Exercise-Mobilized Platelet-Rich Plasma: Short-Term Exercise Increases Stem Cell and Platelet Concentrations in Platelet-Rich Plasma

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Purpose: To evaluate the effects of vigorous short-term exercise on the platelet and other cellular components of 2 point-of-care blood-processing devices: a buffy coat–based platelet-rich plasma (PRP) product and a plasma-based PRP product. **Methods:** Twenty healthy subjects (aged 21-45 years) participated in a 20-minute vigorous exercise regimen on an upright stationary bike at 70% to 85% of maximum target heart rate. Pre- and post-exercise blood was processed in either a plasma-based or automated buffy coat–based PRP system. Complete blood counts were used to compare the cellular components in whole blood and the PRP products. **Results:** Exercise significantly increased the concentrations of platelets by over 20% in whole blood ($P < .001$) and in both PRP products ($P = .002$ and $P = .018$). Both devices performed consistently with pre- and post-exercise blood. Buffy coat–based PRP prepared after exercise was also significantly larger in volume and had a significantly higher concentration of mobilized hematopoietic stem cells (hematopoietic progenitor cells [HPCs], from 1.7/μL to 2.7/μL, $P = .043$). The concentrations of all white blood cell types were increased, which could be differentially collected in the devices studied. **Conclusions:** Exercise can be used to consistently alter the composition of PRP. Twenty minutes of vigorous exercise can increase platelet concentrations in plasma-based and buffy coat–based PRP products and can increase HPC concentrations and volume in buffy coat–based PRP. **Clinical Relevance:** This study shows a nonpharmacologic method to increase platelet and HPC harvests from peripheral blood. This is important because it highlights a method for altering biological therapies with limited comorbidity.

Basic science and early clinical studies on stem cells and point-of-care blood products, such as platelet-rich plasma (PRP), have created optimism and anticipation in the medical community, patients, and industry alike for improved innovations. Although products such as PRP have been applied for decades, controversy remains regarding clinical efficacy. Early shortcomings not only are attributable to the variable outputs of the products themselves but also can be attributed to the variability of cellular components in the blood of individuals. To properly leverage emerging innovations, the orthopaedic community must understand all intricacies of harvest and preparation, including activities of individuals before blood harvest, to optimize the composition of these products for clinical effectiveness.

Current production of PRP is based on 2 principles: (1) the centrifugation of blood creates a density gradient of its constituents, and (2) through selective harvest of a fraction of the density gradient, a product can be created with altered concentrations of blood components. There are 2 predominant types of PRP systems: buffy coat–based systems and plasma-based systems. Buffy coat systems often involve a "hard spin," a long spin time at a high speed, which creates a hard stack of the blood components. Buffy coat–based PRP products are typically low volume and contain high concentrations of platelets and white blood cells (WBCs). Plasma-based PRP products involve a "soft

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spin," a shorter spin at a slower speed, which creates a soft stack of the blood constituents. These products produce a larger-volume PRP that contains a moderate increase in the concentration of platelets compared with blood and a lower concentration of WBCs compared with blood (Fig 1).[1,2] Although studies have evaluated the performance of these systems to process blood, investigation is lacking into how these systems perform when the components of blood are altered with exercise before processing.[3-5]

Exercise, environmental stress, and injury have been established as mechanisms that mobilize components of the immune system to the peripheral circulation, including platelets and hematopoietic stem cells (hematopoietic progenitor cells [HPCs]).[6-13] The ability of mobilized stem cells to home to areas of injury and participate in tissue repair has been documented, and as such, these mobilized cells may serve as part of a repair mechanism after exercise or injury.[8,14,15] Similar to the optimization of centrifugation and selective harvest protocols in the production of PRP, manipulating the immune system with exercise before blood harvest may improve the performance of PRP in clinical practice. To develop the concept of exercise-mobilized PRP, the first step was to evaluate the performance of PRP systems with exercise-mobilized blood, that is, the consistency of exercise-mobilized PRP production. The purpose of this study was to evaluate the effects of vigorous short-term exercise on the platelet and other cellular components of 2 point-of-care blood-processing devices. We hypothesized that a short-term exercise regimen would consistently increase the yield of cells and platelets in a buffy coat–based PRP product and increase the yield of platelets alone in a plasma-based PRP product.

Methods

Subjects and Exercise Protocol

We recruited 20 subjects (10 per device phase) by word of mouth for this study and obtained written informed consent (Salus Institutional Review Board No. 1082). The inclusion criteria included healthy adults with a willingness to donate blood and participate in the indicated exercise regimen. The exclusion criteria included age under 18 years or weight under 110 lb; pregnant women; and a blood donation during the preceding 60 days that, including study draw, would exceed 550 mL. Blood draws by a licensed phlebotomist, exercise protocols, and sample testing were performed in the research laboratory at Arthrex in Naples, Florida, over an 8-month period. The temperature in the laboratory consistently ranged from 70°F to 73°F. Each subject performed his or her entire testing in a single day. For the first 10 subjects, blood processing involved the ACP system (Arthrex, Naples, FL), and for the second 10 subjects, blood processing involved the Angel system (Arthrex). Each donor served as his or her own baseline. In the morning, participants arrived at the laboratory and a pre-exercise blood collection, by antecubital venipuncture, was performed. The volume of blood was determined by device manufacturer's specifications. For the first 10 subjects, both pre- and

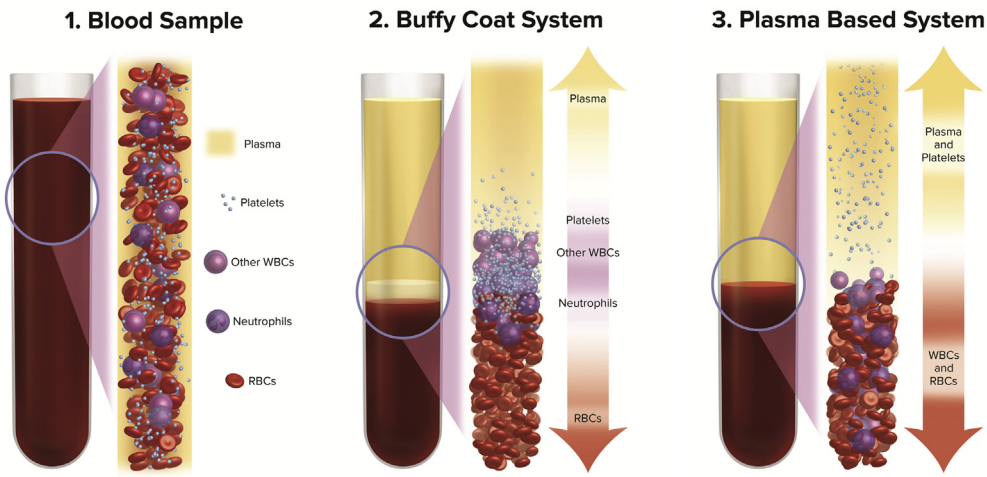

Fig 1. (1) Point-of-care blood products involve the principles of centrifugation to create a density gradient of the blood components and selective harvest from the density gradient. (2) Buffy coat–based products involve performing a longer, faster centrifugation spin to create a hard stack, and isolating the buffy coat produces a low-volume product that contains increased concentrations of platelets (up to 15-fold) and white blood cells (WBCs) (up to 7-fold). (3) Plasma-based systems involve performing a shorter, slower centrifugation spin to create a soft stack, and isolating the layer above the transitional zone produces a larger-volume product that contains an increased concentration of platelets (approximately 2-fold) and a decreased concentration of WBCs (0.04-fold). (RBCs, red blood cells.)

post-exercise blood draws involved the harvest of one sample of 18 mL of blood collected into a 20-mL syringe preloaded with 2 mL of ACDA (anticoagulant citrate dextrose, solution A; Fenwal, Lake Zurich, IL). For the second 10 subjects, both blood draws involved the harvest of 3 samples of 52 mL of blood into a 60-mL syringe preloaded with 8 mL of ACDA.

Four hours after the pre-exercise blood draw and PRP preparation, participants returned to the laboratory and the resting heart rate was recorded. Between the initial pre-exercise blood draw and the following protocol, the subjects were engaged in sedentary office-work activity and did not engage in physical activity. Food and liquid intake was not monitored. The time delay was set to aid in the logistics of the study and for subject comfort. Heart rate was chosen as a biometric measure because it is a simple noninvasive method to monitor and control exercise intensity, and maximum heart rate was determined using the traditional equation of subtracting the participants' age from 220.[16] Participants then followed an exercise regimen on a Schwinn upright bike (model 170; Schwinn Fitness, Vancouver, WA). The exercise regimen involved a 5-minute warm-up period followed by 20 minutes of vigorous exercise determined by maintenance of the target heart rate at 70% to 85% of maximum target heart rate. After completion, the actual maximum heart rate was recorded and a second, post-exercise blood sample was collected from the contralateral arm with a method identical to the pre-exercise blood harvest.

Preparation of ACP or Angel PRP

Pre- and post-exercise blood was processed in an identical fashion within each group. In the plasma-based PRP group, 15 mL of anticoagulated blood was transferred to an ACP double syringe, with the remaining sample used for baseline complete blood count (CBC) analysis. The ACP syringe was processed for 5 minutes at 1,500 rpm in a Hettich Rotofix 32A Centrifuge (Andreas Hettich, Tuttlingen, Germany). The platelet-enriched plasma layer was pulled into the inner syringe until the red blood cell (RBC) layer was reached, per the manufacturer's instructions. The volume of product was recorded, and a sample was taken for CBC analysis. To prepare buffy coat–based PRP, the automated Arthrex Angel system was used. A sample from 1 syringe was taken for baseline CBC analysis, and the anticoagulated blood from the 3 syringes was injected into an Angel concentrated PRP system and processed at a 15% hematocrit setting. Both platelet-poor plasma (PPP) and PRP were collected from the Angel system output, and the volumes were recorded.

Complete Blood Counts

CBCs were obtained from pre- and post-exercise whole blood, autologous conditioned plasma (ACP), PPP, and PRP samples using a Sysmex XE-5000 Hematology Analyzer (Lincolnshire, IL) in HPC mode within 20 minutes of collection. Each sample was read in triplicate, and mean values were used for statistical analysis. Platelet, RBC, overall WBC, and WBC differential (neutrophil, monocyte, lymphocyte, and immature granulocyte [IG]) counts were analyzed. HPC counts were determined from the immature myeloid information channel. Immature cells observed in this channel undergo slower lysis than mature cells owing to a lower lipid content in their membranes. HPCs are gated on this channel based on direct current (size) and radiofrequency (density). The HPC measurements obtained by this method have been shown to correlate with $CD34^+$ cells in peripheral blood samples.[17-19]

Data and Statistical Analysis

The mean and standard deviation were calculated for all values. Fold changes in cell content were calculated on a per-donor basis and averaged. If a zero value was obtained for the pre-exercise but not post-exercise HPC concentration, fold change was assigned as the highest number observed in the group. Statistical analysis was performed in the SigmaPlot software package (version 11.0; Systat, San Jose, CA). Unpaired *t* tests were used to compare data between the 2 cohorts. Paired *t* tests were used to determine any significant differences in pre- and post-exercise donor-matched data, such as volume and cell concentration in whole blood, ACP, and PRP. Significance was set at $P \leq .05$. Normality was confirmed by the Shapiro-Wilk test. Statistical differences in nonparametric data were determined with the Wilcoxon signed rank test. Box-and-whisker plots were prepared in OriginPro (version 9.1; OriginLab, Northampton, MA).

Results

Subject age, sex, resting heart rate, and maximum heart rate are presented in Table 1. Whole blood showed an exercise-induced statistically significant increase in the concentrations of all cellular components except HPCs ($P = .14$, Fig 2). There was a 22% increase in average platelet concentration, from $195 \pm 41 \times 10^3/\mu L$ to $237 \pm 50 \times 10^3/\mu L$ after exercise. The total WBC concentration increased by 50%, from $5.8 \times 10^3/\mu L$ to $8.6 \times 10^3/\mu L$. The WBC differential remained consistent before and after exercise ($P = .26$ to $P = .85$), indicating a consistent mobilization response occurred for all WBC cell types. Furthermore, there was no significant difference in cell concentration fold increase between each phase (ACP vs Angel) of the study ($n = 10$); therefore, each device was evaluated using blood with a similar mobilization response ($P = .11$ to $P = .97$, Table 2).

The PRP systems investigated in this study concentrated cellular constituents differently but consistently

Table 1. Donor Demographic Characteristics and Heart Rates for Each Phase of Study

	Phase 1: ACP	Phase 2: Angel PRP	Total or Average	*P* Value
Sex, n	5 F and 5 M	5 F and 5 M	10 F and 10 M	NA
Age, yr	31.1 ± 8.4 (22-45)	29.9 ± 8.5 (21-43)	30.5 ± 8.3 (21-45)	.71
Resting heart rate, beats/min	78 ± 14 (62-90)	70 ± 13 (58-100)	72 ± 13.1 (58-100)	.34
Maximum heart rate, beats/min	168 ± 10 (158-180)	165 ± 11 (149-181)	166 ± 10 (149-181)	.68

NOTE. Data are presented as mean ± standard deviation (range) unless otherwise indicated.
ACP, autologous conditioned plasma; F, female; M, male; NA, not applicable; PRP, platelet-rich plasma.

regardless of whether the blood was collected and processed before or after exercise (Fig 3). Platelets were concentrated by both systems. The ACP system reduced RBCs and overall WBCs, whereas lymphocytes were the only WBCs that were not significantly reduced. The Angel PRP system increased WBCs, and RBCs were reduced by 20% compared with baseline ($P = .03$).

By use of the ACP system, platelet and WBC concentrations were significantly increased by exercise (Fig 4). The post-exercise platelet concentration in ACP was 562 ± 142 × 10^3/μL compared with 457 ± 123 × 10^3/μL before exercise. The increase in WBCs was attributed to a significantly higher lymphocyte concentration in post-exercise ACP. Neutrophil, monocyte, and RBC concentrations were not significantly increased. IGs and HPCs were reduced in ACP, regardless of exercise.

By use of the Angel system to prepare PRP, a higher cellular content was obtained in post-exercise PRP samples (Fig 5); volume was also significantly higher

Whole Blood

	PLT (k/μl)	HPC (/μl)	WBC (k/μl)	NE (k/μl)	LY (k/μl)	MO (k/μl)	IG (/μl)	RBC (M/μl)
Pre-Exercise	195 ± 40	0.4 ± 0.5	5.8 ± 0.9	3.2 ± 0.8	1.9 ± 0.5	0.5 ± 0.1	19.3 ± 20.6	4.2 ± 0.3
Post-Exercise	237 ± 50	0.6 ± 1.0	8.6 ± 1.9	4.8 ± 1.6	2.9 ± 1.0	0.7 ± 0.2	26.2 ± 25.4	4.3 ± 0.4
p-value	<0.001	0.147	<0.001	<0.001	<0.001	<0.001	0.002	0.018

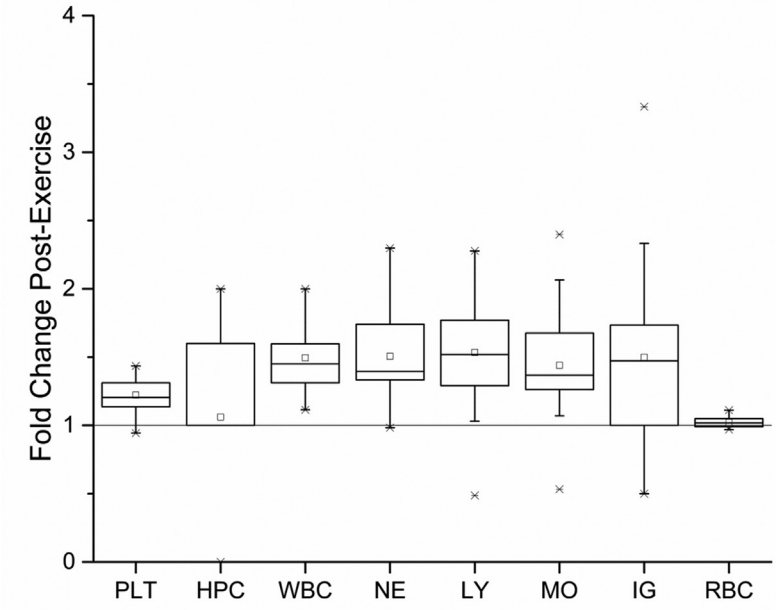

Fig 2. Cell concentrations in whole blood before and after exercise (N = 20). The box plots show donor fold changes in concentrations after exercise. There was a significant increase in all cell types except hematopoietic progenitor cells (HPCs). Boxes depict interquartile ranges, and horizontal lines are median values. Small open squares indicate mean values, and whiskers extend to the range within an interquartile range of 1.5. Small crosshairs are minimum and maximum values. (IG, immature granulocytes; k, thousand; LY, lymphocytes; M, million; MO, monocytes; NE, neutrophils; PLT, platelets; RBC, red blood cells; WBC, white blood cells.)

Table 2. Fold Change in Cell Concentration in Whole Blood after Exercise for Each Cohort (n = 10)

	Fold Change		
	Phase 1: ACP	Phase 2: Angel PRP	*P* Value
PLT	1.2 ± 0.1	1.3 ± 0.1	.23
HPC	1.0 ± 0.7	1.1 ± 0.7	.70
WBC	1.4 ± 0.2	1.5 ± 0.3	.45
NE	1.4 ± 0.2	1.6 ± 0.4	.33
LY	1.5 ± 0.3	1.5 ± 0.5	.97
MO	1.5 ± 0.3	1.4 ± 0.5	.71
IG	1.3 ± 0.5	1.7 ± 0.7	.21
RBC	1.0 ± 0.0	1.0 ± 0.0	.29

NOTE. Data are presented as mean ± standard deviation.

ACP, autologous conditioned plasma; HPC, hematopoietic progenitor cells; IG, immature granulocytes; LY, lymphocytes; MO, monocytes; NE, neutrophils; PLT, platelet; RBC, red blood cells; WBC, white blood cells.

than pre-exercise PRP volume ($P = .02$). The volume of PPP was unchanged ($P = .52$). The post-exercise platelet concentration in PRP was 3,774 ± 1,270 × 10^3/μL compared with 2,953 ± 1,199 × 10^3/μL before exercise ($P = .018$). As an exception to this trend, the Angel system collected PRP to a desired hematocrit setting, so there was no significant difference in RBC concentration after exercise. This PRP system also sequestered the IG and HPC components, resulting in an increase in the concentrations of these cell types in post-exercise compared with pre-exercise samples.

Discussion

The most important finding of this study is that the platelet and other cellular components of PRP can be consistently manipulated with exercise before blood harvest. Whereas previous studies have investigated the effects of exercise on blood, our study investigated the performance consistency of 2 PRP devices with pre- and post-exercise blood and the reliability of exercise to alter a PRP product. The next steps are to optimize exercise regimens for specific blood component mobilization and then evaluate the clinical impact of exercise-mobilized PRP.

Exercise increased the concentrations of platelets in whole blood and PRP products by over 20%. Regarding WBCs, all types were increased in whole blood after exercise. In ACP, the concentration of lymphocytes, but not neutrophils or monocytes, was significantly increased after exercise. In contrast, the concentrations of all types of WBCs after exercise were increased in Angel PRP, including a doubling in the concentration of HPCs in post-exercise PRP when the donor-based

Device Fold Change Compared to Baseline

	PLT (k/μl)	HPC (/μl)	WBC (k/μl)	NE (k/μl)	LY (k/μl)	MO (k/μl)	IG (/μl)	RBC (M/μl)
ACP (Pre) ACP (Post)	2.2 ± 0.2 2.3 ± 0.2	0.2 ± 0.4 0.1 ± 0.2	0.4 ± 0.1 0.4 ± 0.2	0.0 ± 0.0 0.0 ± 0.0	1.0 ± 0.3 0.9 ± 0.4	0.4 ± 0.2 0.4 ± 0.2	0.1 ± 0.3 0.1 ± 0.3	0.0 ± 0.0 0.0 ± 0.0
P-value	0.19	0.54	0.83	0.80	0.59	0.85	0.44	0.15
Angel PRP (Pre) Angel PRP (Post)	15.5 ± 4.9 15.8 ± 3.2	5.6 ± 3.3 6.8 ± 2.3	7.1 ± 1.9 7.8 ± 2.9	3.1 ± 2.1 4.3 ± 3.1	13.3 ± 3.1 12.9 ± 2.7	13.3 ± 3.3 13.4 ± 3.0	14.7 ± 6.3 32.2 ± 39.4	0.9 ± 0.5 0.7 ± 0.3
p-value	0.84	0.45	0.46	0.12	0.78	0.90	0.16	0.36

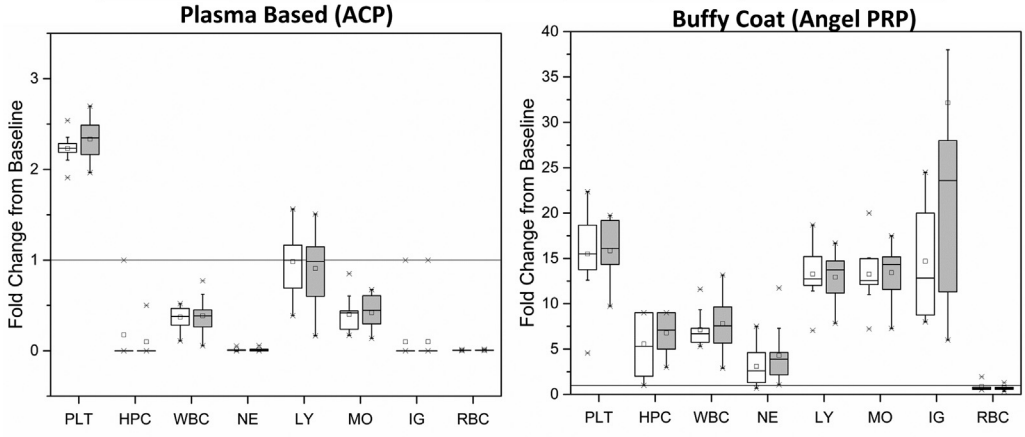

Fig 3. Both platelet-rich plasma (PRP) systems performed consistently, either concentrating or reducing cell types similarly, whether blood was collected before exercise (unshaded) or after exercise (shaded). When pre-exercise blood (Pre) and post-exercise blood (Post) were compared, the ACP system increased the platelet (PLT) concentration by a factor of 2.2 ± 0.2 and 2.3 ± 0.2, respectively, and the Angel device increased the PLT concentration by a factor of 15.5 ± 4.9 and 15.8 ± 3.2, respectively. Boxes depict interquartile ranges, and horizontal lines are median values. Small open squares indicate mean values, and whiskers extend to the range within an interquartile range of 1.5. Small crosshairs are minimum and maximum values. (HPC, hematopoietic progenitor cells; IG, immature granulocytes; k, thousand; LY, lymphocytes; M, million; MO, monocytes; NE, neutrophils; RBC, red blood cells; WBC, white blood cells.)

Plasma Based (ACP)

	Volume (ml)	PLT (k/µl)	HPC (/µl)	WBC (k/µl)	NE (k/µl)	LY (k/µl)	MO (k/µl)	IG (/µl)	RBC (M/µl)
Pre-Exercise	4.0 ± 1.0	457 ± 123	0.1 ± 0.3	2.3 ± 0.8	0.04 ± 0.04	2.0 ± 0.7	0.2 ± 0.1	0.3 ± 1.5	0.03 ± 0.01
Post-Exercise	4.0 ± 1.0	562 ± 142	0.3 ± 0.4	3.4 ± 1.7	0.05 ± 0.07	3.0 ± 1.5	0.3 ± 0.2	7.0 ± 22	0.03 ± 0.02
p-value	0.945	0.002	0.625	0.035	0.585	0.030	0.152	1.000	0.107

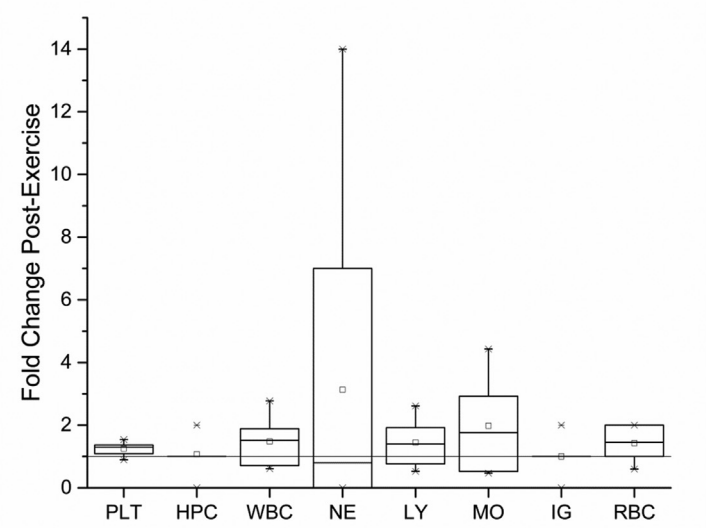

Fig 4. Cellular component concentration in ACP system output before and after exercise (n = 10). Autologous conditioned plasma (ACP) prepared after exercise showed a significant increase in the platelet (PLT) count relative to ACP prepared before exercise. The increase in the white blood cell (WBC) count was attributed to the lymphocyte (LY) increase. The red blood cell (RBC) concentration and total volume were unaffected. Boxes depict interquartile ranges, and horizontal lines are median values. Small open squares indicate mean values, and whiskers extend to the range within an interquartile range of 1.5. Small crosshairs are minimum and maximum values. (HPC, hematopoietic progenitor cells; IG, immature granulocytes; k, thousand; M, million; MO, monocytes; NE, neutrophils.)

increases were averaged. The post-exercise Angel PRP volume was also increased, which is attributed to the automation of the Angel system, in which outputs are based on the presence of cells detected by the sensor. We accept our hypothesis that exercise can be used to manipulate the products of point-of-care blood products. We were able to validate that a short-term exercise regimen would reliably and consistently increase the concentration of cellular components and show the expected change in cellular composition of point-of-care blood products after exercise using the ACP or Angel system at the indicated setting. Each device produced a consistent PRP preparation relative to whole blood regardless of whether the blood was collected before or after exercise.

Previous studies have evaluated the composition of PRP. DeLong et al.[20] reviewed outputs from 7 different commercial systems, showing a range from a 1.3- to 10-fold increase in platelet concentrations over baseline with starting volumes from 9 to 60 mL. Castillo et al.[3] examined the output of 3 different commercial systems and found no significant difference in mean PRP platelet or RBC concentrations but found a significant difference among all systems in the concentrations of WBCs. Sundman et al.[4] compared the cellular and chemokine product composition of a plasma-based system with a buffy coat–based system and found similar results to those in our study, with a 2-fold increase in platelets using the plasma-based system and a higher concentration of platelets and neutrophils using the buffy coat–based system.

Inconsistencies in PRP composition combined with the pathologic variability of differing indications likely contributes to contradictions in clinical outcome studies on PRP. Regardless of the variability, progress is being made through clinical trials. Meta-analysis has determined that leukocyte-poor PRP is an effective treatment for knee osteoarthritis and leukocyte-rich PRP is an effective treatment for tendinopathy.[21,22] Progress highlights the importance of understanding and manipulating the cellular components of PRP. Within the cellular composition, it is also important to consider WBC type. Neutrophils are often associated with an inflammatory and catabolic response, and HPCs and

Buffy Coat (Angel PRP)

	PPP Volume (ml)	PRP Volume (ml)	PLT (k/μl)	HPC (/μl)	WBC (k/μl)	NE (k/μl)	LY (k/μl)	MO (k/μl)	IG (/μl)	RBC (M/μl)
Pre-Exercise	89.8 ± 7.0	9.3 ± 1.7	2953 ± 1199	1.7 ± 2.7	38.4 ± 10.8	8.6 ± 4.8	23.1 ± 8.5	5.6 ± 1.6	292 ± 435	3.5 ± 1.9
Post-Exercise	89.2 ± 7.0	10.8 ± 2.6	3774 ±1270	2.7 ± 3.5	62.6 ± 21.7	19.4 ± 17.0	33.4 ± 14.4	8.1 ± 3.3	646 ± 651	3.0 ± 1.2
p-value	0.515	0.023	0.018	0.043	0.008	0.004	0.047	0.024	0.002	0.379

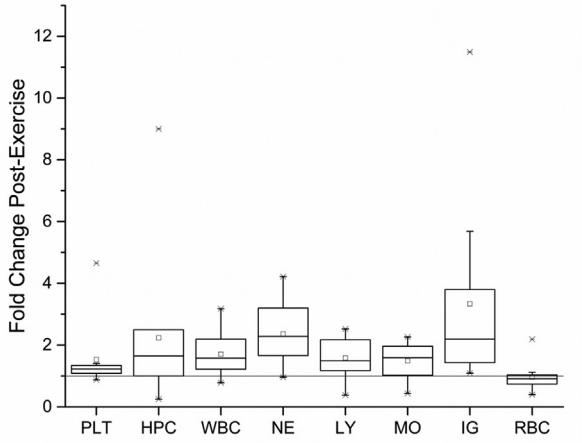

Fig 5. Cellular component concentration in Angel platelet-rich plasma (PRP) before and after exercise (n = 10). PRP collected after exercise was significantly greater in volume compared with PRP collected before exercise owing to the automation of the Angel system. Because the Angel system collected PRP to a desired hematocrit setting, there was no increase in the red blood cell (RBC) concentration. Post-exercise PRP showed significant increases in all cell types. Boxes depict interquartile ranges, and horizontal lines are median values. Small open squares indicate mean values, and whiskers extend to the range within an interquartile range of 1.5. Small crosshairs are minimum and maximum values. (HPC, hematopoietic progenitor cells; IG, immature granulocytes; k, thousand; LY, lymphocytes; M, million; MO, monocytes; NE, neutrophils; PLT, platelets; WBC, white blood cells.)

monocytes are associated with an angiogenic and macrophage response.[23] Further clinical trials are necessary to clarify which cellular components are important for clinical performance and to make clear conclusions.

The Angel system can process 40 to 180 mL of blood at varying hematocrit settings (0%-25%), giving the option for variable cell concentrations in the output. A high hematocrit level (15%) and input volume (180 mL) were chosen for the Angel system to act as a contrast to the leukocyte-reducing ACP system; however, these settings can be manipulated to customize the blood product. For example, a lower hematocrit setting could be used to minimize neutrophil and RBC collection. Although the chosen blood volume input and spin time requirements were greater for the Angel system, there is the ability to obtain higher concentrations of platelets as well as HPCs.

In this study, exercise induced an increase in the average HPC number in whole blood from 0.4/μL to 0.6/μL measured by CBC. This finding was not statistically significant in whole blood, but it was in the Angel PRP preparation ($P = .043$). This is likely due to several of the HPC counts being below the detection limit of the Sysmex system in whole blood samples. Because HPCs were concentrated in the PRP 5-fold over baseline, they were able to be more consistently detected. The HPC content in post-exercise PRP was significantly increased compared with pre-exercise PRP (from 1.7/μL to 2.7/μL on average). This is of similar magnitude to findings in prior studies showing an increase in circulating $CD34^+$ progenitor cells determined by flow cytometry in peripheral blood after exercise.[8-10,24] In addition to HPC mobilization, injury and/or stress may mobilize non-HPC progenitors and growth factors that could be collected in a PRP product.[25-28]

Although studies have illustrated a release of growth factors with endurance exercises,[10,29] there is only one study for comparison that has evaluated the effects of exercise on the platelet content and platelet-derived cytokine content of PRP. In a controlled laboratory study, Hamilton et al.[28] evaluated PRP prepared from post-exercise blood in an activated form with calcium chloride and in a non-activated form in 10 healthy volunteers. Participants performed submaximal exercise, at 50% of their peak power output, for 1 hour, with blood draws immediately before, immediately after, and 18 hours after exercise. Exercise did not have a significant effect on platelet concentrations. It was associated with a significant decrease in both vascular endothelial growth factor and platelet-derived growth factor AB concentrations and showed no significant

effect on insulin-like growth factor 1 or hepatocyte growth factor concentrations. Contrasting results of platelet mobilization in this study are likely due to the difference in the exercise regimens and timing of blood draws: 20 minutes of vigorous exercise at 70% to 85% of maximum target heart rate versus 1 hour of exercise at 50% of peak power output.

Only one exercise protocol and time of blood draw were investigated; nonetheless, a consistent increase in the concentrations of all cellular components was observed. The type and duration of exercise, in addition to the fitness of the participant, may affect the mobilization response.[9] In one study, prolonged (2 hours) endurance exercise produced the largest changes in leukocyte count compared with peak anaerobic (5 minutes) and resistance exercise.[30] Studies in mice have shown circulating HPC peaks after as little as 15 minutes of acute exercise.[31] The optimal timing of blood draws to maximize HPC yield has not yet been defined because a decline in mobilized HPCs has been reported with varied durations from 30 minutes to several hours after exercise.[32] Continued efforts to characterize the effects of differing exercise protocols are the next steps in the development of exercise-mobilized PRP. The most efficient, high-yield, cost-effective way to harvest and clinically apply PRP and stem cells has not yet been clearly established. Future studies are needed to evaluate the potential benefit of exercise-mobilized PRP.

Limitations

A major limitation of this study is the low sample number and the younger age range of participants (range, 21-45 years; average, 30.5 years). However, this study sought to provide proof-of-concept pilot data for further analytical studies of nonpharmacologic methods in maximizing harvests of platelets and HPCs as well other progenitor cells from peripheral blood. Another limitation is that in some whole blood samples, HPC concentrations were below the limit of detection of the Sysmex system; however, this only affected the analysis for whole blood and not for Angel PRP samples. Although the Sysmex HPC mode has been shown to be substantially equivalent to $CD34^+$ detection in peripheral blood samples,[17,33] abundant combinations of markers have been used to identify and investigate specific cells within the HPC hierarchy. Flow cytometric analysis to quantify types of HPCs, as well as other progenitor cell types, and to identify cytokines would have given a more robust data set. There is the possibility that dehydration may have played a role in the change in cellular component concentrations in the post-exercise blood; however, the average fold change in the RBC concentration in baseline blood (1.02 ± 0.04) was insignificant when compared with the fold change observed in the other cell types.

Conclusions

Exercise can be used to consistently alter the composition of PRP. Twenty minutes of vigorous exercise can increase platelet concentrations in plasma-based and buffy coat–based PRP products and can increase HPC concentrations and volume in buffy coat–based PRP.

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