Leg thermotherapy for intermittent claudication

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1.0 Background

Peripheral arterial disease (PAD) is characterized by atherosclerotic obstruction of the arteries in the lower extremities and affects approximately 10% of individuals older than 65 years. The most common clinical presentation of PAD is intermittent claudication (IC), defined as leg pain caused by insufficient blood flow during walking. Individuals with IC have severe exercise intolerance and markedly reduced levels of daily ambulatory activity. It is estimated that up to 40 million people worldwide suffer from IC. Despite the increasing prevalence of this condition, few medical therapies improve mobility and exercise tolerance in these patients. Cilostazol is the only effective medication available to treat IC. Unfortunately, this drug has multiple side effects, and the overall improvement in walking performance after long-term treatment is small. Supervised treadmill exercise is the best single treatment for IC, but the need for frequent visits to a clinical facility over an extended time period makes this option challenging for the vast majority of patients. Endovascular and surgical interventions are also effective alternatives for patients that do not respond sufficiently to medical therapy; however, these procedures are costly, only applicable to patients with certain lesion types and are associated with a high risk of restenosis. An urgent need remains for the development of novel, non-invasive strategies that are more widely accessible and eliminate the need for supervision and frequent traveling to a clinical facility.

2.0 Rationale and Specific Aims

We propose to evaluate the potential of leg thermotherapy (TT) as a non-pharmacological intervention that could improve the mobility and exercise tolerance of patients with IC. Thermotherapy (TT) is a simple, easily applicable therapy that enhances exercise tolerance in patients with chronic heart failure (CHF) by improving peripheral vascular endothelial function. Indeed, recent studies of both healthy individuals and patients with CHF have demonstrated that TT promotes a blood flow-dependent improvement in vascular endothelial function in the microcirculation and in conduit arteries. In a preclinical model of IC, TT accelerates blood flow recovery and increases vascularization in the ischemic muscles. This compelling evidence predicts that TT could have significant benefits for patients with IC. Indeed, since this intervention is non-pharmacological, there is high potential for this work to rapidly translate into clinical practice and improve patients' lives. In preliminary studies (*IRB Protocol #1409063619)*, we have demonstrated application of TT for 90 min increased leg skin temperature by ~7ºC, core body temperature by ~0.6°C and evoked a marked reduction in systolic (~12 mmHg) and diastolic (~6 mmHg) blood pressure when compared to the control intervention (Figure 1). In addition, leg TT application induced a marked increase in leg blood flow in these patients. Based on these compelling preliminary findings, our goal is to evaluate the impact of 6 weeks (3 sessions/week) of TT treatment on vascular function and walking tolerance in patients with IC. *We hypothesize that treatment with TT will enhance walking tolerance in patients with IC by improving endothelial function and therefore increasing leg oxygenation during exercise*. Thermotherapy will be applied using water-circulating 'pants' connected to a water pump. These garments are made of a tight-fitting elastic fabric, with an extensive network of medical grade polyvinyl chloride

tubing sewn onto the fabric, and a portable pump that circulates hot water throughout the garment. This is the same system we employed in our initial study in 16 patients with IC (*IRB Protocol #1409063619*). We have shown that this system is safe and well tolerated by patients with IC. Before and after the treatment, we will examine the following outcomes: 1) cutaneous microvascular function (Laser Doppler flowmetry), 2) reactive hyperemia in the popliteal artery (phase-contrast magnetic resonance imaging of the popliteal artery, 3) calf muscle oxygenation during exercise (near-infrared spectroscopy) and 4) walking distance (6-min walking test).

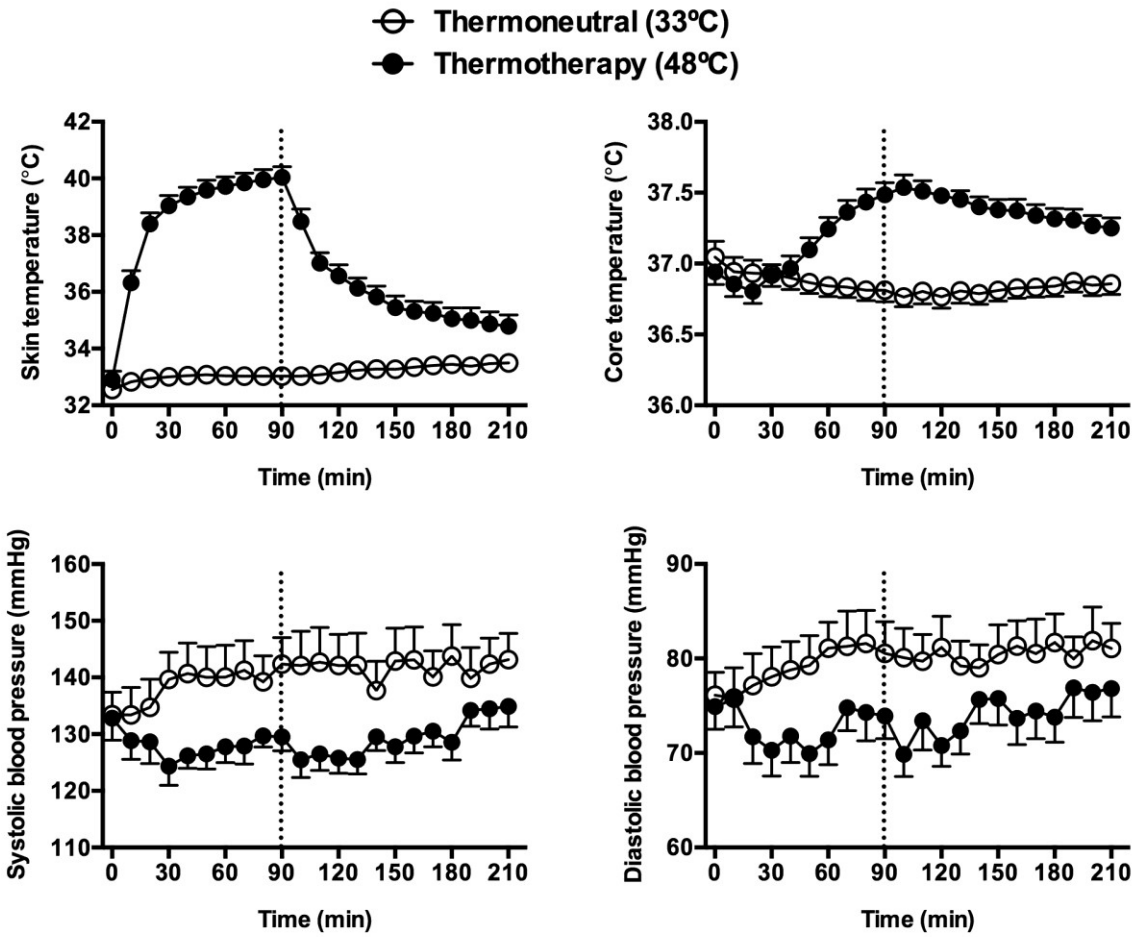

Figure 1: Physiological responses to acute thermotherapy application in patients with intermittent claudication (n=16, IRB# *1409063619).* Patients underwent a single 90-min session of TT or a control treatment and physiological variables were measured before, during and for 2 hours after the intervention.

3.0 Inclusion/Exclusion Criteria

Inclusion criteria

- Men and women with a stable symptomatic claudication for ≥6 months
- Ankle brachial index <0.9

Exclusion criteria

- Uncontrolled Diabetes (HbA1C > 8.5 measured within 3 months prior to date of consent)
- Heart Failure
- COPD
- Critical limb ischemia (ischemic rest pain or ischemia-related non healing wounds or tissue loss
- Prior amputation
- Exercise-limiting comorbidity (i.e., angina, chronic lung disease, or arthritis)
- Recent (<3 months) infrainguinal revascularization (surgery or endovascular revascularization) or revascularization planned during study period.
- Plans to change medical therapy during the duration of the study
- Active cancer
- Chronic kidney disease (eGFR <30 by MDRD or Mayo or Cockcroft-Gault formula).
- HIV positive, active HBV or HCV disease.
- Presence of any clinical condition that in the opinion of the principal investigator makes the patient not suitable to participate in the trial.
- Peripheral neuropathy, numbness, or paresthesia in the legs.
- Morbid obesity BMI > 36 or unable to fit into water-circulating pants.
- Open wounds or ulcers on the extremity.

As this study involves MR imaging, patients that have contraindications to MRI will be included in the study but will not be allowed to participate in the MRI procedure. Information about biomedical devices that may pose a risk to patients undergoing MRI is available on the Internet at www.MRIsafety.com. These exclusions include:

- Cardiac pacemaker
- Implanted cardiac defibrillator
- Aneurysm clips
- Carotid artery vascular clamp
- Neurostimulator
- Insulin or infusion pump
- Implanted drug infusion device
- Bone growth/fusion stimulator
- Cochlear, otologic, or ear implant
- History of claustrophobia or who are unable to lie flat or who do not fit inside the bore of the scanner

4.0 Enrollment/Randomization

Patients that fulfill the inclusion criteria and are interested in participating in the protocol will receive detailed instructions about the experimental set-up and

measurements and will be given a consent form. Once deemed eligible, patients will be asked to complete the baseline assessment. After the initial testing is complete, patients will be randomly allocated into one of two groups: low-heat therapy (control) and high-heat therapy. Medical devices and alternative therapies, such massage and thermal-treatments have shown to be associated with high placebo responses (1). In order to control for this important psychological response, it is imperative to incorporate an appropriate placebo/sham intervention (1). In the present study, we will adopt strategies to minimize the potential confounding effect of psychological and perceptual responses to heat therapy. First, participants will be informed that the purpose of the study is to compare two 'intensities' of heat treatment. In the low-heat group, water at 33ºC will be circulated through the garment during the treatment sessions. In our initial study (*IRB Protocol #1409063619*) we observed that this strategy evokes a small increase (~0.9ºC) in leg skin temperature. In the high-heat group, hot water will be circulated through the garment during the treatment sessions with the goal of increasing skin temperature to 40ºC, as observed in our initial study. A random allocation software (Research Randomizer, Social Psychology Network, http://www.randomizer.org/) will be used to perform the randomization.

5.0 Study Procedures

After enrollment, subjects will be asked to complete two experimental sessions for baseline testing. The procedures involved in each visit, location and estimated duration are listed on Table 1. On **visit 1**, demographic and anthropometric characteristics from participants will be initially recorded. Subjects will then be asked about comorbid conditions, claudication history, and current medications and will fill out two questionnaires to assess generic (36-item Short-Form Health Survey) and disease-specific (Walking Impairment Questionnaire) quality of life. Next, subjects will be escorted to the examination room and will be asked to rest quietly for 15 min in the supine position. During this time, four NIRS (near infrared spectroscopy) sensors (Figure 2) will be attached to subjects' two fingertips and two toes (left and right). These sensors are the same as the pulse oximeters used regularly in the hospital. We will monitor the blood flow during these 15 minutes while subjects are resting. After this rest period, systolic blood pressure will be measured in the arms (brachial artery) and ankles (posterior tibial and dorsalis pedis arteries) for the determination of the ankle brachial index. Subjects will then be familiarized with the 6-min walking test. A near-infrared spectroscopy (NIRS) system will be placed on the calf of the most symptomatic leg for monitoring leg oxygenation during the test. This exercise test consists of walking back and forth along a 100-ft corridor for 6 min. This session will be performed at the CTSI's Clinical Research Center on the 5th floor of Goodman Hall and is expected to last approximately 2 hours.

Figure 2: Multichannel NIRS used to monitor the blood flow at two toes and two fingertips.

Visit 2 will involve the assessment of the primary study outcomes, including vascular function and exercise tolerance. This session is expected to last approximately 4 hrs. Subjects will be asked to fast overnight prior to attending this session but will be permitted to take their usual morning medication regimen. The first exam will be the assessment of leg blood flow responses to circulatory occlusion (reactive hyperemia) using magnetic resonance imaging. This test will be performed at the Imaging Research Facilities in the basement of Goodman Hall. Next, subjects will be escorted to the CTSI's Clinical Research Center on the 5th floor. Subjects will initially be asked to lay supine and a nurse will place a catheter in one antecubital vein in the arm for venous blood sampling. After 10 min of rest, systolic blood pressures in the arms and in the ankles will be measured for the determination of the ankle-brachial index. Once the measurements are complete, a blood sample (20 ml) will be taken and the catheter will be removed. Next, subjects will be instrumented with laser Doppler probes placed inside local heaters for the assessment of cutaneous vascular reactivity. Two probes will be placed on the front portion of the most symptomatic leg and secured in place with tape. The sites where the probes are placed will be marked with a pen to allow for consistent placement in the second and third outcome measurement visits. The protocol consists of heating a site in the skin and measuring the changes in cutaneous red blood cell flux in response to the thermal challenge. Blood pressure and heart rate will be monitored during the test. This procedure will last approximately one hour. Next, the patients will perform the 6-min walking test. A near-infrared spectroscopy system, which measures skeletal muscle oxygenation, will be placed on the calf of the most symptomatic leg, secured in place with tape and covered with aluminum foil and a black Velcro strap. Similar to the laser Doppler probes, pen marks will be used identify the location of the probe. Subjects will be asked to walk back and forth along a 100-ft corridor for 6 min and report when the pain first appears. After the test is completed, subjects will be asked to grade the severity of the pain in the most symptomatic leg using the Borg-scale.

Visit #	Procedures	Location	Duration
1	1) Medical History and Physical Examination 2) Quality of life assessment 3) Measurement of ankle-brachial index 4) Familiarization with the 6-min walking test	CTSI's Clinical Research Center at Goodman Hall	2 hrs
2	1) Leg MRI (reactive hyperemia) 2) Catheter placement and venous blood draw 3) Measurement of ankle-brachial index 4) Assessment of cutaneous vascular function 5) 6-min walking test	Imaging Research Facilities and CTSI's Clinical Research Center at Goodman Hall	4 hrs

Table 1: Procedures involved, location and estimated duration of visits 1 and 2.

After the baseline assessment is complete, subjects will be randomly allocated to one of two groups using a random allocation software. Participants will be asked to

attend ***3 treatments sessions/week for 6 consecutive weeks***. These sessions will be conducted at the CTSI's Clinical Research Center at Goodman Hall and will last approximately 110 min. A research coordinator will supervise the patients during the sessions. On each session, subjects will initially have their body weight and blood pressure measured and recorded. Next, patients will be asked to change into the water-circulating pants (Figure 3) and lay in semi-recumbent position. The garments will be connected to the water pump and the treatment will be applied for 90 min. In the high-heat group, hot water will be circulated through the garment with the goal of increasing skin temperature to 40ºC, while thermoneutral water (33ºC) will be used in the low-heat group. Subjects will be allowed to drink water ad libitum during the sessions but will not be allowed to consume food. At the end of the session, body weight and blood pressure will once again be recorded. The research coordinator will measure temperature (sublingual measurements) and blood pressure every 15 minutes during the treatment and will also inspect the skin in the legs to look for potential signs of injury or adverse reactions to the treatment. The subject will be asked to complete a heat sensation scale every 15 minutes during the treatment. The research coordinator will clean the garment after each use using a hospital-grade disinfectant spray. The garment will also be washed in a washing machine every week.

Figure 3: Water-circulating garment system for thermotherapy application

Outcome measurements will be repeated after 9 sessions (3 weeks) and at the end of the treatment period (18 sessions). The protocol for these visits will be similar to visit 2, with the addition of quality of life assessment. The procedures involved are detailed on table 2. This visit is expected to last approximately 4.5 hrs. Subjects will be asked to fast overnight prior to attending this session but will be permitted to take their usual morning medication regimen.

Visit #	Procedures	Location	Duration
3 and 4	1) Leg MRI (reactive hyperemia) 2) Catheter placement and venous blood draw 3) Measurement of ankle-brachial index 4) Quality of life assessment 5) Assessment of cutaneous vascular function 6) 6-min walking test	Imaging Research Facilities and CTSI's Clinical Research Center at Goodman Hall	4.5 hrs

Table 2: Procedures involved, location and estimated duration of visits 3 and 4.

Outcome Measures:

Reactive Hyperemia in the Popliteal Artery: Blood flow in the popliteal artery of the most symptomatic leg will be measured using MRI. We employed this technique in our initial study (*IRB Protocol #1409063619*). After the knee coil and the occlusion cuff are positioned on the most symptomatic leg, participants will be allowed to rest quietly for 15 min. Resting leg blood flow measurements and brachial systolic blood pressures will be completed every 5 min during this period. Next, a blood pressure cuff firmly wrapped around the lower thigh will be inflated to 75 mmHg above systolic blood pressure, or 250 mmHg, whichever is lower, for 5 min (8). Hyperemic flow will be measured for 10 min after cuff deflation. Flow reserve will be defined as the absolute difference between maximum hyperemic flow after cuff release and resting flow (9).

Cutaneous Microvascular Function: Laser-Doppler flowmetry probes, seated in the center of local skin heaters (Moor Instruments, UK), will be used to measure red blood cell flux, an index of skin blood flow (SkBF). Baseline SkBF will be recorded for 10 min after which local skin temperature will be increased to 39°C (0.1°C/s) and maintained at this level until a plateau is achieved (~40 min). Next, the local skin temperature will be elevated to 43°C, to induce maximal vasodilation. Cutaneous vascular conductance (CVC) will be calculated as laser-Doppler flux divided by mean arterial pressure (MAP), measured throughout the study via brachial artery oscillation, and normalized to maximal vasodilation (%CVC_{max}) (10, 11).

6-min Walking Test.: Participants will receive standardized instructions and will be asked to walk the greatest distance possible by walking back and forth along a 100-ft corridor for 6 min (4).

Measurement of Muscle Oxygenation: The microvascular oxygenation status of the gastrocnemius muscle of the most symptomatic leg will be monitored with near-infrared spectroscopy (NIRS), as described recently by our group (7). The optode holder of a portable NIRS system (The PortaMon Mk II, Artinis Medical Systems, Netherlands) will be placed on the medial gastrocnemius, and the concentration of deoxygenated hemoglobin + myoglobin [deoxy-Hb+Mb] will be measured continuously during the 6-min walking test (7).

Quality of life assessment: The 36-item Short-Form Health Survey domain scores for physical functioning, physical role function (i.e., limitations due to physical problems), bodily pain, and general health perceptions on a scale from 0 (severe limitation) to 100 (no limitation). In addition, self-reported ambulatory ability will be obtained with a validated questionnaire for PAD patients that assesses ability to walk at various speeds and distances and to climb stairs.

Potential risks to subjects:

6-minute walking test: Exercise-induced pain is a hallmark feature of intermittent claudication and therefore exercise tests are used as a diagnostic tool as well as well for the assessment of claudication severity and functional status of the patient (4). Typically patients experience ischemic pain in the foot, calf, thigh or buttocks during the test. The

pain is temporary and subsides within a couple of minutes after the test is terminated (4). The 6-minute walk test has been validated as an outcome measure and is increasingly recognized as a meaningful outcome measure in patients with PAD (4). The tests will be performed according to available guidelines from the American Thoracic Society (3). When performed following these guidelines, the 6-minute walking test is reported to be very safe and reflect walking in the daily life of the patients (4). However, there is a chance that patients may experience: 1) chest pain, 2) intolerable dyspnea, 3) leg cramps, 4) staggering, 5) diaphoresis, and 6) dizziness. The investigators will monitor the patients closely to test. Patients will be asked to stop walking and sit on a chair if any of these symptoms occur.

Leg heating: Hot water (47-50ºC) will be perfused through the water-circulating garment with goal to increase skin temperature in the calf to 40ºC. It is possible that the tubes in contact with the skin could cause discomfort due to a sensation of warmth/heat. Should the tubing that is in contact with the skin cause discomfort we will readjust the suit or turn down the temperature of the water. Ninety minutes of heating will likely lead to skin redness and flushing, which are typical responses to local heating. These effects are temporary and will fade once the heating stimulus is terminated. Once instance of skin being burned to the point of blistering has occurred. The research coordinator will examine the patient's skin during each treatment session for potential signs of injury or adverse reactions to the treatment.

A sophisticated, research-grade heated bath circulator will be used to circulate water through the garment. The actual device we use (SC100) and its technical specifications are displayed in the document entitled "SAHARA S21 Stainless-Steel Heated Bath Circulators." The temperature stability of this heated bath circulator is 0.02ºC. This is a remarkably small variation, which supports the notion that there are no risks of sudden oscillations water temperature.

The actual temporal behavior of skin temperature during 90-minutes of thermotherapy application is shown on the figure on page 4 of the protocol. There are no abrupt changes in temperature. The response follows an exponential rise to a peak of around 39.5ºC at 90 minutes. This temperature is safe and far below the temperature required to burn the skin, which is typically above 43ºC.

Our initial study revealed that the proposed protocol leads to a progressive increase in leg skin temperature from a baseline of 33ºC to ~39.5ºC. A research coordinator will supervise each treatment session. If the patient experiences any discomfort or burning sensations, the coordinator will readjust the suit or turn down the temperature of the water. Subjects will be reminded to remain in a semi-recumbent position during treatment and will also be asked to rate heat sensation every 15 minutes using a thermal sensation scale to monitor for excessive heat levels on the skin. If subject reports a grade of 3 ("Hot") or above, or any discomfort, the coordinator will readjust the suit and turn down the temperature of the water. If, after reducing the temperature and readjusting the suit the patient again reports a grade of 3, we will keep repeating the aforementioned steps until the score drops down and the patient feels comfortable. Water temperature

will be assessed and recorded every 15 minutes using the temperature display on the pump.

In preliminary experiments we observed that thermotherapy application induces a minor reduction in blood pressure in some patients. The research coordinator will measure blood pressure prior to and every 15 minutes throughout the application. If the patients experience dizziness, lightheadedness or any other symptom of hypotension, the therapy will be terminated and the participant will be placed supine with the legs elevated. Blood pressure will be checked every 5 minutes until the symptoms disappear and blood pressure returns to baseline levels.

Venous blood sampling: During catheter insertion there is a risk of pain from the needle. Bleeding, local swelling, bruising and formation of a hematoma might also occur around puncture site. By using appropriate phlebotomy techniques, the risks associated with catheter placement and repeated sampling are minimized.

Blood pressure measurements: There are no risks associated with the blood pressure measurements.

Measurement of calf muscle oxygenation: There are no risks associated with blood pressure measurements.

MRI scan: Potential risks associated with MR imaging include:

- Claustrophobia: the confining conditions of the MR scanner can cause claustrophobia. If the patient experiences claustrophobia the scan will be stopped.
- Nerve stimulation: Some subjects undergoing the rapid scanning procedures which will be used in this scan have experienced minor nerve stimulation effects, such as muscle twitches and tingling sensations.
- Hearing: The MR scanner produces tapping sounds during operation, which may reach objectionable levels. To minimize any discomfort, subjects will be provided with disposable earplugs or headphones
- Collision hazard: The magnetic field near the MR Scanner is strong enough to attract objects containing iron with great force. Near the magnet this force can be strong enough to pull objects in and cause them to fly down into the magnet. Such objects can become projectiles that can cause injury or death. We have established a security zone to prevent objects containing iron from coming into proximity of the magnet.
- Radio-wave effects: If metal wires or electrodes, such as electrocardiograph (ECG) leads are attached to a person being imaged, the radio-wave energy radiated by the imaging coils of the MR scanner may induce sufficient electrical currents in the wires to cause burns where the electrodes or wires contact the skin. The scanner operator is well aware of this risk and knows the proper methods to use to avoid this problem.

Reactive hyperemia: Inflation of a cuff around the thigh to supra-systolic pressures (75 mmHg above brachial systolic blood pressure or 250 mmHg) for 5 min to provoke

ischemia might cause discomfort in some subjects, including pain, numbness and tingling in the tissue located downstream from the occlusion site. These sensations are temporary and disappear immediately after cuff deflation.

6.0 Reporting of Adverse Events or Unanticipated Problems involving Risk to Participants or Others

If the patient is injured due to participation in this study, he/she will be instructed to contact the study investigators. Patients will also be advised to contact the Human Subject Office at Indiana University if they have questions about their rights while taking part in the study.

7.0 Study Withdrawal/Discontinuation

Participation in this study is voluntary. Participants may choose not to participate or, if they agree to participate, they can withdraw their participation at any time without penalty or loss of benefits to which they are otherwise entitled.

8.0 Statistical Considerations

Sample size was estimated based on the ability to detect a significant change in 6-min walk distance from baseline to 6 weeks in the TT-treated group as compared to the control group. The studies of McDermott and co-workers (5) and Gardner and co-workers (2), which investigated the impact of home-based exercise training on walking tolerance in patients with IC, were used as a reference for the sample size calculations. We estimated the variance of improved walk distance by the MSE of Table 3 in the study of Gardner et al (2), which is 48.69 meters. Since both studies reported a non-significant change in 6-min walk in the attention-control group (95% confidence interval for McDermott's study is (-25.4 to 3.2) and for Gardner's study is (-6.33 to 14.33)), we predict that the change in 6-min walk in the control group, in our study, will be negligible (0). As pointed out by Perera et al (6), a change of 50 meters in the 6-min walk test is considered substantial and clinically meaningful in elderly subjects. Using this effect size (50-0)/48.69, we determined that **16 subjects per group** are needed to detect a substantial improvement by TT therapy with a power of 0.802.

9.0 Privacy/Confidentiality Issues

Information produced by this study will be stored in the investigator's file and identified by a code number only. The code key connecting the patient's name to specific information about the patient will be kept in a separate, secure location. Information contained in the patient's records may not be given to anyone unaffiliated with the study in a form that could identify the patient without their written consent, except as required by law.
The results of this study may be published in a medical book or journal or used for teaching purposes. However, the patients' name or other identifying information will not be used in any publication or teaching materials without their specific permission.

The patient's name and social security number will be given to the business office for facilitating payment, but will not be used for any other purposes.

10.0 Follow-up and Record Retention

Records relating to this study will be retained for at least 7 years after completion of the research. Paper records will be shredded and recycled. Records stored on a computer hard drive will be erased using commercial software applications designed to remove all data from the storage device.

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