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Study Protocol

Managing Pain: Testing the dosing and social aspects of exercise therapy using a multi-school collaborative approach with human participants.

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Protocol Summary Form

Managing Pain: Testing the dosing and social aspects of exercise therapy using a multi-school collaborative approach with human participants.

1. Statement of the research question

What is the effect of exercise dose and social interactions on the ability of exercise to reduce acute pain in healthy human participants?

2. Purpose and significance of the study

Chronic pain is a serious problem in the US that affects 116 million adults[1]. Chronic pain (e.g. fibromyalgia, chronic lower back pain) can be linked both epidemiologically and biologically with major depressive disorder (MDD) and various anxiety disorders. Rates of MDD, depressive, or anxiety symptoms in patients with chronic pain are very high (13-82%)[2,3]. As such, the treatment options for patients with pain AND behavioral abnormalities have focused on agents that target this comorbidity. One type of non-pharmacologic therapy that has shown promise in chronic pain is exercise. Regular physical activity and exercise improves many aspects of a person's general health, including cardiorespiratory function, mental health, and pain. Despite the purported benefits of exercise, 1 in 3 Americans are sedentary and do not meet the activity guidelines developed by the Surgeon General[4] (moderate intensity exercise, 30 minutes per day, 5 days per week). Sedentary behaviors are more prevalent in persons with chronic pain. Exercise therapy is an attractive therapy for chronic pain because of its low cost and ability to reduce sedentary behavior, depression, and pain[5,6,7]. One of the challenges at both the research and clinical level in implementing exercise as a therapy are the numerous variables inherent in prescribing exercise to patients with pain which include the best dose of exercise and social anxiety surrounding exercise. Walking has been emphasized because it is an easy exercise that most people can accomplish without special equipment or training. The 2008 Physical Activity Guidelines[4] suggest that even light physical activity is better than none for improving health. Especially for those persons with chronic pain, it may be most beneficial to begin a physical activity exercise protocol at a lower intensity and/or dose and increase physical activity as tolerated. Before a recommendation of low-dose or low-intensity exercise can be made, it is necessary to determine if physical activity at these lower levels will also provide the benefit of exercise-induced analgesia.

In this study we will examine the effect of dose related to exercise therapy for pain in healthy adult humans. We will be using an acute pain model to study the effect of exercise dose on pain. Acute pain models are currently used to study pain (both acute and chronic) in human samples as there is currently no chronic pain model.

We hypothesize that therapy done at a low dose will produce the best cost-to-risk ratio for patients.

3. Research design and procedures

This study is designed as a randomized controlled trial with repeated measures.

Aim – the effect of exercise dose on pain: subjects will be randomly assigned to one of four exercise groups: 1.) no exercise (control); 2.) low dose exercise (3x/week); 3.) moderate dose exercise (5x/week); 4.) high dose exercise (10x/week).

The co-primary investigators (co-PI's) will be involved in the procedures listed below. Graduate research students will assist with recruitment, data collection, and data processing. A PhD student in the Biology department (Anna Polaski) will assume primary responsibility for data collection. The graduate students will be trained on all procedures involved in this study by the co-PI's. Prior to data collection, the prospective subjects will complete a full screening, using a checklist of the inclusion/exclusion criteria and the PAR-Q and AHA health questionnaires (see Instruments) to determine eligibility. The purpose of the study, the methods of data collection and all potential risks will be verbally communicated to prospective subjects at the

initiation of the first data collection session (Day 0) (Figure 1). Prospective subjects will be given an approved informed consent form to read and sign. Subjects will then complete the International Physical Activity Questionnaire long form (IPAQ-long) to assess baseline level of daily physical activity and the Social Interaction Anxiety Scale (SIAS) for a measure of participant's anxiety. The researcher will explain the questionnaires to subjects and will be available to answer any questions while the subjects complete these forms. Basic health information obtained from the subject will assess whether the subject has been diagnosed with any cardiac, respiratory, neurological or musculoskeletal disorder, chronic pain or currently has acute pain. Subjects who report any of the above medical diagnoses or pain during screening will be excused from participation in the study, but will receive compensation for attending the screening session. Any identifying information used to determine eligibility, including the checklist of inclusion/exclusion criteria and the health questionnaires, will be destroyed.

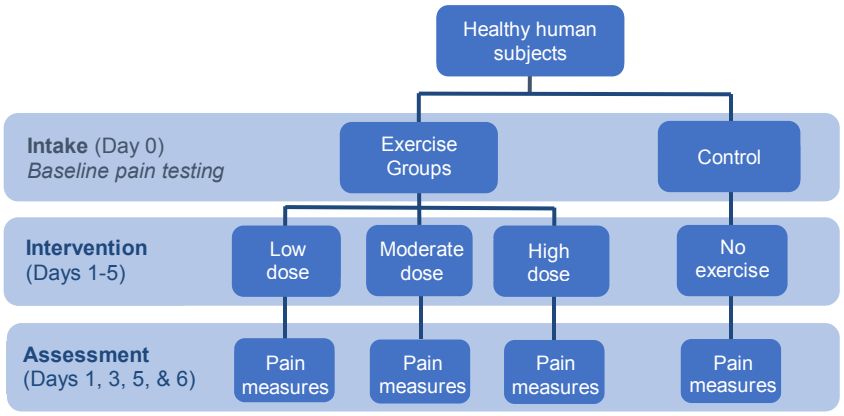

Figure 1: Study Design. Subjects will be randomly assigned to the control, low- dose exercise, or high-dose exercise group. Intervention will occur on Days 1-5 depending on group assignment. Assessment of physical and pain measures occurs on Days 1, 3, 5, and 6.

Subjects will then be set-up for training on the exercise protocol, physical pain measures and pedometer use. Subjects will become familiarized with the exercise protocol, including the physiological measures to be assessed [heart rate (HR)], the Borg Rate of Perceived Exertion (RPE) Scale, and the treadmill speed at which they will be walking (3.5mph). Typical walking speed to engage in moderate intensity physical activity (3-5.99 METs) is a brisk pace of 3-4 mph and anticipated activity heart rate will be 60% of age-adjusted maximal heart rate[8]. The subject will walk on the treadmill for 5 minutes to become comfortable with the exercise procedure. Subjects will be instructed to step up onto the treadmill and stand on the sides not the belt or belt deck. The researcher will slowly raise the speed to 2 mph. The subject will be instructed to step on the treadmill at this time. After 30 seconds, the speed will be ramped up to 2.5 mph and after another 30 seconds, the speed will be ramped up to their prescribed session speed. They will also be instructed to walk with a natural gait pattern, allowing bilateral arm swing. To allow a natural arm swing, subjects are unable to hold onto the treadmill arm rails. Subjects will be introduced to the Borg RPE scale and asked to provide subjective feedback on their level of exertion at the initiation of and during the final minute of this 5-minute training period. Subjects will be given a standard pedometer and instructed to wear it on their wrist during all hours (except showering/bathing) for the 6 consecutive days during the week in which they participate in data collection. The digital screen will be covered as awareness of daily step counts may influence participant's normal daily physical activity. Subjects will be given an instruction sheet for testing sessions and pedometer use.

During the enrollment period subjects will also be given a full description of all sensory and pain measures that will be taken. With initial consent, all subjects will be allowed to experience all sensory/pain measures before full enrollment. All sensory and pain assays are

based on well-established assays used in both healthy human participants and patients with chronic pain[9]. All assays involve either innocuous (non-painful stimuli) or acute noxious stimuli (painful stimuli) that do not damage tissue. Time between different tests will be >5 min to allow the subject to rest. Timing of testing on experimental days will be 5 minutes and 30 minutes following the completion of exercise or 30 minutes of rest (for control subjects and for all subjects on day 6). For all testing days order will proceed in the following manner. Training and description of each measure is as follows:

Cutaneous mechanical sensitivity assay[10]: Enrolled participants will be asked to sit in a chair (chair provided with arm-rests). Assay involves determining the sensitivity threshold for innocuous cutaneous stimulation. Stimulation is provided with standard sensory evaluator filaments (see equipment section below for image of filament). These are small nylon filaments each apply a single force (ranging from 0.008gram to 1.0gram). Before the start of the first experimental trial, subjects will be allowed to feel and manipulate the filaments. Starting with the smallest filament (0.008g; below the sensory threshold for human detection), 5 trials will be directed at the subject's forearm. During testing, subject is asked to look away from their forearm/calf. Experimenter will ask "Can you feel this" while the experimenter applies the filament to the subject's forearm or calf. With each filament, the experimenter will apply the filament 3 times in the "positive" trials. For the other 2 trials, the experimenter will not apply the filament but ask the subject, "Can you feel this?" These 2 "negative" trials will be randomly inserted with the 3 "positive" trials and are designed to test for false responses. If a subject detects ≥2 of the positive trials and 0 negative trials for a filament, that force will be the subject's "mechanical sensory threshold." For a single filament, if the subject detects <2 of the real trials and/or >0 of the false trials, the experiment will start another round of 5 trials with the next biggest filament until the sensory threshold is reached. Typical testing time for each body part (forearm and calf) is about 5 minutes.

Radiant heat sensitivity assay[11]: Assay involves determining the sensitivity threshold for painful thermal stimulation. Stimulation is provided with a radiant heat device[11]. This device uses a focused light beam to slowly heat a subject's skin. Before the start of the training/ experimental trials, subjects will be shown device and allowed to feel the stimulus with their hand. Subject will be asked to rest their forearm or calf on a room temperature glass plate. Using a mirror, the light source will be positioned under a marked area on the subject's forearm or calf. Subjects are told to raise their leg or arm when they feel the stimulus transition from "innocuous warmth or heat" to "painful heat." When the leg or arm is raised from the surface of the glass, the thermal stimulus automatically stops and the time since the beginning of the trial is recorded as "latency to respond." Each trial lasts for a maximum of 20 seconds. The device is set so that typical withdrawal thresholds occur at approximately 10 seconds into the trial. The temperature on the glass at this point is ~47°C (121°F). The maximum temperature of the trial at the 20 second cutoff time point is 50°C which is well below the temperature that causes tissue damage in humans. For each limb, two trials will be completed in two distinct marked areas to avoid re-testing at a single site. Total testing time (2 trials on forearm and 2 trials on calf) is <5 minutes.

A second assay will be used to evaluate the quality and unpleasantness of thermal pain. Each subject will receive a constant 45°C stimulus. 45°C is a standard temperature that is the typical minimal stimulus necessary to feel thermal pain. Stimulus (3cm x5cm heating block) will be applied for 3 seconds. Immediately following stimulus, the subject will be asked to evaluate the intensity and unpleasantness of the pain. This is done with a standard 0-10 visual analog scale (VAS). On the "intensity scale", "0" is represented as "no pain" and "10" is represented as "the worst pain imaginable." On the "unpleasantness scale", "0" is represented as "not unpleasant" and "10" is represented as "the most unpleasant sensation imaginable."

Example instructions for intensity VAS – *"Please use the scale below to tell us how intense your pain is. Place an "X" on the scale that best describes the intensity of your pain."*

Example instructions for unpleasantness VAS – *"Now that you have told us about the physical quality of the pain, we want you to tell us overall how unpleasant your pain is to you. Words used to describe very unpleasant pain include "miserable" and "intolerable." Remember, pain can have a low intensity, but still feel extremely unpleasant, and some kinds of pain can have a high intensity but be very tolerable. With this scale, please tell us how unpleasant your pain feels."*

Mechanical pressure sensitivity assays[10,12]: Assay involves determining the sensitivity threshold for painful pressure stimulation and then determining the quality and unpleasantness of that same pressure in a separate trial. Stimulation is provided with a standard clinical pressure algometer (see below instrument section). This device consists of a 0.5-2cm probe connected to a pressure meter. The probe is placed on the subject's forearm or calf and pressure is gradually applied. Before the first training/experimental trial subjects will be allowed to apply the stimulus to themselves. During a trial, pressure will gradually be applied until the stimulus transitions from "innocuous pressure" to "painful pressure." At this point, subject will say "stop" and the stimulus will be removed from the subject's forearm/calf. When algometer is removed from the subject, the greatest pressure applied is automatically recorded in the device. This is the "pressure pain threshold" for the trial. 2 trials will be applied each to the forearm and calf in two distinct sites (on each limb) to avoid damage to a single area. After the pressure threshold has been determined for a subject, 1 additional trial on each limb will be applied (in a third testing site). In this trial, the subject will be asked to evaluate the intensity and unpleasantness of a pressure stimulus given for 3 seconds. This is done with a standard 0-10 visual analog scale (VAS). On the "intensity scale", "0" is represented as "no pain" and "10" is represented as "the worst pain imaginable." On the "unpleasantness scale", "0" is represented as "not unpleasant" and "10" is represented as "the most unpleasant sensation imaginable." During this trial, the experimenter will tell the subject that they are going to apply a painful stimulus to the subject and then ask the subject to evaluate that pain (on the two VASs). The exact stimulus will be matched to the subject's pain threshold determined during the baseline trials (e.g. if baseline trials for forearm indicated a pressure threshold of 50 N then subject will be asked to evaluate the pain of that stimulus). During subsequent testing days (days 1, 3, 5, 6), the pressure pain threshold will be re-measured to determine if there is a change from baseline. For the intensity/unpleasantness testing, subjects will be tested on experimental days 1, 3, 5, 6 at their original baseline pressure threshold (from day 0) AND the threshold determined on each individual day. This will allow an analysis of change from baseline and pain at threshold for each experimental day.

Procedures: Following the screening and training session, subjects will be randomized to one of four conditions (Control, Low Frequency of Exercise, Moderate Frequency of Exercise, and High Frequency of Exercise) and scheduled for their exercise and data collection sessions (Figure 1). Subjects in the control group will be scheduled for three experimental sessions within a five-day period: Days 1, 3, and 5. Control subjects will complete all of the physical and sensory/pain tests following a 30-minute period of rest (no physical activity) on each of these days. These subjects will be set up in a private room with various reading materials during this time period. Subjects in the low frequency of exercise group will be scheduled for three exercise sessions within a five-day period: Days 1, 3, and 5. Subjects in the moderate frequency exercise group will be scheduled for five exercise sessions within a five-day period: Days 1-5. Subjects in the high frequency exercise group will be scheduled for ten exercise sessions within a five-day period: Days 1-5. For all subjects, physical and sensory/pain measures will be taken on exercise days 1, 3, and 5, 5 and 30 minutes after exercise, and also on Day 6 (no exercise).

Exercise Session

Subjects will arrive at the Health Science Exercise Physiology Lab on their scheduled exercise days. Sessions will be scheduled at the same approximate time each day to control for factors that may influence exercise performance. At the initiation of each exercise session, HR measures will be taken. At the initiation of the session on days 1, 3, and 5, pain measurement will be made using the 3 assays described in detail above. For each assay, pain will be individually defined on a 0-10 visual analog scale (VAS), a common scale used in human pain testing[13]. Each assay is safe and produces only mild escapable acute (<1 min) pain. Pain measurements will be completed on both the calf and volar non-dominant forearm for each subject. On days 2 and 4 for subjects in the moderate frequency and high frequency of exercise group, sensory and pain measures are not collected.

The heart rate monitor will be strapped to the subjects' chest to monitor HR throughout the exercise session. The purpose of monitoring HR is two-fold: 1.) to ensure the subject is working at a moderate level of intensity, and 2.) to ensure the subject is safely participating in physical activity with no abnormal or adverse changes in HR. Subjects will provide a rating of their perceived exertion on the Borg RPE scale before beginning the exercise task. Subjects will then be set-up to begin walking on the treadmill. Subjects will be reminded of the instructions for how to step onto the treadmill and the process for ramping up the speed as described in the training session. Once walking on the treadmill at the prescribed speed, subjects will be instructed to walk until the researcher returns (30 minutes). Subjects will be told that they can stop walking if they become excessively fatigued (≥17 on the Borg RPE scale) or experience symptoms of overexertion, such as lightheadedness and excessive sweating. The Borg RPE scale will be positioned in front of them for a visual reminder of the fatigue scale and the treadmill safety key will be secured to the subject's shorts. After 30 minutes, the researcher will return to the room. At this time, perceived exertion on the Borg RPE scale will be assessed again. The researcher will then slow the treadmill speed down to approximately 2 mph for a 2-minute cool-down. Pain measures will be completed as described above following completion of the treadmill walking at 5 minutes and 30 minutes.

Subjects will perform their exercise session alone, with no close supervision by study staff. To ensure safety during exercise, the subject will be monitored with 2 wireless video cameras. Camera 1 will be focused on the subject to visually observe the subject's performance and ensure there are no visual signs that the subject is over-exerted. Camera 2 will be focused on the treadmill interface to monitor real-time HR and ensure the subject does not adjust the speed. Video recordings from the cameras will not be saved and is only used to monitor the subject's physical response to exercise. Subjects will be unaware that they are being monitored via camera. All subjects will return on Day 6 to complete a final round of pain/sensory testing.

4. Instruments

Screening Questionnaires

PARQ and You will be used to determine if a participant is not healthy enough to participate in the study due to a presence of a heart conditions, chest pain, dizziness, joint problems, and drug use.

AHA/ACSM Health Fitness Facility Preparticipation Screening Questionnaire collects past medical history, current health issues, and cardiovascular risk factors that would indicate exclusion from participation.

Physical performance

For all conditions, we will evaluate daily normal physical activity and intensity with a pedometer.

The International Physical Activity Questionnaire long version[14] (IPAQ-long) will be used to obtain estimates of weekly physical activity. Based on subjects' responses, the total energy

expenditure for participation in different physical activities can be calculated. The total energy expenditure is used to categorize subjects based on the amount of vigorous, moderate, or low intensity activity they engage in.

The Borg RPE scale[15] is used to collect a subjective measure of fatigue. The Borg RPE scale ranges from 6 (no exertion at all) to 20 (maximal exertion). This scale is based on expected heart rate during minimal, moderate, and vigorous intensity exercise. A subject who rates their exertion level as a 12 on this scale is working at a moderate intensity and should have a heart rate of 120 beats per minute (12 x 10).

All exercise sessions will be monitored using two wireless cameras (Dropcam Wireless Video Camera) to avoid influence of social observation while continuing to monitor subject's performance and physiological measures (HR) of exercise. Video will be transmitted via a secure Wi-Fi signal to a university-owned laptop computer. Either a PI or graduate student will view the real-time video to ensure subject's safety and continued optimal performance. Video will be transmitted via secure wireless signal to ensure confidentiality.

Pain measures –As described above, the three tests are for tactile sensitivity, thermal pain, and pressure pain.

Cutaneous Stimulators –

For cutaneous mechanical sensation, we will use standard von Frey filaments (Touch Test Sensory Evaluators; North Coast Medical). These filaments (ranging from 0.008g to 1.0g) are used to measure threshold for sensation to innocuous stimulation. See above for full description of assay.

Radiant Heat –

For radiant thermal heat measurements, we will use a standard "Hargreaves" apparatus (IITC Thermal Stimulus Apparatus) (see image to right).

Pressure Algometer –

For pressure pain testing, we will use a standard mechanical pressure algometer (Wagner Instruments).

Anxiety measures

All groups will be assessed for anxiety with questionnaire methods using the Social Interaction Anxiety Scale (SIAS)[16]. The SIAS measures anxiety with respect to social interactions. It is one of the more often-used self-report measures of social anxiety, with numerous evidence-based studies demonstrating its reliability and construct validity[17]. The SIAS contains 20 items which are rated from 0 (not at all characteristic or true of participant) to 4 (extremely characteristic or true of participant). Items are self-statements describing one's typical cognitive, affective, or behavioral reaction to a variety of situations requiring social interaction in dyads or groups (e.g., going to a party, talking to an attractive member of the opposite gender, expressing one's feelings).

5. Sample selection and size

Healthy adults will be recruited for participation in this study. The PAR-Q and You form and the AHA screening questionnaire will be administered during the screening process to determine whether potential subjects meet the inclusion/exclusion criteria.

Inclusion criteria include:

- Between age 18-40
- Normal BMI (18.5-25)
- HR 60 to 100
- BP less than or equal to 140/90
  *HR and BP will be taken as part of the screening process to determine eligibility and also taken at the beginning of each exercise session.

Exclusion criteria include as indicated by the PAR-Q and You and AHA screening forms:

- Age less than 18 or greater than 40 years
- Cardiac, respiratory, neurological or musculoskeletal disease
- Acute pain
- Chronic pain condition
- Diabetes
- BMI ≥ 25.1 or ≤ 18.4
- Regular participation in high intensity athletic/sporting activities
- Sedentary
- Anxiety or depression disorders.
- Tape allergy
- Currently pregnant

A power analysis of pain outcome indicates that a minimum of 10 people/condition will be sufficient to detect statistical differences in our primary dependent variable (pain sensitivity), but to account for variability, our recruitment goal is 15 people/condition. When 10 participants per group have completed the study, a preliminary analysis will be conducted for statistical power, if we reach 0.80, recruitment will stop.

6. Recruitment of subjects

Participants will be recruited using flyers and announcements in class. Students, staff, and faculty from Duquesne University will be invited to participate in this study. All recruitment efforts are expected to take place on the campus of Duquesne University. Student recruitment will include announcements made at the beginning of class time. After securing approval of a course's instructor, a general announcement describing the study will be made to students in the class. The purpose, procedures, and inclusion/exclusion criteria will be briefly described. A hand-out further detailing the study with the PI's contact information will be given. Students will be asked to contact the PI for a more detailed explanation if interested in participating in this study. A flyer detailing the study and with the PI's contact information will be displayed within the Health Sciences Building and other campus buildings in student areas and faculty/staff areas. These recruitment methods will maintain confidentiality of potential subject information and limit coercion.

When prospective subjects contact the PI, the PI will conduct a brief eligibility screening then invite eligible subjects to enroll. Prior to participation, the subject's BP and HR will be taken as well. Should a potential participant be disqualified, screening forms, the checklist, and any identifying information will be destroyed. Recruitment and enrollment will be conducted without regard for gender, race or ethnic background. Participation in this study will not affect a student's standing in any course, their department or school, and staff or faculty's relationships within their school or university.

7. Informed consent procedures

At the initiation of the data collection session, the PI will explain the purpose of the study, the methods of data collection and all potential risks associated with participation to prospective subjects. Prospective subjects will then be given an approved informed consent form to read. If

they agree to participate, they will be asked to sign two copies of the form; one copy to be retained by the PI and the second copy for the subject's records. Subjects will be made aware during the consent process that they may withdraw from participation at any time during their enrollment with no repercussions.

8. Collection of data and method of data analysis

The collection of data has been described in detail under "Procedures". The primary outcome is a change in Quantitative Sensory Testing. This includes a series (8) of Quantitative Sensory Tests measured at baseline and at the completion of the 1-week intervention period. These tests are measured at two sites (calf and forearm). Significant differences between groups for 7 of the tests (per body site) will be identified using a one-way ANOVA comparing percent change from baseline (post-test (i.e. day 6)/baseline test (i.e. day 0)). Given the number of statistical tests that will be required for the primary outcome measurements, $p<0.01$ will be utilized for each body site. An additional test for mechanical sensitivity will be measured at each site using a rank sum test at $p<0.05$.

The secondary outcomes for this study include (1) HR during exercise, (2) Borg RPE before and after exercise and (3) testing "acute" effects of exercise on QST testing. HR during exercise and Borg RPE are measured to make sure that exercise causes a change in HR and/or perceived exertion. HR and Borg RPE data will be analyzed using percent change from start of exercise (post-exercise/pre-exercise) with two-way ANOVA for 3 treatment groups (3-day, 5-day, 10 day) across time (day 1, 3, 5) with an adjusted p value of $p<0.03$. Control group will not be included in that analysis as HR was not measured on the control days and perceived exertion is at "0" on all days. For acute effects of exercise, participants were given 8 QST tests (on each forearm and calf) 5 min and 30 min after each exercise (or control session) on days 1, 3, and 5. Significant differences between groups for these tests (per body site) will be identified using a two-way MANOVA comparing percent change from baseline (post-exercise/baseline test (i.e. day 0)) across the three days (day 1, day 3, day 5) and at the 5 min and 30 min time QST measuring point. Given the number of statistical tests ($n=8$) that will be required for the QST secondary outcome measurements, $p<0.01$ will be utilized for each body site.

The following demographic variables will be collected and compared between groups to further check against potential bias; age, handedness, body mass index (BMI), baseline heart rate (HR), baseline blood pressure (BP), baseline IPAQ-short, and STAI (state-trait anxiety). A significant difference in the proportion handedness will be tested using the Chi-Square test where a $p<0.05$ will be considered significant. All other continuous variables will be tested using the one-way ANOVA to test for significance differences between the four study groups ($p<0.05$).

9. Dissemination of Results

Results of this study will be submitted to a peer-reviewed journal for publication and will be available to the public.

10. Emphasize issues relating to interactions with subjects and subjects' rights

Subjects will be notified of their rights, all risks and benefits of participation in this study during the informed consent procedure. Subjects will be informed that they may withdraw from participation at any time. Data collection sessions will be scheduled at times that are mutually convenient for the subject and the researchers and all efforts will be made to work with the subject's schedule. All personal and health information will be kept confidential as data will be coded. Identifiers will be stored in a locked filing cabinet and all coded data will be stored in either a separate locked filing cabinet or a password protected computer. Only researchers and staff directly involved on this study will have access to this information. Health information obtained for this study will come directly from the participant and not their healthcare providers

or medical records. This information will be obtained during screening occurring in the Rangos School of Health Sciences exercise physiology lab and therefore is not under HIPAA.

Subjects will be instructed that they are able to stop the exercise and pain measurements at any time due if they experience excessive discomfort/pain. Treadmill exercise will be monitored visually by a researcher in the adjacent room using a wireless camera system. Physiological measures, including heart rate, will also be monitored in real-time to ensure no issues during exercise. Subjects will be encouraged to stop exercise if they experience any discomfort or symptoms of over-exertion (≥ 17 on the Borg RPE scale). These symptoms will be explained during the informed consent process. There is a risk of falling off the treadmill. Subjects will be instructed to hold onto the rails if they feel like they are losing their balance or are going to fall. The safety key will be on the waist of their pants to turn off the treadmill if they move too far back on the belt. Also, in case they do fall off the machine, padded mats will surround the treadmill to minimize risk of injury. Because individuals recruited for this study will be young and healthy, the risk of falling is minimal.

Risk of injury related to cutaneous mechanical testing is extremely rare and unlikely. Mechanical testing is widely used and safe. Risks to the individual are minimal, because 1) this is not a painful/noxious stimulus; 2) subjects are instructed that they may stop any procedure at any time with no adverse consequences; and 3) the level of sensation experienced by subjects is well below their tolerance level and threshold for pain.

Risk of injury related to thermal pain testing is minimal. Thermal testing is widely used and safe. While thermal testing does produce pain, risks to the individual are minimal, because 1) the pain is transient in nature and generally subsides immediately after the procedure; 2) subjects are instructed that they may stop any procedure at any time with no adverse consequences; and 3) the level of pain experienced by subjects is below their tolerance level. With thermal stimulation there is a very slight risk of a burn, but this is minimized by the following: 1) positive lockout of stimulus parameters above 50°C; 2) the stimulator has built in a shut-down system to prevent the delivery of prolonged or high intensity stimuli (20 sec); 3) and before each use, temperature at glass surface will be verified with an electronic thermometer. Trials will proceed only if temperature detected at 20 sec cutoff is $\leq$ 50°C. Moreover, risk is further mitigated by limiting enrollment to healthy young individuals. Colleagues who have used this pain paradigm inform us that they have completed thermal testing with this stimulator on over 1000 subjects without producing a single burn. However, skin will be monitored for changes consistent with burns, such as redness that persists greater than one day and blistering. If a burn occurs, standard medical treatment will be recommended.

Risk of injury related to pressure pain testing is minimal. Pressure testing is widely used and safe. While pressure testing does produce pain, risks to the individual are minimal, because 1) the pain is transient in nature and generally subsides immediately after the procedure; 2) subjects are instructed that they may stop any procedure at any time with no adverse consequences; 3) the level of pain experienced by subjects is below their tolerance level; and 4) the pain applied is never more than that subjects pain threshold, which is well below any pressure that could cause damage. Moreover, risk is further mitigated by limiting enrollment to healthy individuals.

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