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Official Title: Heat Therapy to Reduce Leg Pain and Improve Walking Tolerance in Patients With Symptomatic Peripheral Artery Disease

STUDY PROTOCOL AND STATISTICAL ANALYSIS PLAN

Experimental Design

A schematic of the experimental protocol is depicted in Figure 1. To familiarize participants with the symptom-limited cardiopulmonary treadmill exercise test and assess the test-retest reliability of walking tolerance, two tests were completed at baseline, at least 72 hours apart. On visit 1, participants underwent a lower extremity arterial examination to determine the ankle-brachial index (ABI). Next, participants were escorted to an adjacent room and underwent resting pulmonary function testing, followed by a graded cardiopulmonary treadmill exercise test. Pulmonary oxygen uptake ($\dot{V}O_2$) and calf muscle oxygenation were measured continuously throughout the test. Similar procedures were followed on visit 2, with the exception of the lower extremity arterial exam. If the difference in peak walking time (PWT) during the exercise tests between visits 1 and 2 was greater than 20%, participants were asked to complete a third exercise test, at least 72 hrs after visit 2. Once the variation in PWT was deemed acceptable (i.e. <20%), participants were assigned, using a randomized, crossover design, to undergo a single session of either HT or a control treatment (CON) prior to a symptom-limited cardiopulmonary treadmill exercise test. The primary study outcome was the difference in peak walking time (PWT) between HT and the CON conditions. Secondary outcomes included the differences between visits in claudication onset time (COT), blood pressure and calf muscle oxygenation at rest and during exercise, pulmonary oxygen uptake ($\dot{V}O_2$) and plasma ET-1, IL-6 and TNF-a concentrations post-treatment and post-exercise. Prior to the experimental visits, participants were asked to report to the laboratory in a fasted state (>8 hrs postprandial), refrain from exercise (24 hrs), smoking (>4 hrs), and take their usual medications.

Pulmonary function tests

Pulmonary function tests were performed following the American Thoracic Society and European Respiratory Society guidelines [1]. FVC and FEV1 were measured and reported as both raw and %predicted values [2].

Symptom-limited cardiopulmonary exercise test

Exercise testing was performed in a motorized treadmill (Pro 27, Woodway, St. Paul, Minnesota, United States) following the Gardner-Skinner protocol, which consists of walking at a constant speed (2 mph) with a 2%-grade increase every 2 min [3]. A 12-lead electrocardiogram (ECG) was registered continuously. Blood pressure was measured in the left arm using a stethoscope and sphygmomanometer prior and during exercise and for 10 min during recovery. Expired respiratory gases were collected breath-by-breath via a facemask attached to a gas analyzer (MedGraphics, CardiO2, and CPX/D system using Breeze EX Software, 142090-001, ReVia; MGC Diagnostics, St. Paul, MN, United States). Calf muscle oxygenation was assessed using a near infrared spectroscopy device (NIRS; Portomon, Medis, the Netherlands), which was affixed on the skin over the medial portion of the gastrocnemius of the leg of which the participants self-described as having the more severe pain during exercise. Participants received standardized instructions and were asked to indicate when they first began to feel leg pain with a "thumbs up" signal (defined as COT), and then give a "thumbs down" signal when they could no longer continue with the test (defined as PWT). Participants were allowed to use the handrails for balance, but were not allowed to use them as an aid for walking (i.e. pulling themselves up).

Heat and control treatments

Participants were instructed to ingest a core temperature sensor (CorTemp, HQ Inc., Palmetto, Florida, United States) the night before the experimental visits. Upon arrival, participants were allowed to rest for 15 min and were then instrumented with leg skin thermistors (MLT422; ADInstruments, Colorado Springs, CO, United States) and a blood pressure cuff on the upper arm that was connected to an automated monitor (Suntech CT40, Suntech Medical, Morrisville, North Carolina, United States). An intravenous catheter was placed in an antecubital vein of the left arm for blood sampling. After 30 min of rest in the semi-recumbent position, participants were fitted with liquid-circulating trousers as described previously [4]. In the CON session, water at 33°C was circulated through the trousers for 90 min using a water pump (HTP-1500, Adroit Medical, Louden, Tennessee, United States). We have previously demonstrated that this regimen elevates skin temperature by approximately 2ºC, but does not evoke measurable changes in core body temperature, heart rate and blood pressure [4]. In the HT session, water at 43°C was circulated through the tube-lined trousers using a heated bath circulator (HT; Aqua Relief Systems, Akron, Ohio, United States) with the goal of increasing leg skin temperature to 37-38ºC. The following parameters were assessed every 5 min during baseline and throughout the 90-min intervention: intestinal temperature (HQ Inc., Palmetto, Florida, United States), leg skin temperature, blood pressure, and heart rate. Prior to the onset of the treatment and at 45 and 90 min, patients were asked to subjectively grade thermal comfort scores using on an 11-point feeling scale [5]. After the completion of the treatment, participants were promptly escorted to an adjacent room and performed a symptom-limited exercise test as in visits 1 and 2. Blood samples were taken at baseline, at the end of the 90-min treatment and 10 min after the end of the exercise test.

Assessment of tissue oxygenation

The tissue saturation index (TSI%) of the most symptomatic leg was assessed with a commercially available NIRS system (Portamon, Artinis Medical Systems, The Netherlands). Initially, the skin over the medial gastrocnemius of the most symptomatic leg was cleaned and, if necessary, shaved. The device was affixed using medical tape and covered with a dark cotton sleeve. The location of the monitor was traced with permanent marker to ensure consistent placement in subsequent visits. The tracings were covered with an adhesive dressing (Tegaderm, 3M, Maplewood, MN, United States) after the exercise test and re-drawn at each subsequent visit. Data were exported at 1Hz and the final 20-second average of each stage was utilized for analysis.

Analysis of blood markers

Samples were drawn into tubes containing EDTA (BD Vacutainer, BD, Ontario, Canada). One tube was transported to the Indiana University Health Pathology Laboratory for the assessment of a routine basic metabolic panel. All other plasma samples were centrifuged at 4ºC for 10 min at 1,100 g (ST16, Thermo Scientific, Waltham, MA, United States). Samples were then aliquoted and immediately placed into a -80°C freezer. Commercially available enzyme-linked immunosorbent assay kits were used to measure the plasma concentrations of ET-1 (DET100, Endothelin-1 Quantikine ELISA Kit, R&D Systems, Minneapolis, MN, United States), TNF-α (HSTA00E, Human TNF-alpha Quantikine HS ELISA kit, R&D Systems, Minneapolis, MN, United States) and IL-6 (HS600C, Human IL-6 HS ELISA Kit, R&D Systems, Minneapolis, MN, United States). Blood biomarker values were corrected for potential changes in plasma volume during heating and exercise as described elsewhere [6].

Statistical Analysis

All data were analyzed using SAS v9.4. Data are presented as mean ± SD. Variables assessed during exposure to HT or CON (blood pressure, intestinal temperature, HR, and skin temperature) were compared using a two-way repeated measures analysis of variance (ANOVA). PWT and COT times were compared using a paired t-test. All measurements taken at COT and PWT were time-aligned (isotime) between visits 3 and 4 at the point of the earlier COT and PWT. Blood pressure during exercise was compared between groups using a two-way repeated measures ANOVA. Missing values during the exercise test were imputed by linear interpolation, using the mean of the values from the two adjacent stages. If the value for the final stage was missing, then it was recorded as a repeat of the previous stage. TSI% and $\dot{V}O_2$ are represented using the mean of the final 20 seconds for each stage. Staged averages were compared using a two-way repeated measures ANOVA. When a group-by-time interaction was detected, post-hoc analysis compared values at each time point. A Bonferroni adjustment was applied to correct for multiple comparisons when appropriate.

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