Passive heating as an accessible and tolerable strategy to improve the inflammatory profile and cardiometabolic health in people with spinal cord injury

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Significance

Persons with spinal cord injury (SCI) are at an increased risk for T2DM and CVD compared to able-bodied (AB) individuals. [3] This may be related to factors directly associated with the injury, such as the loss of muscle mass and autonomic dysfunction.[2] It is, however, increasingly recognized that the physical inactivity among people with SCI is but one of several contributors to the increased risk of CVD compared to the AB population. [20] An increase in absolute[21] and relative[22] total body fat (visceral adipose tissue in particular),[4] occurs as a result of a chronically positive energy balance and contributes to other disorders associated with poor glucose utilization and endothelial dysfunction.[23] Furthermore, structural adaptations of *large conduit* arteries occur as soon as 3-6 weeks after SCI.[6,7] Specifically, resting diameter of the common femoral artery is 30-50% smaller,[7,24] limb blood flow is reduced by 30-40%[25,26] and responses to reactive ischemia decreased.[24,27] One year post-injury, arterial stiffening is documented,[28,29] which is an independent risk factor for CVD.[30,31] The etiology of such changes is yet to be elucidated but chronic inflammation has been proposed.[15]

Cardiometabolic disorders such as T2DM and CVD **share a common and likely causative etiology; chronic low grade inflammation**.[4,32,33] Indeed, the **increased concentration of circulating pro-inflammatory factors** such as interleukin-6 (IL-6), C-reactive protein (CRP) and tumor necrosis factor-α (TNF- α) in persons with chronic low-grade inflammation **can directly interfere with insulin signaling**[4], potentially through inhibition of C-Jun N-Terminal Kinase (JNK). [34] *Animal studies show that a reduction in markers of inflammation, such as c-Jun N-Terminal Kinase (JNK) activity as well as TNF-α expression, enhances insulin sensitivity.*[34-36] For CVD, these **pro-inflammatory cytokines, facilitate the infiltration of macrophages through the vascular wall to form atherosclerotic plaques**,[37,38] which compromises vascular integrity. In mice, gene knockout studies provide evidence that the actions of TNF-α lead to an increase of plaque formation.[39] This aligns with observational epidemiological data that show a positive association between pro-inflammatory markers and future CVD events.[40]

Individuals with SCI have chronically elevated serum concentrations of pro-inflammatory markers (e.g.; CRP, IL-6 and TNF-α) compared with able-bodied (AB) individuals.[3,5,41-43] Reasons for this are not fully elucidated but two theories include 1) The increase in visceral fat that leads to an increased production of pro-inflammatory cytokines by adipose tissue and 2) The decrease in sympathetic activity after SCI leads to an attenuation of lipolysis, dyslipidemia, and increased macrophage infiltration in adipose tissue, which contribute to chronic inflammation.[23] In addition, after SCI, **endothelial function**[15] of the brachial artery, an important risk factor for CVD, **and glucose tolerance**[44] **is impaired compared to AB individuals**. As an intervention, exercise training can serve as a non-pharmacological, low-cost intervention to reduce chronic low-grade inflammation.[10] Its positive effects may be related to the anti-inflammatory environment created in the circulation following each exercise session, characterized by an **immediate increase in IL-6 concentration that in turn stimulates the sustained elevation of anti-inflammatory cytokines, IL-1ra and IL-10**.[10]

It should be noted that IL-6 is a pleiotropic cytokine, having both pro-and anti-inflammatory properties as well as affecting metabolism. **Chronic elevation of IL-6 concentration is associated with chronic diseases** such as T2DM and inflammatory conditions such as rheumatoid arthritis.[45] In contrast, the **acute, transient increase in IL-6 concentration as observed following exercise has predominantly metabolic and anti-inflammatory effects**, as evidenced by the **accompanied increase in the concentration of anti-inflammatory cytokines** IL-1ra and IL-10 **in the absence of changes in pro-inflammatory cytokines** TNF-α and IL-1β.[14] Furthermore, **transient elevations of IL-6 concentration have been shown to stimulate fat and glucose metabolism**.[46,47]

It should further be pointed out that, exercise in the heat results in a greater inflammatory (i.e.; acute IL-6 increase) response when compared with exercise in thermoneutral or cold conditions.[48-52] Moreover, clamping core temperature (Tcore) by cycling in cold water can abolish the acute IL-6 response.[51] The amplified acute cytokine response following exercise in the heat may be partly

mediated by the increased plasma catecholamine concentrations[51] and carbohydrate utilization when compared with exercise in thermoneutral or cold conditions.[53] In conclusion, **the increase in body temperature plays a large role in the exercise-induced acute inflammatory response**. Interestingly, similar to exercise, **heat stress raises antegrade shear stress and, importantly, it also reduces retrograde shear stress**.[54,55] More specifically, in the femoral artery, passive heating can increase antegrade shear stress on the endothelium by a greater magnitude than can exercise.[56] Thus, the rise in Tcore alone (in absence of exercise) not only has anti-inflammatory, but also vascular health benefits.

Despite the appeal of exercise to lower the risk for chronic disease, the **reduced physical capacity of persons with SCI and their inability to achieve maximum workloads**[13] **comparable to AB persons, often precludes or limits participation in regular exercise**.[57] Additionally, persons with SCI do not demonstrate the same positive acute and chronic physiologic responses to exercise. For example, the acute increase in IL-6 following exercise is dampened in people with a cervical SCI,[12] (possibly due to decreased lean muscle mass) potentially interfering with its anti-inflammatory benefits. In addition, we have demonstrated that a seven-day exercise program in the heat does not lead to typical heat acclimation adaptations (i.e.; decreased core temperature and heart rate) in persons with SCI, possibly due to autonomic dysfunction.[13] In conclusion, considering the limitations of the ability to exercise, the ability to achieve similar workloads to AB persons, and the lack of anti-inflammatory and physiologic benefits of exercise in the SCI population, **alternative interventions that increase body temperature and provide the same anti-inflammatory and cardiometabolic benefits are highly warranted**.[9] Passive heating, a practical and accessible intervention even for those at the lowest end of the physical capacity-spectrum, such as post-SCI,[14] may be such a therapy.

Innovation

Several methods exist to passively elevate body temperature in humans. Most stem from ancient cultures worldwide, such as the Romans and ancient Scandinavian and Asian cultures. Sauna bathing and hot water immersion (HWI) are most widely studied. While both demonstrate improvements in vascular health in AB persons, emerging evidence is showing anti-inflammatory effects as well. A brief overview of evidence is presented in Table 1 however, for an exhaustive list of studies see Hoekstra et al. 2020.[9] The current status of the field of passive heat therapy to improve inflammation and cardiometabolic health is now discussed followed by gaps in the SCI literature and how we intend to test its impact in persons with SCI.

Table 1. Summary of a few passive heating studies in AB (see Hoekstra et al[9] for exhaustive list of AB studies) and of *all studies* (n=3) in persons SCI (in grey shade) that report inflammatory, glucose metabolism and vascular health outcomes. Note, only 3 studies of acute responses to passive heating have been performed in persons with SCI and no studies have examined the chronic responses to repeated passive heating sessions over a longer term.

			OUTCOMES		
Author	**Population**	**Intervention**	**Inflammation**	**Glucose Metabolism**	**Vascular Health**
ACUTE (one heat stress session)					
Laing et al.[50]	AB (n=13)	2h HWI	Acute increase in IL-6, IL-10, IL-1ra and Hsp72	NT	NT
Oehler et al.[58]	AB (n=12)	2h HWI	Acute increase in IL-6, IL-10, IL-1ra and Hsp72	NT	NT
Hoekstra et al.[16]	AB (n=10)	60min HWI	Acute increase in IL-6	lower fasting glucose and insulin	Increase NO bioavailability

Hashizaki et al.[59]	**SCI (n=19)**	60min hot water perfused suit (lower body)	Acute increase in IL-6	**NT**	NT
Leicht et al.[14]	**SCI (n=7)**	60min HWI	Acute increase in IL-6, IL-8 and IL-1ra	**NT**	NT
Coombs et al.[15]	**SCI (n=15)**	60min HWI (lower limbs only)	NT	**NT**	1.elevated CD62e+ 2.unchanged macrovasculature AND microvascular function
CHRONIC (repeated)					
Brunt et al.[17]	AB (n=12)	8 wks. of 60min HWI (4-5x/wk.)	increase in Hsp90	NT	improved microvascular function
Ely et al.[60]	AB (n=18)	8-10 wks. of 60min HWI (3-4x/wk.)	decrease in CRP	NT	1.improved FMD 2. decreased carotid and femoral artery thickness
None to date in SCI	**SCI**	**?**	**?**	**?**	**?**

Acute responses to passive heating

Important in the context of passive heating, the acute inflammatory response after exercise is greater when exercising in the heat compared with thermoneutral conditions.[50] Recent studies have built on this notion by investigating whether passively elevating body temperature can also acutely induce the production and secretion of inflammatory markers. Laing et al[50] and Oehler et al[58] showed that 2h of HWI leads to an increased plasma concentration of IL-6, IL-10, IL-1ra and heat shock protein 72 (Hsp72). In search for a more tolerable, shorter and applicable passive heating method, Hoekstra et al. showed that *just 60 min* HWI also increases plasma concentrations of these markers.[16] Therefore, the **acute responses to passive heating suggest that, in keeping with exercise, this strategy also has potential to reduce chronic low-grade inflammation when engaging in it on a regular basis.**

Additionally, elevation of core body temperature increases nitric oxide (NO) production through enhanced NO synthase activity (NOS),[17,61] that is possibly mediated by increased expression of Hsp90.[62] (Figure 1.) **NO not only reflects endothelial function but also impacts glucose uptake within tissues.[63-66]** Furthermore, higher NO bioavailability has anti-inflammatory effects on human leukocytes[64] and increases the expression of iHSP72 in monocytes.[67] This suggests some signaling between NO and the immune system. Further, supporting the possible benefits of passive heating on blood vessel health, both sauna bathing and HWI can acutely increase blood flow in the brachial as well as femoral artery.[8] Indeed, Hoekstra et al. demonstrated that HWI can acutely elevate NO bioavailability in AB individuals.[16]

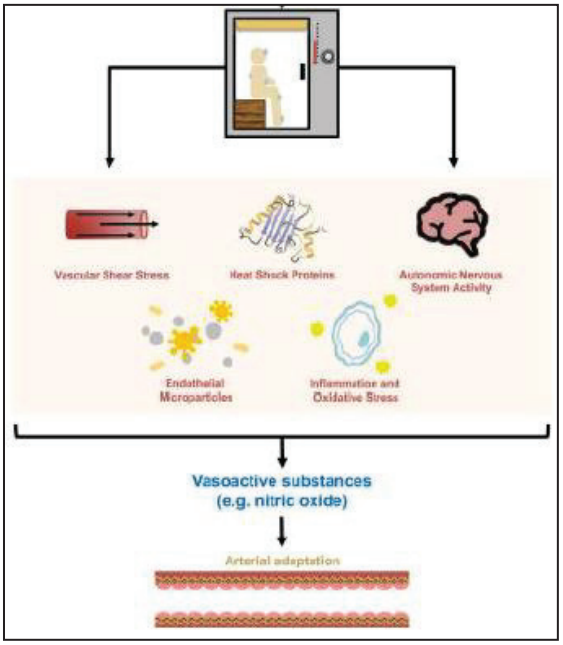


Figure 1. Potential mechanisms for the effect of heat stress on the vasculature [8]

In 2015, Leicht, et al. published the **first study <u>in persons with SCI,</u> showing that one episode of passive heating elicits an acute anti-inflammatory response** (increase in IL-6, IL-8 and IL-1ra) **comparable to that of AB persons.**[14] Hashizaki et al later confirmed this finding of increase in IL-6 in persons with SCI using another method of passive heating, a water-perfused suit.[59] Regarding vascular function effects of passive heating, Coombs et al[15] showed that a **single** 60 minute session of hot water lower limb immersion at 40°C (rise in core temperature of 0.7°C) **acutely increased arterial blood flow** in the

femoral and brachial artery **in persons with SCI**. Furthermore, one passive heating session **reduced the circulating concentrations CD62+** microparticles to the levels found in healthy control participants. **As elevated circulating CD62+ microparticles indicate endothelial activation** and potentially inflammation and atherosclerosis at the vessel wall, repeated passive heating sessions may improve vascular health over the long-term. Importantly, these studies indicate that **passive heating can be safely implemented in this population and may exert protective anti-inflammatory and vascular health effects**.

Despite the short-term/transient changes after *acute (one bout)* passive heating in AB and SCI persons, it is unknown how long these benefits are sustained and if the same responses are seen with *chronic* (*repeated bouts*) passive heating. Furthermore, *acute* changes in inflammation from one passive heating session are likely insufficient to decrease long-term risk of DM and CVD, and their associated morbidity and mortality in persons with SCI. Coombs et al concluded by suggesting the following: **"the effects of long term controlled heating (in SCI persons) warrant further testing."**[15] This proposal aims to pursue this recommendation.

Chronic responses to passive heating

There exist **limited data on the effects of a *chronic* repeated (2-8 weeks) passive heating** intervention on cardiometabolic health in AB individuals, let alone in persons with SCI, where there is none. Nonetheless, the available AB studies show promise. For instance, **eight weeks of passive** heating (HWI 4-5x/week to maintain rectal temperature >38.5°C for 60 min/session) in young, healthy participants **elevates the expression of the anti-inflammatory protein Hsp72 in peripheral blood mononuclear cells,** while also **improving endothelial function (assessed via local thermal hyperaemia)**.[17] Furthermore, in a pilot study to inform chronic interventions in persons with a disability, we showed that repeated **passive heating** (39°C for 1 h x 10 sessions over 2 weeks) **improves glucose metabolism** in sedentary, overweight AB males.[16] This finding was later corroborated in obese women with polycystic ovary syndrome, where 30 one-hour sessions of heat stress over 10 weeks significantly **improved flow mediated dilation (FMD) (i.e.; NO activity), decreased C reactive protein (CRP) and carotid and femoral wall thickness**.[68] Together, this provides tentative evidence that in **AB individuals, chronic repeated bouts of passive heating has the potential to improve the inflammatory profile, blood vessel health as well as glucose metabolism.** Whether persons with SCI experience these benefits is unknown.

In summary, passive heat stress appears to be an easily feasible and practical preventative health intervention with evidence to potentially improve underlying pathophysiology that leads to T2DM and CVD in persons with SCI. Whether persons with SCI receive similar benefits to AB persons during an intervention of *chronic* repeated bouts of a heat stress is unknown. At present, **no long-term/chronic intervention study** investigating the effects of **passive heating on immunological, metabolic and endothelial responses in people with SCI exists. This pilot proof-of-concept study is the first step** addressing such issues.

This study aims to address the current gaps in the literature and be the **first to test the impact of chronic passive heat therapy in persons with SCI** and the **first to test its impact on glucose metabolism** acutely and chronically. We will **test the hypothesis that *8 weeks* of a chronic passive heating method** of electrical heating blankets will **1) decrease chronic inflammation, 2) improve glucose utilization and 3) improve endothelial function in persons with SCI.** This proposed research project aims to take the first step to address a gap in the literature by providing proof-of-concept and preliminary evidence of therapeutic benefit.

Given HWI is more cumbersome and less feasible/practical for persons with paralysis from SCI, we will utilize a more practical and safe method of electrical heating blankets *and water perfused suits*, used extensively in our lab over the past 3 years for over 100 sessions of passive heat stress.[11] *If effective, these heating methods can be more easily implemented than HWI in clinical settings as well*

as in the home setting.

Significance to Veterans: The United States Veterans Health Administration (VA) provides primary and chronic care to over 27,000 patients with SCI and is the largest health care system in the world providing such persons` lifelong care. Medical care for the SCI population is improving and as such life expectancy of this population is slowly approaching that of persons without SCI, with cardiovascular disorders as the leading cause of mortality. Primary providers within the VA manage long-term sequela of chronic SCI such as CVD and type T2DM and would thus benefit from additional interventions to prevent such conditions. Indeed, in a population with many of its members unable to engage in regular exercise due to barriers such as a low physical capacity, the creation of an evidence-based alternative to improve cardiometabolic health can have a major impact. As passive heating can be performed by a practical accessible intervention, such as heating blankets, if effective, it can be widely implemented in clinical settings as well as in the home setting. On an individual level, passive heat therapy may reduce disease-risk, enhancing mobility and ultimately quality of life.

Preliminary Studies: This is a new study born out of mutual scientific interest and recently findings of studies conducted by the PIs and collaborators (Hoekstra and Leicht). Both of the involved labs have conducted studies related to heat therapy to promote health in non-injured individuals and persons with SCI, evidenced by multiple publications[12-14,16,69,70] Over the past 3 years of Dr. Trbovich's VA Career Development Award entitled "Vasomotor and sudomotor activity during heat stress in persons with SCI" has studied 24 persons with SCI (10 with tetraplegia and 14 with paraplegia) with passive whole body heat stress (core temperature rise of up to 1.2°C) using electrical heating blankets over approximately 100 sessions total. The proposed heat stress protocol using electrical heating blankets has not only been perfected over this time, but also been shown to be safe (i.e.; no significant medical complications such as burns, autonomic dysreflexia, hypotension) and tolerable as has also been seen in HWI protocols in SCI persons.[71] This proposed study is the first next step to evaluate the therapeutic impact of passive heat stress with Dr. Hoekstra who has expertise in studying the inflammatory outcome measures proposed.[14] Furthermore, Drs. Kellogg and Trbovich have conducted local thermal hyperaemia studies on over 10 persons with chronic SCI and demonstrated this to be safe in persons with SCI, without risk of burns in the protocol proposed.[72] The combination of experience with whole body passive heat stress protocols[11] in persons with SCI coupled with clinical SCI expertise from Dr. Trbovich, expertise in local skin heating protocols of Dr. Kellogg [72-74] and expertise in inflammation changes with passive heat therapy (via HWI) of Dr. Hoekstra[12] and Leicht,[14] make this team well poised to carry out this proposal. The ultimate goal of the team is to determine if this non-invasive, practical therapy can ultimately impact the underlying pathophysiology of the leading causes of morbidity and mortality in persons with SCI.

Research Design and Methods

Participants: Ten adults with sensorimotor complete (AIS A) SCI *levels C2-T6.*

Inclusion criteria: *1)* Spinal cord injury of >1 year of age; *2)*18-60 years old and; *3)* with a clinical history of sensorimotor complete (AIS A) SCI with level ranging from the C2 cervical level to the *T10 thoracic* level. All subjects with SCI will have their neurological level of injury (LOI) established by Dr. Trbovich at the time of enrollment via the International Standards of Neurological Classification after SCI (ISNCSCI) exam.[75,76] The "International Standards to document remaining Autonomic Function after Spinal Cord Injury (ISAFSCI)" form will also be filled out by Dr. Trbovich on all participants.[76]

Exclusion criteria: 1) Subjects who smoke; 2) Daily administration of anti-inflammatory medications; 3) Daily administration of vasoactive medications (e.g.; beta or alpha antagonists or agonists); 4) Current pressure ulcer or skin breakdown; 5) History of heat related illness (e.g.; heat stroke); and

6) Any active acute illness, 7) A resting haemoglobin concentration of less than 11 g/dl, as obtained from clinical records within 6 months of study enrolment or the baseline blood sample at Visit 1.

A schematic of the study protocol is given in Fig. 3. Outcome measurements will be collected at three time points: at baseline (BL), after 8 weeks without intervention (control=CON) and following *an 8-week* passive heating protocol (intervention=INT), allowing participants to serve as their own control. *We will ask participants to not make any major changes in exercise frequency or intensity or dietary changes (e.g.; switching to vegan diet) during participation. If they do for medical reasons (ie: doctors' recommendation) they need to inform us*

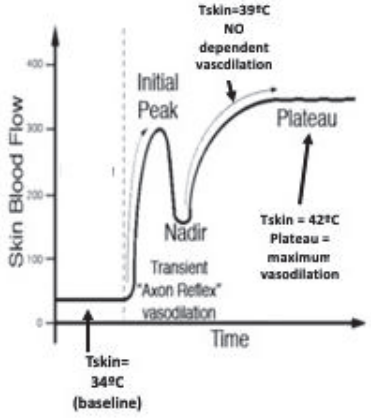
Baseline measurements

Visit 1 (V1)

(a). Inflammatory markers assessment: A resting blood sample will be collected to assess the expression of inflammatory markers in plasma (i.e. CRP, IL-6, IL-1β, TNF-α and IL-1ra) and circulating monocytes using flow cytometry (Hsp72 and TLR4), according to previously developed methods.[16] This group of inflammatory markers are strongly associated with impaired glucose utilization and vascular disease; moreover, mechanistic animal studies support their direct detrimental effects on insulin signaling and vascular health. As previously mentioned, IL-6 can have anti-inflammatory (transient increase after exercise/heat stress) and pro-inflammatory (chronic levels) effects. We will measure chronic inflammatory marker concentrations by measuring *before initial (V1), after control period (V3)* and *3 to 5 days after* the final (V29) heat stress session (see figure 3). Flow cytometry is a powerful method to phenotype specific immune cells. Using the surface markers CD14 and CD16, the expression of Hsp72 and TLR4 in one of the major sources of circulating inflammatory markers, namely monocytes, will be investigated.

Figure 2. Change in skin blood flow during local heating to 42°C

(b). Glucose metabolism testing: An oral glucose tolerance test (OGTT) will be conducted to assess glucose metabolism. After a 10-12 hour fast overnight, the participants will consume a 75-g glucose drink, where after blood samples will be collected at -10, -5, +15, +30, +45, +60, +90, +120, +150 and +180 min to measure glucose, insulin, C-peptide and glucagon. This method has been validated against the more invasive insulinemic - and glycaemic clamp methods and is widely used to test glucose tolerance.[77] BRU will be used for the OGTT and the blood draw for inflammatory and circulating vascular markers assessments. Together, a maximum of 200 ml blood will be drawn at Visit 1 (and Visit 2,3 and 29)



(c). Endothelial function testing: Human skin is an easily accessible tissue for studying both physiological and pathophysiological mechanisms of neural and endothelial control of blood vessels.[18,74,78] Changes in the skin circulation are similar to those of other vascular beds in hypertension, diabetes, and aging, thus making the skin an excellent model for human in vivo pathophysiological studies.[78,79] The application of skin-specific methods of study makes this vascular bed particularly useful as a safe model for in vivo studies. Endothelial function in this protocol will be assessed via nitric oxide (NO) dependent cutaneous vasodilation responses (local thermal hyperaemia) over the right ventral forearm *and right posterior calf* with local skin heating to 39°C[19] using laser Doppler flowmetry (LDF) to measure skin blood flow. The following equation will be used to measure cutaneous vascular conductance (CVC): CVC = LDF/mean arterial blood pressure). Values will be measured at 34°C (baseline), and 39°C (NO dependent vasodilation), then 42°C (maximum vasodilation) so the 39°C values can normalized to maximum CVC obtained at skin temperatures of 42°C.[18] (Fig. 2) Persons with SCI have tolerated this well in our lab without adverse events (e.g.: burns).[70,71]

(d) Evaluation of impact of PHS on mental health, general physical health, sleep, pain, mood and physical activity via 7 surveys as follows:

1. The International Spinal Cord Injury Pain Extended Data Set (Version 1.0)
2. 5-item Mental Health Inventory
3. Epworth Sleepiness (ESS)-Adult version

(e) Participants will be asked to report the foods and drinks consumed in the 24 hours prior to the visit using a food diary, which they will then use to replicate their diet in the 24 hours prior to Visit 3 and 29.

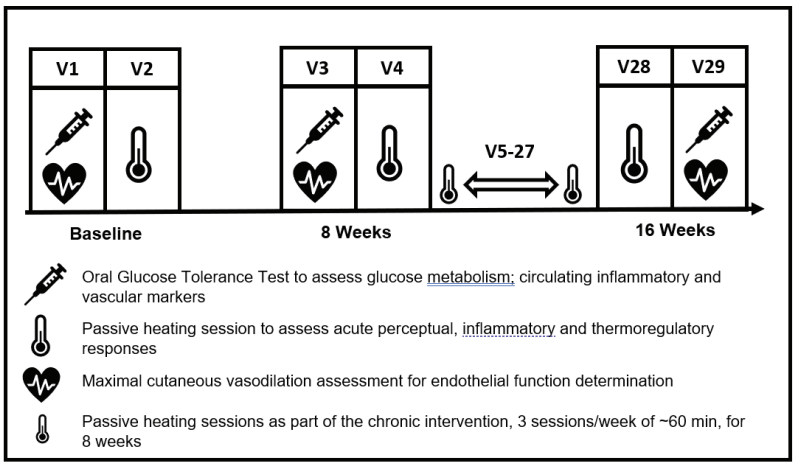


Figure 3. Schematic of the 16-week chronic passive heating protocol. Participants are their own control for the first 8 wks, followed by 8-wk passive heating intervention.

Visit 2 (V2) (within 1 week of V1)

Passive heat stress session 1: The acute thermo-physiological, perceptual and inflammatory response to passive heating will be assessed in this first passive heat stress session. To enhance the impact of the work and facilitate its implementation in practice, a highly accessible and tolerable method of passive heating (i.e. *electrical heating blankets and water perfused suits*) will be used. This method has been safely utilized on over twenty persons with SCI (~100 sessions) in our lab over the past three years. [11] *During a one-hour passive heating session, participants will rest in a supine position while a warm fleece lined electrical blanket set at 43°C is placed over insensate skin areas while a water perfused suit (water temp 42°C) is placed over sensate areas.* [11] *Area of ventral arm and post calf of endothelial function testing (Visit 1) will not have blanket or suit touching them during passive heat stress to enable the measurement of skin blood flow during the passive heat stress session, using laser Doppler flowmetry. Starch iodine test will be conducted over the arms, chest and lower limbs to measure the number of dermatomes with intact sweating as in our previous studies*.[11] Previous studies found beneficial impact on inflammation, metabolism and vascular health with core temperature (Tcore) rise of 0.7-1°C. *In our experience, induction of passive heat stress using electric heating blankets and water perfused suits, one hour is sufficient time period to elevate Tcore by 1°C.* During passive heat stress, Tcore will be measured by an oral temperature probe that is placed under the tongue and *Deep tissue temperature at the vastus lateralis will be measured using the validated* [80] *non-zero heat flux method (Bair Hugger, 3M, Minnesota, US).*[81] Skin temperature (Tskin) will be measured by local thermocouples over the forehead, chest, upper arm, upper and lower leg; blood pressure (BP) and heart rate (HR) will be measured via Portapres; and perceived thermal strain responses will be measured on a 9 point Likert scale from 0-8.[82]If Tcore rises above 39°C (sufficient Tcore to obtain heat therapy CVD benefits,[60] *while a Tcore >39.6°C increases the risk for heat illness*[83,84]*), Tcore rises 1°C, or the participant becomes uncomfortable prior to one hour, heating will cease.*

Control phase (CON): *Visit 3 (8 weeks* after V2) will repeat V1. BRU will be used for the OGTT and the blood draw for inflammatory and circulating vascular markers assessments. *Visit 4* (within 1 week of V3) will be the same as V2 excluding the blood draw.

Intervention phase (INT): *Visits 5-29*

The passive heat stress protocol will be identical to V2; *without blood collection and starch iodine test,* but *with* cardiovascular (i.e.; HR and BP) and temperature monitoring (oral) for safety. This will occur 3 times per week for *8 weeks*. On the final passive heating session *(V28),* repeat measurements will be obtained using the procedures described for V2 (except there will be no blood draw). The V29 procedures (within 2-7 days *of V28*[17]) will be identical to V1 (Fig. 3). BRU will be used for the OGTT and the blood draw for inflammatory and circulating vascular markers assessments.

Statistical Analyses: Hsp72 and TLR4 expression in monocytes will be assessed by subtracting the geometric mean fluorescence intensity of the appropriate isotype control from that of the antibody of interest. Inflammatory cytokine levels (CRP, IL-6, IL-1β, TNF-α and IL-1ra) will be compared between baseline, CON and INT values via repeated measures ANOVA. The same analysis will be conducted to test the acute change in inflammatory marker concentrations in response to a single passive heat stress session (Visit 2).Nitric oxide dependent vasodilation data will be analysed as follows: CVC at 39°C will be normalized to maximum CVC at 42°C and expressed as % CVC max at the arm and the leg. Ten blood samples collected during the 2h OGTT will be used to calculate the glucose and insulin incremental area under the curve (iAUC) by the trapezoid method, where a lowered iAUC signifies improved glucose tolerance**.** To test for differences between BL (V1-2), CON (V3-4) and INT *(V28-29)* visits, repeated measures ANOVAs will be conducted on all normally distributed data; while their nonparametric counterparts will be used when the assumption of normality is violated. *Effect of lesion level (i.e.: tetraplegia vs. paraplegia) on all outcome measures and effect of limb (arm vs. leg) on CVCs will be compared. Dermatomes with intact sweating on starch iodine test in V2 vs. V4 vs. V28 will be compared.*

Table 2. Study timeline

ACTIVITY	Year 1				Year 2			
Quarter (Q)	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Recruit and enroll 5 participants								
Begin and complete protocol for 5 participants								
Recruit and enroll 5 more participants								
Begin and complete protocol for 5 more participants								
Analyze preliminary data and start article writing								
Submit abstract to national meeting and paper for publication								

Future directions: This study is the first step needed to determine if repeated bouts of heat stress has potential reduce inflammation in persons with SCI. If this proof-of-concept study is deemed feasible and once power analysis provides a number of subjects needed for a larger trial, we will pursue a larger randomized control trial (via a funding mechanism such as an NIH RO1). Next steps would also include 1) Comparing the impact of passive heating with electrical blankets in the laboratory-controlled setting vs. home setting (to evaluate feasibility and efficacy in the most practical setting) on the same outcome measures, to see if similar results are obtained and 2) Measuring impact of long term/chronic passive heat therapy on the incidence of DM and T2DM and its associated morbidity and mortality. Finally, this work takes the first step towards evidence-based guidelines for the use of passive heat treatment to improve cardiometabolic health in individuals with SCI, comparable to the SCI-specific exercise guidelines as produced by international partners.[85]