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Mark C. Callanan, The Orthopedic Clinic
Hillary A. Plummer, Andrews Research & Education Foundation
Garrett L. Chapman, Beaver Medical Group
Tyler J. Opitz, Andrews Research & Education Foundation
Nicole Rendos, Emory University
Adam W. Anz, Andrews Research & Education Foundation

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Blood Flow Restriction Training Using the Delfi System Is Associated With a Cellular Systemic Response

Mark C. Callanan, M.D., Hillary A. Plummer, Ph.D., A.T.C., Garrett L. Chapman, M.D., Tyler J. Opitz, D.P.T., Nicole K. Rendos, Ph.D., A.T.C., and Adam W. Anz, M.D.

Purpose: To determine the effects of blood flow restriction (BFR) exercise on CD34+ cells, platelets, white blood cells, neutrophils, lymphocytes, lactate, and glucose. Methods: Healthy participants aged 20 to 39 years who were able to perform the exercise sessions were recruited. Participants underwent an experimental (EXP) occluded testing session and a control (CON) session using the Delfi Personalized Tourniquet System. Blood draws were performed prior to testing and immediately after the exercise session. Blood analysis consisted of a complete blood count as well as flow cytometry to measure peripheral CD34+ counts as a marker for hematopoietic progenitor cells. Results: Fourteen men (aged 30.8 ± 3.9 years) volunteered. There was a significant increase in average CD34+ counts immediately after the EXP session only (3.1 ± 1.2 cells · µL⁻¹ vs 5.2 ± 2.9 cells · µL⁻¹, P = .012). Platelet counts were significantly elevated after both sessions, with the average increase being higher after the EXP session (mean difference [MD], 34,200/µL; P < .002) than after the CON session (MD, 11,600/µL; P < .002). White blood cell counts significantly increased after both the EXP (8,400 ± 2,200/µL vs 6,300 ± 1,600/µL; P < .001) and CON (MD, 900/µL; P < .001) sessions. There was a significant increase from baseline to immediately after exercise in the average number of lymphocytes (MD, 6.3%; P < .001) and, conversely, a significant decrease in the average neutrophil count (MD, 6.5%; P < .001) in the EXP session only. Lactate levels significantly increased in the EXP (MD, 6.1 mmol · L⁻¹; P = .001) and CON (MD, 3.6 mmol · L⁻¹; P = .001) groups. No changes in glucose levels were observed. Conclusions: Exercise with BFR causes a significant post-exercise increase in peripheral hematopoietic progenitor cells and platelets, beyond that of standard resistance training. Clinical Relevance: BFR can be considered a way to manipulate point-of-care blood products such as platelet-rich plasma to increase product yield.

Blood flow restriction (BFR) therapy is becoming a part of orthopaedic rehabilitation, showing promise in muscle recovery as well as limb salvage after injury and orthopaedic surgery.¹⁻⁴ BFR is associated with functional, physiological, and cellular expression of genes related to muscle upregulation, similarly to heavy-load strength training.⁵,⁶ Low-load BFR can result in increases in muscular size and strength, even in proximal muscle groups that are not directly occluded. The same ability to achieve increases in proximal muscle size and strength has not been shown in matched controls undergoing traditional training methods.⁷ Even in well-trained athletes, BFR has been shown to increase strength and hypertrophy using submaximal loads that otherwise would not have the same response in a control group.⁸⁻¹¹ BFR has been studied in the postoperative care of patients after knee arthroscopy and anterior cruciate ligament reconstruction, with improved strength and patient-reported outcomes compared with conventional therapy, as
well as diminishment in the degree of disuse atrophy.\textsuperscript{3,12} BFR is a viable option to improve muscle strength in patients unable to perform high-intensity exercise who have ultimately not improved with traditional therapy.\textsuperscript{2,4,11}

Although the mechanism of action of increased muscle strength and hypertrophy from BFR is not completely understood, lactate and growth hormone levels increase from 0 to 40 minutes after BFR.\textsuperscript{13-17} Exercise with BFR is associated with low skeletal muscle tissue oxygenation saturation levels (<10\%) as measured by near-infrared spectroscopy, representing severe hypoxia in the working tissue.\textsuperscript{18} The metabolic overload from the accumulation of hydrogen and lactate, in combination with the hypoxia, may activate IL-6, macrophages, and neutrophils, leading to an overall anabolic environment without the mechanical muscle damage that occurs with high-intensity training.\textsuperscript{19} Increased signaling and proliferation of local myogenic stem cells in post-therapy muscle biopsy samples have been observed after BFR therapy.\textsuperscript{20-22} BFR has also been shown to induce a local angiogenic response through upregulation of vascular endothelial growth factor, another proposed mechanism for the noted efficacy of BFR therapy.\textsuperscript{23}

Another rapidly growing area of interest in orthopaedic surgery and recovery science is the clinical use of stem cells. Adult stem cells have the ability to monitor their local and systemic environment for stimuli, mobilize locally and/or systemically in the setting of an environmental insult such as exercise, interact with their surrounding environment through paracrine effects, and differentiate to end-stage cells if necessary.\textsuperscript{24-26} In rat models, heat, hypoxia, and cold can stimulate stem cells to mobilize, with hypoxia-induced factors being upregulated as a key factor in peripheral migration of mesenchymal stem cells.\textsuperscript{27} An increase in the peripheral mobilization of platelets as well as hematopoietic stem cells after vigorous exercise in humans has also been observed.\textsuperscript{25,26,28,29} Exercise using BFR may be a less invasive method to mobilize stem cells to optimize the physiology of recovering orthopaedic patients, as well as to manipulate point-of-care blood and bone marrow products in orthopaedics.\textsuperscript{28}

Despite the previously studied mechanisms of efficacy for BFR therapy, the degree of mobilization of the cellular components of blood including hematopoietic progenitor cells (HPCs) to the peripheral circulation after exercise with BFR is unclear. The purpose of this study was to determine the effects of BFR exercise on CD34\textsuperscript{+} cells, platelets, white blood cells (WBCs), neutrophils, lymphocytes, lactate, and glucose. It was hypothesized that BFR training would stimulate a systemic cellular response to increase CD34\textsuperscript{+} cells, platelets, WBCs, neutrophils, lymphocytes, lactate, and glucose.

### Methods

A randomized crossover-design study was performed with the Delfi PTS Personalized Tourniquet System (Owens Recovery Science, San Antonio, TX). A complete blood count (CBC) with WBC differential, flow cytometry to quantify the number of CD34\textsuperscript{+} HPCs, and blood lactate and glucose levels were measured prior to the exercise protocols (PRE) and at various time points after the exercise protocols.

Healthy adults aged 20 to 39 years were recruited to participate in this study. Participants were excluded if they had a history of uncontrolled hypertension, diabetes, autoimmune disorders, blood disorders, disorders requiring immunosuppression, or cancer; an ongoing infectious disease; use of steroids; or significant cardiovascular, renal, hepatic, or pulmonary disease. Furthermore, participants were excluded if they had a history of an orthopaedic injury within the past 6 months. All participants had to be medically fit to perform 20 minutes of intense exercise.

All procedures were approved by the Baptist Hospital-Pensacola institutional review board. Prior to data collection, all testing procedures, risks, and benefits of the specific study were explained to each participant and written informed consent was obtained. Each participant underwent a standard physical examination, including the completion of a medical history and assessment of activity level with the Tegner Activity Level scale. Once all screening processes were passed, the participants were enrolled for a testing appointment. Participants were asked to refrain from strenuous exercise for 24 hours and from alcohol and caffeine for 12 hours prior to each testing session.

An a priori power analysis (G\textsuperscript{*}Power, version 3.1.9.3) revealed that a sample size of 10 participants was necessary to detect large effects (200\%) with a power of 0.9 and \(\alpha\) of .05. Sufficient power has been confirmed in previous mobilization studies.\textsuperscript{10} The sample size of our study was increased to 14 to account for potential participant withdrawal. Fourteen participants completed the study. Participant characteristics are provided in Table 1.

Participants rested in the sitting position for 15 minutes prior to each testing session. A 6-mL volume of venous blood was drawn from an antecubital vein into

### Table 1. Demographic Characteristics of Participants Undergoing Exercise with Blood Flow Restriction Using the Delfi System

| Characteristic     | Data       |
|--------------------|------------|
| Age, yr            | 30.8 ± 3.7 |
| Height, m          | 1.8 ± 0.07 |
| Weight, kg         | 89.6 ± 16.5|
| Tegner score       | 5.5 ± 1.1  |

NOTE: Data are presented as mean ± standard deviation.
Table 2. Complete Blood Count With Differential and Flow Cytometry Results

| Variable | PRE | T0   | T20  | T40  | T60  |
|----------|-----|------|------|------|------|
| WBC count |     |      |      |      |      |
| Experimental, 1,000 • μL⁻¹ |     |      |      |      |      |
| Participant 1 | 4.5 | 5.8  | 4.3  | 3.9  | 4.8  |
| Participant 2 | 5.2 | 8.4  | 5.6  | 5    | 4.7  |
| Participant 3 | 7.3 | 8    | 7.6  | 6.5  | 6.4  |
| Participant 4 | 3   | 4.4  | 3.2  | 3.1  | 3.3  |
| Participant 5 | 6.7 | 9.4  | 6.2  | 5.9  | 5.7  |
| Participant 6 | 8.1 | 10.3 | 7.6  | 7.3  | 7    |
| Participant 7 | 7.3 | 11.7 | 8.6  | 7.1  | 7.2  |
| Participant 8 | 6.2 | 9.2  | 6.7  | 5.7  | 5.7  |
| Participant 9 | 6.5 | 8    | 5.9  | 5.2  | 5.4  |
| Participant 10 | 3.6 | 5.3  | 3.8  | 3.3  | 3.2  |
| Participant 11 | 7.6 | 9.4  | 7.7  | 7.3  | 7.3  |
| Participant 12 | 4.7 | 6.1  | 4.5  | 4.1  | 4    |
| Participant 13 | 7   | 10.7 | 7.5  | 6.6  | 6.5  |
| Participant 14 | 5.7 | 9    | 6.8  | 5.1  | 4.8  |
| Mean ± SD | 6.0 ± 1.6 | 8.3 ± 2.2 | 6.3 ± 1.7 | 5.6 ± 1.5 | 5.6 ± 1.5 |
| 95% CI | 5.2-7.0 | 7.2-9.7 | 5.4-7.2 | 4.8-6.4 | 4.8-6.4 |
| Range | 3.8-11.7 | 6.4-11.7 | 3.2-8.6 | 3.1-7.3 | 3.2-7.3 |
| Δ from PRE for experimental, % | 37.7 | 3.3 | 95% CI  | 5.2-7.0 | 7.2-9.7 | 5.4-7.2 | 4.8-6.4 | 4.8-6.4 |

Control, 1,000 • μL⁻¹

| Participant 1 | 7.9 | 8.8  | 8.1  | 8.2  | 7.2  |
| Participant 2 | 5.6 | 6.7  | 5.9  | 5.7  | 5.7  |
| Participant 3 | 6.7 | 7.4  | 6.2  | 6    | 5.9  |
| Participant 4 | 4.3 | 4    | 3.5  | 3.5  | 3.5  |
| Participant 5 | 7.1 | 7.6  | 7.2  | 7.3  | 7.3  |
| Participant 6 | 7.7 | 8.6  | 7.3  | 6.8  | 7.4  |
| Participant 7 | 9.2 | 11.5 | 9.5  | 9    | 9.1  |
| Participant 8 | 6   | 5.9  | 5    | 5.1  | 5.4  |
| Participant 9 | 6.8 | 7.1  | 6.2  | 6.2  | 6.6  |
| Participant 10 | 5.7 | 6.9  | 5.9  | 5.4  | 5.2  |
| Participant 11 | 7.8 | 9.1  | 7.3  | 7.2  | 6.9  |
| Participant 12 | 3.8 | 4.4  | 4    | 4.4  | 4.5  |
| Participant 13 | 8.4 | 9.1  | 7.7  | 7.3  | 7.2  |
| Participant 14 | 5.4 | 7.5  | 6.4  | 6.3  | 6.3  |
| Mean ± SD | 6.6 ± 1.5 | 7.5 ± 1.9 | 6.4 ± 1.5 | 6.3 ± 1.4 | 6.2 ± 1.4 |
| 95% CI | 5.8-7.5 | 6.5-8.6 | 5.6-7.3 | 5.5-7.1 | 5.5-7.0 |
| Range | 3.8-9.2 | 4-11.5 | 3.5-9.5 | 3.5-9 | 3.5-9.1 |
| Δ from PRE for control, % | 13.6 | −3.0 | 95% CI  | 5.8-7.5 | 6.5-8.6 | 5.6-7.3 | 5.5-7.1 | 5.5-7.0 |

Platelets

| Variable | PRE | T0   | T20  | T40  | T60  |
|----------|-----|------|------|------|------|
| Experimental, 1,000 • μL⁻¹ |     |      |      |      |      |
| Participant 1 | 250 | 288  | 254  | 247  | 260  |
| Participant 2 | 255 | 306  | 271  | 270  | 268  |
| Participant 3 | 203 | 153  | 203  | 190  | 197  |
| Participant 4 | 182 | 202  | 172  | 165  | 172  |
| Participant 5 | 254 | 294  | 251  | 232  | 238  |
| Participant 6 | 227 | 265  | 238  | 237  | 236  |
| Participant 7 | 364 | 439  | 388  | 350  | 354  |
| Participant 8 | 227 | 289  | 230  | 222  | 229  |
| Participant 9 | 208 | 246  | 195  | 196  | 193  |
| Participant 10 | 187 | 216  | 197  | 181  | 173  |
| Participant 11 | 197 | 225  | 199  | 194  | 184  |
| Participant 12 | 169 | 154  | 132  | 141  | 63   |
| Participant 13 | 245 | 325  | 275  | 254  | 237  |
| Participant 14 | 257 | 299  | 260  | 238  | 230  |
| Mean ± SD | 230.4 ± 48.6 | 264.6 ± 72.2 | 234.8 ± 58.6 | 224.3 ± 50.3 | 218.8 ± 62.9 |
| 95% CI | 206.1-298.6 | 226.6-306.6 | 202.4-267.2 | 196.5-252.2 | 184.0-253.6 |
| Range | 169-364 | 153-439 | 132-388 | 141-350 | 63-354 |
| Δ from PRE for experimental, % | 14.7 | 1.03 |
| Control, 1,000 • μL⁻¹ |     |      |      |      |      |
| Participant 1 | 215 | 215  | 293  | 290  | 307  |
| Participant 2 | 279 | 244  | 283  | 281  | 273  |

(continued)
Table 2. Continued

| Variable | PRE | T0 | T20 | T40 | T60 |
|----------|-----|----|-----|-----|-----|
| Participant 3 | 173 | 174 | 167 | 165 | 164 |
| Participant 4 | 183 | 182 | 168 | 167 | 174 |
| Participant 5 | 255 | 265 | 259 | 257 | 252 |
| Participant 6 | 223 | 251 | 231 | 230 | 230 |
| Participant 7 | 428 | 458 | 419 | 397 | 412 |
| Participant 8 | 227 | 245 | 230 | 223 | 247 |
| Participant 9 | 203 | 198 | 201 | 206 | 208 |
| Participant 10 | 209 | 234 | 208 | 201 | 198 |
| Participant 11 | 264 | 289 | 267 | 254 | 249 |
| Participant 12 | 133 | 152 | 152 | 148 | 160 |
| Participant 13 | 267 | 267 | 271 | 259 | 254 |
| Participant 14 | 224 | 251 | 236 | 221 | 228 |
| Mean ± SD | 235.9 ± 66.1 | 247.5 ± 71.2 | 242.5 ± 71.2 | 236.7 ± 61.2 | 239.1 ± 62.8 |
| 95% CI | 199.2-272.5 | 208.1-286.9 | 206.3-278.7 | 202.8-270.6 | 204.3-273.9 |
| Range | 133-428 | 152-458 | 152-419 | 201-397 | 160-412 |
| Δ from PRE for control, % | 4.9 | 2.3 | 0.3 | 1.4 | |

Neutrophils

| Experimental, % | | | | |
| Participant 1 | 51.1 | 45.5 | 52.2 | 55.3 | 63.4 |
| Participant 2 | 51.5 | 36.8 | 49.5 | 49.7 | 52.4 |
| Participant 3 | 66.6 | 64.6 | 66.9 | 68.5 | 68.3 |
| Participant 4 | 46.1 | 44.6 | 50 | 49.9 | 50.9 |
| Participant 5 | 44.5 | 41.2 | 47.7 | 49.7 | 52.5 |
| Participant 6 | 47.3 | 39.6 | 46.6 | 48.8 | 50.5 |
| Participant 7 | 54.9 | 44.6 | 48.4 | 54 | 58.9 |
| Participant 8 | 49.2 | 43.6 | 45.1 | 46 | 48 |
| Participant 9 | 46 | 43.2 | 48.4 | 53.2 | 52.9 |
| Participant 10 | 65.5 | 53.7 | 61.5 | 65.7 | 66.5 |
| Participant 11 | 55.4 | 53.1 | 56.1 | 59.3 | 60.7 |
| Participant 12 | 61.7 | 53.9 | 63.1 | 62.5 | 60.5 |
| Participant 13 | 50.8 | 43.1 | 49.3 | 52.7 | 56.7 |
| Participant 14 | 46.6 | 36.2 | 40.6 | 45.8 | 50.1 |
| Mean ± SD | 52.7 ± 7.3 | 46.3 ± 7.6 | 52.0 ± 7.2 | 54.6 ± 6.9 | 56.8 ± 6.3 |
| 95% CI | 48.9-56.7 | 42.1-50.6 | 48.0-56.0 | 50.7-58.4 | 53.3-60.3 |
| Range | 46.6-66.6 | 36.2-64.6 | 40.6-66.9 | 45.8-68.5 | 48.6-88.3 |
| Δ from PRE for experimental, % | −12.3 | −1.5 | 3.4 | 7.6 | |

Lymphocytes

| Experimental, % | | | | |
| Participant 1 | 60.4 | 58.5 | 58.7 | 59.2 | 61.2 |
| Participant 2 | 55.2 | 49.1 | 53 | 55.2 | 53.1 |
| Participant 3 | 56.4 | 56.3 | 57.4 | 56.7 | 56.9 |
| Participant 4 | 48.7 | 53.3 | 54.9 | 56.7 | 53 |
| Participant 5 | 48.4 | 48.2 | 47.3 | 47.7 | 47.2 |
| Participant 6 | 44.1 | 42.4 | 46.4 | 45.7 | 44.1 |
| Participant 7 | 60.3 | 55.3 | 56.7 | 57.3 | 57.1 |
| Participant 8 | 51.6 | 53.4 | 56.2 | 58.3 | 60.2 |
| Participant 9 | 48.6 | 47.9 | 49.1 | 49.3 | 51.1 |
| Participant 10 | 63.4 | 59.2 | 63.7 | 62.7 | 62.1 |
| Participant 11 | 55.3 | 53.8 | 55.9 | 56.8 | 56.3 |
| Participant 12 | 52.6 | 55.1 | 60.6 | 59.3 | 60 |
| Participant 13 | 48 | 46.8 | 51.2 | 51.4 | 52 |
| Participant 14 | 62.7 | 56.9 | 65.3 | 66.4 | 65.9 |
| Mean ± SD | 52.7 ± 7.6 | 51.5 ± 6.5 | 54.6 ± 6.5 | 55.0 ± 6.5 | 54.9 ± 6.6 |
| 95% CI | 48.5-56.9 | 47.9-55.0 | 51.0-58.2 | 51.4-58.6 | 51.3-58.6 |
| Range | 44.1-63.4 | 42.4-59.2 | 46.4-65.3 | 45.7-66.4 | 44.1-65.9 |
| Δ from PRE for control, % | −2.3 | 3.6 | 4.4 | 7.8 | |

(continued)
| Variable | PRE | T0  | T20 | T40 | T60 |
|----------|-----|-----|-----|-----|-----|
| Participant 6 | 37.5 | 45.8 | 39.2 | 36.9 | 35.1 |
| Participant 7 | 30.4 | 40 | 36.3 | 30.9 | 26.8 |
| Participant 8 | 34.5 | 40.2 | 37.8 | 37.7 | 35.7 |
| Participant 9 | 42 | 45 | 39.9 | 35.8 | 35.3 |
| Participant 10 | 17 | 26.5 | 19.9 | 17.2 | 16.6 |
| Participant 11 | 31.8 | 34.9 | 32.2 | 29.6 | 27.2 |
| Participant 12 | 31.2 | 37 | 29.1 | 30 | 30.1 |
| Participant 13 | 41.1 | 47.8 | 40.1 | 37.7 | 34.3 |
| Participant 14 | 40.7 | 50 | 46.6 | 41.4 | 37.1 |
| Mean ± SD | 34.3 ± 7.3 | 40.4 ± 7.8* | 34.9 ± 6.9 | 32.6 ± 6.7 | 30.4 ± 6.3 |
| 95% CI | 30.2-38.1 | 36.0-44.7 | 31.1-38.8 | 28.9-36.3 | 26.9-33.9 |
| Range | 17-42.6 | 25.5-52.2 | 19.9-46.6 | 17.2-41.4 | 16.6-37.1 |

Δ from PRE for experimental, %

| Control, % |
|----------|-----|-----|-----|-----|-----|
| Participant 1 | 26.6 | 29 | 29.2 | 28.1 | 26.9 |
| Participant 2 | 33.3 | 39.7 | 35.7 | 33.7 | 36.6 |
| Participant 3 | 31.1 | 31.9 | 30.8 | 31.3 | 32.8 |
| Participant 4 | 40 | 36.4 | 34.5 | 32.9 | 36.2 |
| Participant 5 | 35 | 36.1 | 35.8 | 35.8 | 35.6 |
| Participant 6 | 43.6 | 45.7 | 41.7 | 41.6 | 43.8 |
| Participant 7 | 29.9 | 34.6 | 33.6 | 32.6 | 33.5 |
| Participant 8 | 33.4 | 32.3 | 29.9 | 27.3 | 26.1 |
| Participant 9 | 40.8 | 40.6 | 39.8 | 39.8 | 38.2 |
| Participant 10 | 24.7 | 28.5 | 23.8 | 23.6 | 24 |
| Participant 11 | 31.7 | 32.2 | 30.9 | 29.9 | 30.1 |
| Participant 12 | 40 | 36.6 | 31 | 30.8 | 31.3 |
| Participant 13 | 40 | 41.7 | 38.4 | 37.5 | 38 |
| Participant 14 | 28.6 | 32.4 | 24.9 | 23.5 | 24.6 |
| Mean ± SD | 35.1 ± 6.7 | 36.4 ± 5.7 | 33.4 ± 5.5 | 32.6 ± 5.7 | 33.1 ± 5.9 |
| 95% CI | 31.4-38.8 | 33.2-39.5 | 30.4-36.5 | 29.4-35.8 | 29.9-36.4 |
| Range | 24.7-43.6 | 28.5-45.7 | 24.9-41.7 | 23.5-39.8 | 24.6-38.2 |

Δ from PRE for control, %

| CD34⁺ |
|--------|
| Experimental, cells · μL⁻¹ |
| Participant 1 | 5.5 | 4 | 2.5 | 4.5 | 7 |
| Participant 2 | 3 | 3.5 | 2.5 | 2.5 | 3.5 |
| Participant 3 | — | — | — | — | — |
| Participant 4 | 1.5 | 2 | 3 | 1.5 | 1.5 |
| Participant 5 | 4.5 | 7 | 4 | 4 | 4.5 |
| Participant 6 | 4 | 6.5 | 4.5 | 4 | 5 |
| Participant 7 | 3.5 | 9.5 | 4.5 | 4.5 | 4 |
| Participant 8 | 3 | 4.5 | 2.5 | 2.5 | 2 |
| Participant 9 | 3.5 | 4 | 3.5 | 2.5 | 2 |
| Participant 10 | 1.5 | 1.5 | 1.5 | 1.5 | 1 |
| Participant 11 | 9 | 12.5 | 9 | 7 | 6 |
| Participant 12 | 2 | 11.5 | 4.5 | 3 | 4 |
| Participant 13 | 2.5 | 5 | 2 | 2.5 | 2 |
| Participant 14 | 2.5 | 2.5 | 1.5 | 1 | 1 |
| Mean ± SD | 3.1 ± 1.2 | 2.5 ± 2.9* | 3.1 ± 1.1 | 2.9 ± 1.2 | 3.2 ± 1.8 |
| 95% CI | 2.5-3.8 | 3-6.7 | 2.6-3.7 | 2.3-3.5 | 2.3-4.1 |
| Range | 1.5-9 | 1.5-12.5 | 1.5-9 | 1-7 | 1-7 |

Δ from PRE for experimental, %

| Control, cells · μL⁻¹ |
|----------|-----|-----|
| Participant 1 | 3.5 | 11.5 |
| Participant 2 | 3.5 | 4 |
| Participant 3 | 8 | 7.5 |
| Participant 4 | 2 | 2 |
| Participant 5 | 5.5 | 4 |
| Participant 6 | 5 | 6 |
| Participant 7 | 5 | 5.5 |
| Participant 8 | 5 | 3.5 |
| Participant 9 | 2 | 3 |

(continued)
two 3-mL blood collection tubes (Vacuette [454246]; Greiner Bio-One, Monroe, NC) before (PRE) and at various time points after the testing protocol. Three milliliters of whole blood was used to obtain a CBC with WBC differential using a Sysmex automated hematology analyzer (Sysmex America, Lincolnshire, IL). Flow cytometry (Cytomics FC500 Flow Cytometer; Beckman Coulter Life Sciences, Indianapolis, IN) was used to quantify the number of CD34+ HPCs present in the peripheral blood.

Finger-stick capillary samples were used to evaluate lactate and blood glucose levels. A Lactate Plus portable lactate analyzer (Nova Biomedical, Waltham, MA) and Contour Next blood glucose meter (Ascensia Diabetes Care US, Parsippany, NJ) were used to measure blood lactate and blood glucose levels, respectively. The fingers were cleaned with an alcohol swab; then, a single-use lancet was used to puncture the finger for blood testing. Both sides of the puncture site were pressed gently as needed to develop a drop of blood. The first drop of blood was wiped off using a sterile cotton swab to avoid contamination with interstitial fluid. When the second drop of blood had developed, the test strip for each meter was touched to the blood drop until the unit beeped. Different testing fingers were used for each finger stick. All samples were handled under universal precautions.

Each participant attended 3 testing sessions: a familiarization session followed by 2 testing sessions. The familiarization session occurred between 3 days and up to 2 weeks before the first experimental testing session. The 2 experimental testing sessions occurred within a minimum of 48 hours and a maximum of 2 weeks between sessions. Each participant completed an experimental (EXP) testing session using the Delphi system and a control (CON) testing session using the same exercise protocol without the Delphi system. The order for EXP and CON sessions was randomized among participants.

Height, weight, and blood pressure were obtained on presentation for the familiarization session. All participants were then introduced to each of the exercise machines and proper use was demonstrated. The exercise machines used during testing included a seated leg extension machine, a semi-reclined leg press machine, and a seated hamstring curl machine.

The 1-repetition maximum (1-RM) for each exercise was determined during the first familiarization session using a standard algorithm. The resistance of each exercise machine was subsequently increased until the participant was only able to perform a single repetition to determine the participant’s maximum. This process was repeated for each exercise (seated leg extension, semi-reclined leg press, and seated hamstring curl) until all 1-RM values were determined.

Participants completed 2 testing sessions separated by a minimum of 48 hours and within 2 weeks of the familiarization session in a randomized order. The standardized blood draw protocol was used to obtain PRE blood samples. Participants completed the EXP and CON sessions under the supervision of an investigator (T.J.O.) trained in use of the Delphi system. During the EXP session, bilateral proximal thigh tourniquets were applied and inflated to a pressure of 80% of occlusive pressure as determined by the automated tourniquets. During the CON session, participants completed the same exercise protocol without the use of the Delphi system. Each participant then completed the 3 exercises (seated leg extension, prone hamstring curl, and semi-reclined leg press) with a format of 1 set each of 30, 15, and 15 repetitions per exercise with 30 seconds of rest between sets while using the Delphi system at 80% limb occlusion pressure. The resistance for each exercise was set at 30% of the predetermined 1-RM. The tourniquets were deflated between exercises for 1 minute after the 4 sets had been completed. The tourniquets were reinflated at 80% occlusion prior to beginning each subsequent exercise until all exercises
| Variable | PRE | T0 | T10 | T20 | T30 | T40 | T50 | T60 |
|----------|-----|----|-----|-----|-----|-----|-----|-----|
| **Lactate, mmol · L⁻¹** |     |    |     |     |     |     |     |     |
| **Experimental** |     |    |     |     |     |     |     |     |
| Participant 1 | 3.1 | 4.7 | 4.7 | 2.3 | 4.2 | 3.5 | 1.4 | 1.7 |
| Participant 2 | 3.2 | 7.6 | 6.0 | 4.2 | 4.1 | 3.8 | 2.5 | 3.1 |
| Participant 3 | 1.8 | 5.4 | 5.2 | 4.5 | 2.4 | 2.5 | 1.7 | 2.1 |
| Participant 4 | 1.1 | 4.7 | 3.4 | 2.3 | 2.3 | 2.3 | 1.2 | 1.1 |
| Participant 5 | 1.3 | 6.2 | 4.5 | 3.4 | 2.3 | 2.4 | 1.7 | 2.2 |
| Participant 6 | 1.8 | 5.5 | 5.6 | 3.1 | 3.2 | 2.8 | 1.3 | 1.1 |
| Participant 7 | 2.0 | 11.5 | 12.0 | 10.0 | 8.1 | 6.0 | 4.4 | 4.9 |
| Participant 8 | 1.9 | 6.3 | 5.4 | 4.4 | 3.2 | 2.2 | 2.1 | 1.3 |
| Participant 9 | 0.5 | 8.0 | 7.9 | 7.2 | 6.3 | 6.2 | 5.8 | 3.1 |
| Participant 10 | 1.6 | 10.7 | 11.0 | 8.3 | 5.4 | 5.3 | 3.7 | 2.6 |
| Participant 11 | 2.5 | 5.8 | 5.1 | 5.9 | 2.8 | 2.4 | 2.8 | 2.1 |
| Participant 12 | 1.6 | 8.0 | 5.8 | 5.7 | 4.6 | 2.6 | 2.4 | 2.6 |
| Participant 13 | 1.7 | 9.8 | 10.3 | 8.7 | 4.4 | 3.9 | 4.1 | 2.5 |
| Participant 14 | 1.7 | 13.8 | 13.1 | 11.2 | 9.5 | 6.6 | 6.1 | 4.2 |
| **Mean ± SD** | 1.8 ± 0.7 | 7.87 ± 2.8* | 7.1 ± 3.0* | 6.0 ± 2.8* | 4.5 ± 2.1* | 3.7 ± 1.6* | 3.0 ± 1.6* | 2.48 ± 1.06* |
| **95% CI** | 1.4-2.2 | 6.4-9.4 | 5.0-8.8 | 4.4-7.5 | 3.4-5.7 | 2.8-4.6 | 2.1-3.8 | 1.9-3.1 |
| **Range** | 0.5-3.2 | 4.7-13.8 | 3.4-13.1 | 2.3-11.2 | 2.3-9.5 | 2.3-6.6 | 1.2-6.1 | 1.1-4.9 |
| **Glucose, mg · dL⁻¹** |     |    |     |     |     |     |     |     |
| **Experimental** |     |    |     |     |     |     |     |     |
| Participant 1 | 135 | 93 | 91 | 107 | 125 | 123 | 117 | 101 |
| Participant 2 | 102 | 108 | 112 | 96 | 101 | 108 | 111 | 112 |
| Participant 3 | 103 | 100 | 101 | 98 | 98 | 93 | 100 | 97 |
| Participant 4 | 111 | 104 | 109 | 108 | 112 | 111 | 110 | 110 |
| Participant 5 | 133 | 83 | 88 | 102 | 113 | 115 | 102 | 99 |
| Participant 6 | 88 | 80 | 87 | 90 | 94 | 95 | 101 | 100 |
| Participant 7 | 82 | 94 | 99 | 90 | 89 | 87 | 82 | 79 |
| Participant 8 | 80 | 82 | 85 | 86 | 96 | 95 | 85 | 86 |
| Participant 9 | 106 | 125 | 119 | 102 | 100 | 98 | 106 | 96 |
| Participant 10 | 84 | 99 | 109 | 102 | 96 | 92 | 94 | 89 |
| Participant 11 | 104 | 88 | 96 | 90 | 92 | 96 | 98 | 91 |
| Participant 12 | 120 | 128 | 90 | 91 | 100 | 101 | 100 | 90 |
| Participant 13 | 131 | 96 | 99 | 91 | 99 | 110 | 123 | 132 |
| Participant 14 | 91 | 97 | 99 | 86 | 84 | 82 | 85 | 82 |
| **Mean ± SD** | 104.7 ± 18.4 | 98.2 ± 14.0 | 98.9 ± 10.0 | 96.3 ± 7.6 | 99.9 ± 10.2 | 100.5 ± 11.1 | 101.0 ± 11.6 | 97.6 ± 13.3 |
| **95% CI** | 94.5-114.9 | 90.5-105.9 | 93.4-104.4 | 92.0-100.5 | 94.3-105.6 | 94.3-106.6 | 94.6-107.5 | 90.2-104.6 |
| **Range** | 80-135 | 80-128 | 87-119 | 86-108 | 84-125 | 82-123 | 82-123 | 79-132 |
| **Control** |     |    |     |     |     |     |     |     |
| Participant 1 | 94 | 82 | 98 | 93 | 96 | 93 | 90 | 91 |
| Participant 2 | 118 | 112 | 117 | 121 | 113 | 116 | 106 | 109 |
| Participant 3 | 101 | 93 | 93 | 94 | 93 | 94 | 91 | 97 |
| Participant 4 | 117 | 114 | 111 | 119 | 110 | 114 | 110 | 111 |
| Participant 5 | 93 | 97 | 98 | 105 | 105 | 93 | 94 | 94 |

(continued)
were completed. The exercise bout of a specific exercise
was terminated prematurely if participants reached
failure and were unable to complete 3 repetitions in a
row; participants were then instructed to complete the
subsequent exercise set.

Post-exercise blood samples were collected immediately
after exercise (T0) and again at the 20-minute
(T20), 40-minute (T40), and 60-minute (T60) time
points from a peripheral intravenous line that was
placed immediately after the training session. Finger-
stick blood lactate and blood glucose measurements
were also taken at T0 and at 10-minute intervals for 60
minutes after the training session (10 minutes [T10],
T20, 30 minutes [T30], T40, 50 minutes [T50], and
T60). The remaining testing session (EXP or CON) was
repeated on a second testing day using the same pro-
tocol. A baseline blood sample was also taken on the
second day of testing.

Repeated-measures analyses of variance (ANOVAs)
were used to detect differences between the EXP and
CON sessions and among time points for each outcome
variable. Dependent variables included the WBC count
(per microliter), platelet count (per microliter), per-
centages of neutrophils and lymphocytes in the WBC
differential, CD34⁺ count (cells per microliter), blood
lactate level (millimoles per liter), and blood glucose
level (milligrams per deciliter). Statistical significance
was set a priori at \( P < .05 \), and all analyses were per-
formed using IBM SPSS Statistics software (version
24.0; IBM, Armonk, NY).

Separate 2 (session) \( \times 5 \) (time) repeated-measures
ANOVAs were used to detect differences between the
EXP and CON sessions among the 5 time points (PRE, T0,
T20, T40, and T60) for WBC count, platelet count, per-
centage of neutrophils, percentage of lymphocytes, and
CD34⁺ count. Additional 2 (session) \( \times 8 \) (time)
repeated-measures ANOVAs were used to detect differ-
ences between the EXP and CON sessions among the 8
time points (PRE, T0, T10, T20, T30, T40, T50, and T60)
for lactate and glucose levels. If the Mauchly test of
sphericity was statistically significant (\( P < .05 \)), a Huynh-
Feldt adjustment was used to correct for the violation of
sphericity. Simple effects were used to investigate a 2-
way interaction, and pair-wise comparisons with a Bonferroni correction for multiple comparisons were
used with a significant main effect of time.

### Results

Fourteen healthy men (age, 30.8 ± 3.9 years; height,
179.7 ± 7.3 cm; and weight, 89.6 ± 17.1 kg) vol-
unteered to participate. The mean Tegner Activity Level
score for the participants was 5.5 ± 1.1 (Table 1). There
was a significant increase in average CD34⁺ counts
immediately after the EXP session at T0 only (3.1 cells \( \cdot \mu L^{-1} \) vs 5.2 cells \( \cdot \mu L^{-1} \); PRE range, 1.5-9 cells \( \cdot \mu L^{-1} \);
T0 range, 1.5-12.5 cells \( \cdot \mu L^{-1} \); \( P = 0.012 \)). These
values normalized by 20 minutes and beyond after the
exercise session (Table 2). One participant’s CD34⁺ data
for the EXP session were removed because of outliers
greater than 3 standard deviations above the mean.

There was a significant increase in platelet counts
immediately after the exercise session (T0) for both the
EXP (232,400/\( \mu L \) vs 266,600/\( \mu L \); PRE range, 169,000-
364,000/\( \mu L \); T0 range, 153,000-439,000/\( \mu L \); \( P < .002 \))
and CON (235,900/\( \mu L \) vs 247,500/\( \mu L \); PRE range, 133,000-428,000/\( \mu L \); T0 range, 152,000-458,000/\( \mu L \); \( P < .002 \)) testing sessions. The average increase was
higher after the EXP session versus the CON session
(mean difference [MD], 8,000/\( \mu L \)). These values
normalized at T20 after exercise for both the EXP and
CON testing sessions. After the EXP session only, a
significant decrease in average platelet values was
observed from baseline to T40 after the exercise session
(232,400/\( \mu L \) vs 224,300/\( \mu L \); T0 range, 141,000-
350,000/\( \mu L \); \( P < .01 \)), which again normalized by T60
after the session (Table 2).
There was a significant increase in the average WBC counts from baseline to T0 after both the EXP (8,400/µL vs 6,300/µL; PRE range, 3,000-8,100/µL; T0 range, 4,400-11,700/µL; P < .001) and CON (PRE range, 3,000-9,200/µL; T0 range, 4,000-11,500/µL; P < .001) sessions (Table 2). This increase in WBC counts was higher after the EXP session versus the CON session (MD, 900/µL; P < .001) (Table 2). There was a significant increase from baseline to T0 in the average number of lymphocytes (34.1% vs 40.4%; PRE range, 17%-42.6%; T0 range, 25.5%-52.2%; P < .001) and, conversely, a significant decrease in the average neutrophil count (52.8% vs 46.3%; PRE range, 46.6%-66.6%; T0 range, 36.2%-64.6%; P < .001) in the EXP session only. These findings initially normalized by T20, but then a significant decrease in average lymphocyte count from baseline was observed at T60 (34.1% vs 30.4%; T60 range, 16.6%-37.1%; P < .001). A significant increase in average neutrophil count at both T40 (52.8% vs 54.6%; T40 range, 45.8%-68.5%; P < .001) and T60 (52.8% vs 56.8%; T60 range, 48%-68.3%; P < .001) was also observed after the EXP session.

There were no significant changes from baseline to post-workout glucose levels after either training session at any time point (Table 3). There was a significant increase in lactate levels immediately after the workout for both the EXP (MD, 6.1 mmol · L⁻¹; P = .001) and CON (MD, 3.6 mmol · L⁻¹; P = .001) training sessions, which remained significantly elevated until T40, when the values normalized. The noted average increase in lactate levels was higher after the EXP training session at all time points up to T40 (Table 3).

**Discussion**

The most important findings of this study were the significant elevations in CD34⁺ cells and platelets above CON values immediately after the EXP exercise session, which could represent another potential mechanism for the noted efficacy of BFR. The results suggest that resistance exercise in men using the DelPhi system produces a statistically significant mobilization of HPCs (72% vs 4.3%) and platelets (14% vs 4.9%) to the peripheral circulation, beyond that of the CON session. This finding is consistent with findings in previously published literature showing a general rise in peripheral HPCs after standard non-BFR exercise.²⁵,²⁶,²⁸,²⁹ The significant lactate elevation was noted immediately after exercise and from 0 to 40 minutes after the exercise session, which is consistent with previously published findings.¹³⁻¹⁷ This finding shows that the participants were exercising at a high enough level to cause a desired systemic metabolic response.

The higher average platelet count should also be taken into consideration if one wishes to alter the components of a point-of-care blood product.²⁸ Previous literature has shown variability in the platelet product yield among commercially available platelet-rich plasma kits.³⁰ BFR may be potentially leveraged as a way to noninvasively increase peripheral platelet release prior to blood draw to improve the platelet-rich plasma yield that would be administered. The rise in platelets after the EXP session was consistent with recent findings showing an increase in peripheral mobilization of platelets after vigorous exercise. However, these studies focused on traditional training methods not using BFR.²⁵,²⁶,²⁸,²⁹ These results may explain the noted efficacy of BFR versus traditional therapy methods and show that BFR may be leveraged to improve the physiology of the rehabilitating athlete and potentially manipulate point-of-care blood products. Additionally, it is important to consider the individual variability in blood levels, as well as the variability in blood levels at different time points in the same individual.

Lymphocytes and neutrophils were also examined because we hypothesized that these cells could potentially represent indirect markers for the peripheral release of stem cells. There was a significant increase in lymphocyte numbers and, conversely, a significant decrease in average neutrophil numbers immediately after the exercise session. The finding of a significant decrease in average lymphocyte numbers at T60 after the EXP session and a significant increase in neutrophil numbers at both T40 and T60 may represent physiological overcompensation to correct the noted post-exercise changes in an attempt for the body to re-achieve homeostasis. The physiological overcompensation could also explain the significant increase in average platelet count noted after the EXP session only at T40 after exercise. It is speculated that the significant rise in lymphocyte numbers and converse decrease in neutrophil numbers for the EXP session may represent the release of progenitor cells that were registered as lymphocytes by the automated processing that was used for the CBC analysis.

**Limitations**

A limitation of this study was the relatively low number of participants included in each evaluation. This number was due to the selection criteria, as well as the fairly invasive nature of the assessments. The use of manual differentiation of the CBC for post-training blood draws versus our automated processing may also have potentially clarified some of the significant changes noted, specifically the elevation of lymphocytes and, conversely, the significant decrease in average neutrophils. Another limitation of this study was that only male participants were included. The results may differ in female participants.

**Conclusions**

Exercise with BFR causes a significant post-exercise increase in peripheral HPCs and platelets, beyond that of standard resistance training.
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