



Quantification of Peripheral Hematopoietic Progenitor Cell Circulation Following Acute, Vascular Restriction Resistance Exercise Using the Delfi Tourniquet System of the Upper Extremity

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Abstract

Background: Stem cell harvesting techniques historically require invasive methods to gather stem cells. Advancements in technology and research urge the need to find minimally invasive methods of stem cell harvesting.

Purpose: The primary purpose is to quantify the amount of stem cells harvested from peripheral venipuncture after blood flow restriction exercises. The secondary purpose of this research is to identify a minimally invasive method to harvest stem cells. The overall purpose is to determine the effects of blood flow restriction training with the upper extremity on systemic stem cell quantity post training with a single bout of exercise while identifying a minimally invasive method of harvesting stem cells in significant quantities and being universally achievable by most individuals in the population.

Study Design: This study follows a controlled intervention design. All participants follow a structured protocol in controlled conditions. Quantitative data is measured and gathered to test against the hypothesis.

Methods: Participants enrolled in this study underwent a structured exercise protocol consisting of 3 bilateral upper extremity exercises. Each participant was physically evaluated for the ability to safely perform weighted exercises. A Delfi blood flow restriction device was used to maintain constant venous occlusion in the upper extremities to create a temporary localized hypoxic environment of the upper limbs. Venipuncture is performed at time points before, during, and after the exercise protocol for quantitative measurement of stem cells in the peripheral venous system.

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Abbreviations

AE	Adverse Event
ANOVA	Analysis of Variance
AREF	Andrews Research and Education Foundation
BFR	Blood Flow Restriction
CBC	Complete Blood Count
Cc	Cubic Centimeter
CD	Cluster Differentiation
CFR	Code of Federal Regulations
EDC	Electronic Data Capture
G-CSF	Granulocyte Colony Stimulating Factor
HIPAA	Health Insurance Portability and Accountability Act
HSC	Hematopoietic Stem Cell
ICF	Informed Consent Form
IRB	Institutional Review Board
mL	Milliliters
MSC	Mesenchymal Stem Cells
NIRS	Near-infrared Spectroscopy
NSAIDS	non-steroidal anti-inflammatory drugs
PI	Primary Investigator
1-RM	1 Repetition Maximum
SAE	Serious Adverse Event

Introduction

The utilization of Hematopoietic Stem Cells (HSC's) in orthopedic and sports medicine has increased in prevalence in recent years. Currently, mainstream research is focused on HSC differentiation, understanding of immunologic properties, and identification of markers to aid in recovery from injury. Despite this ongoing research, there is a lack of standardized, repeatable, and minimally invasive procedures for collection of HSC's persists [1]. Hematopoietic stem cells have traditionally been used in therapeutic transplantation for patients with myelosuppressive and aplastic conditions, however with this

realization of their flexible regenerative capabilities, an increase in stem cell demand is expected. The conventional methods for collection of HSC's, such as bone marrow aspiration, are invasive procedures that are more prone to complications than non-invasive procedures due to the use of general anesthesia and complications from open wounds. In addition to surgical complications, invasive HSC harvesting procedures may not yield an adequate amount of stem cells for clinical or therapeutic use [2].

To fulfill the gap in stem cell harvesting techniques, research has been focused on improving the quantity of cells harvested using less invasive methods of collection. Recent research suggests that peripheral hematopoietic stem cell levels are increased and become more mobilized in episodes of acute hypoxia, increased heat, and elevated stress [3]. To date, no research has been conducted that utilizes the technology of Blood Flow Restriction (BFR) as a method of harvesting peripheral HSC's. BFR can be used as an artificial method of creating the hypoxic and hypothermic intramuscular environment that induces peripheral HSC mobilization. This physiologic response to localized stress within the body is an internal measure to protect against muscle damage and serves to improve recovery after trauma [4].

The methods by which BFR can improve tissue healing and muscle repair is unknown, but we hypothesize that it is related to increased HSC concentration when the body is placed under the artificial physiologic stress that BFR creates as well as the physiological changes, that have been well established, that occur such as increases in growth hormone, white blood cell count, vasoendothelial growth factor and decreases in catabolic components. The purpose of this study is to analyze the relationship between BFR of the upper extremities and peripheral HSC concentration. It is through this connection that we hope to create a safe, universal, and minimally invasive way to harvest

HSC's for future clinical and therapeutic use.

Methods

Subjects who are enrolled in this study utilized the OxeFit XP1 training platform to perform the prescribed upper body exercises. This training platform utilizes tension-loaded cables for resistance training with micro-adjustable resistance loading for enhanced safety and accuracy of exercise protocols. This minimizes the risk of injury commonly associated with free weights and ensures that even the most novice participants can use exercise equipment without fear of injury.



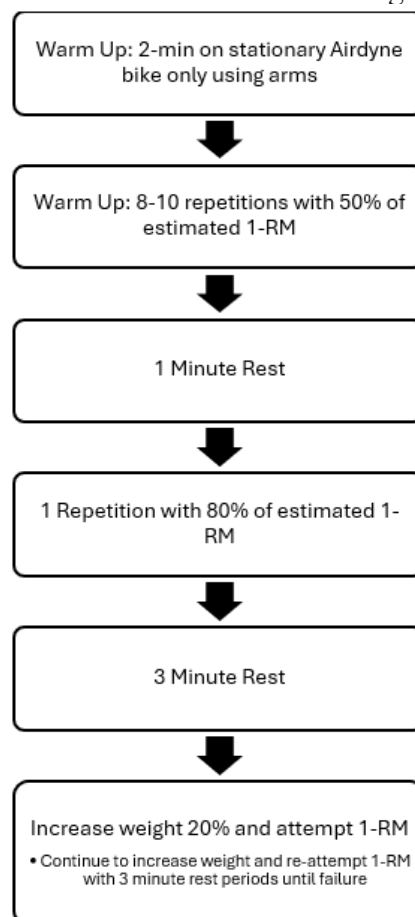
Figure 1: OxeFit XP1 Training Platform

During the prescribed exercises, participants wore Delfi BFR cuffs on the most proximal portion of each of their upper extremities. Each participant underwent the personalized tourniquet pressure (PTP) measurement reading to determine limb occlusion pressure (LOP). The LOP is what pressure is required to obtain 100% occlusion of the limb and the PTP will be the individual pressure for each specific subject used during exercise. For standardization the PTP will be set at 50% of the LOP, which according to Delfi and current literature is the industry standardized pressure to safely perform UE exercises. The utilization of the Delfi BFR system maintains a constant state of restriction on the extremities, even during movement, to ensure that the artificially created hypoxic environment is maintained throughout the duration of the exercises. The purpose of this is to restrict oxygenated blood flow into the muscle tissue during the exercise which may counteract the desired effects of the hypoxic conditions needed to create an increase in peripheral HSC's.

Each participant completed 3 training sessions. After the initial screening visit, the subject attended a familiarization session to understand the use and application of the Delfi BFR system, OxeFit XP1, and establish a 1-RM for each exercise. The experimental testing session occurred between 1-7 days after the familiarization session. During the testing session, each subject performed the exercises with the Delfi BFR tourniquet system. Before and after the testing session, subjects' blood was collected via venipuncture to collect data for a CBC with differential data and for HSC quantification.

All participants followed the same exercise protocol with the use of the Delfi BFR Tourniquet system. The three exercises (double arm bicep cable curl, cable triceps extension, and flat barbell bench press) were performed with a format of 4 total sets. The first set was a total of 30 repetitions, while the last 3 sets were 15 repetitions for each set. The resistance of the OxeFit machine was set to 30% of the 1-RM for each exercise after the appropriate 1-RM is identified for each participant following the protocol below.

Figure 2: Protocol for Determining 1 RM



Results

A total of 22 participants enrolled in the study, with 20 completing the entire study. 10 males and 10 females with an average age of 25.05 ± 3.96 . Table 1 demonstrates the demographic characteristics of the study sample.

Table 1: Study Demographics

Characteristic	Overall N = 20 ¹	Female N = 10 ¹	Male N = 10 ¹
Age	25.05 ± 3.96 (20.00,37.00)	24.10 ± 2.96 (20.00,29.00)	25.91 ± 4.66 (21.00,37.00)
Race			
Asian	1 (4.5%)	0 (0%)	1 (9.1%)
Hispanic or Latino/White	1 (4.5%)	1 (9.1%)	0 (0%)
Other: Black and White Mixed	1 (4.5%)	0 (0%)	1 (9.1%)
White	19 (86%)	10 (91%)	9 (82%)
Tegener Activity Level	5.82 ± 1.65 (2.00,9.00)	5.09 ± 1.22 (3.00,6.00)	6.55 ± 1.75 (2.00,9.00)
¹ n (%); Mean $\pm$ SD (Min,Max)			

For each subject, blood lactate measurements were recorded at 0-min, 10-min, and 20-min. Blood lactate analysis revealed a statistically significant difference in the blood lactate levels measured in patients at the 0-, 10-, and 20-minute post-exercise time points from 7.09 ± 2.71 at 0 minutes (pre-exercise) to 4.85 ± 2.41 at 20 minutes post-exercise. Table 2 demonstrates the distribution of the blood lactate across the three time points. The average blood lactate decreases over time from 0- to post exercise returning to pre-exercise levels.

Table 2: Blood Lactate Comparison

Characteristic	0-min N = 22 ¹	10-min N = 22 ¹	20-min N = 22 ¹	p-value ²
Blood Lactate Score	7.09 ± 2.71 (3.30, 13.90)	6.10 ± 2.94 (2.50, 15.30)	4.85 ± 2.41 (1.90, 12.80)	0.008
No reads	2	2	2	
¹ Mean $\pm$ SD (Min, Max)				
² Quade test				

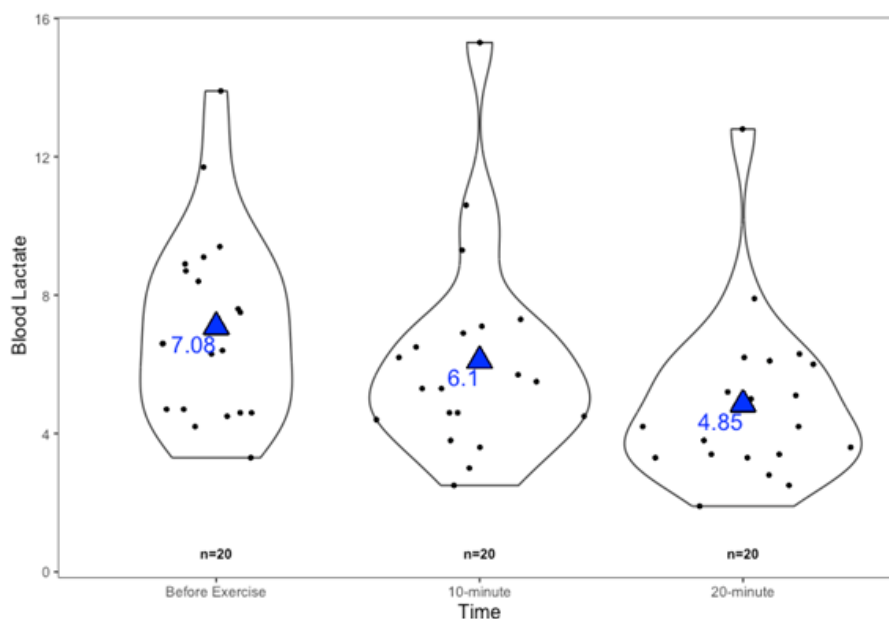


Figure 2: The Blood Lactate Distribution Across Time Points

CBC analysis demonstrated statistically significant differences in the following values: White Blood Cell (WBC), Red Blood Cell (RBC), Hemoglobin (HGB), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC), Platelets (PLT), Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils, and Immature Granulocytes (Figure 2). Most values, whether statistically significant or not, showed either a spiking increase or decrease, followed by a rebound towards baseline at the 0-minute and 20-minute post-exercise time points, respectively. Values for HCT, HGB, Lymphocytes, Monocytes, PLT, RBC, RDW-SD, & WBC all showed a statistically significant decrease in values from the Pre-exercise time point to the 20-minute post exercise time point. Values for MCV, MCHC, Neutrophils, & Eosinophils all showed a statistically significant increase in value. Components of the CBC that did not show any statistically significant changes were Mean Corpuscular Hemoglobin (MCH), Red Cell Distribution Width-Coefficient of Variation (RDW-CV), Red Cell Distribution Width-Standard Deviation (RDW-SD), and Mean Platelet Volume (MPV).

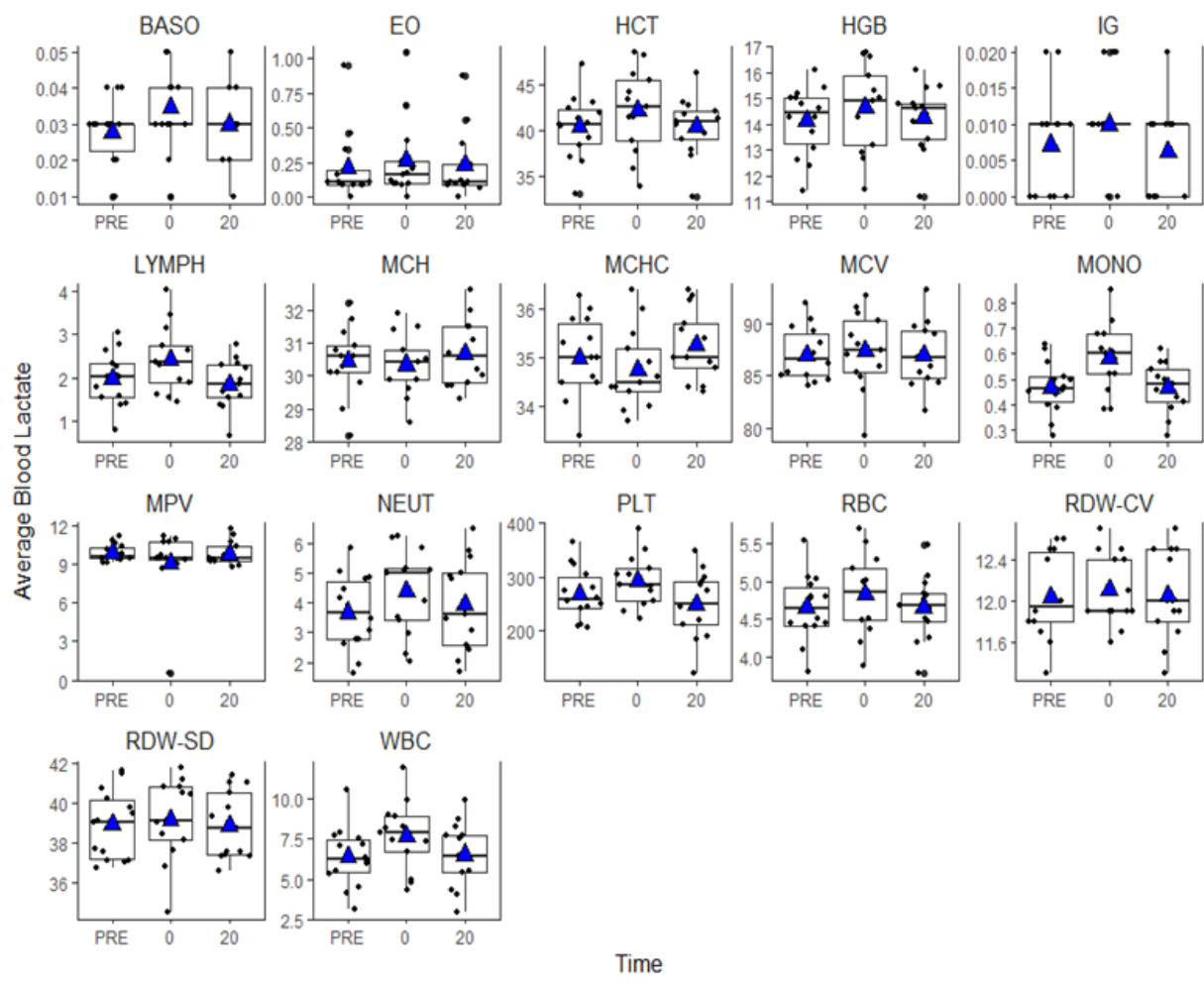


Figure 2: CBC Metrics Distributions Across Time Points

Table 3: CBC Descriptive Statistics

Characteristic	Time			p-value ²
	PRE N = 14 ¹	0 N = 14 ¹	20 N = 14 ¹	
WBC	6.55 ± 1.86 (3.07, 10.54)	7.76 ± 2.10 (4.34, 12.00)	6.67 ± 1.96 (2.94, 9.95)	<0.001
RBC	4.70 ± 0.48 (3.79, 5.55)	4.90 ± 0.61 (3.88, 5.88)	4.70 ± 0.46 (3.77, 5.49)	0.003
HGB	14.34 ± 1.38 (11.40, 16.10)	14.87 ± 1.85 (11.50, 17.60)	14.35 ± 1.42 (11.20, 16.20)	0.002
HCT	41.02 ± 3.88 (33.00, 47.20)	43.06 ± 5.00 (33.90, 51.50)	40.91 ± 3.68 (32.70, 47.10)	<0.001
MCV	87.27 ± 2.34 (84.10, 91.90)	88.01 ± 2.61 (83.70, 92.60)	87.05 ± 2.88 (81.70, 93.10)	<0.001
MCH	30.51 ± 0.84 (29.00, 32.20)	30.35 ± 0.81 (29.30, 31.90)	30.51 ± 1.00 (29.20, 32.60)	0.2
MCHC	34.95 ± 0.77 (34.10, 36.30)	34.51 ± 0.79 (33.40, 36.40)	35.06 ± 0.87 (33.50, 36.40)	<0.001
PLT	274.29 ± 54.19 (206.00, 366.00)	293.64 ± 53.04 (224.00, 390.00)	254.14 ± 63.49 (121.00, 350.00)	<0.001
RDW-SD	39.55 ± 1.48 (37.00, 41.60)	39.84 ± 1.51 (36.80, 41.80)	39.37 ± 1.60 (36.60, 41.40)	0.055
RDW-CV	12.19 ± 0.37 (11.60, 12.60)	12.19 ± 0.34 (11.60, 12.70)	12.16 ± 0.35 (11.50, 12.70)	0.7
MPV	9.84 ± 0.65 (9.10, 11.20)	9.84 ± 0.78 (8.70, 11.20)	9.80 ± 0.91 (8.80, 11.80)	0.47
NEUT	3.80 ± 1.21 (1.63, 5.84)	4.36 ± 1.38 (2.06, 6.21)	4.02 ± 1.47 (1.69, 6.48)	<0.001
LYMPH	1.98 ± 0.62 (0.79, 3.05)	2.46 ± 0.73 (1.44, 4.01)	1.87 ± 0.55 (0.68, 2.78)	<0.001
MONO	0.50 ± 0.09 (0.32, 0.64)	0.62 ± 0.13 (0.38, 0.85)	0.50 ± 0.09 (0.33, 0.67)	<0.001
EO	0.23 ± 0.24 (0.00, 0.95)	0.27 ± 0.28 (0.00, 1.05)	0.23 ± 0.23 (0.00, 0.88)	<0.001
BASO	0.03 ± 0.01 (0.01, 0.04)	0.04 ± 0.01 (0.01, 0.07)	0.03 ± 0.01 (0.01, 0.05)	0.016
IG	0.01 ± 0.01 (0.00, 0.02)	0.01 ± 0.01 (0.00, 0.02)	0.01 ± 0.01 (0.00, 0.02)	0.038

¹Mean ± SD (Min, Max)²Kruskal-Wallis rank sum test

Cytometry data are presented in Table 4. It demonstrates descriptive statistics (mean, SD, max, and min) of the numbers of events, the percentages out of the total, and the percentage out of parent.

Table 4: Cytometric Data Descriptive Statistics

Characteristic	PRE N = 14 ¹	0 N = 14 ¹	20 N = 14 ¹	p-value ²
Singlets.Events	4,321.57 ± 2,418.61 (843.00, 7,752.00)	4,359.29 ± 2,198.61 (1,263.00, 7,933.00)	3,656.21 ± 2,392.62 (729.00, 7,150.00)	0.078
Singlets.Total(%)	45.56 ± 21.64 (11.53, 77.52)	45.32 ± 20.40 (19.54, 79.33)	38.41 ± 22.05 (11.66, 71.50)	0.139
Singlets.Parent(%)	45.56 ± 21.64 (11.53, 77.52)	45.32 ± 20.40 (19.54, 79.33)	38.41 ± 22.05 (11.66, 71.50)	0.139
CD45+.Events	3,256.36 ± 1,723.50 (630.00, 5,775.00)	3,434.57 ± 1,595.00 (1,117.00, 5,865.00)	2,597.29 ± 1,668.21 (426.00, 5,213.00)	0.331
CD45+.Total(%)	34.43 ± 15.19 (8.61, 57.75)	35.85 ± 14.64 (17.28, 58.65)	27.42 ± 15.35 (6.81, 52.13)	0.337
CD45+.Parent(%)	79.19 ± 7.72 (59.28, 88.82)	82.64 ± 8.26 (61.12, 91.55)	75.25 ± 13.57 (39.56, 86.96)	0.013
CD34+.Events	2,381.29 ± 1,404.67 (385.00, 4,299.00)	2,513.00 ± 1,241.45 (747.00, 4,466.00)	1,880.36 ± 1,306.78 (328.00, 4,034.00)	0.022
CD34+.Total(%)	24.98 ± 12.83 (5.26, 42.99)	26.09 ± 11.48 (11.55, 44.66)	19.76 ± 12.19 (5.25, 40.34)	0.027
CD34+.Parent(%)	69.87 ± 10.26 (48.59, 83.13)	71.79 ± 6.90 (57.56, 83.03)	70.34 ± 9.55 (52.63, 82.29)	0.961
HSC-HPC.Events	2,071.79 ± 1,326.98 (274.00, 3,960.00)	2,119.43 ± 1,206.61 (404.00, 4,176.00)	1,611.14 ± 1,229.81 (248.00, 3,747.00)	0.061
HSC-HPC.Total(%)	21.59 ± 12.36 (3.75, 39.60)	21.83 ± 11.47 (6.25, 41.76)	16.79 ± 11.66 (3.97, 37.47)	0.074
HSC-HPC.Par-ent(%)	96.34 ± 2.92 (89.57, 100.00)	95.66 ± 3.13 (88.21, 98.61)	95.42 ± 3.14 (87.93, 98.57)	0.368

¹Mean ± SD (Min, Max)

²Quade test

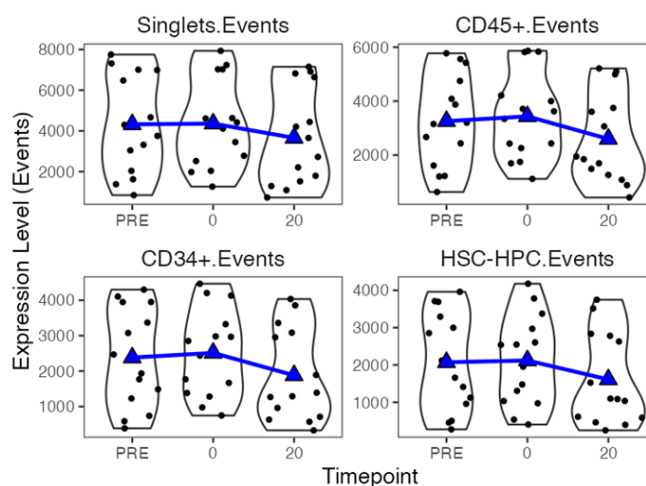
Distribution of Cytometry Markers**Figure 3:** The Counts Distributions of Cytometry Markers

Figure 3 shows the number of events from single-cell to CD34+ maker and HSC-HPC events. The makers' distribution at each time point appears similar. There is a slight decrease in the expression level (counts) from pre-exercise to post-exercise.

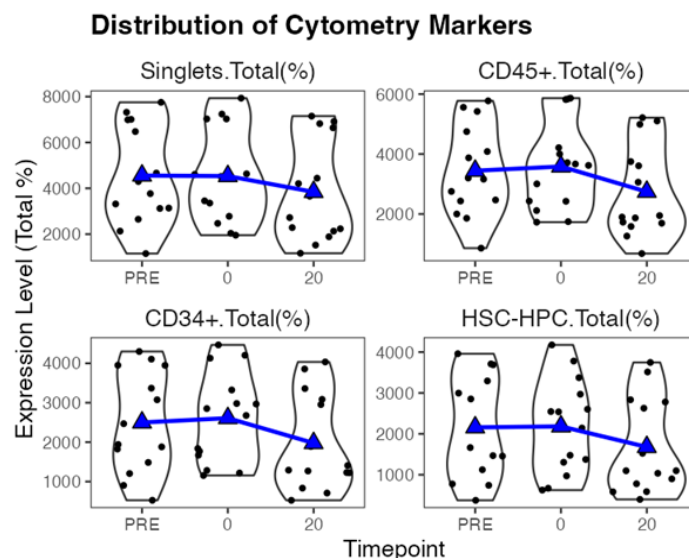


Figure 4: The Percentage Total Distribution of Cytometry Markers

Figure 4 shows the total percentage of events from single-cell to CD34+ maker and HSC-HPC percentage total. There is a slight decrease in the expression level (Total %) from pre-exercise to post-exercise.

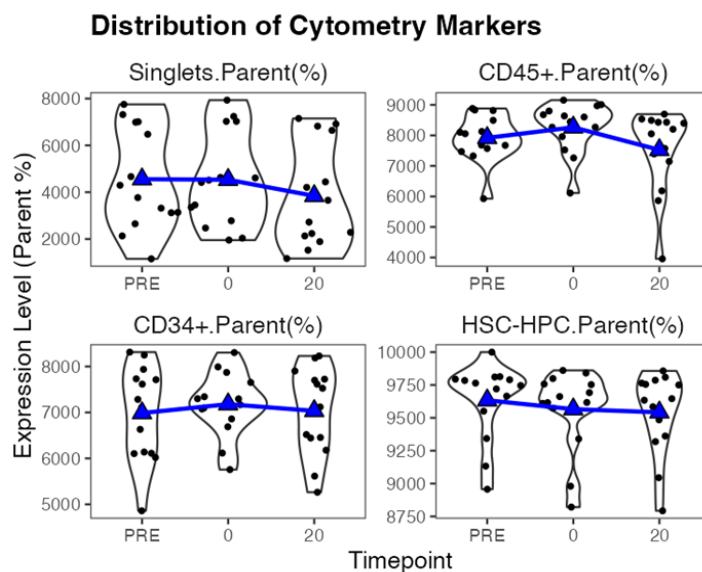


Figure 5: The Parents Percentages Distributions of Cytometry Markers

Figure 5 shows the parent percentages from single-cell to CD34+ maker and HSC-HPC percentage total. The markers distribution at each point time looks similar. There is a decrease in the expression level in Singlet and CD45+ Parent % from pre-exercise to post-exercise.

Limitations

There were few limitations during this study that should be noted. Due to the technology of the OxeFit XP1, participants may not have performed an exercise at exactly 30% on their one rep max. An example of this on certain occasions is that for bicep curls, the OxeFit machine has a minimum of 5 pounds for the bicep curls exercise. If a participant's calculated weight for 30% of their one rep max was less than 5 pounds, then they would have to perform this exercise at a minimum of 5 pounds, which would have been greater than their prescribed weight. This occurred with the bench press exercise as well. With a minimum of 31 pounds for the bench press, some participants required a lower weight for this exercise. These participants had to use a 31-pound minimum even if their prescribed weight was less than 31 pounds.

Limitations of the current study, such as the difference between prescribed and actual resistance levels due to the OxeFit XP1's minimum weight settings, suggest areas for technical refinement. These inconsistencies may have influenced the uniformity of the hypoxic stimulus across participants, potentially underestimating the protocol's full effect. Future iterations could employ more adaptable equipment or personalized resistance adjustments to ensure precision. Additionally, expanding the sample size and extending post-exercise monitoring beyond 20 minutes could provide deeper insights into the mechanisms and longevity of BFR-induced mobilization. Previous studies have shown that blood marker levels peak in the immediate time post exercise and the 20-minute interval, then return to normal pre-exercise levels by the 40-minute post-exercise time point and remain unchanged at the 60-minute post-exercise time point [5].

Discussion

This study sought to investigate the potential of blood flow restriction (BFR) exercise as a minimally invasive, safe, and universally applicable method for mobilizing and harvesting hematopoietic stem cells (HSCs) from peripheral blood. The results provide promising evidence that an upper extremity exercise protocol under controlled BFR conditions induces significant physiological responses, as reflected in both complete blood count (CBC) and cytometry analyses. Statistically significant changes were

observed in numerous CBC parameters—including white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), neutrophils, lymphocytes, monocytes, eosinophils, and basophils. This demonstrates a positive response to the hypoxic intramuscular environment created by BFR. The initial spike or decline followed by a partial rebound toward baseline by the 20-minute post-exercise time point highlights the acute and transient nature of the body's reaction to a hypoxic cellular environment. Flow Cytometry data further illustrated the impact of BFR exercise on stem cell mobilization. Notably, populations of CD34+ cells and HSC-HPC (hematopoietic stem and progenitor cells) exhibited a modest increase immediately following exercise, with mean event counts rising from $2,381.29 \pm 1,404.67$ pre-exercise to $2,513.00 \pm 1,241.45$ at the 0-minute post-exercise mark for CD34+ cells, and from $2,071.79 \pm 1,326.98$ to $2,119.43 \pm 1,206.61$ for HSC-HPC. However, by the 20-minute post-exercise time point, these counts declined to $1,880.36 \pm 1,306.78$ and $1,611.14 \pm 1,229.81$, respectively, suggesting that the mobilization effect may be short-lived. While these changes did not consistently reach statistical significance across all metrics, they align with prior research indicating that acute hypoxia and stress can enhance peripheral HSC circulation, further supporting our hypothesis that BFR could serve as an artificial trigger for this process (Callanan et al., 2021). Prior Studies utilized BFR on the LE, showing increased HSC circulation, but to date, no studies have evaluated the effects of BFR on the UE and the relationship with HSC circulation count. Additionally, blood lactate levels decreased significantly from 7.09 ± 2.71 mmol/L pre-exercise to 4.85 ± 2.41 mmol/L at 20 minutes post-exercise ($p = 0.005$), reflecting a metabolic response to the hypoxic conditions and providing further evidence of the physiological stress induced by the protocol.

Conclusion

In conclusion, this study establishes evidence to support that BFR exercise can safely and effectively mobilize HSCs into peripheral blood, laying the foundation for a novel and minimally invasive harvesting strategy. While this method requires optimization to rival established invasive techniques, the potential for future improvements is vast. Enhanced HSC harvesting through refined BFR protocols could expand

access to stem cell-based treatments, reduce patient burden, and accelerate therapeutic innovation.

Despite these promising findings, the quantities of HSCs mobilized into peripheral blood in this study may not meet the threshold required for practical therapeutic harvesting, particularly when compared to traditional methods like bone marrow aspiration or cytokine-stimulated mobilization. Nevertheless, the absence of serious adverse events (SAEs) and the successful participation of a diverse cohort of individuals affirm the safety and feasibility of this approach. This is a critical step toward achieving the study's broader goal of developing a minimally invasive harvesting technique that avoids the complications of invasive procedures such as infection, anesthesia-related risks, and insufficient yield while being accessible to a wide population, including those unable to undergo more intensive interventions.

Looking forward, the potential for BFR-based HSC harvesting to revolutionize regenerative medicine is substantial, particularly if future research can enhance both the quantity and persistence of mobilized stem cells for longer periods of time post-exercise. Advances in exercise protocol design to amplify the hypoxic stimulus and sustain higher HSC concentrations in peripheral blood could be investigated. Additionally, optimizing the timing of venipuncture to capture peak mobilization could maximize harvestable yields. Coupling BFR with adjunctive techniques such as cryo or thermal application or pharmacolog-

ical agents like granulocyte colony-stimulating factor (G-CSF) might further boost mobilization. Such advancements would be transformative, offering a scalable, cost-effective alternative to current harvesting methods and expanding access to stem cell therapies for an infinite number of clinical applications.

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