Cover Page for Study Protocol and Statistical Analysis Plan

Official title	Clinical Effectiveness of Boxing Training in Individuals with Elevated Blood Pressure or Stage 1 Hypertension
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Study Protocol

Participants

Participants will be recruited from the University of Texas at El Paso and its surroundings. They will be identified by a preliminary blood pressure screening and a health questionnaire. The inclusion criteria will consist of: (1) ≥18 years old, (2) SBP between 120-139 mmHg or DBP between 80-89 mmHg obtained from 2 different days, (3) an estimated 10-year risk of Cardiovascular Diseases ≤10%, calculated by the American College of Cardiology/Amerian Heart Association Pooled Cohort Equations, and (4) no current participation in 3 or more days per week of endurance or resistance exercise training. Exclusion criteria will include non-controlled cardiac, pulmonary, or metabolic diseases, smoking, consumption of nutritional supplements containing antioxidants, and any physical impairment to exercise.

Protocol

An initial brachial blood pressure screening will be performed to identify potential participants for the present study. Those with systolic blood pressure readings between 120-139 mmHg or diastolic blood pressure between 80-89 mmHg will be instructed to assist to the Clinical Physiology Lab at the University of Texas at El Paso in fasting conditions for a second blood pressure assessment to confirm the diagnosis of elevated blood pressure or stage 1 hypertension. Then, they will complete a health questionnaire to disregard any cardiac, pulmonary, or metabolic condition. Once they are cleared to participate, they will start the inform consent process. After obtaining the consent, their height and weight will be measured using a stadiometer (Seca 225, Seca Medical Measuring Systems and Scales, Hamburg, Germany) and a digital scale (Tanita WB-110A, Tanita Corporation, Tokyo, Japan), respectively. Then, they will remain seated for 5 minutes followed by an assessment of central blood pressure through Pulse Wave Analysis (Sphygmocor, Xcel, West Ryde, Australia). Thereafter, participants will be asked to lay down in supine position over an examination table for 10 minutes where Pulse Wave Velocity (Sphygmocor, Xcel, West Ryde, Australia), brachial and popliteal Flow Mediated Dilation, and strain gauge venous occlusion plethysmography (AI6 Arterial Inflow System, D.E. Hokanson Inc., Bellevue, Washington) will be performed. At the end of the first lab visit, they will complete the SF-36 questionnaire and they will be asked to comply with a low nitrate diet for 48 hours. In a second visit, a blood samples will be collected from the antecubital vein of the participants, followed by a body composition analysis through dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy, GE Healthcare, Madison, WI) and cardiopulmonary testing using a crank-arm ergometer (Angio V2, Lode, Groningen, Netherlands) that will determine their VO_2max (Parvomedics Inc., Sandy, UT). Then participants will be randomly assigned into a boxing or a control group using an online number generator (https://www.graphpad.com/quickcalcs/randomize1/). Finally, all the measurements that were taken in the first two visits will be repeated in the same order after a 6-week intervention.

Interventions

Boxing Training

First, in a familiarization visit, participants will learn how to wrap their hands and basic boxing techniques, such as stance and punches, while wearing 14 oz gloves. This visit will finish with an incremental boxing test that consists of punching a 100 lb heavy bag (Ringside soft filled leather, Ringside-CSI Fitness 1st, Lenexa, KS) at a fixed force that will be tracked with a sensor attached to the bottom of the heavy bag (UFC Force Tracker) and with increments on the punching cadence every 3 minutes. The test will start at a cadence of 60 punches per minute (ppm) that is controlled by a metronome (Pro Metronome by EUMLab, Xanin Tech. GmbH.), and will increase 15 ppm every 3 minutes, until fatigue. As depicted in Figure 2, at the end of each 3-minute workload, oxygen uptake, and heart rate will be measured.

The boxing training intervention will consist of three exercise sessions per week in nonconsecutive days for six weeks. The workout will begin with a 3-minute warm up period where participants will actively move their shoulders, elbows, wrists, and finger joints. Then participants will be instructed to complete 10 rounds of three minutes with one-minute resting period interspersed. Four rounds will consist on heavy bag punching (e.g. straight, jab, hook) at 60% VO_2max and three rounds at 90-95% VO_2max, while the remaining 3 rounds will be focus on mitt work at 60% VO_2max. Heart rate will be constantly monitored to ensure that each participant will be exercising at the desire intensity.

Flexibility and Balance Training (control intervention)

The control group will perform three days per week 10 minutes of dynamic articular movement, five minutes of unipedal stance, and five minutes of stretching of the upper limbs for six weeks.

Statistical Analysis

Data will be analyzed by SPSS version 24.0. Normal distribution of the data will be examined with the Shapiro-Wilk test and visual inspection. If the data is not normally distributed a transformation will be attempted prior the use of non-parametric statistics. Normally distributed data will be presented as mean (standard deviation) while non-normally distributed will be presented as median (interquartile range). Paired t-tests will be conducted to compare pre- and post-variables from both groups. The magnitude of the effect will be assessed through Hedges's gs corrected effect size[246]. Significance will be established at $\alpha \leq 0.05$.

The sample size will be determined using the software G*Power 3.1. Based on Izadi et al. results on the effects of six weeks of High Intensity Interval Training on systolic blood pressure (effect size = 1.73), establishing α at 0.05 and β at 0.2, and assuming a 30% drop out rate, the estimated number of participants per group will be 12.

University of Texas at El Paso (UTEP) Institutional Review Board
Informed Consent Form for Research Involving Human Subjects

Protocol Title: Effects of Physical Activity on Cardiovascular Risk and Endothelial Function in Individuals with Elevated Blood Pressure or Stage 1 Hypertension

Principal Investigator: Francisco Morales

UTEP: Rehabilitation Sciences Department

In this consent form, "you" always means the study subject. If you are a legally authorized representative (such as a parent or guardian), please remember that "you" refers to the study subject.

1. Introduction

You are being asked to take part voluntarily in the research project described below. Please take your time making a decision and feel free to discuss it with your friends and family. Before agreeing to take part in this research study, it is important that you read the consent form that describes the study. Please ask the study researcher or the study staff to explain any words or information that you do not clearly understand.

2. Why is this study being done?

You have been asked to take part in a research study to determine the effects of physical activity on cardiovascular risk and endothelial function in individuals with elevated blood pressure or stage 1 hypertension.

A total of 24 participants will be recruited from the University of Texas at El Paso and its surroundings. They will be identified by a preliminary blood pressure screening and a health questionnaire. The inclusion criteria will consist on: (1) 18-40 years old, (2) Systolic Blood Pressure between 120-139 mmHg or Diastolic Blood Pressure between 80-89 mmHg obtained from 2 different days, (3) an estimated 10-year risk of CVD ≤10%, calculated by the ACC/AHA Pooled Cohort Equations, and (4) no current participation in 3 or more days per week of endurance or resistance exercise training. Exclusion criteria will include non-controlled cardiac, pulmonary, or metabolic diseases, smoking, consumption of nutritional supplements containing antioxidants, and any physical impairment to exercise. If you decide to enroll in this study, your involvement will last about 8 weeks. The first and last weeks, you will be asked to come to the

laboratory for testing 2 times per week for around 1 hour each time. The other 6 weeks, you will be asked to exercise three days per week (e.g. Monday, Wednesday, and Friday) for a maximum of 1 hour each time.

3. What is involved in the study?

If you volunteer to participate in this study, you will be asked to do the following things:
You will attend some testing and training sessions for 8 weeks to Room 454, located at the 4th floor of the Health Science Building of UTEP. During these visits, you will need to wear loose shorts or pants and short sleeve t-shirt.

During your first visit, you will discuss your participation with the investigator. Then, if you agree to participate, you will sign this informed consent form. Afterwards, you will answer some questions about you (like your date of birth) and your height and weight will be measured. Then, you will remain seated for 5 minutes followed by an assessment of blood pressure with an automatic cuff. Thereafter, you will be asked to *lay down on an examination table* for 10 minutes. Next, we will attach some electrodes on your chest and your baseline blood pressure will be taken. *After resting on the table for 15 minutes*, your blood pressure will be taken again. Then, a transducer holder, which is a plastic structure attached to the arm to keep the position of the ultrasound wand, will be attached to your arm and ultrasound pictures will be recorded of the large artery of your upper arm. A blood pressure cuff will be placed below your elbow and will be inflated at 200 mmHg; as it is inflated at the doctor office, for 15 - 20 seconds and then deflated. The Ultrasound pictures will be repeated immediately following deflation of the cuff for the next 3 minutes. Then, the same procedure will be repeated, but this time the ultrasound wand will be placed behind your knee and the cuff will be placed below the knee. Additionally, we will place a tonometer (pen-like device) on your neck to measure the velocity of propagation of the blood pressure wave. At the end of the first lab visit, *you will perform an exercise test* using a crank-arm ergometer that will determine their maximum oxygen uptake (VO_2max). For this last test, we will put a mouth piece connected via a long tube to a metabolic cart, and a nose-clip to prevent air leakage. Immediately before starting their exercise tests, your earlobe will be poked with a micro-lancet (similarly as blood glucose sampling from the finger) and the

baseline blood micro-sample will be draw with a micro-hematocrit capillary (i.e. very small glass straw). Additionally, you will be asked to comply with a low nitrate diet for 48 hours.

In a second visit, *21 ml of blood will be collected from your antecubital vein* followed by a body composition analysis through dual-energy X-ray absorptiometry (DXA), an echocardiographic assessment to determine cardiac dimensions and function, and you will complete a questionnaire of 36 questions related to quality of life. Then participants will be randomly assign to a 6-week intervention of physical activity and you will need to come 3 times per week for 6 weeks for the training sessions. Finally, all the measurements that were taken in the first two visits will be repeated in the same order after the intervention.

4. What are the risks and discomforts of the study?

Exercise stress tests have inherent risks involved. The risk is about 1 nonfatal event in 10,000 maximal cycle tests.[1] In fact, *the risk of dying during a supervised exercise test*, as the ones you will be performing, is lower than dying from being bitten by a Dog (i.e. 1 in ~100,000 sub-maximal exercise tests). Your risk will be kept as low as possible because all personnel involved are experienced with exercise testing, and emergency treatment is readily available. Your exercise test will be supervised by a licensed physical therapist. Shortness of breath or fatigue is common during testing. Muscle soreness may follow testing but is normal and should not interfere with other activities. Finally, there is a small chance of falling off the bicycle; however, at least two people will be overseeing your exercise test to prevent that from happening.

Your electric heart activity will be continuously monitored during exercise and we will stop the test if your heart shows any abnormal rhythm (e.g. ST segment depression, multiple premature ventricular contractions (PVCs), or atrial fibrillation). We will also stop the test if you have a sudden drop in blood pressure, chest pain, significant leg pain, or significant shortness of breath (dyspnea). All these signs can be related to a cardiac problem.

In the event that you suffer a decompensation of your heart condition (e.g. an acute cardiac event or myocardial infarction), the lab is equipped with an external automatic defibrillator, all personnel are CPR-certified, and we will immediately call 911. Any hospital expenses due to a decompensation will be billed to you or your insurance provider. You will be responsible for any deductible, co-insurance, or co-payments.

There is minimal risk associated with drawing blood from an earlobe. The risks include discomfort and possible bruising or swelling around the drawing site; rarely an infection; and, uncommonly, faintness from the procedure. This risk will be minimized by the use of sterile tips and alcohol pads. In addition, we ask you to put some ice on it when you get back home. If you request to stop the procedure because it is painful or highly uncomfortable, blood draws will be stopped immediately.

There is no risk when using ultrasound imaging techniques. Ultrasound does not produce any radiation and it is extremely safe. However, if you experience any discomfort for having your arm straight for 10-20 minutes, images acquisition will be stopped immediately.

DXA scanning radiation is considered minimal. For example, the total radiation of a 7-hour flight is 0.04 mSv and for a chest x ray is 0.04 mSv, meanwhile, for a single DXA evaluation the exposure is 0.002 mSV.[2]

There is a slight risk that information given by you could be revealed inappropriately or accidentally. However, every effort will be made to protect the information attained from subjects.

1. Fletcher GF, Ades PA, Kligfield P, Arena R, Balady GJ, Bittner VA, et al. Exercise standards for testing and training: a scientific statement from the American Heart Association. Circulation 2013; 128 (8):873-934.
2. Australian Radiation Protection and Nuclear Safety Agency. https://www.arpansa.gov.au/search/DEXA.

5. What will happen if I am injured in this study?

The University of Texas at El Paso and its affiliates do not offer to pay for or cover the cost of medical treatment for research related illness or injury. No funds have been set aside to pay or reimburse you in the event of such injury or illness. You will not give up any of your legal rights by signing this consent form. You should report any such injury to Dr. Alvaro Gurovich at (915) 747-7248 and to the UTEP Institutional Review Board (IRB) at (915) 747-7693 or irb.orsp@utep.edu.

6. Are there benefits to taking part in this study?

Physical activity has shown to be beneficial for cardiovascular health. Being involved in this study will improve your health. This research could improve the treatment and prevention of cardiovascular diseases, such as high blood pressure.

7. What other options are there?

You have the option not to take part in this study. There will be no penalties involved if you choose not to take part in this study.

8. What are my costs?

There are no direct costs. You will be responsible for travel to and from the research site and any other incidental expenses.

9. Will I be paid to participate in this study?

You will not be compensated for taking part in this research study.

10. What if I want to withdraw, or am asked to withdraw from this study?

Taking part in this study is voluntary. You have the right to choose not to take part in this study. If you do not take part in the study, there will be no penalty or loss of benefit.

If you choose to take part, you have the right to skip any questions or stop at any time. However, we encourage you to talk to a member of the research group so that they know why you are leaving the study. If there are any new findings during the study that may affect whether you want to continue to take part, you will be told about them.

The researcher may decide to stop your participation without your permission, if he or she thinks that being in the study may cause you harm.

11. Who do I call if I have questions or problems?

You may ask any questions you have now. If you have questions later, you may contact Dr. Alvaro Gurovich at (915) 747-7248, agurovich@utep.edu or Francisco Morales at (812) 917-8661, fmoralesac@miners.utep.edu.

If you have questions or concerns about your participation as a research subject, please contact the UTEP Institutional Review Board (IRB) at (915-747-7693) or irb.orsp@utep.edu.

12. What about confidentiality?

Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your permission or as required by

law. Confidentiality will be maintained through 3 methods: 1) coding, 2) data storage, and 3) confidentiality training.

1) Coding: after signing this informed consent, a study code will be assigned to your name. Your name and code will be together only on one form. All other forms will use only your code.

2) Data storage: this informed consent, the preliminary screening questionnaire and the form with your name and code will be kept in a locked cabinet on Dr. Gurovich office. Only Dr. Gurovich will have access to all study forms.

3) Training: all the researchers, including principal investigators and research assistants, are trained on health information privacy guidelines.

These measures will minimize the risk of any confidentiality break.

13. Authorization Statement

I have read each page of this paper about the study (or it was read to me). I know that being in this study is voluntary and I choose to be in this study. I know I can stop being in this study without penalty. I will get a copy of this consent form now and can get information on results of the study later if I wish.

Participant Name: _____ Date: _____

Participant Signature: _____ Time: _____

14. Voluntary Follow-Up

Can we contact you if there are other studies that you might qualify?

Yes No

Participant Signature: _____ Date: _____