RESEARCH PLAN

Title: Blood flow restriction-enhanced platelet-rich plasma: a pilot study.

Specific Aims

The present study aims to assess platelet-rich plasma (PRP) changes in platelet and leukocyte count, IGF-1, and IL-6 concentration after bilateral low-load knee extensions under blood flow restriction (BFR). The hypothesis is that bilateral low-load knee extensions under BFR will increase platelet and leukocyte count, IGF-1, and IL-6 in PRP prepared after the exercise bout.

Finding changes in platelet and leukocyte count, IGF-1, and IL-6 concentration in PRP is a promising starting point for potentially modifying its composition for disease-specific formulations.

Background and Significance

PRP is a portion from autologous blood with increased platelet concentration obtained by centrifugation [1]. This orthobiologics therapy has been increasingly implemented during the last two decades, especially in knee osteoarthritis and tendinopathies, with promising outcomes [2-4]. Although the wide variety of available PRP products and the poor reporting quality of the PRP preparations have limited its standardization, platelet, and leukocyte dose have been established as key factors in this orthobiologics therapy [5, 6].

Among PRP components, IGF-1 and IL-6 have critical roles in their potential mechanisms of action. IGF-1 has been associated with the proliferation and maturation of chondrocytes and inhibiting the apoptosis of osteoarthritic chondrocytes (via PDCD5 downregulation) [7-9]. On the other hand, IL-6 is a pro-inflammatory cytokine that has been linked with cartilage matrix degradation (via metalloproteinases) [10] but potentially promotes muscle growth by activating satellite cells to repair damaged muscle fibers [11], neovascularization [12], and induces tendons repair by collagen production [13-16].

BFR training, a rehabilitation modality with local and systemic effects in the musculoskeletal system [17], has also been associated with the release of IGF-1 [18-20] and IL-6 [18, 21-24] in the bloodstream. This training modality consists of the application of a tourniquet in the proximal area of the upper or lower limb with partial occlusive pressures (from 40%-80%) to create a venous stasis and promote a metabolic stress environment that triggers the release of multiple hormones and growth factors with local and remote effects [25-29].

BFR training allows implementing loads as low as 20-30% of the one-repetition maximum (1-RM) (the weight load that would allow performing only one successful repetition of a specific exercise), compared to the 70% of the 1-RM of the traditional resistance training [28, 29]. The BFR standard protocol comprises a 30-15-15-15 repetition consecutive sets with 30-second rest intervals for a total of 75 repetitions with 20-30% of the one-repetition maximum load [28, 29].

The combination of the tourniquets with low-load training results in similar muscle strength and hypertrophy gains as the traditional weight resistance training with high loads, attracting much interest for postoperative knee rehabilitation [30, 31]. This muscle hypertrophy is mediated by the growth hormone [32], IGF-1, and myokines, specifically IL-6 (promoting muscle regeneration and increased protein synthesis), driven by anaerobic glycolytic metabolism, glycogen depletion, and intramuscular hypoxia [33]. The increase in these factors has been reported as early as in the first 30 minutes post-exercise [18, 19, 21].

Different exercise modalities consistently alter plasma composition [34-36] and increment platelet concentration by over 20% [37] (Table 1). Analyzing PRP composition after BFR training supposes a potentially enhanced PRP formulation with increased platelet count, IGF-1, and IL-6 levels that could benefit patients with cartilage, tendon, and muscle pathologies. BFR can be performed safely [38] and at a low cost in an office setting and easily implemented before blood draw for PRP preparation, resulting in an easy and fast intervention to improve PRP composition for treating sports-related injuries.

Thus, the present study aims to assess PRP changes in platelet and leukocyte count, IGF-1, and IL-6 concentration after bilateral low-load knee extensions under BFR. The hypothesis is that bilateral low-load knee extensions under BFR will increase platelet and leukocyte count, IGF-1, and IL-6 in PRP prepared after the exercise bout.

STUDY	N° of patients	EXERCISE MODALITY	RESULT
Hamilton et al. 2015	10	Modified submaximal cycling test on an electronically braked cycle ergometer at 50% of peak power output for 1 hour.	Increased platelet, leukocyte, and platelet-derived growth factor-AB in PRP. No effect on IGF-1 and hepatocyte growth factor.
Baria et al. 2020	10	After a 5-minute warm-up, the participants engaged in interval exercise using an exercise bike, completing eight intervals. Each interval consisted of 20 seconds of work followed by 10 seconds of rest at strenuous or maximum intensity according to Borg's rate of perceived exertion.	Increased platelet count and TGF-β levels in PRP. The other cellular components (leukocytes, red blood cells, and mean platelet volume) and growth factors (PDGF, IGF-1, and VEGF) were not significantly changed.
Callanan et al. 2021	16	Testing was performed on the recumbent cross-trainer wearing a cooling vest set at a temperature of 8.3°C. Blood flow restriction cuffs were applied bilaterally on the upper arm and upper leg at 40- and 65-mm Hg of compression throughout exercise testing. The participants sat on a cooling pad at 8.3°C (47°F). Testing was performed with the participant barefoot because the foot panels of the machine also provided cooling. The participants were placed on the machine for 20 minutes of exercise. Once the warm-up was completed, the participants performed six sprint intervals, alternating 30-second and 60-second sprints at maximal exertion effort. Following the 30-second sprints, the participants had 1.5 minutes of active recovery before the 60-second sprint. Two minutes of active recovery followed each 60-second sprint (Fig 2).	Increased leukocyte count is marked by an increase in lymphocyte differential and a decrease in neutrophil differential in blood samples. Increased platelet count and CD34+ cells but decreased monocyte count. No differences in IL-10, IL-6, granulocyte-macrophage colony-stimulating factor, IL-1ra, TNF-α, or IL-2 were observed.
Anz et al. 2019	20	Participants followed an exercise regimen on an upright bike. The exercise regimen involved a 5-minute warm-up period followed by 20 minutes of vigorous exercise determined by maintaining the target heart rate at 70% to 85% of the maximum target heart rate.	Increased platelet count (by over 20%), leukocytes, and hematopoietic progenitor cells in PRP.

Table 1. Exercise modalities and their effects on plasma and PRP composition.

Research Design and Method

1. Type of study:

   Randomized controlled trial.

2. Research population or the sampling frame:

   Twenty-two healthy individuals with Tegner scale scores > 5.

3. How were sample size and grouping decided upon:

   A power analysis of continuous endpoint in two independent samples anticipating a 25% increase of IGF-1 with α = 0.05 and 80% power validates our sample size. Additionally, previous studies have been conducted using a similar sample size [34, 35, 37]. Participants will be randomly allocated in the intervention and control groups (1:1).

4. Gender: Males.
5. Age: 18-40 years.
6. Language of participants: Spanish.
7. How long will an individual subject participate in the research?

   Two hours.

8. How long will it take to enroll in all needed subjects?

   3-4 weeks.

9. Inclusion Criteria
   a. Healthy individuals undergoing routine health screening, with
   b. ages between 18 to 40 years,
   c. no musculoskeletal conditions that would interfere with exercise.
10. Exclusion criteria
    a. Individuals with systemic inflammatory diseases,
    b. or cardiovascular risk factors,
    c. any blood dyscrasia,
    d. with Tegner Activity scale scores < 5, or
    e. under nonsteroidal anti-inflammatory drugs and aspirin treatment within one week before testing, or
    f. that had previously performed exercises on the testing day.
11. Withdrawal criteria

    Patients can withdraw from the study at any time.

12. Procedures and persons responsible for recruitment.

    The principal investigator will assess patients undergoing routine screening. Eligible participants will be offered enrollment with previous informed consent.

13. Methodology & Project Duration
    a. Clinical or Non-clinical interventions

    Before the intervention, participants will have a pre-exercise peripheral vein catheterization, blood sample draw, and PRP preparation for baseline measurements. Each participant will undergo standard venipuncture in the

antecubital fossa by a single phlebotomist under sterile conditions for a total blood draw of 15 ml in a BD vacutainer and undergo a single centrifugation at 1500 rpm for five minutes. The plasma portion and buffy coat will be separated from the red blood cells, and samples will be sent to the laboratory and divided into two aliquots, one for cell counts (automated cell counter), the other for IGF-1 and IL-6 immunoassays analysis within 6 hours.

The participant will then perform the low-load BFR protocol. The low-load bilateral knee extensions under BFR (using tourniquets at the proximal end of both thighs) will follow the standard protocol of four sets consisting of 30-15-15-15 repetitions, with 30-second rest intervals at 80% of limb occlusive pressure (calculated using arteria pedis ultrasound) and 30% of 1-RM load (using Holten diagram). Individuals will be randomly assigned to the intervention or control groups (performing the low-load knee extension protocol without BFR) at recruitment (1:1). Eleven participants will be recruited into each group (Figure 1).

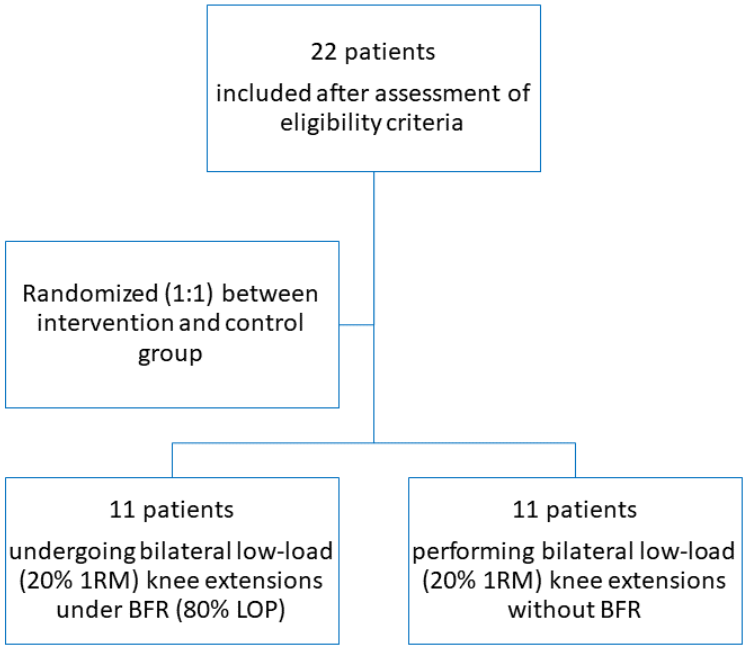

Figure 1. Participant recruitment, randomization, and group allocation flowchart.

A staff physician will monitor the entire exercise protocol and recovery period for safety and adverse events. Once the exercise protocol is completed, participants will be allowed a recovery period (including rest, walking if desired, and fluid intake) before undergoing the consecutive blood draw. The maximum recovery permitted time will be 5 minutes. The subsequent blood draws, and PRP processing will be performed identically to the first at 10-, 20-, and 30-minutes post-intervention.

The expected outcome is that the standard protocol of low-load bilateral knee extensions under BFR will increase the platelet and leukocyte count, IGF-1, and IL-6 in the PRP preparation.

b. Average time taken per intervention/procedure (minutes, hours, or days)

   Tests will last around 2 hours.

c. Description of the drug/device/vaccine/dietary intake that is being tested or in social sciences for example providing training or information to groups of individuals.

Low-load BFR bilateral knee extensions are a form of exercise that involves the partial occlusion of the venous return to promote a metabolic stress environment in the muscle tissue. This training modality can achieve similar muscular gains to traditional resistance exercises using low loads (30% 1-RM) [28, 29].

d. Details of who will conduct the intervention/procedure, and where it will take place.

A multidisciplinary medical team (the investigators) will conduct the intervention. It will take place in the Avilab Laboratory.

e. Procedures

- Evaluation of eligibility in athletes undergoing routine screening, including Tegner Activity Scale assessment.
- Informed consent.
- Demographic data collection.
- Pre-BFR blood samples collection.
- Low-load BFR bilateral knee extension intervention.
- Post-BFR blood samples collection at 10', 20' and 30'.
- Laboratory testing of collected samples.

Details were previously described in the preceding text.

f. Duration of the Project: 6 months

g. Timeline of data collection:

	1° month	2° month	3° month	4° month	5° month	6° month
Piloting the procedure						
Participant recruitment						
Data collection						
Data Analysis						
Dissemination of results						

14. Research Risks and Problems Anticipated

BFR training is safe, with risks consistent with traditional exercise modalities [38]. However, sparse reports of common side effects include pain or discomfort during exercise, delayed-onset muscle soreness, and cardiac stress (increased heart rate, increased blood pressure, decreased stroke volume). In contrast, more serious, less common

side effects include numbness or nerve injury, bruising or ischemic injury, dizziness or fainting, thrombus formation, muscle damage, and rhabdomyolysis. These risks are negligible when BFR is performed under current standards.

Contraindications for BFR implementation include a history of or the potential for deep vein thrombosis, blood clotting disorder, poor circulation, hypertension, inadequate lymphatic system, history of endothelial dysfunction, varicose veins, peripheral vascular disease, diabetes, easy bruising, active infection, cancer, renal compromise, pregnancy, and intervention intolerance.

15. Difficulties that the investigators anticipate in successfully completing their projects within the time frame and solutions to deal with these difficulties.

    None expected.

16. Potential for a benefit to research participants.

    Patients will benefit from the systemic and local musculoskeletal effects of BFR training. Patients will also be given their blood test results for annual control purposes.

17. Safety/ Ethics Considerations and Follow-up for Adverse Events

    No ethical concerns are to be disclosed. Informed consent will be obtained in the screening clinic, a previous comprehensive explanation of the clinical trial. Potential adverse effects will be recorded in a logbook and promptly reported to the IRB. A follow-up call will be done 72 hours after the blood sample collection to assess for late presentation of adverse effects, if any.

18. Informed Consent Forms (Adults/Children under 18)

    As per IRB requirements.

19. Data Type and Management

    The medical record numbers will be paired with a unique research ID number and stored on a separate spreadsheet to protect against a breach of privacy. Other data mentioned below will be stored on another spreadsheet. Also, the link between code and identifier will be destroyed after the study is finished, and de-identified data will be kept for at least five years.

    Investigators and the laboratory staff responsible for blood sample analysis are listed in this template.

    The leading investigator will be responsible for monitoring the data collected, including the data related to unanticipated problems and adverse events.

    Data will be stored as per department policy.

    Studies will be performed on each variable with repeated-measures analysis of variance to test the hypothesis at a confidence level of 95% ($P < .05$). The results will be presented as mean ± standard deviation. Statistical significance is set a priori at $P < .05$. All analyses will be conducted using statistical software available in the department.

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