University of Kansas Medical Center
RESEARCH PROTOCOL INVOLVING HUMAN SUBJECTS
TEMPLATE WITH GUIDANCE

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Principal Investigator: Paige Geiger, PhD

Study Title: Heat Shock Therapy to Improve Mitochondrial Function in Neuropathy (HOTFUN)

Co- Investigator(s): Douglas Wright, PhD; Mamatha Pasnoor, MD; Andrea Nicol, MD

I. Purpose, Background and Rationale

A. Aim and Hypotheses

1. Sensory dysfunction as a result of peripheral nerve damage is a significant problem that leads to reduced quality of life for patients. The prevalence of sensory dysfunction in peripheral neuropathy associates with epidemic increases in prediabetes and diabetes, but also is relevant to chemotherapy treatments and genetic disorders. Clinical approaches to treat peripheral neuropathy and to stimulate axon growth in settings of peripheral axon loss are limited. Although new drugs will hopefully be forthcoming, the most promising approaches likely involve behavioral and lifestyle interventions. Mitochondrial dysfunction is emerging as a key cellular contribution to peripheral axon health and peripheral neuropathy. Mitochondrial deficiencies contribute to neuropathy and include impaired mitochondrial problems with trafficking, mitophagy, fission, and biogenesis. All of these are thought to lead to a bioenergetic crisis, ending in distal axonal degeneration, sensory dysfunction and pain. Heat shock proteins play a critically important role in cellular homeostasis and increasing heat shock protein functions within cells leads to a range of positive improvements, particularly in mitochondria. In addition, new evidence suggests that increasing heat shock protein responses in peripheral nerves has powerful, positive impacts on sensory function and neuropathy.

2. Our interdisciplinary team will investigate the role of mitochondrial dysfunction in peripheral neuropathy and translate these approaches to improve treatment for patients with peripheral neuropathy. We hypothesize that novel heat treatment interventions that improve mitochondrial function will improve metabolic symptoms and peripheral nerve mitochondria, leading to improvements in sensory function, via heat shock protein induction. We will employ immersion heat treatment to elevate heat shock protein responses that induce positive changes in peripheral nerve mitochondria. One aspect is to confirm the efficacy, safety, and potential for heat treatment to improve sensory dysfunction in human patients with prediabetes. The goal of this proposal is 1) to test the breadth of heat treatment on various forms of neuropathy, 2) identify mechanisms in which heat treatment improves mitochondrial function, and 3) test the efficacy, safety, and potential for heat treatment to improve sensory dysfunction in human patients with prediabetes.

B. Background and Significance

1. Study Significance: Diabetic neuropathy (DN) is a common prediabetic and diabetic complication and is a leading cause for disability due to foot ulceration and amputation, gait disturbance, and fall-related injury. Sensory dysfunction in peripheral neuropathy (PN) is also is relevant to chemotherapy treatments and genetic disorders. Compared to

diabetic patients without neuropathy, patients with DN experience a twofold increase in health-care costs, and those with severe painful peripheral neuropathy experience a fourfold increase. In addition, 25% of patients with prediabetes also develop PN[1]. Currently, there are no treatments for diabetic and prediabetic PN other than pain control. This problem must be addressed as our global population with diabetes increases in epidemic proportions. The scientific premise of our application is based on the evidence from both preclinical and clinical studies.

2. The reliance of peripheral neurons on proper mitochondrial support for their maintenance has emerged as a key mechanism underlying neuropathy. In diabetic neuropathy, a number of recent studies have reported deficient mitochondrial support. Thus, approaches to improve mitochondrial function and stimulate new mitochondrial biogenesis are logical directions to treat peripheral neuropathy. Heat shock is one of the best-known interventions that lead to changes in gene expression known to improve mitochondrial function. New evidence is emerging that heat treatment leads to a significant enhancement of heat shock protein effects in a number of organ systems. A key step in leading to this proposal was the successful implementation of a hot tub in the CTSU that now allows us to heat treat patients and test the hypothesis that heat treatment stimulates heat-shock protein mediated mitochondrial improvements related to glucose control and nerve function.

3. Literature Review: **Peripheral neuropathy is strongly associated with metabolic syndrome and diabetes**. Current strategies to prevent diabetic PN focus on glucose control and lifestyle modifications. Improved glucose control dramatically reduces the incidence of PN in type 1 and type 2 diabetes. Current clinical recommendations focus on optimizing glucose control to prevent or delay the development of diabetic PN. New approaches that contribute to better glucose control are needed[2].

   Peripheral neuropathy is associated with mitochondrial dysfunction. Mitochondrial dysfunction is a key cellular contributor to peripheral axon health. Mitochondrial deficiencies contribute to neuropathy and include impaired mitochondrial problems with trafficking, mitophagy, fission, and biogenesis. Together, this leads to a bioenergetic crisis, ending in distal axonal degeneration and sensory dysfunction. Heat shock proteins play a critically important role in cellular homeostasis and increasing heat shock proteins improves neuronal health[3].

   Heat treatment reduces abnormal metabolic changes in diabetes. Several studies have reported metabolic benefits in type 2 diabetes patients with heat therapy. In these studies, 3 weeks of heat treatment reduced plasma glucose and HbA1c levels. Additional studies using heat therapy in obese patients report improvements in quality of life and reduced weight and fat. Thus, an additional benefit of heat treatment may be to reduce detrimental metabolic changes that contribute to PN[4].

   Heat treatment stimulates heat shock protein levels and improves mitochondrial function in patients. Ongoing studies are assessing the ability of heat therapy to impact mitochondria in human subjects (ages 28-45, n=5). A skeletal muscle biopsy was performed at the KU Clinical Research Center (CRC). One week later, subjects underwent 60 min of heat exposure (hot tub) located in the CRC. Subjects then returned 24 h after the heat therapy for a second muscle biopsy. Muscle samples were analyzed for heat shock protein levels and respiratory measures. These studies reveal that skeletal muscle respiratory capacity increases following acute heat therapy. Moreover, these studies suggest that heat treatment increases heat shock proteins in muscle that positively

correlate with increased mitochondrial respiration. These findings are consistent with our preclinical studies of heat therapy in rodents.

C. Rationale

1. Literature from our own studies and others reveal that heat treatment leads to beneficial heat-shock-mediated improvements in glucose control and mitochondrial function. Studies now reveal that deficient energy support from mitochondria in peripheral nerves is a significant contributor to impaired nerve function and neuropathy.
2. Success in this project will reveal a new intervention for patients suffering from nerve damage and sensory dysfunction. Currently, there are no treatments other than pain control for patients with peripheral neuropathy. Heat treatment likely can be combined with healthy lifestyle and exercise to benefit patients suffering from neuropathy. The project will also provide new information about how heat treatment may improve glucose control in humans with prediabetes or diabetes.
3. The information gained in this research study will be useful in developing fundamental knowledge about heat therapy and determining if heat therapy can be a novel treatment of peripheral neuropathy related to prediabetes patients.

II. Research Plan and Design

A. Study Objectives: To see if heat therapy can be used to improve metabolic symptoms and peripheral nerve mitochondria, leading to improvements in sensory function.

B. Study Type and Design: This prospective, observational cohort pilot study will recruit 20 patients with prediabetes with or without peripheral neuropathy (painful and non-painful) to participate in a 4-week heat therapy intervention. After screening, informed consent, and enrollment into the study, subjects will undergo a pre-intervention evaluation that will assess metabolic biomarkers and heat shock protein levels (blood), peripheral neuropathy, and epidermal evaluation (skin biopsy). After the skin biopsy has the appropriate time to heal (approximately 7-14 days), the subjects will undergo 45-minute heat treatments in 40$^{\circ C}$ water, three times weekly, for 4 weeks. After completion of heat treatments, subjects will undergo a repeated post-intervention evaluation and skin biopsy. Statistical approaches will compare pre– and post-heat therapy measures.

C. Sample size, statistical methods, and power calculation

1. Subjects will not be randomized as our study is a pilot intervention of subjects with prediabetes and neuropathy. Analyses of pre- treatment vs. post-treatment outcomes will be performed using ANOVA. The primary goal of this pilot study will be to provide necessary data for a power analysis to estimate the effect size for design of a larger future study.
2. N/A
3. 20 subjects will receive intervention. As this is a pilot study which will be used to inform future, larger studies, power calculations will not be needed. Statistical approaches will compare pre- and post-heat therapy measures.

D. Subject Criteria (See Vulnerable Populations appendix, if applicable):

1. Inclusion criteria: Subjects will include both males and females, ages 45-75, that have suspected or diagnosed prediabetes with or without neuropathy (to be confirmed at pre-intervention evaluation).

2. Exclusion criteria: (1) skin conditions, circulatory insufficiency, or open wounds in the leg that would interfere with healing from a biopsy;
   (2) stroke or other significant nervous system pathology;
   (3) lidocaine allergy;
   (4) anticipated difficulty with blood clotting due to disorder or use of a blood thinner such as Coumadin, Xarelto, or Eliquis;
   (5) use of any medication used to treat abnormal blood glucose such as Metformin;
   (6) body weight > 350 lbs.;
   (7) history of anemia or vitamin b12 deficiency;
   (8) clinical anemia (hematocrit <32 for women, <36 for men);
   (9) abnormal SPEP result;
   (10) history of cancer or chemotherapy treatment;
   (11) current or recent use (within the last 6 months) of artificial fingernails / nail enhancements that would interfere with quantitative sensory testing;
   (12) no special classes of subjects such as fetuses, neonates, pregnant women, prisoners, institutionalized individuals, non-English speaking individuals, or other who may be considered vulnerable populations will be included in this study.
3. Withdrawal/Termination criteria: Prediabetes will be determined using the American Diabetes Association (ADA) Diabetes Management Guidelines. The subject will meet the criteria for a diagnosis of pre-diabetes if the subject has one or more positive test(s) for either A1c (5.7-6.4%), fasting glucose (100-125 mg/dl), or 2-hr oral glucose tolerance test (140-199 mg/dl). If at pre-intervention evaluation, the subject does not meet the criteria for prediabetes (lower than the above-mentioned range), meets the ADA criteria for diabetes (higher than the above-mentioned range), the subject will be considered a screen fail and will not progress to skin biopsy or intervention.
4. Subject may participate in other observational studies but cannot participate in other studies in which they receive an intervention.

E. Specific methods and techniques used throughout the study

1. After screening, informed consent, and enrollment into the study, subjects will be assessed for the following at the CTSU:

   Blood draw (approximately 8 tubes of blood): complete blood count, complete metabolic panel, lipid panel, inflammatory panel, fasting glucose, 1 hour and 2 hour glucose (oral glucose tolerance test), insulin, glycosylated hemoglobin (HbA1c), serum protein electrophoresis (SPEP), vitamin B12, point of care glucose finger stick (pre- and post-OGTT), heat shock protein (HSP) 72 and 90
   Urine Analysis: pregnancy test, if applicable

2. Study Procedures: All study visits will be completed at the University of Kansas Clinical and Translational Science Unit (CTSU) by study personnel and CTSU staff. Prior to the first visit, the inclusion and exclusion criteria will be reviewed with a phone screen (included in appendix).

   Visit 1 (pre-intervention evaluation): During the pre-intervention evaluation (approx. 3.5 hours), inclusion and exclusion criteria will be confirmed via medical history, medication review, and the above listed laboratory tests. Vitals, demographic

information, social and lifestyle information, and questionnaires on lifestyle will also be collected.

Medical History: prediabetes diagnosis and duration, neuropathy diagnosis and duration, family history of diabetes or neuromuscular disorders, general medical history
Medication Review
Vitals: heart rate, blood pressure, height, weight, waist circumference, body mass index (BMI)
Demographic Information: date of birth, sex, race, ethnicity, recruitment source, education level
Social/Lifestyle: marital status, employment status, smoking history
Questionnaires: PROMIS Emotional Distress and Depression, PROMIS Sleep Disturbance (included in appendix).

Following an overnight fast, participants will have blood drawn (6 teaspoons) for analysis at Quest Diagnostics. After initial fasting blood samples (listed above), patients will drink a sweet and concentrated solution of glucose (Azer Scientific Glucola, 75 g) within 5 minutes. After this, patients will wait for 1 hour and blood will be redrawn to test glucose and insulin levels (2 teaspoons). A final blood draw for glucose and insulin will occur after another hour (2 teaspoons). Patients will complete a point of care glucose finger stick to ensure blood glucose is within a safe range before start and after completion of OGTT for safety.

Visit 2 (pre-intervention evaluation): Once inclusion and exclusion criteria are confirmed by laboratory results, the presence and type of neuropathy will be confirmed with a clinical examination and nerve function testing (Drs. Mamatha Pasnoor or Andrea Nicol, approx. 1.5 hours).

Neuropathy and Pain Assessment: medical evaluation by Drs. Pasnoor and Nicol, Utah Early Neuropathy Scale (UENS), Quantitative Sensory Testing (QST). Questionnaires: Brief Pain Inventory (BPI), PROMIS Physical Function short form.
Skin Biopsy: Patients will undergo a punch skin biopsy from the proximal thigh. Total dermal and epidermal innervation will be quantified from skin biopsies. The punch skin biopsy will be performed using a 3 mm disposable circular punch (Miltex, York, PA) after injection of 2% lidocaine under a sterile technique. No suture will be used. The skin will be placed in freshly prepared Zamboni's fixative for one hour. The tissue will be sectioned at 50 μm and processed for immunohistochemistry using rabbit anti-PGP9.5 primary antibody (1:3000; Chemicon, Temecula, CA). Sections will be coded in order to carry out the analysis in a blinded fashion and quantified. The biopsy will be used for quantification of GLO1 enzymatic activity.

Visits 3-14 (heat therapy intervention x 12 treatments): After the skin biopsy has appropriate time to heal (approximately 7-14 days), the subjects will undergo 30-minute heat treatments in 40°C water, three times weekly, for 4 weeks (approx. 1 hour each).

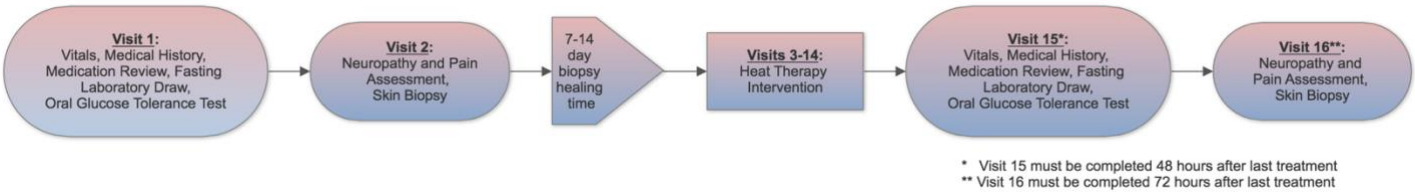
Subjects will be immersed up to the shoulder in a 40°C hot tub until rectal temperature (T_{re}) increases by 1°C (~20 minutes). Subjects will then remain in the water bath submerged to waist level to maintain T_{re} between 38.5 and 39.0°C for another 30 minutes (approx. total time submerged ~50 minutes). Following hot water immersion, subjects will be monitored for another 10 min, or until T_{re} falls below 38.5°C. Core temperature will be monitored using either 1) a rectal probe with sterile disposable sheaths or 2) a sterile disposable rectal thermistor probe (401 A/C, Advanced Industrial Systems, Inc., Harrods Creek, KY) to be inserted ~1 inch past the anal sphincter

(inserted by participant). Heart rate and blood pressure will be monitored throughout the treatment. Subjects will be continually monitored and removed from the hot bath if T_{re} exceeds 39.5°C.

Visit 15 (post-intervention evaluation): After completion of heat therapy intervention, subject will return to assess any change in fasting laboratory tests, oral glucose tolerance test, and questionnaires as listed in Visit 1 (approx. 2.5 hours). In addition to the two questionnaires completed at Visit 1, subject will also complete a Patient Global Impression of Change (PGIC) survey (included in appendix). A single question asks patients "After my treatment, my pain is:" with a 7-point scale ranging from -3 (very much worse) to 0 (no different) to +3 (very much improved). This measure will be a secondary determinant of analgesic response to treatment and will only be administered at the completion of heat therapy intervention

Visit 16 (post-intervention evaluation): Subject will return for a final visit to assess any change in the presence or type of neuropathy by completing the same clinical examination and nerve function testing as in Visit 2 (Drs. Mamatha Pasnoor or Andrea Nicol, approx. 1.5 hours).

3. All procedures will be performed solely for research purposes. None should be considered standard of care or billed to insurance.
4. Blood will be processed at the CTSU and sent to Quest Diagnostics for resulting. Subjects will have the option to save leftover samples for future deidentified research. Otherwise, any leftover samples will be destroyed. Skin biopsy samples will be housed at the University of Kansas Medical Center in a secure laboratory for processing. Only the study team will have access to any samples. All samples will be marked only with subject ID and date.
5. Timeline:



Subjects are expected to complete the study in approximately 11-13 weeks.

F. Risk/benefit assessment:

1. Physical risk: Participants will be asked to fast overnight for the blood draw. The study visits will be scheduled in the morning when possible, and subjects will be provided with a snack immediately following testing. Risks of blood draws include temporary discomfort from the needle being inserted into the skin, bruising, swelling, fainting, or in rare cases, infection or blood clot at the injection site.

   During the oral glucose tolerance test, there is a very small risk of developing symptoms of low blood sugar, which include rapid heartbeat, sweating, and headache. Nausea and vomiting are also rare, but known, risks of the oral glucose tolerance test. Patients will complete a point of care glucose finger stick to ensure blood glucose is within a safe range before leaving the CTSU.

   Quantitative sensory testing (QST) may cause minor but temporary physical discomfort.

Study personnel will be trained by the investigators to be sensitive to participant discomfort and concerns. Participants will be instructed that they can stop any QST procedure anytime that the pain or unpleasantness of the task becomes unbearable. There have been no significant adverse events associated with any of these procedures in the experience of its use at the Chronic Pain and Fatigue Research Center at the University of Michigan. Specifically, MAST testing may cause some temporary physical discomfort on the thumbnail. The MAST System incorporates a series of redundant mechanical, electrical, and software safety features to prevent patient injury in the event of user error or device failure, including a safety pin that the subject can turn to immediately remove the pressure actuator from his or her thumb. The test is terminated at or before 10 kg/cm2 of pressure which is a commonly used maximum pressure level in human sensory testing and does not result in physical injury. Participants will always have personal control over the stimulus and can stop it at any time or express instructions to stop the stimuli. They can also withdraw their thumb from the device. However, these instruments may cause minor physical discomfort in the areas of testing that is expected to resolve within minutes of test completion.

The 3mm skin biopsy procedure is not painful. However, when the Lidocaine is injected, there may be a brief pinprick pain followed by a few seconds of burning form the anesthesia. A small percentage of patients can have an allergic reaction to the Lidocaine. If an allergic reaction to the Lidocaine occurs, it will be treated immediately with medicines in the clinic. Following the biopsy there will probably be some soreness. There is a risk that the biopsy site will bleed or become infected. The risk for infection is low at the biopsy site. The biopsy will leave a small scar that will fade in intensity over time. 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.

2. Psychological risk: Some questions related to medical history, weight, or pain status may result in feelings of discomfort or embarrassment. Participants may decline to answer any questions.

3. Social risk: N/A

4. Economic risk: N/A

5. Potential benefit of participating in the study
    a. Potential benefits for individuals including learning new information based on laboratory testing and medical examination, at no cost to the subject. This information may be of use to the participant and their physician by providing updated information on important parameters of prediabetes and/or neuropathy. Subjects will have the opportunity to receive heat therapy, which may offer multiple health benefits related to the cardiovascular system and metabolic health. Physiological benefits to the cardiovascular system in healthy subjects has been documented and those studies were performed by Dr. Chris Minson, from the University of Oregon. In addition, quality of life improvements have commonly been reported by patients undergoing chronic heat exposure (e.g. hot tub, sauna, etc.).
    b. The information gained in this research study will be useful in developing novel treatments of peripheral neuropathy in the future related to prediabetes patients.
    c. The proposed research will be helpful to develop fundamental knowledge about treatment approaches that will help reduce the burdens of disease and disability. This knowledge should offset the very minor risk to participation by individual subjects, given the protections that are in place and the low likelihood of a serious adverse event in this study.

G. Location where study will be performed:

Pre-screening data will be securely kept via REDCap.
All study visits will take place at the University of Kansas Clinical and Translational Science Unit (CTSU) "Fairway" at 4350 Shawnee Mission Parkway, Fairway, KS or the CTSU "Rainbow" at 3901 Rainbow Boulevard, Kansas City, KS.
Consent forms will be kept in a separate folder from subject study worksheets, and kept in a secure, locked location at the University of Kansas Medical Center - Hemenway.

H. Collaboration (with another institution, if applicable): N/A

I. Single IRB Review for a Multi-site study (if applicable): N/A

1. For which sites will KUMC serve as the IRB of record? N/A
2. Indicate which study activities will occur at each site. If all study procedures will be identical across study sites, state this. N/A
3. Describe how you will assess the capacity of each site to perform the research (e.g., expertise, staffing, space, equipment, etc.) If applicable, include site evaluation tools in your IRB submission. N/A
4. Describe how the lead investigators will ensure that all participating sites use the IRB-approved version of the protocol, consent, recruitment materials and other study documents. N/A
5. Describe how the lead investigators will communicate with and disseminate new information to other sites (e.g., training meetings, regularly-scheduled conference calls, notifications, etc.) N/A
6. Describe how the lead investigator will assess protocol compliance, unanticipated problems and adverse events at other sites. N/A
7. Name the member of the KUMC study team who will be the point of contact to coordinate oversight and communication with the sites. N/A

J. Community-Based Participatory Research (if applicable)

1. Participants and the nature of their involvement: N/A
2. Cultural issues: N/A
3. Origin of the research question: N/A
4. Risks and Benefits: N/A
5. Study Description and Process: N/A
6. Return of results: N/A
7. Sustainability: N/A

K. Personnel who will conduct the study, including:

1. Indicate, by title, who will be present during study procedure(s):
    a. Paige Geiger, PI
    b. Douglas Wright, co-I
    c. Mamatha Pasnoor, co-I
    d. Andrea Nicol, co-I
    e. Michelle Vitztum, regulatory and study coordinator
    f. Miranda McMillan, study coordinator
    g. Janelle Ryals, lab manager and study coordinator
    h. Josh Miller, study coordinator
    i. Daniel Elliott, lab technician and study coordinator
2. Primary responsibility for the following activities, for example:
    a. Determining eligibility: PI, co-Is, and study coordinators
    b. Obtaining informed consent: PI, co-Is, and study coordinators
    c. Providing on-going information to the study sponsor and the IRB: Study coordinators
    d. Maintaining participant's research records: Study coordinators
    e. Completing physical examination: Co-Is and resident
    f. Taking vital signs, height, weight: CTSU Nursing Staff, PI, co-Is, and study coordinators
    g. Drawing / collecting laboratory specimens: CTSU Nursing Staff
    h. Performing / conducting tests, procedures, interventions, questionnaires: PI, co-Is, and study coordinators
    i. Completing study data forms: PI, co-Is, and study coordinators
    j. Managing study database: PI, co-Is, and study coordinators

L. Assessment of Subject Safety and Development of a Data and Safety Monitoring Plan

1. Elements of the plan include:
    a. Persons/groups who will review the data (study team; independent safety monitor, data monitoring committee or formal DSMB): The PI or Co-Is will monitor AEs monthly, grade them, and indicate if the AE is related to the study procedure (definite, probably, possible, unlikely, not related). For each AE, the PI will determine if the study procedure should be continued. Patients will be instructed to call the PI's office (or on-call resident/coordinator if at night or on the weekend) to report events that occur between study visits. All SAEs will be reported to the HSC and Clinical Research Center (if applicable) and FDA guidelines, as soon as possible, but no later than five working days.
    b. Data/events that will be reviewed: Any serious adverse events (SAE) will be reviewed as soon as possible and reported to the IRB if applicable.
    c. Frequency of review: Data safety will only be monitored if necessary (unusually high frequency of adverse events (AE) or at any SAE).
    d. Types of analyses to be performed: N/A
    e. Safety-related triggers that would cause the PI to stop or alter the study: N/A - Study activities are considered low risk and any adverse symptoms will be treatment promptly, including temporary removal from heat treatment. Will assess if further safety-related triggers need to be identified as needed.
2. Nursing 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. AEs will be classified as follows: related to study procedure as determined by the PI or unrelated to study procedure (related instead to underlying disease, pre-existing conditions, or other factors), anticipated (included in consent form) or unanticipated (not included in consent form), serious (SAE, Grade 4 using CTCAE v3.0) or not serious (AE, Grade 1, 2 or 3 using CTCAE v3.0)
3. AEs that are not related to study procedures or are otherwise considered anticipated, low-risk events (ie: bruising or bleeding at injection site) will be monitored and recorded, but not necessarily reported to the medical monitor or the HSC unless frequency of event is considered unanticipated.
   Any AEs that are related to study procedure, are unanticipated, and are considered serious will be reported to the medical monitor and to HSC. A report that summarizes the frequency of all AEs will be provided to the medical monitor as needed. If a patient experiences a serious adverse event, they will be discontinued from the study.

III. Subject Participation

A. Recruitment:

1. Participants will be recruited for this study through flyers posted at the University of Kansas Medical Center, in the community, and electronically via websites and email (included in appendix). Subjects may also be recruited from Drs. Pasnoor's and Nicol's clinics. Michelle Nguyen will utilize the KU Diabetes Institute to identify potential participants from ongoing research studies. We will also utilize the Frontiers Research

Participant Registry after approval from the Data Request Committee (DRC). Participants will be offered a $25 for completing Visits 1, 2, 15 and 16; and $15 for completing Visits 3-14; via ClinCard after completing each of the visits ($280 total) to aid with time and transportation.

2. Michelle Nguyen and Miranda McMillan will perform the majority of recruitment. Individual mailings and phone calls will be made to applicable subjects from the Frontier's Research Participant Registry.
3. Attached.
4. Attached.

B. Screening Interview/questionnaire: Attached.

C. Informed consent process and timing of obtaining of consent

1 PIs and/or study coordinators authorized to review Informed Consent

2 Subjects will be given a copy of the consent form when their first visit (pre-intervention evaluation) is scheduled. This will give them ample time to review and prepare questions for the study team before their visit. The consent form will be reviewed with the subject by the PI and/or study coordinator in a private room at the CTSU at the start of their first visit. The subject will have the opportunity to ask any and all questions to the coordinator, or an investigator if requested, before signing. A signed copy will be given back to the subject.

3 All subjects should be English speaking, of consenting age (> 18 years old) and of sound mind (no cognitive or decisional impairment). If there is any question of this, the study coordinator will have an investigator assess ability to consent.

D. Alternatives to Participation: The consent form includes the following statement: "Research is voluntary, and you may change your mind at any time. There will be no penalty to you if you decide not to participate, or if you start the study and decide to stop early. Either way, you can still get medical care and services at the University of Kansas Medical Center (KUMC).

The consent form explains what you have to do if you are in the study. It also describes the possible risks and benefits. Please read the form carefully and ask as many questions as you need to, before deciding about this research.

You can ask questions now or anytime during the study. The researchers will tell you if they receive any new information that might cause you to change your mind about participating."

E. Costs to Subjects: There will be no cost to the subject for participation in the study, including all labs and procedures.

F. How new information will be conveyed to the study subject and how it will be documented: Michelle Vitztum, Miranda McMillan and Josh Miller will review screening results (laboratory) with subject only to assess eligibility for continuation. Drs. Pasnoor or Nicol will further discuss screening results with the subject by request.

G. Payment, including a prorated plan for payment: Participants will be offered a $40 for completing Visits 1, 2, 15 and 16; and $20 for completing Visits 3 - 14; via ClinCard after

completing each of the visits ($400 total) to aid with time and transportation. Subjects will not continue to receive payments after they've withdrawn from the study.

H. Payment for a research-related injury: We will include the following text in the consent form: "If subjects have a serious side effect or other problem during this study, they should immediately contact Dr. Mamatha Pasnoor at (913) 588-6970. If it is after 5:00 p.m., a holiday or a weekend, subjects should call (913) 588-5000 [24-hour phone number], and this number will contact Dr. Pasnoor. A member of the research team will decide what type of treatment, if any, is best for them at that time. If the subjects have a bodily injury as a result of participating in this study, treatment will be provided for them at the usual charge. Treatment may include first aid, emergency care and follow-up care, as needed. Claims will be submitted to their health insurance policy, their government program, or other third party, but they will be billed for the costs that are not covered by the insurance. The subjects do not give up any legal rights by signing this form."

IV. Data Collection and Protection

A. Data Management and Security:

1. Paige Geiger, Douglas Wright, Mamatha Pasnoor, Andrea Nicol, Michelle Vitztum, Miranda McMillan
2. Following a phone call pre-screening, potential subjects will be emailed or mailed the consent form prior to the first visit and the consent discussion. Pre-screening data will be securely kept via RedCAP. Consent forms will be kept in a separate folder from subject study worksheets, and kept in a secure, locked location at the University of Kansas. All subject study worksheets, labs, and biopsies will be de-identified and marked only with a subject ID. This information will be kept for 15 years according to the KUMC Records Retention Policy.
3. Through coded information.
4. Only the study team will have access to the key to the code.
5. Subjects will be assigned a study ID and this number will be used to follow subjects throughout the study. Only the consent forms (housed separately from the study worksheets) will be able to link identifiable data to study IDs.
6. Paper data (consents, study worksheets) will be stored in a designated and locked storage cabinet, to be overseen by the PI, co-Is, and study coordinators. No one except for the study team will have access to the storage cabinet. Electronic data will be stored on a KUMC-supported network drive (S- or P-drive) and in a RedCAP database.
7. N/A
8. N/A

B. Sample / Specimen Collection: Blood will be collected at the CTSU by nursing staff and analysis of blood samples will be performed externally my Quest Diagnostics.

C. Tissue Banking Considerations: N/A

D. Procedures to protect subject confidentiality: Subject confidentiality will not be at risk during any time in the study.

E. Quality Assurance / Monitoring

1. The data to be collected in this study will be reviewed in a timely fashion by the study team. We will provide self-assessments on a regular basis to determine that the data values are within normative values.
2. No

V. Data Analysis and Reporting

A. Statistical and Data Analysis: To understand how heat therapy impacts glucose control and sensory function in prediabetic patients with or without neuropathy, we compare pre-treatment versus end-of-treatment measures of fasting glucose and neuropathy measures using intraepidermal nerve fiber (IENF) density as our two primary outcome measures. We will also make comparisons between secondary outcome measures such as the Utah Early Neuropathy Scale (UENS), Quantitative Sensory Testing (QST), blood and metabolic biomarkers. As pre- and post-treatment measurements are available for each patient, a two-sided paired t-test will be used to test the null hypothesis of no difference in conductance between pre- and post- conductance measures. As the paired t-test assumes normally distributed data and because of the modest number of subjects in this pilot study (e.g., N = 20), we will also explore the use of non-parametric methods for testing the aforementioned null hypothesis, such as a Wilcoxon signed-rank tests and/or a randomized-based test. We will also introduce variables for key clinical characteristics of the patient (e.g., patient age, sex, ethnicity, disease severity, etc.) into the linear mixed effects model and formally statistically test whether there is a significant interaction between these variables and time. In other words, if there a significant relationship between the temporal trajectory of conductance over the course of treatment and patient age at baseline.

B. Outcome: The hypothesis will test whether prediabetic patients with or without neuropathy exhibit any improvements in metabolic symptoms and peripheral nerve mitochondria, leading to improvements in sensory function, via heat shock protein induction.

C. Study results to participants: Laboratory results and medical evaluations will be given to the subject if they request it.

D. Publication Plan: Data from this intervention will be published by Drs. Geiger, Wright, Pasnoor, and Nicol in appropriate journals, likely focusing on diabetes and/or neuropathy and pain. Publications will be developed in a timely fashion and are the responsibility of the PI and co-Is.

VI. Bibliography / References / Literature Cited

1. Juster-Switlyk K and Smith AG. Updates in diabetic peripheral neuropathy. F1000Research 2016; 5(F1000 Faculty Rev):738.
2. Rodica-Popsui et al. Diabetes Care 2017; 40:136–154
3. Fernyhough P. Mitochondrial dysfunction in diabetic neuropathy: a series of unfortunate metabolic events. Curr Diab Rep. 2015; 15(11):89.
4. Krause M, et al. Heat shock proteins and heat therapy for type 2 diabetes: pros and cons. Curr Opin Clin Nutr Metab Care. 2015; 18(4):374-80.

APPENDIX I: VULNERABLE POPULATIONS

I. The recruitment plan will not include any of the vulnerable groups below, except possibly employees of the University or Health System.

II. Cognitively or decisionally impaired individuals: N/A

III. Children: N/A

IV. Pregnant women: N/A

V. Prisoners: N/A

VI. Students and/or Employees:

A. N/A

B. Our study will not specifically target employees of the University or Health System. However, it is not outside the realm of possibility that employees may contact the study team for pre-screening after having seen recruitment postings or that they are not consenting participants of the Frontiers registry.

C. N/A