Title: Effects of passive heat therapy on oxygen consumption and cardiovascular fitness in adults.

Key Personnel:

Brigid Flynn, MD. Associate Professor, Department of Anesthesiology, Division of Critical Care
Paige Geiger, PhD. Professor, Department of Molecular and Integrative Physiology

Background:

Endothelial Disease and Cardiovascular Risk

Cardiovascular disease is the leading cause of death for both men and women in the United States.[1,2] Millions of people world-wide suffer from cardiovascular disease, with 84 million people suffering in the United States alone.[3] It is estimated that one American dies of cardiovascular disease every 40 seconds.[3]

Compromised endothelial function has been implicated with the development and progression of cardiovascular disease. The arterial endothelium is recognized as a smart barrier and key regulator of blood flow in micro- and macrovascular beds.[4] Healthy, distensible arteries allow smoothing of blood pressure variations and ensure blood flow in one direction.

Endothelial dysfunction presents as arterial stiffness, inflammation and lack of vascular relaxation. As arteries stiffen and lose elasticity, pulsatility is dampened causing wider blood pressure fluctuations within the micro- and macrovasculature.[5] These fluctuations and the resultant low diastolic blood pressure place vital organs such as the heart, brain and kidneys at risk. There is a reduction in coronary artery perfusion[6] and increased risks of stroke, renal impairment and hypertrophic heart disease.[7] Furthermore, the effects of shear stress on these stiff vessels promote vascular remodeling and thickening.[8] Endothelial thickening coincides with a decreased production of endogenous vasodilatory mediators, such as nitric oxide, further creating non-distensible arteries without a propensity for vascular dilation. Ultimately this thickening and lack of elasticity fail to properly regulate blood flow and further vital, end-organ damage results.

Heat Therapy and Endothelial Function

In humans, previous work has demonstrated that heat exposure can improve arterial endothelial function.[9-11] In sedentary adults, heat therapy via water immersion had widespread beneficial effects on vascular dilatation and a reduction in arterial stiffness.[10] After eight weeks (4-5 times/week) of heat therapy, participants had several markers of improved vascular reactivity including increased flow-mediated dilatation (FMD), improved arterial compliance, reduced aortic pulse wave velocity, decreased carotid intima media thickness and decreased blood pressure.

Other studies utilized infrared, dry sauna therapy daily for eight weeks in patients with cardiovascular risk. In patients with at least one coronary risk factor, flow-mediated dilatation was improved by 5.3%.[11] This improvement translates into substantial improvements in vascular health as improvements of just 2% have been correlated with a 15% reduction in cardiovascular risk.[10,12] A follow up study showed that in patients with congestive heart failure, heat therapy not only improved flow-mediated dilatation but also decreased plasma brain natriuretic peptide (BNP) levels indicating less congestive heart failure burden.[9]

Mechanism of Endothelial Improvement: Heat Shock Proteins

A major mechanism thought to be involved in the improvements in vascular tone with heat therapy are the expression of heat shock proteins (HSP).[13] Heat shock proteins are cytoprotective molecular chaperones that accelerate cellular repair and block cell death.[14] Heat shock proteins are also thought to be beneficial to arterial integrity in that there is association of anti-inflammatory[13] and anti-oxidative effects with HSP response.[15] In addition, in bovine coronary endothelial cells, one particular HSP, Hsp90, has been shown to be associated with the critical step by which endothelial nitric oxide is produced.[16] Nitric oxide is an important

and potent arterial vasodilator. Another HSP, Hsp72, is thought to be the HSP most important for thermotolerance by decreasing gut-associated endotoxin translocation and by reducing the body's inflammatory response.[17] Transgenic mice overexpressing Hsp72 display a 2-fold increase in running capacity relative to wildtype mice and exhibit a 50% increase in mitochondria.[18] Increased expression of Hsp72 could therefore contribute to an increase in aerobic capacity following chronic heat therapy.

Study Aim:

Translation of Endothelial Modifications using Heat Therapy into Cardiovascular Fitness Improvement

In this study, we will assess if the reported improvements in endothelial function translate into improved cardiovascular function. Since heat therapy has been shown to illicit several molecular markers of benefit for endothelial function including enhancement of FMD and other markers of arterial distensibility, there is a potential direct benefit for cardiovascular health and fitness. As of today, these benefits remain theoretical.

Improvements in cardiovascular function are measured by increased exercise tolerance with various strategies of fitness testing. Historically, the gold standard of fitness testing relies on measurements of maximal oxygen consumption (VO_{2max}). There are numerous methods to obtaining VO_{2max}, however some methods are invasive, require significant equipment and/or require intense exercise by the participant. The methods chosen for this study are validated, utilize two different mechanisms of VO_{2max} measurements in order to enhance reliability, are noninvasive and require minimal exercise in which the participant can rest when needed to ensure safety.

Oxygen consumption testing (VO_2) will be measured with a treadmill exercise respirometry study. This test measures VO2 indirectly by measuring energy expenditure. This method is known as indirect calorimetry and is the gold standard for measuring energy expenditure[19]. Study participants will complete the treadmill exercise respirometry on the day of their first hot tub immersion, prior to the hot tub session. This exercise will also be performed on within 24 to 48 hours of the final hot tub experience.

We will also measure and record various hemodynamic data points prior to, during and after hot tub sessions utilizing the Clearsight© (Edwards Lifesciences, Irvine, CA) monitor.[20] This noninvasive monitor incorporates a disposable, single use finger-tip blood pressure cuff which accurately calculates blood pressure, stroke volume, stroke volume variation, cardiac index and systemic vascular resistance.[23] It is important to measure these data points in order to investigate the effect of heat therapy on the cardiovascular system since all of these data points are functions of arterial distensibility. Alterations to these hemodynamic variables is directly reflective of cardiovascular tone. This information will further the understanding of the relationship of heat therapy and endothelial tone in a real-time and an *in vivo* setting.

Clearsight finger probe and monitor read out.

Methods:

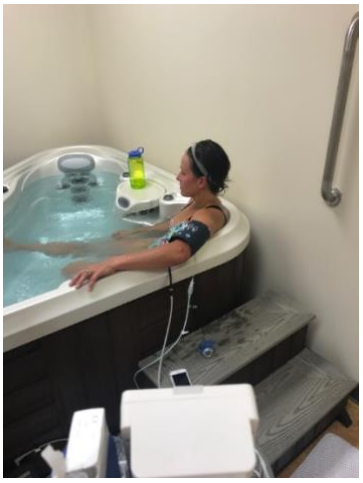
All experimental procedures will be approved through TUKH institutional review board prior to advertisement for volunteers. Participants will volunteer in response to advertisements placed in public areas in The University of Kansas Health Systems. All subjects will provide oral and written informed consent prior to participation. Monetary compensation of $100 will be offered upon completion of the study. The hot tub used to provide immersion is located in the Clinical and Translational Science Unit (CTSU), Fairway, KS.

Study Cohort and Safety

Twenty adults, age 50 or older, will be included in the study. Demographic data required for pertinent calculations will be obtained from each participant including height, weight, age and gender. Exclusion criteria includes current major cardiovascular disease known to the patient including recent myocardial infarction, stroke, angina pectoris, high grade coronary vasculopathy or atherosclerosis, severe valvular disease, current cardiac dysrhythmia that requires treatment (i.e. atrial fibrillation, atrial flutter, frequent ventricular premature beats and/or implantation of permanent pacemaker/defibrillator). Patients with a history of coronary artery bypass grafting, valvular surgeries and coronary interventions will be assessed as to current exercise tolerance and symptomology. Other exclusion criteria include current treatment with steroids or immunosuppressive agents, current malignancy, serious liver disease, end-stage renal disease or judgement by a medical provider that water or heat therapy poses an undue burden or risk upon the individual. All females of child bearing age will consent to pregnancy testing and will be excluded if pregnant. Should a participant become hemodynamically or cognitively impaired during any portion of the study activities, 911 will be called for evaluation and possible transport to the emergency department at TUKH. Volunteers can choose to recuse themselves from any or all of the study activities at any time.

Hot Water Immersion Therapy

Previous reports have demonstrated that benefits from heat therapy require 5-14 days to become apparent.[9-11,14] Thus, participants in the present study will undergo 8-10 shoulder-high hot water immersions of 40.5°C for 45 minutes/session during a 14-16-day period. Either 1) a rectal probe with sterile disposable sheaths or 2) a sterile rectal thermistor probe will be used to monitor the subject's temperature (T_{re}). These probes are inserted approximately 2.54 cm past the anal sphincter. The subject will be removed from the hot tub if T_{re} is 38.5°C or higher. Participants will be asked to sit on a bench of the hot tub with until T_{re} is < 38.0°C at which time they may return to the hot tub. Participants are able to drink water *ad libitum* while in the hot tub. T_{re} will be monitored for 10 additional minutes following emersion from the hot tub or until T_{re} < 38.0°C.


Hot tub located in the CRC at KUMC.

Subjects will have dry weights measured prior to and after hot water therapy. If body weight loss following therapy is > 1%, the participant will be asked to drink additional fluids to make up the difference. Heart rate will be monitored continuously during heat therapy. If heart rate is altered > 50% from baseline, the participant will

be assessed for neurologic and hemodynamic changes. If there are changes concerning these assessments, the subject will be removed from the hot tub, temperature monitored, and a medical provider notified. Blood pressure will be obtained prior to and after hot water therapy. Participants with pre-therapy systolic blood pressure < 75mmHg or > 180mmHg and/or diastolic blood pressure < 40mmHg or > 90mmHg, will not be allowed to participate in hot water therapy. If post-hot tub blood pressure varies by > 20% lower or > 30% higher than baseline values, the subject will be monitored until return to < 20% deviation from baseline. If blood pressure values do not return to baseline after 10 minutes, discussion with a medical provider will take place prior to discharge from CTSU. If at any time, should a participant become hemodynamically or cognitively impaired during any portion of the study activities, 911 will be called for evaluation and possible transport to the emergency department at TUKH.

Cardiovascular performance: Oxygen consumption measurements

Study participants will complete the treadmill exercise respirometry at their first study visit. This exercise will also be performed on within 24 to 48 hours of the final hot tub experience. This waiting period will obviate the transient enhancements of cardiac output and systemic vascular resistance due to water immersion that may alter VO_{2max} assessments.[22-25] These effects are normalized shortly after removal from the water and heat sources, thus should be normalized and not have impact on VO_{2max} by this time. This waiting period also allows for muscle rest prior to the exercise step test. Importantly, waiting until the next day to perform the laboratory and exercise testing also ensures that the changes seen are not acute benefits of heat therapy, but are chronic.

Measurements:

The following data will be collected at two separate time points: T1 = beginning of the study, prior to hot water immersion; and T2 = at the end of the study, 24-48 hours after the last hot tub experience.

1. Blood pressure, stroke volume, stroke volume variation, cardiac index and systemic vascular resistance, heart rate with the Clearsight© (Edwards Lifesciences, Irvine, CA) monitor
2. VO_2 as measured with treadmill exercise respirometry
3. Blood collection tube of approximately 4 tablespoons of blood to assess complete blood count, complete metabolic panel, lipid panel, and serum heat shock protein measurements (Hsp72 and Hsp90)

Risks:

Whole-Body Heat Therapy:

There are some risks associated with heat exposure, including: fatigue, light-headedness, muscle cramps, dehydration, and neurological detriments (i.e. heat stroke). However, these symptoms do not typically occur until core temperature rises above 40°C. Core temperature (rectal thermistor) will be continuously monitored and recorded. Subjects will be removed from the hot bath immediately if either core temperature reaches 39.5°C or the subjects experience any symptoms of heat-related illness. All symptoms subside upon lowering core temperature. Ice packs will be on hand if rapid cooling is necessary (see Rapid Cooling SOP). Heart rate will also be continuously monitored and recorded throughout heating. If HR increases > 60 beats/min above resting or increases > 20 beat/min with a 5 min time period, subjects will be moved to a seated position if they were previously fully submerged, or removed from the hot tub if they were already sitting up. Additionally, heat exposure may have detrimental effect on a developing fetus in females. Thus, subjects who are pregnant or trying to conceive will be excluded from the study. This will be confirmed with a pregnancy test.

Rectal temperature probes:

The use of rectal probes to measure core temperature carries minimal risk. The primary risk is of damage to the lining of the rectum; however, this risk is very slight as we use a flexible thermistor that is designed for this

purpose. We will exclude any subjects who have had recent rectal, anal, vaginal, or prostate surgery. In addition, we will exclude any subjects who have a personal history of heart disease, as the use of a rectal thermometer can cause a vagal reaction, increasing the potential for arrhythmias and fainting. There is also the risk of infection, either by the subject not washing their hands properly or exposure by others to a poorly cleaned probe. We will use clean, sterile disposable probes to minimize this risk. The risk of infection is similar to that of having a bowel movement, and is considered minimal (similar to daily experience). There is also the risk of embarrassment. The approach is typically well tolerated by subjects, and the investigative team is professional in regard to their treatment of the subjects.

Recruitment:

Participants will be recruited for this study through IRB-approved flyers posted at the University of Kansas Medical Center, in the community, and through electronic communications via websites and email. The study team will also utilize the Pioneers/Frontiers Research Participant Registry after approval from the Data Request Committee (DRC). Individual mailings and phone calls will be made to applicable subjects from the Pioneer/Frontiers Research Participant Registry.

Vulnerable Populations:

The recruitment plan will not include any of the vulnerable groups below, except possible students or employees of the University or Health System:

I. Cognitively or decisionally impaired individuals
II. Children
III. Pregnant women
IV. Prisoners
V. Students and/or employees: While our study does not specifically seek to recruit this subgroup, it is not outside the realm of possibility that students or employees may contact the study team after having seen recruitment postings or if they have previously consented to participate in the Pioneers/Frontiers research registry.

Assessment of Subject Safety and Development of a Data and Safety Monitoring Plan:

Nursing and study staff will continually monitor participants for adverse events. Any adverse event will be immediately reported to the PI and study coordinator per KUMC Human Subject Committee reporting policies. This procedure will be scheduled when a CTSU safety monitor is available. The Principal investigator will assess safety data after every 5 subjects are enrolled. If a trend of adverse events related to heat-related illness occurs, the study will pause or stop until the safety issue is assessed and addressed by the study team.

Definition of Adverse Experiences:

Adverse events are defined as any untoward medical occurrence in study participants or others, which does not necessarily have to be a causal relationship with the study treatment. The seriousness of the adverse event will be determined using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) v3.0,[95] Adverse events (AE) will be reported if they are reported by the patient or observed by research staff to cause a noticeably different experience than is typical for the individual (CTCAE v3 Grade 1-2) Serious adverse events (SAE) will be deemed those AEs significant enough to interfere with activities of daily living (CTCAE v3 Grade 3-5). An adverse event in any category rated 3 or greater according to CTCAE v3.0, will result in immediate termination of the procedure.

Expected Adverse Experiences:

Expected adverse experiences include potential bruising and soreness at the site of blood draw.

Data Protection:

All data will be deidentified by giving participants a random number that will unable to be traced to identity or demographic data. All data will be stored in a database on the PI's password protected, hospital computer in a password protected spreadsheet. This computer is housed in a locked hospital office.

Objectives

The main objective of this pilot study is to understand the relationship between the molecular mechanisms of cardiovascular improvements demonstrated in previous studies due to heat therapy to functional cardiovascular improvements in humans. The translation of enhanced endothelial arterial distensibility into improved oxygen consumption, or VO_{2max}, has yet to be studied. If indeed, the benefits of heat therapy do translate into better cardiovascular health, the implications of this would be far reaching for millions of people who suffer from cardiovascular disease throughout the world.

The current pilot study will measure cardiovascular function following hot water therapy in patients without significant cardiovascular disease. However, the findings of this study will hopefully set the framework for hot water therapy in patients with NYHA Class II -III heart failure in a future study. The benefits in these patients may not only be in VO_{2max} and biomarker measurements of cardiac function improvements, but also in activities of daily living. This would have a very meaningful impact on society due to the large and ever-increasing number of patients with heart failure. Currently the National Institute of Health recognizes heart failure as major public health problem with a prevalence over 5.8 million Americans and 23 million people worldwide.[30] Heart failure has been singled out as an emerging epidemic.[31] Furthermore, the need for research is increasing as each year more than 550,000 new cases of heart failure are diagnosed in the United States.[32]

Importantly, the amount of healthcare expenditure on heart failure is staggering. More than one million hospitalizations for heart failure occur yearly.[33] Additionally, commonly prescribed medications to treat the symptoms associated with this disease are expensive and many are not covered by major health insurance companies.

Ultimately, with results from this and other pilot studies, the breadth of knowledge in this field of endothelial translational research will dramatically increase. However, it is necessary to continue the pursuit of understanding heat therapy and the implications in patients with cardiovascular disease. Unlike other morbid disease processes, heart failure can often be prevented and treated such that patients live higher quality lives. This in turn, allows freedom from hospital admission and costly medications. Utilizing the human body's own molecular mechanisms to combat cardiovascular disease is the ideal prevention and therapeutic strategy. We believe heat therapy will be an important stepping stone in understanding these molecular pathways and possibly in treating cardiovascular disease.

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