (x10^6/μL)	0.21 ± 0.16	0.30 ± 0.16	$P = .019$
White blood cells (x10^3/μL)	2.4 ± 1.2	6.1 ± 4.5	$P = .010$
Lymphocytes (%)	87.3 ± 7.6	89.3 ± 5.9	—
Monocytes (%)	9.3 ± 5.1	7.4 ± 3.8	—
Neutrophils (%)	3.4 ± 3.6	3.3 ± 3.6	—
TGF (pg/mL)	8237.2 ± 7676.5	21 535.7 ± 4062.6	$P = .004$*
PDGF (pg/mL)	5663.8 ± 3830.0	9407.3 ± 5117.1	$P = .076$
VEGF (pg/mL)	123.6 ± 120.0	132.1 ± 129.9	$P = .28$
IGF (pg/mL)	70.4 ± 17.9	83.5 ± 22.9	$P = .097$

Presented as mean ± standard deviation. TGF = transforming growth factor; PDGF = platelet-derived growth factor; VEGF = vascular endothelial growth factor; IGF = insulin-like growth factor. Asterisk (*) indicates significance (set at ≤.006 after the post-hoc Bonferroni correction was applied to correct for family-wise error rate).

The impact of HIIE on the cellular and growth factor content of PRP is displayed in Table 2. A significant difference in cell count between pre- and postexercise PRP concentrations was noted for platelets only. Preexercise mean platelet count was 367.4 ± 57.5 k/μL and increased to 497.7 ± 93.3 k/μL postexercise ($P < .001$). Considering the strenuous group only (as they accounted for the majority of volunteers and to ensure that changes are not driven by outliers in the maximum exertion group), their preexercise platelet count was 370.3 ± 56.9 k/μL and increased to 487.9 ± 83.3 ($P = .001$). Exercise did not significantly change mean platelet volume, RBCs, leukocyte count, or leukocyte differential.

A significant increase in GF concentration between pre- and postexercise PRP was noted only for TGF-β. Prior to exercise, the mean concentration was 8237.2 ± 7676.5 pg/mL and increased to 21 535.7 ± 4062.6 pg/mL postexercise ($P = .004$). For the strenuous group alone, TGF-β increased from 6975.54 ± 6992.93 pg/mL to 22 362.81 ± 1509.47 pg/mL ($P < .0001$). Differences in values for the other three growth factors were not statistically significant.

Three adverse events occurred in the 3 participants in the maximal exertion group. One of these participants had an exacerbation of previously diagnosed exercise-induced asthma, which was completely treated with use of a rescue bronchodilator. The other 2 experienced approximately 24 hours of coughing that resolved spontaneously without need for further treatment. No adverse events occurred in the strenuous group. All participants in both groups tolerated blood draws well. Specifically, there were no vasovagal events during the postexercise draws.

Discussion

The most important finding of this study is that a short bout of HIIE significantly increased the total platelet count and TGF-β concentration in PRP. No other cellular components (RBCs, leukocytes, MPV, or the differential) were significantly changed. Similarly, the other 3 GFs analyzed did not change significantly. This is the first study to analyze the effects of HIIE on PRP content and the first work to demonstrate an increase in GF concentration in response to exercise. These data show that a short exercise protocol increases the yield of key cellular and anabolic GF components of PRP, which has the potential to enhance its clinical effect.

The platelet count increased by 35% after HIIE in this study, which confirms that exercise-induced thrombocytosis changes PRP content. In addition to thrombocytosis, it is important to recognize that HIIE also leads to increased peripheral leukocytes (ie, exercise-induced leukocytosis), which should be considered especially if using a separation system that produces leukocyte-rich PRP.[32] The single-spin device used in this present study

maintained its ability to effectively eliminate leukocytes in the post-HIIE PRP, thereby minimizing the impact of exercise-induced leukocytosis on PRP created using this specific device.

At the GF level, the baseline concentrations are consistent with previously reported concentrations for ACP.[3,15,33] The postexercise PRP concentration of TGF-β increased over 160%. The reason for this large increase is not entirely understood. Certainly, the thrombocytosis accounts for some of the increase. It is also possible that the platelets in the splenic pool have larger GF reserves because they are not active in peripheral circulation and have not been depleted from activity in circulation, but this needs additional research to confirm. This increase has potentially significant clinical implications. TGF-β has an important and complex role in joint health. With regard to cartilage, in vitro models have shown that TGF-β has both anabolic and anticatabolic effects. It stimulates type II collagen and extracellular matrix synthesis, while blocking harmful inflammatory cytokines and degradative enzymes, like IL-1 and MMPs, respectively.[34–37] It also further antagonizes MMP activity by inducing production of tissue inhibitors of metalloproteinases (TIMP).[37] Though the effect of TGF-β on cartilage is positive, its effects are not exclusive to cartilage and concerns have been raised about potential adverse effects of TGF-β on other joint structures. Bakker et al found that prolonged exposure to TGF-β produced by an adenoviral vector induced osteophyte formation in murine knees.[38] Van Beuningen et al performed repeated pure TGF-β injections into murine joints and observed osteophyte formation and synovial hyperplasia.[39] Although these effects are important to consider, the models used are not reflective of the complex milieu of PRP. These murine models relied on repeated, and even constant, exposure to a single GF whereas PRP contains a multitude of GFs and more limited joint exposure. Therefore, the TGF- β from PRP does not necessarily have the same impact clinically as what was seen in the murine studies. Furthermore, a relatively new orthobiologic intervention, autologous protein solution (APS), has TGF-β concentrated 4-fold and in a pilot study, repeat magnetic resonance imaging of patients who underwent an APS injection demonstrated stable osteophyte size at 1 year compared to the control group, who experienced osteophyte enlargement.[40,41] The elevated TGF-β in APS did not worsen osteophytes, further demonstrating that TGF-β as part of a larger orthobiologic intervention does not have deleterious effects. Regarding the elevated TGF-β achieved by HIIE in this current work, prospective studies with clinical and imaging outcomes are necessary to define the effect on clinical outcomes including symptoms and structure.

The effect of HIIE on the other GFs analyzed in this study can be explained by considering the different sources of each GF. PDGF, as the name implies comes from platelets, and although the increase in PDGF did not reach statistical significance, there was an upward trend that likely is from the post-HIIE increase in platelet count.[42] The sample size of this study may have been too small to detect a statistically significant change. There was no significant change in VEGF, which corresponds to how the ACP system processes blood. VEGF is more abundant in leukocytes, so the unchanged VEGF concentration reflects the plasma-based system's ability to eliminate leukocytes.[17] Finally, although IGF is found in platelets, it is more abundant in plasma as it is excreted by the liver.[43] Because this system concentrates platelets and not the plasma, an increase in IGF specifically was not expected.

Two other studies have analyzed the effect of exercise on the cellular and GF content of PRP. Hamilton et al had 10 healthy volunteers perform 1 hour of cycling at 50% of their peak power output. They found no impact on cellular content and suppression, rather than elevation, of PDGF and VEGF concentration immediately and up to 18 hours after exercise.[44] Using a higher intensity protocol, Anz et al had 20 healthy participants perform 20 minutes of exercise at 70%-85% of their predicted maximum heart rate, which resulted in elevated platelets and hematopoietic progenitor cells in PRP.[45] The current data are most comparable to the work of Anz et al as both used higher intensity exercise, similar exercise equipment, and the ACP system to process blood. Platelet elevation was similar between studies; 23% in the work by Anz et al compared to 35% in the current work. Two additional useful findings are presented in this work. First, it demonstrated that a much shorter exercise protocol (4 minutes vs 20 minutes) can be used to increase platelet yield, which is important if a preprocedural exercise protocol were adopted into clinical practice. Second, it demonstrates that TGF-β concentrations increase after HIIE. This was critical to confirm because Hamilton et al showed a paradoxical effect of long duration exercise on GF and no GF analysis was performed by Anz et al.

Three adverse events occurred during the study. There was 1 exercise-induced asthma exacerbation that was effectively treated with the participant's inhaler. The other 2 bouts of coughing lasted approximately 24 hours and resolved spontaneously. Neither participant had known asthma or other respiratory condition and, therefore, these likely were episodes of exercise-induced bronchospasm. All 3 adverse events occurred in the maximal exertion group. No complications were observed in the strenuous group. Our largest concern prior to the study was the potential for vasovagal events during the blood draw, but none occurred. Each patient was instructed to arrive well hydrated and the blood draws were performed in the supine position, which likely helped them tolerate the blood draw.

Although participants in this work were advised to train before testing to allow for acclimation, it has been shown that sedentary participants also experience

exercise-induced thrombocytosis.[27] Because platelet elevation has been observed in a sedentary population and this current work found submaximal exercise yielded increased platelets, preparatory training and maximal exertion are not necessary to elicit this platelet response. Because the submaximal, strenuous protocol was more easily tolerated and still resulted in a significant increase in platelets, we recommend its use for future research and clinical use.

Study Limitations

There are several limitations of this work that should be considered. First, the sample size was small and potentially limited the ability to detect a change in parameters such as PDGF and MPV. However, the size is comparable to other similar work.[44,45] Second, this study lacks an objective measure of workload and instead relied upon RPE, which is subject to significant variability in the workload actually performed for each individual. Although quantitative measures of work are more reproducible and yield information on workload needed to induce a change, we selected the Borg RPE scale because of its widespread use in exercise science and simplicity in clinical application.[46] Future work could use an objective workload measure to determine what level of physical stress is needed to induce the thrombocytosis. Third, there was an uneven distribution between intensity groups, which precludes any comparison between groups. The intention was to have 5 volunteers in each group, but because all testing was performed on a single day, technical and procedural difficulties with the blood draw and processing prevented 2 highly trained volunteers from participating. Therefore, 2 less trained volunteers were enrolled who could only tolerate the strenuous protocol. No statistical comparison can be made between the groups, but each participant had an increase in their platelet count, which indicates that maximal exertion may not be necessary to induce a positive hematological response. The fourth limitation is that of a true control group that could account for any changes potentially induced by 2 blood draws in the absence of exercise. Finally, because no baseline fitness testing was performed, there may exist heterogeneity in fitness levels among participants. This could affect the amount of work performed at the assigned RPE, with more highly trained individuals potentially achieving higher workloads. Future research should quantify the workload associated with each RPE level needed to induce the hematologic response observed.

Conclusions

This work demonstrates that a short bout of HIIE effectively increases platelet count and TGF-β concentration in PRP. Prospective, controlled trials with patient-reported and imaging outcomes will further define the clinical efficacy and structural impact of post-HIIE PRP.

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References

1. Marx RE. Platelet-rich plasma (PRP): what is PRP and what is not PRP? *Implant Dent*. 2001;10(4):225-228.
2. Mazzucco L, Balbo V, Cattana E, Guaschino R, Borzini P. Not every PRP-gel is born equal. Evaluation of growth factor availability for tissues through four PRP-gel preparations: Fibrinet, RegenPRP-kit, Plateltex and one manual procedure. *Vox Sang*. 2009;97(2): 110-118.
3. Sundman EA, Cole BJ, Fortier LA. Growth factor and catabolic cytokine concentrations are influenced by the cellular composition of platelet-rich plasma. *Am J Sports Med*. 2011;39(10): 2135-2140.
4. Cross JA, Cole BJ, Spatny KP, et al. Leukocyte-reduced platelet-rich plasma normalizes matrix metabolism in torn human rotator cuff tendons. *Am J Sports Med*. 2015;43(12):2898-2906.
5. Yang J, Lu Y, Guo A. Platelet-rich plasma protects rat chondrocytes from interleukin-1beta-induced apoptosis. *Mol Med Rep*. 2016;14 (5):4075-4082.
6. Zhang L, Chen S, Chang P, et al. Harmful effects of leukocyte-rich platelet-rich plasma on rabbit tendon stem cells in vitro. *Am J Sports Med*. 2016;44(8):1941-1951.
7. Xie X, Ulici V, Alexander PG, Jiang Y, Zhang C, Tuan RS. Platelet-rich plasma inhibits mechanically induced injury in chondrocytes. *Arthroscopy*. 2015;31(6):1142-1150.
8. Cole BJ, Karas V, Hussey K, Pilz K, Fortier LA. Hyaluronic acid versus platelet-rich plasma: a prospective, double-blind randomized controlled trial comparing clinical outcomes and effects on intra-articular biology for the treatment of knee osteoarthritis. *Am J Sports Med*. 2017;45(2):339-346.
9. Filardo G, Di Matteo B, Di Martino A, et al. Platelet-rich plasma intra-articular knee injections show no superiority versus Viscosupplementation: a randomized controlled trial. *Am J Sports Med*. 2015;43(7):1575-1582.
10. Mishra AK, Skrepnik NV, Edwards SG, et al. Efficacy of platelet-rich plasma for chronic tennis elbow: a double-blind, prospective, multicenter, randomized controlled trial of 230 patients. *Am J Sports Med*. 2014;42(2):463-471.
11. Patel S, Dhillon MS, Aggarwal S, Marwaha N, Jain A. Treatment with platelet-rich plasma is more effective than placebo for knee osteoarthritis: a prospective, double-blind, randomized trial. *Am J Sports Med*. 2013;41(2):356-364.
12. Sanchez M, Fiz N, Azofra J, et al. A randomized clinical trial evaluating plasma rich in growth factors (PRGF-Endoret) versus hyaluronic acid in the short-term treatment of symptomatic knee osteoarthritis. *Arthroscopy*. 2012;28(8):1070-1078.
13. Smith PA. Intra-articular autologous conditioned plasma injections provide safe and efficacious treatment for knee osteoarthritis: an FDA-sanctioned, randomized, double-blind, placebo-controlled clinical trial. *Am J Sports Med*. 2016;44(4):884-891.
14. Xu Z, Luo J, Huang X, Wang B, Zhang J, Zhou A. Efficacy of platelet-rich plasma in pain and self-report function in knee osteoarthritis: a best-evidence synthesis. *Am J Phys Med Rehabil*. 2017;96(11):793-800.

15. Mazzocca AD, McCarthy MB, Chowaniec DM, et al. Platelet-rich plasma differs according to preparation method and human variability. *J Bone Joint Surg Am*. 2012;94(4):308-316.
16. Castillo TN, Pouliot MA, Kim HJ, Dragoo JL. Comparison of growth factor and platelet concentration from commercial platelet-rich plasma separation systems. *Am J Sports Med*. 2011;39(2):266-271.
17. Oudelaar BW, Peerbooms JC, Huis in't veld R, AJH V. Concentrations of blood components in commercial platelet-rich plasma separation systems: a review of the literature. *Am J Sports Med*. 2018;47(2): 479-487.
18. Taniguchi Y, Yoshioka T, Sugaya H, et al. Growth factor levels in leukocyte-poor platelet-rich plasma and correlations with donor age, gender, and platelets in the Japanese population. *J Exp Orthopaed*. 2019;6(1):4.
19. Evanson JR, Guyton MK, Oliver DL, et al. Gender and age differences in growth factor concentrations from platelet-rich plasma in adults. *Mil Med*. 2014;179(7):799-805.
20. Xiong G, Lingampalli N, Koltsov JCB, et al. Men and women differ in the biochemical composition of platelet-rich plasma. *Am J Sports Med*. 2018;46(2):409-419.
21. Tian J, Lei XX, Xuan L, Tang JB, Cheng B. The effects of aging, diabetes mellitus, and antiplatelet drugs on growth factors and anti-aging proteins in platelet-rich plasma. *Platelets*. 2019;30(6):773-792.
22. Mannava S, Whitney KE, Kennedy MI, et al. The influence of naproxen on biological factors in leukocyte-rich platelet-rich plasma: a prospective comparative study. *Arthroscopy*. 2019;35 (1):201-210.
23. Jayaram P, Yeh P, Patel SJ, et al. Effects of aspirin on growth factor release from freshly isolated leukocyte-rich platelet-rich plasma in healthy men: a prospective fixed-sequence controlled laboratory study. *Am J Sports Med*. 2019;47(5):1223-1229.
24. Dawson AA, Ogston D. Exercise-induced thrombocytosis. *Acta Haematol*. 1969;42(4):241-246.
25. Shephard RJ. Responses of the human spleen to exercise. *J Sports Sci*. 2016;34(10):929-936.
26. 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.
27. Belviranli M, Okudan N, Kabak B. The effects of acute high-intensity interval training on hematological parameters in sedentary subjects. *Med Sci*. 2017;5(3):15.
28. Tabata I, Nishimura K, Kouzaki M, et al. Effects of moderate-intensity endurance and high-intensity intermittent training on anaerobic capacity and VO2max. *Med Sci Sports Exerc*. 1996;28 (10):1327-1330.
29. Borg G. Perceived exertion as an indicator of somatic stress. *Scand J Rehabil Med*. 1970;2(2):92-98.
30. Zimmermann R, Arnold D, Strasser E, et al. Sample preparation technique and white cell content influence the detectable levels of growth factors in platelet concentrates. *Vox Sang*. 2003;85(4):283-289.
31. Schnabel LV, Mohammed HO, Miller BJ, et al. Platelet rich plasma (PRP) enhances anabolic gene expression patterns in flexor digitorum superficialis tendons. *J Orthop Res*. 2007;25(2): 230-240.
32. Steel JM, Steel CM, Johnstone FD. Leukocytosis induced by exercise. *Br Med J*. 1987;295(6606):1135-1136.
33. Magalon J, Bausset O, Serratrice N, et al. Characterization and comparison of 5 platelet-rich plasma preparations in a single-donor model. *Arthroscopy*. 2014;30(5):629-638.
34. Redini F, Daireaux M, Mauviel A, Galera P, Loyau G, Pujol JP. Characterization of proteoglycans synthesized by rabbit articular chondrocytes in response to transforming growth factor-beta (TGF-beta). *Biochim Biophys Acta*. 1991;1093(2–3):196-206.
35. Redini F, Galera P, Mauviel A, Loyau G, Pujol JP. Transforming growth factor beta stimulates collagen and glycosaminoglycan biosynthesis in cultured rabbit articular chondrocytes. *FEBS Lett*. 1988;234(1): 172-176.
36. Redini F, Mauviel A, Pronost S, Loyau G, Pujol JP. Transforming growth factor beta exerts opposite effects from interleukin-1 beta on cultured rabbit articular chondrocytes through reduction of interleukin-1 receptor expression. *Arthritis Rheum*. 1993;36(1):44-50.
37. Hui W, Rowan AD, Cawston T. Modulation of the expression of matrix metalloproteinase and tissue inhibitors of metalloproteinases by TGF-beta1 and IGF-1 in primary human articular and bovine nasal chondrocytes stimulated with TNF-alpha. *Cytokine*. 2001;16(1): 31-35.
38. Bakker AC, van de Loo FA, van Beuningen HM, et al. Overexpression of active TGF-beta-1 in the murine knee joint: evidence for synovial-layer-dependent chondro-osteophyte formation. *Osteoarthr Cartil*. 2001;9(2):128-136.
39. van Beuningen HM, van der Kraan PM, Arntz OJ, van den Berg WB. Transforming growth factor-beta 1 stimulates articular chondrocyte proteoglycan synthesis and induces osteophyte formation in the murine knee joint. *Lab Investig*. 1994;71(2): 279-290.
40. Kon E, Engebretsen L, Verdonk P, Nehrer S, Filardo G. Clinical outcomes of knee osteoarthritis treated with an autologous protein solution injection: a 1-year pilot double-blinded randomized controlled trial. *Am J Sports Med*. 2018;46(1): 171-180.
41. O'Shaughnessey K, Matuska A, Hoeppner J, et al. Autologous protein solution prepared from the blood of osteoarthritic patients contains an enhanced profile of anti-inflammatory cytokines and anabolic growth factors. *J Orthop Res*. 2014;32(10):1349-1355.
42. Miyazono K, Takaku F. Platelet-derived growth factors. *Blood Rev*. 1989;3(4):269-276.
43. Ionita CR, Troillet AR, Vahlenkamp TW, Winter K, Brehm W, Ionita JC. Comparison of humoral insulin-like growth factor-1, platelet-derived growth factor-BB, transforming growth factor-beta1, and interleukin-1 receptor antagonist concentrations among equine autologous blood-derived preparations. *Am J Vet Res*. 2016; 77(8):898-905.
44. Hamilton B, Tol JL, Knez W, Chalabi H. Exercise and the platelet activator calcium chloride both influence the growth factor content of platelet-rich plasma (PRP): overlooked biochemical factors that could influence PRP treatment. *Br J Sports Med*. 2015;49(14): 957-960.
45. Anz AW, Parsa RS, Romero-Creel MF, et al. Exercise-mobilized platelet-rich plasma: short-term exercise increases stem cell and platelet concentrations in platelet-rich plasma. *Arthroscopy*. 2019;35(1):192-200.
46. Russell WD. On the current status of rated perceived exertion. *Percept Mot Skills*. 1997;84(3 Pt 1):799-808.

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Written consent obtained from all volunteers.

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