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Blood Flow Restriction Using the BStrong Tourniquets is Not Associated with a Cellular Systemic Response

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Abstract:	Purpose: The purpose of this study was to determine the effects of blood flow restriction (BFR) using B Strong tourniquets on CD34+ cells, platelets, white blood cells, neutrophils, lymphocytes, lactate, and glucose. Methods: Healthy participants aged 20-39 were recruited. Participants underwent an experimental (EXP) occluded testing session using the B Strong tourniquets on all four extremities and a control (CON) session. The exercise protocol concluded after 9-minutes or when participants reached a rating of perceived exertion of 20. Blood draws were performed prior to testing, and immediately post exercise session. Blood analysis consisted of complete blood counts as well as flow cytometry to measure peripheral CD34+ counts as a marker for hematopoietic progenitor cells (HPCs). Results: Fifteen adults (8 males, 28.6±3.6 years) volunteered to participate. A significant increase from pre to post exercise values was observed in both the EXP and CON groups with CD34+, WBC counts, platelets, and lymphocytes however no differences existed between EXP and CON group for any variable. CD34+ increased in the EXP (3.1±1.6 vs. 4.3±1.8 cells×mL-1; p<0.001) and CON (3.3±1.9 vs. 4.4±1.4 cells×mL-1;p<0.001) sessions. White blood cells also significantly increased in both the EXP (7.8±1.4 vs. 11.8±2.5K×mL-1;p<0.001) and CON (7.5±1.8 vs.11.3±3.0 K×mL-1; p<0.001) sessions. Platelets also increased in both the EXP (258.6±52.5 vs. 309.9± 52.7K×mL-1;p<0.001) and CON (263.1±44.7 vs. 316.1± 43.9 K×mL-1;p<0.001) sessions and conversely a significant decrease in the average neutrophil counts in the EXP (MeanDifference=-13.7%;p<0.001) and CON (MeanDifference=-13.2%;p<0.001) sessions was observed. Lymphocyte counts in the EXP (MeanDifference=22.8%;p<0.001) and CON (MeanDifference=19.3%;p<0.001) sessions increased significantly. Conclusions: BFR therapy can be considered as a way to manipulate point of care blood products like platelet rich plasma to increase product yield, but we did not demonstrate significant differences in systemic cellular response when undergoing aerobic based exercise with and without B Strong. Level of Evidence: 2

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Blood Flow Restriction Using the B Strong Tourniquets is Not Associated with a Cellular Systemic Response

Abstract

Purpose: The purpose of this study was to determine the effects of BFR using B Strong tourniquets on CD34+ cells, platelets, white blood cells, neutrophils, lymphocytes, lactate, and glucose.

Methods: Healthy participants aged 20-39 that were able to perform the exercise sessions on a VersaClimber were recruited. Participants underwent an experimental (EXP) occluded testing session using the B Strong tourniquets on all four extremities and a control (CON) session. The exercise protocol concluded after 9-minutes or when participants reached a rating of perceived exertion of 20. Blood draws were performed prior to testing, and immediately post exercise session. Blood analysis consisted of complete blood counts as well as flow cytometry to measure peripheral CD34+ counts as a marker for hematopoietic progenitor cells (HPCs).

Results: Fifteen adults (8 males, 7 females, 28.6 ± 3.6 years) volunteered to participate. A significant increase from pre to post exercise values was observed in both the EXP and CON groups with CD34+, WBC counts, platelets, and lymphocytes however no differences existed between EXP and CON group for any variable. CD34+ increased in the EXP (3.1 ± 1.6 vs. 4.3 ± 1.8 cells·μL^{-1}; $p<0.001$) and CON (3.3 ± 1.9 vs. 4.4 ± 1.4 cells·μL^{-1}; $p<0.001$) sessions. White blood cells also significantly increased in both the EXP (7.8 ± 1.4 vs. 11.8 ± 2.5 K·μL^{-1}; $p<0.001$) and CON (7.5 ± 1.8 vs. 11.3 ± 3.0 K·μL^{-1}; $p<0.001$) sessions. Platelets also increased in both the EXP (258.6 ± 52.5 vs. 309.9 ± 52.7 K·μL^{-1}; $p<0.001$) and CON (263.1 ± 44.7 vs. 316.1 ± 43.9 K·μL^{-1}; $p<0.001$) sessions and conversely a significant decrease in the average neutrophil counts in the EXP (MeanDifference=-13.7%; $p<0.001$) and CON (MeanDifference=-13.2%; $p<0.001$) sessions was observed. Lymphocyte counts in the EXP (MeanDifference=22.8%; $p<0.001$) and CON (MeanDifference=19.3%; $p<0.001$) sessions increased significantly.

Conclusions: BFR therapy can be considered as a way to manipulate point of care blood products like platelet rich plasma to increase product yield, but we did not demonstrate significant

differences in systemic cellular response when undergoing aerobic based exercise with and without the B Strong torniquet system.

Level of Evidence: 2.

Key Words: Cell biology; physical therapy; platelet rich plasma; rehabilitation; stem cells.

Introduction

Exercise with Blood Flow Restriction (BFR) is becoming a popular modality of use for both strength and conditioning as well as orthopedic rehabilitation.[1, 2-5] Compared to traditional strength training paradigms, BFR is advantageous because it allows for the utilization of submaximal loads to increase muscular size and strength with less stress placed on the joints.[6] Systemic cellular responses such as increases in CD34+ and cellular expression of genes related to muscle up regulation occur during exercise with BFR, which may contribute to increases in muscular size and strength to proximal muscle groups that are not directly occluded.[7-10] The same ability for increases in proximal muscle size and strength have not been demonstrated in matched controls undergoing traditional training methods.[6] The increases in proximal muscle size and strength with the use of BFR is ideal for orthopedic rehabilitation in patient populations who are unable to perform high intensity exercise and who have failed to improve with traditional therapy.[2,5,11,12]

The occlusion of blood flow provided by commercial BFR devices results in severe hypoxia to the working tissue that likely leads to a cascade of systemic cellular response that contribute to increased muscle size and strength.[13] Lactate and growth hormone levels have been shown to increase from 0-40 minutes after BFR[14-18] and metabolic overload from the accumulation of hydrogen and lactate may activate IL-6, macrophages and neutrophils.[19] BFR has also been shown to induce a local angiogenic response through upregulation of VEGF, another proposed mechanism for the noted efficacy of BFR therapy.[20] The amount of blood flow occlusion that is induced may vary between BFR devices and potentially limits the amount of systemic cellular responses that occurs with exercise. If the occlusion provided does not create a hypoxic environment in the working tissues, there may be limited efficacy for increasing muscle size and strength. Most of the scientific literature on the cellular responses to BFR has been performed using pneumatic BFR devices that adjust in real-time to ensure a consistent limb occlusion pressure throughout the full range of motion of an exercise. These types of devices are more cumbersome and restrictive in their use, and can be more expensive. Pneumatic devices, however, have been shown to ensure consistent occlusion is provided throughout the exercise.[21,22] The B strong system is

a more portable tourniquet system in which the cuffs are manually inflated prior to exercise but the pressure is not monitored or adjusted electronically during the exercise. The portability of the B Strong system makes it more advantageous to use in clinical settings however there is currently a gap in knowledge regarding its efficacy in creating beneficial systemic cellular responses.

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 following exercise with BFR using B Strong tourniquets is unclear. The purpose of this study was to determine the effects of BFR using B Strong tourniquets on CD34+ cells, platelets, white blood cells, neutrophils, lymphocytes, lactate, and glucose. It was hypothesized that exercise with BFR using B Strong tourniquets would stimulate a systemic cellular response to increase CD34+ cells, platelets, white blood cells, neutrophils, lymphocytes, lactate, and glucose that would not be observed during regular exercise alone.

Methods

A repeated-measures randomized crossover design was performed with the B Strong Training System ((B)STRONG, Park City, UT, USA). A complete blood count (CBC) with white blood cell (WBC) differential, flow cytometry to quantify the amount of CD34+ HPCs, and blood lactate and glucose levels were measured prior to (PRE) and immediately following (POST) exercise.

Healthy adults aged 20-39 were recruited to participate. Participants were excluded if they had a history of uncontrolled hypertension, diabetes, autoimmune disorders, blood disorders, disorders requiring immunosuppression, 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 orthopedic injury within the past 6 months. All procedures were approved by the hospital's 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 exam, including the completion of a medical history and assessment of activity level with the Tegner Activity Level Scale.[23] 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*Power 3.1.9.3) revealed a sample size of 10 participants was necessary to detect large effects (200%) using a power of 0.9 and alpha of 0.05. Sufficient power has been confirmed on previous mobilization studies.[10] The sample size of this study was increased to 15 to account for potential participant withdrawal.

The exercise protocol is summarized in Figure 1. Participants rested in the sitting position for 15 minutes prior to each testing session. A volume of 6 mL of venous blood was drawn from an antecubital vein into two 3 mL blood collection tubes (VACUETTE® 454246 Blood Collection Tube, Greiner Bio-One, Monroe, NC, USA) before (PRE) and after exercise (POST). Three mL of whole blood was used to obtain a complete blood count (CBC) with a white blood cell (WBC) differential using a Sysmex automated hematology analyzer (Sysmex America, Inc. Lincolnshire, IL, USA). Flow cytometry (Cytomics FC500 Flow Cytometer, Beckman Coulter Life Sciences, Indianapolis, IN, USA) was used to quantify the amount of CD34+ hematopoietic progenitor cells present in the peripheral blood.

Finger stick capillary samples were used to evaluate blood lactate and glucose levels. A Lactate Plus portable lactate analyzer (Nova Biomedical, Waltham, MA, USA) and Contour® Next blood glucose meter (Ascensia Diabetes Care US, Inc., Parsippany, NJ, USA) were used to measure blood lactate and blood glucose, respectively. Fingers were cleaned with an alcohol swab and 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 contaminant 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 meter beeps. Different testing fingers were used for each finger stick. All samples were handled under Universal Precautions.

Participants completed two testing sessions. The second testing session occurred within a minimum of 48 hours and a maximum of two weeks following the first testing session and the order of the sessions was randomized. Each participant completed a testing session using the B Strong BFR Tourniquet System during the exercise protocol (EXP) and completed a second testing session utilizing the same protocol without the B Strong BFR Tourniquet System (CON).

The standardized blood draw protocol was used to obtain PRE blood draw samples. After resting blood samples (PRE) were obtained, proximal arm and proximal thigh circumference were measured to determine the appropriate B Strong tourniquet band size for each participant. Participants then completed the randomly assigned EXP or CON exercise session. During EXP, B Strong tourniquets were applied bilaterally on the proximal arm and proximal thigh and inflated to pressures recommended by B Strong software for a healthy individual at a hard intensity level. Participants completed the CON session with the same exercise protocol without the B Strong tourniquets.

Exercise protocols were completed on the VersaClimber SM (VersaClimber, Santa Ana, CA, USA). Participants completed 3 sets of 3 minutes of exercise on the VersaClimber separated by 1-minute rest periods. Participants were instructed to maintain a loose hand grip, avoid a static squatting position during climbing, avoid hanging on the arms, and maintain full use of the lower body throughout the climbing bout.[24] During both rest periods of EXP, B Strong tourniquet pressures were checked and re-adjusted to the recommended pressure if needed. Borg rating of perceived exertion (RPE)[25] was recorded every minute of exercise. The 9-minute exercise bout was terminated early if the participant reached failure (RPE = 20). Total accumulated exercise time and number of stairs climbed were recorded. Immediately following the exercise protocol, an additional 6 mL of venous blood was collected for POST exercise. Finger sticks were performed to assess blood lactate and glucose. The remaining condition (EXP or CON) was a repeated on a second testing day with at least 48 hours of recovery between sessions. Since the change in cellular components were found at the PRE to POST interval blood draws.

Repeated-measures analyses of variance (ANOVAs) were used to detect differences between EXP and CON and among time points for each outcome variable. Dependent variables included: WBC count ($K \cdot \mu L^{-1}$), platelet count ($K \cdot \mu L^{-1}$), percent of neutrophils and lymphocytes in the WBC differential (%), CD34+ count ($cells \cdot \mu L^{-1}$), blood lactate level ($mmol \cdot L^{-1}$) and blood glucose level ($mg \cdot dL^{-1}$). Statistical significance was set *a priori* at $p < 0.05$. Two (session) x 2 (time) repeated-measures ANOVAs were used to detect differences between EXP and CON sessions among PRE and POST exercise for all dependent variables. All analyses were performed using IBM SPSS Statistics Version 24.0 software (International Business Machines Corp., Armonk, NY, USA).

Results

Fifteen healthy adults (8 males, 7 females, 28.6 ± 3.6 years; 172 ± 11 cm; 74.3 ± 16.1 kg) volunteered to participate in this study (Table 1). One female participant was removed from the data set due to abnormally high, above two standard deviations from the mean pre-exercise CBC and flow cytometry results, leaving 14 total participates for the study. The mean Tegner activity level for the participants was 5.5 ± 0.9 (Table 1). A significant increase from pre to POST exercise values was observed in both the EXP and CON groups with respect to WBC counts ($p<0.001$) platelets ($p<0.001$) lymphocytes ($p<0.001$), CD34+ ($p<0.001$) and blood lactate ($p<0.001$) (Table 2). Conversely, a significant decrease in peripheral neutrophils ($p<0.001$) from pre to POST exercise following both the experimental and control sessions was observed. Despite the significant increases noted at POST in both the EXP and CON exercises respectively, repeated-measures ANOVAs revealed no difference between EXP and CON group values for any of the variables. There were no differences in blood glucose levels between PRE and POST for either session (Table 2).

Discussion

The most important finding of this study was a significant increase from baseline in CD34+ markers post exercise in both the EXP (38.7% increase) and CON sessions (33.3% increase). However, despite the greater increase noted in the EXP group, there was no statistically significant difference between the overall increase in both groups. Similarly, while there was a significant increase in peripheral platelets following exercise in both groups, there was no difference in the degree of increase between EXP and CON sessions (19.8% vs. 20.1%). This is consistent with previously published literature demonstrating a general rise in peripheral HPC's following standard non BFR exercise.[26-29] The significant lactate elevation noted immediately post-exercise is consistent with previously published findings.[14-18] This elevation in lactate does demonstrate that the participants were exercising at a high enough level to cause a desired systemic metabolic response.

An emerging area of interest in orthopedics is to utilize exercise both with and without BFR to potentially optimize point of care blood products.[9,10,26] BFR may be potentially leveraged as a way to non-invasively increase peripheral platelet release prior to blood draw to improve the platelet rich plasma (PRP) yield that would be administered. The overall higher average platelet count noted in the EXP group should be taken into consideration if one wished to alter the components of a point of care blood product.[26] Previous literature has demonstrated variability in platelet product yield among commercially available platelet rich plasma (PRP) kits.[30] The rise in platelets the EXP session was consistent with recent findings showing an increase in peripheral mobilization of platelets following vigorous exercise.[9,10,26] These studies however focused on traditional training methods not employing BFR, which could explain the similar yet significant platelet elevation (19.8% vs. 20.1%) noted in both the EXP as well as CON sessions.[26-29] 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.

Anz et al[26] found that 20 minutes of vigorous exercise increases platelet concentration by over 20% in PRP products and buffy coat-based PRP prepared after exercise had significantly higher concentrations of mobilized hematopoietic progenitor cells. Callanan et al[10] recently reported significant

elevations of CD34+ cells and platelets above control values immediately following an exercise session that included 4 sets of 30-15-15-15 repetitions for the seated leg extension, prone hamstring curl, semi-reclined leg press using the Delfi PTS Personalized Tourniquet System. Their results suggest that a statistically significant mobilization of hematopoietic progenitor cells (72% vs. 4.3%) and platelets (14% vs. 4.9%) to the peripheral circulation occurs with BFR, beyond that of the control session.[10] Lymphocytes and neutrophils were examined in this study as we hypothesized that these cells could potentially represent indirect markers for the peripheral release of stem cells. There was a significant increase in lymphocytes, and conversely, a significant decrease in average neutrophils immediately following exercise in both the EXP and CON sessions. We speculate that the significant rise in lymphocytes, and the converse decrease in neutrophils may represent the release of progenitor cells which were registered as lymphocytes by the automated processing that was used for the CBC analysis. We did not however observe the similar mobilization of hematopoietic progenitor cells or platelets using the B Strong system for full body aerobic exercise on the VersaClimber. Possible explanations for this would be that the exercise with the VersaClimber, focused more on aerobic exercise versus pure resistance training. Another possible reason for the difference noted in this study would be that the B Strong cuffs did not have as great or consistent of an effect on occluding blood flow compared to the Delfi system to elicit a similar systemic response. These two factors should be taken into consideration, both the unit specifics and the selection of BFR exercise if there was a goal to manipulate point of care products.

The B Strong system uses a 5 cm cuff width and a detachable pressurizing system allowing for multiple cuffs to be inflated at the same time and does not restrict the participant to a certain area, but due to utilization of lower pressures, this system can be more tolerable to the user during exercise than electronic systems. Furthermore, unlike electronic systems that carry a significant financial burden and are cost prohibitive, the B Strong can be a more affordable alternative system. As previously mentioned, future studies should continue to investigate the influence of differing training modalities using BFR on platelet and HPCs release. Ideally these results could also be compared across other commercially

available BFR systems to further determine the optimal training method and system to achieve the most desirable systemic metabolic response. Further investigation should also be undertaken to identify and delineate patient specific factors that may correlate to a greater mobilization of platelets and HPCs following exercise with BFR. This would further allow for determination of who may benefit most from exercise with BFR for both rehabilitation purposes, as well as potentially leveraging point of care blood products.

Limitations

The similar increase in platelets and CD34+ counts noted following the B Strong EXP and CON sessions, may ultimately be attributable to several factors. This study focused on a systemic anaerobic/aerobic cardiovascular workout with the Versaclimber™ versus traditional weight training exercises. In addition, males and females were included in the study sample and while they were equally distributed, the role of gender on the metabolic response to exercise could also be a factor to consider. This number was secondary to the selection criteria, as well as the fairly invasive nature of the study. The use of manual differentiation of the CBC for post training blood draws vs 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.

Conclusion

BFR therapy can be considered as a way to manipulate point of care blood products like platelet rich plasma to increase product yield, but we did not demonstrate significant differences in systemic cellular response when undergoing aerobic based exercise with and without the B Strong torniquet system.

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Tables

Table 1. Participant demographics.

Characteristic	B Strong Protocol
Sex	8 M, 7 F
Age (years)	28.6 ± 3.8
Height (m)	1.7 ± 0.11
Weight (kg)	74.3 ± 16.1
Tegner Score	5.5 ± 1.0

Table 2. Results of the cellular analysis.

Variable	Experimental PRE	Experimental POST	Control PRE	Control POST
WBC ($K \cdot \mu L^{-1}$)	7.8 ± 1.4	11.8 ± 2.5[a]	7.5 ± 1.8	11.3 ± 3.0[a]
95% CI	7.0, 8.5	10.3, 13.2	6.5, 8.5	9.5, 13.0
Δ from PRE		*51.3%*		*50.7%*
Platelets ($K \cdot \mu L^{-1}$)	258.6 ± 52.5	309.9 ± 52.7[a]	263.1 ± 44.7	316.1 ± 43.9[a]
95% CI	228.4, 288.9	279.5, 340.4	237.3, 289.0	290.8, 341.5
Δ from PRE		*19.8%*		*20.1%*
Neutrophils (%)	56.8 ± 6.6	49.0 ± 9.8[b]	52.1 ± 5.6	45.2 ± 6.5[b]
95% CI	53.0, 60.6	43.4, 54.7	48.9, 55.3	41.4, 48.9
Δ from PRE		*-13.7%*		*-13.2%*
Lymphocytes (%)	32.4 ± 6.6	39.8 ± 9.8[a]	36.2 ± 5.5	43.2 ± 6.7[a]
95% CI	28.6, 36.3	34.1, 45.5	33.0, 39.4	39.3, 47.0
Δ from PRE		*22.8%*		*19.3%*
CD34+ ($cells \cdot \mu L^{-1}$)	3.1 ± 1.6	4.3 ± 1.8[a]	3.3 ± 1.9	4.4 ± 1.4[a]
95% CI	2.2, 4.0	3.3, 5.4	2.2, 4.4	3.5, 5.2
Δ from PRE		*38.7%*		*33.3%*
Lactate ($mmol \cdot L^{-1}$)	1.8 ± 0.8	10.7 ± 3.9[a]	1.7 ± 0.7	9.9 ± 3.2[a]
95% CI	1.3, 2.3	8.5, 13.0	1.3, 2.1	8.0. 11.7
Glucose ($mg \cdot dL^{-1}$)	105.4 ± 19.8	108.4 ± 14.2	102.6 ± 18.8	96.1 ± 9.5
95% CI	93.9, 116.8	100.2, 116.5	91.8, 113.5	90.7, 101.6

WBC = white blood cells; a = significant increase from PRE; b = significant decrease from PRE

Figure Legend

Figure 1. B Strong Exercise Session Flowchart.

Acknowledgements

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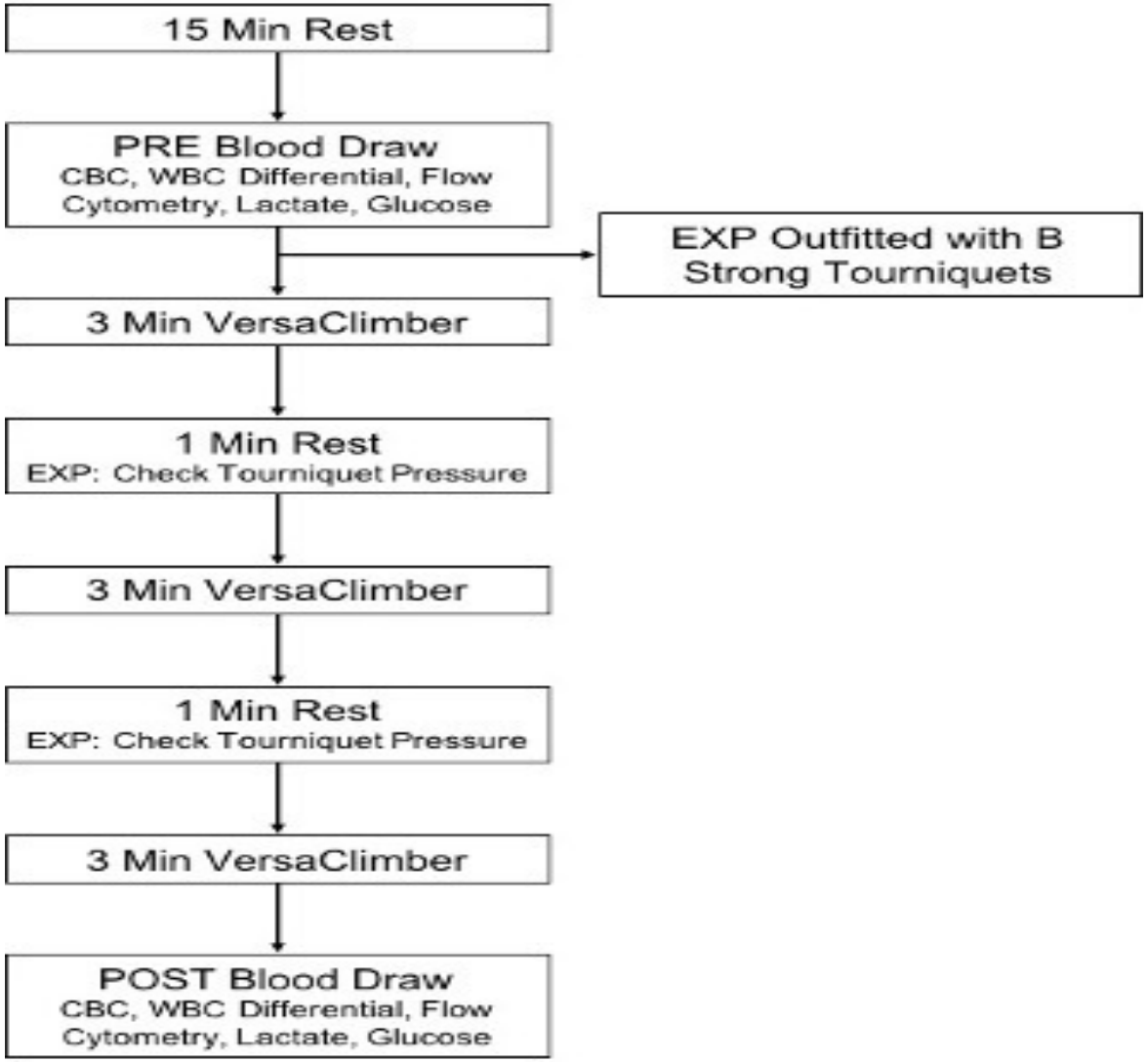
Figure 1


15 Min Rest
PRE Blood Draw
CBC, WBC Differential, Flow Cytometry, Lactate, Glucose
EXP Outfitted with B Strong Tourniquets
3 Min VersaClimber
1 Min Rest
EXP: Check Tourniquet Pressure
3 Min VersaClimber
1 Min Rest
EXP: Check Tourniquet Pressure
3 Min VersaClimber
POST Blood Draw
CBC, WBC Differential, Flow Cytometry, Lactate, Glucose

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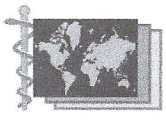

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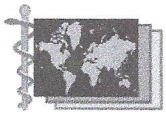

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Issued: The patent has been issued by the agency
Licensed: The patent has been licensed to an entity, whether earning royalties or not
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1. Given Name (First Name): Thaddeus
2. Surname (Last Name): Broderick
3. Date: 30-April-2021

4. Are you the corresponding author? ☐ Yes ☑ No
Corresponding Author's Name: Hillary Plummer

5. Manuscript Title
Blood Flow Restriction Using the B Strong Tourniquets is Associated with a Cellular Systemic Response

6. Manuscript Identifying Number (if you know it)

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Did you or your institution **at any time** receive payment or services from a third party (government, commercial, private foundation, etc.) for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.)?

Are there any relevant conflicts of interest? ☐ Yes ☑ No

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Are there any relevant conflicts of interest? ☐ Yes ☑ No

Section 4. Intellectual Property -- Patents & Copyrights

Do you have any patents, whether planned, pending or issued, broadly relevant to the work? ☐ Yes ☑ No

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Mr. Broderick has nothing to disclose.

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This section asks for information about the work that you have submitted for publication. The time frame for this reporting is that of the work itself, from the initial conception and planning to the present. The requested information is about resources that you received, either directly or indirectly (via your institution), to enable you to complete the work. Checking "No" means that you did the work without receiving any financial support from any third party – that is, the work was supported by funds from the same institution that pays your salary and that institution did not receive third-party funds with which to pay you. If you or your institution received funds from a third party to support the work, such as a government granting agency, charitable foundation or commercial sponsor, check "Yes".

3. Relevant financial activities outside the submitted work.

This section asks about your financial relationships with entities in the bio-medical arena that could be perceived to influence, or that give the appearance of potentially influencing, what you wrote in the submitted work. You should disclose interactions with ANY entity that could be considered broadly relevant to the work. For example, if your article is about testing an epidermal growth factor receptor (EGFR) antagonist in lung cancer, you should report all associations with entities pursuing diagnostic or therapeutic strategies in cancer in general, not just in the area of EGFR or lung cancer.

Report all sources of revenue paid (or promised to be paid) directly to you or your institution on your behalf over the 36 months prior to submission of the work. This should include all monies from sources with relevance to the submitted work, not just monies from the entity that sponsored the research. Please note that your interactions with the work's sponsor that are outside the submitted work should also be listed here. If there is any question, it is usually better to disclose a relationship than not to do so.

For grants you have received for work outside the submitted work, you should disclose support ONLY from entities that could be perceived to be affected financially by the published work, such as drug companies, or foundations supported by entities that could be perceived to have a financial stake in the outcome. Public funding sources, such as government agencies, charitable foundations or academic institutions, need not be disclosed. For example, if a government agency sponsored a study in which you have been involved and drugs were provided by a pharmaceutical company, you need only list the pharmaceutical company.

4. Intellectual Property.

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1. Given Name (First Name): Nicole
2. Surname (Last Name): Rendos
3. Date: 30-April-2021

4. Are you the corresponding author? ☐ Yes ☑ No
Corresponding Author's Name: Hillary Plummer

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Dr. Rendos has nothing to disclose.

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Section 1. Identifying Information

1. Given Name (First Name): Adam
2. Surname (Last Name): Anz
3. Date: 30-April-2021

4. Are you the corresponding author? ☐ Yes ☑ No
Corresponding Author's Name: Hillary Plummer

5. Manuscript Title: Blood Flow Restriction Using the B Strong Tourniquets is Associated with a Cellular Systemic Response

6. Manuscript Identifying Number (if you know it):

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Are there any relevant conflicts of interest? ☑ Yes ☐ No

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Arthrex Inc.	☑	☑	☐	☑	Consulting fees, speaking fees, educational and research support, royalties
CGG Medical	☑	☐	☐	☐	Educational and research support
Smith & Nephew	☐	☑	☐	☐	Speaking fees
Bioventus	☐	☑	☐	☐	Consulting fees

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Dr. Anz reports grants, personal fees and other from Arthrex Inc., grants from CGG Medical , personal fees from Smith & Nephew, personal fees from Bioventus, during the conduct of the study .

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