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2019-03-01

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Coombs G., Otto Barak, Phillips A., Mijacika T., Sarafis Z., et al. 2019. Acute heat stress reduces biomarkers of endothelial activation but not macroor microvascular dysfunction in cervical spinal cord injury. American Journal of Physiology - Heart and Circulatory Physiology 316(3): H722–H733. doi: 10.1152/ajpheart.00693.2018.
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Am J Physiol Heart Circ Physiol 316: H722–H733, 2019.
First published December 21, 2018; doi:10.1152/ajpheart.00693.2018.

RESEARCH ARTICLE | *Integrative Cardiovascular Physiology and Pathophysiology*

Acute heat stress reduces biomarkers of endothelial activation but not macro- or microvascular dysfunction in cervical spinal cord injury

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Submitted 22 October 2018; accepted in final form 18 December 2018

Coombs GB, Barak OF, Phillips AA, Mijacika T, Sarafis ZK, Lee AH, Squair JW, Bammert TD, DeSouza NM, Gagnon D, Krassioukov AV, Dujic Z, DeSouza CA, Ainslie PN. Acute heat stress reduces biomarkers of endothelial activation but not macro- or microvascular dysfunction in cervical spinal cord injury. *Am J Physiol Heart Circ Physiol* 316: H722–H733, 2019. First published December 21, 2018; doi:10.1152/ajpheart.00693.2018.—Cardiovascular diseases (CVD) are highly prevalent in spinal cord injury (SCI), and peripheral vascular dysfunction might be a contributing factor. Recent evidence demonstrates that exposure to heat stress can improve vascular function and reduce the risk of CVD in uninjured populations. We therefore aimed to examine the extent of vascular dysfunction in SCI and the acute effects of passive heating. Fifteen participants with cervical SCI and 15 uninjured control (CON) participants underwent ultrasound assessments of vascular function and venous blood sampling for biomarkers of endothelial activation (i.e., $CD62e^+$) and apoptosis (i.e., $CD31^+/42b^-$) before and after a 60-min exposure to lower limb hot water immersion (40°C). In SCI, macrovascular endothelial function was reduced in the brachial artery [SCI: 4.8 (3.2)% vs. CON: 7.6 (3.4)%, $P = 0.04$] but not the femoral artery [SCI: 3.7 (2.6)% vs. CON: 4.0 (2.1)%, $P = 0.70$]. Microvascular function, via reactive hyperemia, was ~40% lower in SCI versus CON in both the femoral and brachial arteries ($P < 0.01$). Circulating concentrations of $CD62e^+$ were elevated in SCI versus CON [SCI: 152 (106) microparticles/μl vs. CON: 58 (24) microparticles/μl, $P < 0.05$]. In response to heating, macrovascular and microvascular function remained unchanged, whereas increases (+83%) and decreases (−93%) in antegrade and retrograde shear rates, respectively, were associated with heat-induced reductions of $CD62e^+$ concentrations in SCI to levels similar to CON ($P = 0.05$). These data highlight the potential of acute heating to provide a safe and practical strategy to improve vascular function in SCI. The chronic effects of controlled heating warrant long-term testing.

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NEW & NOTEWORTHY Individuals with cervical level spinal cord injury exhibit selectively lower flow-mediated dilation in the brachial but not femoral artery, whereas peak reactive hyperemia was lower in both arteries compared with uninjured controls. After 60 min of lower limb hot water immersion, femoral artery blood flow and shear patterns were acutely improved in both groups. Elevated biomarkers of endothelial activation in the spinal cord injury group decreased with heating, but these biomarkers remained unchanged in controls.

heat therapy; reactive hyperemia; tetraplegia; thermoregulation

INTRODUCTION

It is well appreciated that a spinal cord injury (SCI) can result in motor, sensory, and autonomic deficits. The loss of supraspinal sympathetic inputs to the heart commonly causes acute cardiac complications in SCI. Additionally, the risk of cardiovascular diseases (CVD) in chronic SCI is approximately threefold greater than the general population (18), and CVD account for ~40% of deaths in SCI (28). Moreover, the peripheral vasculature is also affected by many factors including the loss of sympathetic innervation, physical inactivity, and repeated exposures to autonomic dysreflexia (74). Because of extreme physical inactivity after SCI, rapid deconditioning of the vasculature occurs. For example, within weeks of SCI, the common femoral artery diameter and leg blood flow have been reported to both be ~40% lower compared with uninjured controls (CON) (19). Impaired macrovascular (71) and microvascular (53) functions have been reported below the level of injury in SCI, but previous studies have also found that flow-mediated dilation (FMD) of the femoral artery was preserved (67) or even increased (34) after thoracic SCI. Conversely, arterial structure and function are generally maintained above the level of injury (66, 71), possibly indicating that preserved physical activity and intact sympathetic innervation preserves

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macrovascular function of local conduit arteries. However, cutaneous microvascular function has also been reported to be impaired in both the upper and lower limbs in thoracic SCI (73), which could indicate dysfunction of the small arterioles that precedes impairment of the conduit arteries. In uninjured individuals, acute reductions in physical activity were associated with impaired FMD in the lower limb and endothelium-derived apoptotic microparticles (MPs) (10), highlighting a potential relationship between endothelial MPs and macrovascular function. However, the types and concentrations of circulating endothelium-derived MPs have not been characterized in SCI. The majority of previous studies have tested low-level SCI (i.e., lumbar or thoracic injuries); therefore, the extent of potential peripheral vascular dysfunction is currently unknown in high-level SCI (i.e., cervical injuries) in both the upper and lower limbs.

Regular exercise maintains or improves vascular function (20) and is dependent on greater levels of arterial shear stress (31, 44). Two main types characterize shear stress in arteries: antegrade, which is typically observed in physiologically forward-moving laminar flow, and retrograde, which is oscillating low flow typically observed in turbulent regions such as artery bifurcations. Similar to exercise, heat stress raises antegrade shear stress, and, importantly, it also reduces retrograde shear stress (61, 69). Indeed, it has been demonstrated that passive heating can increase antegrade shear stress on the endothelium by a greater magnitude than exercise (69) in the femoral artery. Several studies have reported both acute and long-term benefits of heat stress on important vascular measures, including blood pressure (12, 46), central artery stiffness (17, 68), FMD (15, 17, 61), microvascular function (14, 61), and MPs (i.e., lowered concentrations) (5). Moreover, recent evidence indicates that long-term repeated exposures to heat (i.e., sauna) is associated with lower fatal cardiovascular and all-cause mortality in middle-aged men (45). Passive heating, therefore, might be a useful strategy for improving vascular health in those with compromised exercise capacity, such as in SCI where physical activity levels are reduced (72a). In a recent randomized control trial, following the physical activity guidelines specific to SCI was reportedly insufficient to improve vascular health (72), suggesting that alternative strategies are needed to improve vascular function in SCI. The potential of heat therapy to improve vascular health has been demonstrated in various populations, including older individuals (61), peripheral artery disease (68), and heart failure (42), but limited testing of this approach has been performed in individuals with SCI. Yet, existing evidence suggests that the acute cytokine response to hyperthermia is intact in SCI despite attenuated adrenergic activation (47).

The aims of the present study were to determine the influence of cervical SCI on vascular function and whether the practical approach of lower limb heating has the potential to improve vascular function in SCI. It was hypothesized that individuals with SCI would display greater vascular dysfunction compared with uninjured CON participants and that acute responses to a single bout of heating would improve these outcomes in the SCI group.

MATERIALS AND METHODS

Approval of this study was obtained by the Ethics Committee of the School of Medicine at the University of Split, and all procedures conformed with the Declaration of Helsinki. Fifteen individuals with chronic cervical SCI and 15 sex- and age-matched uninjured (i.e., able-bodied) CON individuals were recruited. Participants in the SCI group were screened in accordance to the American Spinal Injury Association Impairment Scale (43). All participants provided written informed consent before completion of any data collection and were of Croatian nationality. Participant characteristics and medications are shown in Table 1.

Experimental design. Participants were instructed to arrive at the laboratory fasted for at least 6 h and well hydrated. The protocol comprised 60 min of passive lower limb heating in the supine position using a manually circulated water bath at 40°C (legs immersed to midcalf level, ~30 cm) and an electric heating blanket covering the upper body. This protocol was chosen to be comparable in length with previous studies (13, 24, 47) as well as a practical method of heating similar to Romero et al. (61). Considering the length of the protocol, participants with SCI rested supine with their knees flexed (~45°) and feet placed downward into the water bath to avoid hyperextension of

Table 1. *Participant characteristics, risk factors, and medications*

Category	SCI Group	CON Group	*P* Value
Sex (men/women)	10/5	10/5	
Age, yr	43 (12)	42 (11)	0.74
Mass, kg	72 (13)	80 (12)	0.12
Body mass index, kg/m^2	22 (3)	25 (3)	0.01
Time since injury, yr	21 (13)		
Level of injury/severity			
C3			
A			
B	2		
C			
C4			
A	1		
B			
C			
C5			
A	4		
B	1		
C	1		
C6			
A			
B	3		
C	1		
C7			
A	1		
B			
C	1		
Risk factors			
Total cholesterol, mg/dl	147 (27)	147 (38)	0.73
High-density lipoprotein, mg/dl	44 (13)	42 (10)	0.77
Low-density lipoprotein, mg/dl	85 (18)	90 (29)	0.69
Triglycerides, mg/dl	93 (43)	88 (46)	0.89
Glucose, mg/dl	86 (11)	89 (19)	0.62
Insulin, mIU/ml	9.8 (7.9)	9.1 (6.3)	0.91
HOMA-IR	2.1 (1.9)	2.1 (1.6)	1.00
Medications			
Corticosteroids (asthma)	1	1	
Alprazolam	1		
Acetylsalicylic acid		1	
Euthyrox		1	
Lyrica	1		
Tramadol	1		
Ventolin	1		
Baclofen	1		

Data are presented as means (SD). Spinal cord injury (SCI) severity was assessed using the American Spinal Injury Assessment impairment scale. Risk factors are based on $n = 11$ SCI partipants and $n = 15$ uninjured control (CON) participants. $P < 0.05$, difference between groups.

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the hips and consequent autonomic. Plastic covers were also placed over the feet and legs for appropriate skin protection. Before the heating procedure, participants rested supine for ≥20 min before baseline measures of peripheral vascular function. Immediately before immersion in the 40°C bath, baseline thermo- and hemodynamics were measured after 5 min of leg immersion in thermoneutral water (33°C) to minimize the effects of hydrostatic pressure on these measurements. Central hemodynamics as well as peripheral blood flow patterns were measured at baseline and every 15 min thereafter until completion of the heating. Tests of vascular function were repeated ~30 min after the end of heating.

Thermometry. Core body temperature was measured via telemetric pill (HQ, Palmetto, FL) ingested ≥2 h before data collection. Skin temperatures were measured using thermistor probes (AD Instruments, Colorado Springs, CO) adhered to the skin with medical tape on the lateral deltoid (arm) and on the medial calf (leg), which was immersed in the water bath and covered with Tegaderm (3M, St. Paul, MN). Thermal sensation was assessed with a modified visual analog scale (36) where −1 represents "slightly cool" and 5 represents "extremely hot."

Central hemodynamics. Heart rate was recorded via three-lead electrocardiogram, and beat-by-beat measurements of blood pressure were recorded using finger plethysmography (Finometer PRO, Finapres Medical Systems, Amsterdam, The Netherlands) backcalibrated to manual or automated cuff readings of the brachial artery (BA). Mean arterial pressure (MAP) was calculated as 2/3 × diastolic blood pressure + 1/3 × systolic blood pressure.

Ultrasonography. Peripheral hemodynamics were obtained simultaneously from the BA and superficial femoral artery (SFA) using duplex ultrasonography interfaced with a 10-MHz linear array probe (Terason uSmart 3300/T3200, Teratech, Burlington, MA). Artery diameter was measured in B-mode while simultaneous recording of peak blood velocity occurred in pulse-wave mode, with the insonation angle of 60° maintained constant. Approximately 1-min recordings, resulting in ≥20 cardiac cycles, were saved using screen capture software (Camtasia Studio, TechSmith, Okemos, MI) for future offline analysis using automated edge detection software (FMD/BloodFlow software, version 5.1, Reed C) (75). Regions of interest were placed around the highest quality portion of the B-mode longitudinal image of the artery, and a second region of interest surrounded the Doppler strip to record blood velocity. This software tracks the vessel walls and peak envelope velocity trace within their respective regions of interest at 30 Hz (75). Brachial and femoral blood flow were calculated from the 1-min ultrasound recordings as follows: flow = (peak envelope blood velocity/2) × [π (0.5 × diameter^2]. Brachial and femoral conductance were calculated as follows: conductance = flow/MAP.

Shear stress. Shear stress, in the absence of blood viscosity values, was estimated as shear rates from the product of (4 × peak envelope blood velocity/arterial diameter) (29, 64). Antegrade and retrograde shear rates are reported. The oscillatory shear index (OSI) was calculated as follows: |retrograde shear rates|/(|antegrade shear rates| + |retrograde shear rates|) (51). The OSI provides an indication of the degree of oscillations in blood flow between both antegrade and retrograde directions. Low mean and high retrograde shear have been associated with impaired endothelial function and atherosclerotic development (41, 49, 64).

Microvascular and macrovascular dilator function. Macrovascular dilator function was determined by endothelium-dependent FMD as detailed in previously published guidelines (63). Briefly, the artery was occluded (≥220 mmHg) for 5 min after a 1-min baseline period. Upon cuff release, data were collected for another 5 min. As mentioned above, peak diameter was automatically detected using a moving window smoothing function (smoothed median across time) after cuff deflation. FMD was calculated as the relative and absolute difference between peak and baseline diameter. The FMD stimulus was quantified as the shear rate area under the curve (SRAUC) from cuff deflation to peak diameter (58). Microvascular dilator function was determined via peak and total reactive hyperemia, where peak artery conductance was averaged in 1-s bins over the 5 min after cuff release. The maximum value was selected as the peak and the total AUC above baseline values was considered total hyperemia. The hyperemic response to a period of occlusion/ischemia is governed by the resistance of downstream vasculature, and the upstream increase in blood flow therefore provides an index of microvascular dilator capacity. Peak reactive hyperemia has been reported as a predictor of adverse cardiovascular events (2, 39).

Blood sampling. One 6-ml sample and three 5-ml samples of whole blood were drawn into vacutainers containing EDTA and sodium citrate, respectively, at baseline and at the end of heating via an intravenous catheter. The whole blood sample (EDTA) was processed immediately for hematological parameters (AcT 8 Hematology Analyzer, Beckman Coulter, Brea, CA). Sodium citrate samples were centrifuged at 1,550 *g* for 10 min at room temperature to separate and freeze plasma at −80°C for future batch analysis. Plasma metabolic biomarkers (i.e., glucose, insulin, lipids, triglycerides, etc.; see Table 1) were assessed using standard techniques at a clinical laboratory, and MPs were analyzed using flow cytometry (BD Biosciences FACSAria I High Speed Cell sorter and flow cytometer) as previously described in detail elsewhere (5, 26).

To characterize and quantify circulating MPs, plasma was centrifuged at 13,000 *g* for 2 min, and 200 μl were transferred to a TruCount tube (BD Biosciences). The endothelial MP phenotype was determined by incubating samples with fluorochrome-labeled antibodies (BioLegend, San Diego, CA) indicative of activation ($CD62e^+$) and apoptosis ($CD31^+/42b^-$) for 20 min at room temperature in a dark room. Samples were then fixed with 2% paraformaldehyde (ChemCruz Biochemicals, Santa Cruz, CA) and diluted with RNase-free PBS. The size threshold for MPs was established using MegamixPlus SSC calibrator beads (Biocytex, Marseille, France), and only events <1 μm in size and positively expressing markers of $CD62e^+$ and $CD31^+/CD42b^-$ were counted. Total numbers of circulating platelet, monocyte, and leukocyte-derived MPs were also counted using platelet-specific (CD62P, $CD31^+/42b^-$), monocyte-specific, and leukocyte-specific antibodies. Concentrations of MPs were determined using the following formula: [(number of events in a region containing MPs/number of events in absolute count bead region) × (total number of beads per test/total volume of sample)] (57).

Statistics. All cardiovascular and skin temperature measurements were sampled at 1,000 Hz using a digital-to-analog data-acquisition system (PowerLab 880, AD Instruments) interfaced with LabChart Pro software (version 7.2, AD Instruments) and saved for offline analysis. Data were extracted from LabChart as 5-min averages at each 15-min interval. Core temperature was monitored continually via a handheld device, and values were recorded at 15-min intervals throughout heating.

Baseline participant characteristics, vascular, hemodynamic, and temperature values were compared between groups with a Mann-Whitney *U*-test. All hemodynamic and thermodynamic values were compared with linear mixed-model analysis with a compound symmetry covariance structure (fixed factors: time and group, i.e., SCI vs. CON). Logarithmically transformed diameter values were entered into the model for allometric scaling of FMD, where baseline artery diameter and SRAUC were entered as covariates (3, 58). Corrected FMD values and SDs were back calculated from the linear mixed-model estimated means (EM) and SEs with the following equation: $[(e^{EM}-1) \times 100)]$. Pre- and postheating values for uncorrected FMD, MPs, and blood biomarkers were compared with a mixed-model ANOVA with the repeated factor of time. When significant interactions were detected, a Bonferroni correction was applied to multiple comparisons. Relationships between key variables were examined with simple linear regressions. All statistics were performed using SPSS (version 24, IBM, Armonk, NY) or GraphPad (version 6.0,

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Table 2. *Baseline vascular characteristics*

Group	Diameter, mm	Velocity, cm/s	Flow, ml/min	Conductance, ml·min^{-1}·mmHg^{-1}	Antegrade Shear, s^{-1}	Retrograde Shear, s^{-1}	Oscillatory Shear Index
Femoral artery							
SCI group	4.8 (0.8)	16.4 (10.8)	88 (58)	1.05 (0.71)	197 (95)	−44 (58)	0.17 (0.15)
CON group	6.5 (1.0)	9.7 (6.3)	98 (54)	1.12 (0.62)	124 (50)	−47 (23)	0.31 (0.18)
P value	<0.01	0.07	0.53	0.59	0.02	0.19	0.02
Brachial artery							
SCI group	3.5 (0.7)	27.6 (12.0)	83 (59)	0.97 (0.60)	355 (158)	−2 (5)	0.02 (0.04)
CON group	4.2 (0.8)	17.9 (10.1)	81 (58)	0.95 (0.74)	232 (105)	−12 (21)	0.05 (0.09)
P value	<0.01	0.03	0.98	0.89	0.07	<0.01	0.32

Data are means (SD) based on $n = 15$ spinal cord injury (SCI) participants and $n = 15$ uninjured control (CON) participants for femoral hemodynamics and $n = 13$ SCI participants and $n = 13$ CON participants for brachial hemodynamics.

Prism, La Jolla, CA). The level of statistical significance was set a priori at $P < 0.05$. All data are presented as means (SD).

RESULTS

Baseline variables. Participant characteristics and risk factors are shown in Table 1. SCI and CON groups were matched for age, but body mass index was significantly lower in SCI. Cholesterol, high-density lipoprotein, low-density lipoprotein, triglycerides, glucose, insulin, and homeostatic model assessment-insulin resistance (HOMA-IR) were not different between groups (all $P > 0.05$). Diameters of both the BA ($P < 0.01$) and femoral artery ($P < 0.01$) were significantly smaller in the SCI group by 26% and 17%, respectively, compared with the CON group (Table 2). Blood velocity tended ($P = 0.07$) to be higher in the femoral artery and was higher ($P = 0.04$) in the BA of the SCI group, whereas blood flow and conductance were not different between groups in either artery (all $P > 0.05$). Consequently, antegrade shear rates were higher in the femoral artery ($P = 0.02$) and tended to be higher in the BA ($P = 0.07$) in the SCI versus CON group. However, retrograde shear rates were greater in the CON versus SCI group in the BA ($P = 0.02$), whereas retrograde shear rates were not different between groups in the femoral artery ($P = 0.19$). Although the OSI in the BA was not different between groups ($P = 0.32$), OSI was greater in the CON group in the femoral artery compared with the SCI group ($P = 0.02$; Table 2).

Thermodynamics. Core temperature was 0.73°C lower during the resting baseline in SCI compared with CON ($P < 0.01$; Table 3) and increased to a greater extent in SCI than CON after 60 min of heat exposure [SCI: +0.68 (0.17)°C vs. CON: 0.34 (0.17)°C, interaction $P < 0.01$; Fig. 1]. Skin temperature on the leg increased in both groups during heating [SCI: +6.2 (2.9)°C vs. CON: +6.3 (1.6)°C] with no between-group difference (time $P < 0.01$, interaction $P = 0.59$). Skin temperature on the arm did not significantly change in either group with heating ($P = 0.18$). The heating protocol was well tolerated in both groups, with the SCI group reporting a thermal sensation of "warm" compared with "hot" in the CON group. There was a main group effect where thermal sensation was lower (i.e., felt colder) in the SCI group ($P = 0.04$). Core temperature data are absent from one CON participant because of connectivity issues with the telemetric pill, and skin temperature data are absent from two SCI participants and one CON participant because of technical difficulties.

Hemodynamics. Data for central and femoral hemodynamics are presented for all participants in both SCI and CON groups. Brachial hemodynamics are based on $n = 13$ for each group because of inadequate wall tracking of arterial diameters. Although heart rate increased slightly during heating in both groups (time $P < 0.01$, interaction $P = 0.17$), systolic blood pressure ($P = 0.41$) and MAP ($P = 0.18$) did not change in either group, whereas diastolic blood pressure decreased by 7 mmHg in the CON group only ($P = 0.02$; Table 3).

Femoral blood flow (time $P < 0.01$, interaction $P = 0.68$) and conductance (time $P < 0.01$, interaction $P = 0.61$) increased by ~100% after heating in both the SCI and CON groups. In the BA, blood flow (interaction $P < 0.01$) and conductance (interaction $P < 0.01$) increased with heating in the CON but not SCI group (Table 3). Femoral antegrade shear

Table 3. *Hemodynamic and thermodynamic values before and after heating*

	SCI Group		CON Group	
Category	Baseline	End heating	Baseline	End heating
Hemodynamics				
Heart rate, beats/min	55 (10)	58 (9)*	59 (11)	66 (9)*
Systolic blood pressure, mmHg	120 (18)	118 (24)	126 (21)	121 (26)
Diastolic blood pressure, mmHg	68 (16)	66 (17)	73 (15)	65 (18)*
Mean arterial pressure, mmHg	81 (10)	84 (15)	87 (12)	85 (10)
Brachial flow, ml/min	83 (59)	83 (44)	81 (58)	108 (51)
Femoral flow, ml/min	88 (58)	171 (81)*	98 (54)	199 (84)*
Thermodynamics				
Core temperature, °C	36.37 (0.62)	37.05 (0.63)*	37.10 (0.26)†	37.45 (0.24)*†
Skin temperature (shoulder), °C	32.12 (1.35)	32.67 (1.47)	32.84 (1.34)	32.85 (1.40)
Skin temperature (calf), °C	31.78 (1.19)	38.81 (0.91)*	32.48 (0.92)	38.73 (0.88)*

Data are means (SD). SCI group, spinal cord injury group; CON group, uninjured control group. *Different from baseline ($P < 0.05$); †different from the SCI group ($P < 0.05$).

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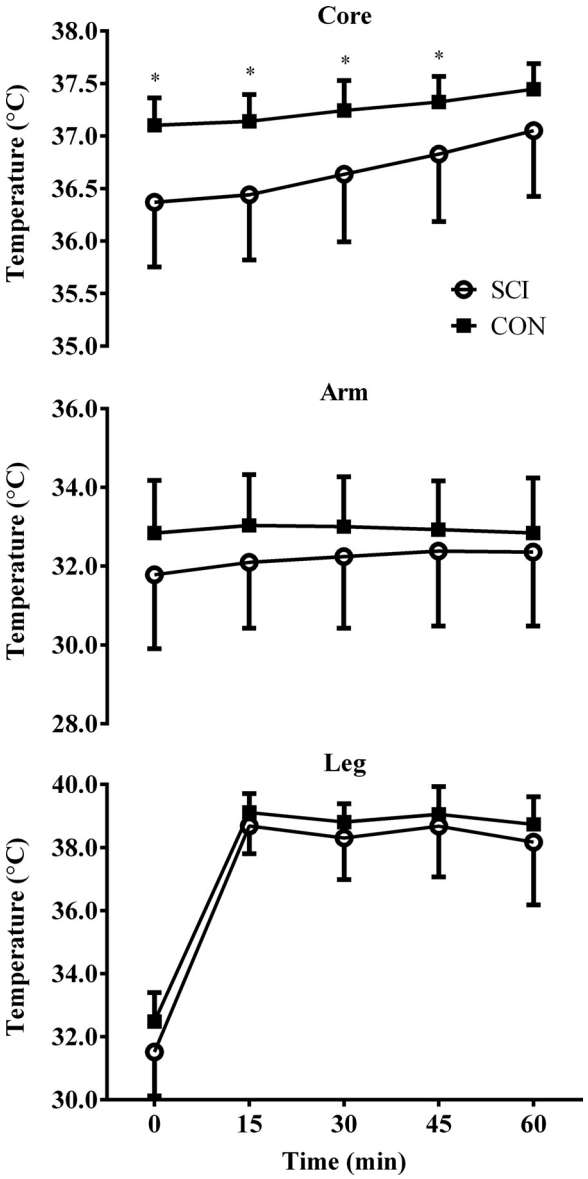

Fig. 1. Core (*top*), shoulder (*middle*), and calf (*bottom*) skin temperatures at 15-min intervals during lower leg hot water immersion (40°C). Data are based on $n = 15$ participants in the spinal cord injury (SCI) group and $n = 14$ participants in the uninjured (UI) group. *Statistical significance ($P < 0.05$). CON, uninjured control group.

rates increased with heating to a greater extent in the SCI versus CON group (+83% vs. +45%, respectively, interaction $P = 0.02$; Fig. 2). Similarly, greater increases in antegrade shear rates were also apparent in the BA of the SCI group with heating (interaction $P = 0.04$). Although retrograde shear rates in the femoral artery were reduced after heating in both groups, it was nearly abolished at 60 min of heating in the SCI compared with CON group [SCI: −3 (6) s^{-1} vs. CON: −23 (16) s^{-1}, time $P < 0.01$, group $P = 0.03$). Conversely, BA retrograde shear rates remained near zero in the SCI group throughout heating, whereas greater baseline retrograde shear in the CON group was attenuated with heating (interaction $P = 0.03$). The femoral artery OSI decreased similarly in both groups but was lower in the SCI versus CON group throughout heating (time $P < 0.01$, group $P = 0.01$). In the BA, the OSI was greater at baseline in the CON compared with SCI group and decreased with heating in the SCI group only (time $P = 0.02$, interaction $P = 0.07$).

Macro- and microvascular function. Five FMD/reactive hyperemia measurements were excluded from the SCI group because of poor wall tracking and/or because of involuntary muscle spasms. Three and four participants for the femoral artery and BA, respectively, were excluded from the CON group because of inadequate wall tracking or excessive angle shifts of the B mode image. There were no group differences in femoral FMD at baseline or after heating (interaction $P = 0.63$, Fig. 3). When corrected for baseline diameter and SRAUC there were no between-group differences in femoral FMD (interaction $P = 0.70$, Table 4). Conversely, BA FMD was not different between groups or after heating ($P = 0.27$), but it was 39% lower at baseline in SCI versus CON after correction for baseline diameter and SRAUC (group $P = 0.04$, Table 4). Brachial FMD did not change following heating in either group (interaction $P = 0.64$).

Peak reactive hyperemia (Fig. 4) was ~40% lower in the SCI group at baseline in both the femoral artery [SCI: 7.5 (3.8) $ml \cdot min^{-1} \cdot mmHg^{-1}$ vs. CON: 11.6 (2.9) $ml \cdot min^{-1} \cdot mmHg^{-1}$, group $P < 0.01$] and BA [SCI: 2.7 (1.1) $ml \cdot min^{-1} \cdot mmHg^{-1}$ vs. CON: 4.4 (1.6) $ml \cdot min^{-1} \cdot mmHg^{-1}$, group $P < 0.01$). Peak brachial conductance tended to increase with heating in the CON group only (time $P = 0.01$, interaction $P = 0.15$), whereas peak femoral conductance did not change with heating (interaction $P = 0.59$). The 5-min area under the curve of reactive hyperemia (Table 4) was not different between groups in either the femoral artery [SCI: 13.4 (7.3) ml/mmHg vs. CON: 15.2 (6.8) ml/mmHg, interaction $P = 0.92$] or BA [SCI: 7.6 (3.9) ml/mmHg vs. CON: 8.1 (4.9) ml/mmHg, interaction $P = 0.12$] and were unchanged with heating.

Microparticles. Pre- and postheating blood samples were unable to be obtained from five participants in the SCI group and three participants in the CON group because of difficulties with the intravenous catheter insertion. Baseline concentrations of total circulating platelet-derived MPs [SCI: 217 (105) MPs/μl vs. CON: 208 (201) MPs/μl, $P = 0.98$], monocyte-derived MPs [SCI: 147 (82) MPs/μl vs. CON: 110 (86) MPs/μl, $P = 0.46$], and leukocyte-derived MPs [SCI: 582 (174) MPs/μl vs. CON: 583 (310) MPs/μl, $P = 0.90$] were not different between groups and were unaffected by heating. Additionally, there was no between-group difference of total endothelium-derived MPs [SCI: 105 (102) MPs/μl vs. CON: 56 (22) MPs/μl, $P = 0.10$]; however, when participants taking medications were excluded, a difference between groups emerged [SCI: 152 (106) MPs/μl vs. CON: 58 (24) MPs/μl, $P = 0.02$]. Considering such an effect of medications, subtype analyses of endothelial-derived MPs were performed only on participants not taking medications. After stratifying for endothelial MP phenotype (i.e., activation vs. apoptosis) and removal of participants with potentially interactive medications, activation-derived MPs ($CD62e^+$; Fig. 5) were markedly elevated in the SCI compared with CON group [152 (106) vs. 58 (24) MPs/μl] and were reduced by 62% to values similar to the CON group after heating [58 (18) vs. 43 (42) MPs/μl, interaction $P = 0.05$]. Conversely, apoptosis-derived MPs ($CD31^+/42b^-$) were not different between groups at baseline [SCI: 39 (17) MPs/μl vs. CON: 62 (30) MPs/μl] and did not change

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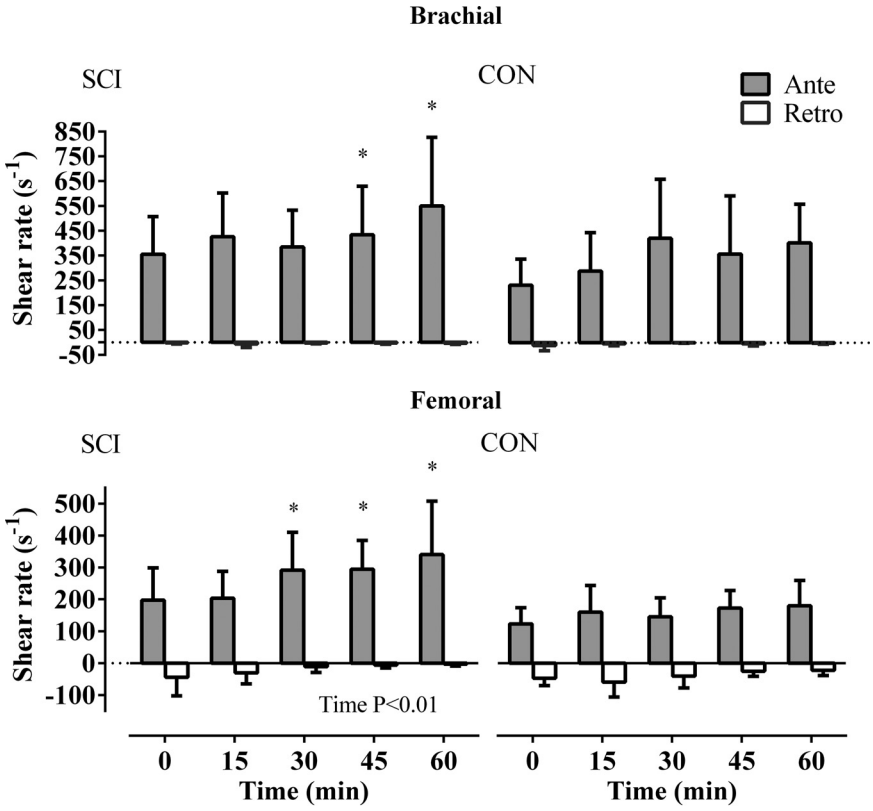

Fig. 2. Brachial (*top*) and femoral (*bottom*) artery shear rates at 15-min intervals during heating. Data are based on $n = 15$ participants in the spinal cord injury (SCI) group and $n = 14$ participants in the uninjured control (CON) group. *Bonferroni-adjusted pairwise comparison between SCI and CON groups ($P < 0.05$).

with heating [SCI: 52 (21) MPs/μl vs. CON: 68 (31) MPs/μl, interaction $P = 0.70$].

Metabolism. As previously mentioned, all blood-based biomarkers of cardiovascular risk factors were not different between groups (all $P > 0.05$; Table 1). After heating, however, in both groups, circulating triglycerides were lowered by ~15–20% (time $P < 0.01$), insulin was lower by ~25% (time $P = 0.03$), and therefore HOMA-IR was lowered by ~20–30% (time $P = 0.02$). Glucose levels were reduced with heating by 15% in the SCI group but did not change in the CON group (interaction $P = 0.03$).

Relationship between selected variables. Linear regressions were calculated in participants in which complete data for both MPs and shear rates were present (SCI: $n = 6$ and CON: $n = 10$). Negative correlations existed between the change in circulating $CD62e^+$ and the change in femoral retrograde shear rate ($P =$

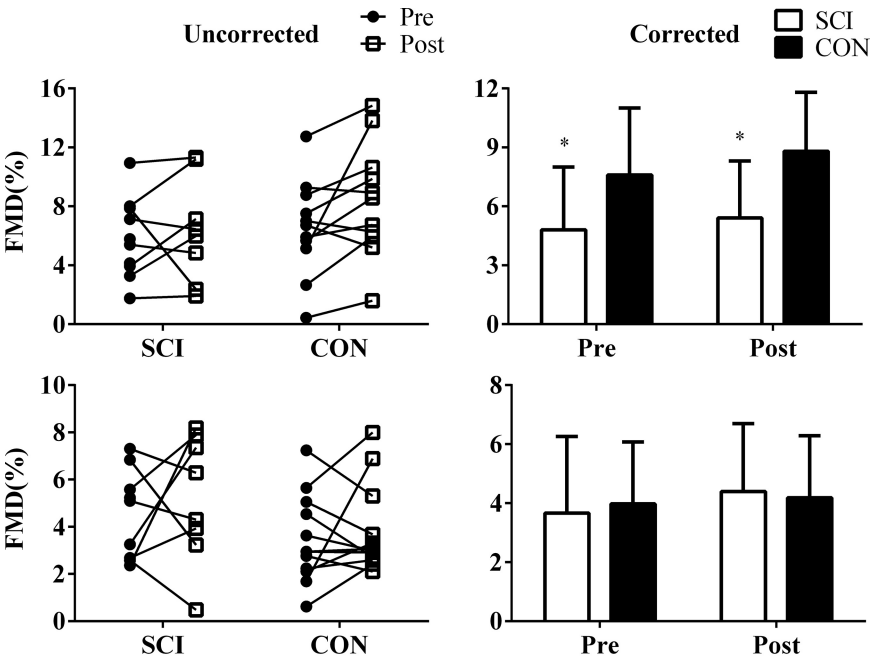

Fig. 3. Endothelium-dependent vasodilation measured via flow-mediated dilation (FMD) before and after 60 min of lower limb heating in the brachial (*top*) and femoral (*bottom*) arteries. Uncorrected individual data (*left*) and grouped data corrected for baseline artery diameter and shear rate area under the curve (SRAUC; *right*) are shown. Data are based on $n = 10$ participants in the spinal cord injury (SCI) group and $n = 12$ participants in the uninjured control (CON) group. *Different from the CON group ($P < 0.05$).

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Table 4. *Endothelium-dependent vasodilation measured via FMD*

Category	Preheat		Postheat		*P* Values		
	SCI group	CON group	SCI group	CON group	Time	Group	Interaction
Femoral artery							
Baseline diameter, mm	4.80 (0.71)	6.32 (0.92)	4.94 (0.71)	6.20 (0.87)	0.77	<0.01	0.10
Peak diameter, mm	4.99 (0.72)	6.54 (0.93)	5.19 (0.68)	6.43 (0.85)	0.89	<0.01	0.09
SRAUC	26,705 (12,885)	22,344 (8784)	44,330 (31,640)	24,473 (10,098)	0.01	0.08	0.05
Time to peak, s	73 (59)	77 (48)	97 (51)	70 (30)	0.45	0.45	0.19
FMD, %	4.2 (2.1)	3.5 (1.9)	5.2 (2.7)	3.8 (1.9)	0.26	0.17	0.63
Corrected FMD, %	3.7 (2.6)	4.0 (2.1)	4.4 (2.3)	4.2 (2.1)	0.41	0.95	0.70
Brachial artery							
Baseline diameter, mm	3.64 (0.64)	4.28 (0.73)	3.68 (0.65)	4.20 (0.76)	0.60	0.10	0.04
Peak diameter, mm	3.85 (0.63)	4.55 (0.75)	3.91 (0.65)	4.54 (0.77)	0.14	0.06	0.08
SRAUC	45,439 (16,385)	24,250 (10,130)	43,907 (24,585)	37,543 (12,835)	0.03	0.04	0.05
Time to peak, s	92 (22)	51 (15)	92 (42)	84 (34)	0.06	0.02	0.08
FMD, %	5.8 (2.7)	6.5 (3.3)	6.4 (3.5)	8.4 (3.8)	0.09	0.30	0.27
Corrected FMD, %	4.8 (3.2)	7.6 (3.4)	5.4 (2.9)	8.8 (3.0)	0.15	0.04	0.64

Data are means (SD) based on $n = 10$ spinal cord injury (SCI) participants and $n = 12$ uninjured control (CON) participants for the femoral artery and $n = 10$ SCI participants and $n = 11$ CON participants for the brachial artery. SRAUC, shear rate area under the curve; FMD, flow-mediated dilation.

0.02) and the OSI ($P = 0.05$), and there was a trend toward a relationship with the change in femoral antegrade shear rate ($P = 0.08$). No relationships existed with the BA. Moreover, no relationships existed between baseline MPs and age, body mass index, or FMD, nor did one exist between the change in MPs and the change in core temperature with heating.

DISCUSSION

The goals of this study were as follows: *1*) to determine the influence of chronic cervical SCI on vascular function and *2*) to determine the effects of acute lower limb heating on peripheral vascular function in SCI compared with healthy CON individuals. We found that macrovascular function (i.e., FMD) was selectively reduced in the BA but not SFA in those with SCI. In both the SFA and BA, downstream microvascular function measured via reactive hyperemia was ~40% lower in SCI participants versus CON participants. Additionally, circulating biomarkers of endothelial activation ($CD62e^+$) but not apoptosis ($CD31^+/42b^-$) were significantly elevated in SCI. In response to heating, macrovascular and microvascular function remained unchanged, whereas increases and decreases in antegrade and retrograde shear rates, respectively, were associated with reductions of endothelial activation (i.e., $CD62e^+$). The results of this study highlight the potential of acute heat therapy as a novel intervention to reduce biomarkers of endothelial disturbances in chronic SCI.

Conduit artery structure and function. The acute phase of SCI (i.e., 3–4 wk postinjury) is characterized by inward vascular remodeling reflected by smaller artery diameters below the level of injury (i.e., lower limb) (19, 34), whereas the diameter of arteries above the level of injury (e.g., BA) have been reported to be unchanged (19) or even increased (71). However, most of these studies tested participants who were paraplegic (i.e., thoracic injury, T1–T12). Our study recruited cervical (level of injury C3–C6) participants with SCI, and we observed that both SFA and BA diameters were lower in the SCI compared with CON group by 26% and 17%, respectively (Table 2). De Groot et al. (34) reported that SFA diameter and leg volume were reduced by ~25% within 3 wk after thoracic SCI, suggesting that vascular remodeling might be determined by metabolic demands of local tissues. Consistent with previous studies (19, 53), we also found that blood velocity was higher in SCI, and blood flow was thus preserved despite smaller artery diameters (Table 2). Because of the reductions in artery diameter and increases in blood velocity, elevated shear stress on the artery walls below the level of injury is widely reported in SCI (9, 19, 34, 67). Consistently, in the present study, antegrade shear rates in both the SFA and BA were higher in the SCI versus CON group (Table 2). Thus, despite structural remodeling, peripheral hemodynamics appear to be relatively well maintained in chronic SCI.

Exposure to elevated arterial wall shear stress and inward vascular remodeling have been speculated as potential mechanisms of preserved endothelial function in SCI (67). For example, Thijssen et al. (67) reported that mean wall shear rate in the superficial femoral artery was approximately four times higher in thoracic SCI versus CON groups. Moreover, in that study, both endothelium-dependent (i.e., FMD) and endotheli-

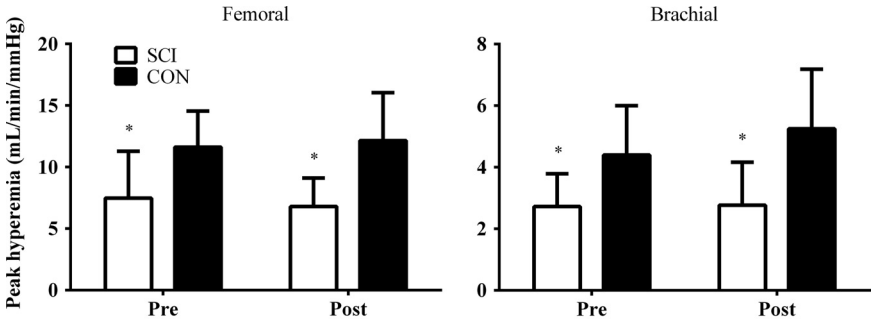

Fig. 4. Peak vascular conductance in the femoral (*left*) and brachial (*right*) arteries measured via Doppler ultrasound during reactive hyperemia before and after 60 min of lower limb heating. Data are based on $n = 10$ participants in the spinal cord injury (SCI) group and $n = 12$ participants in the uninjured control (CON) group. *Different from the CON group ($P < 0.05$).

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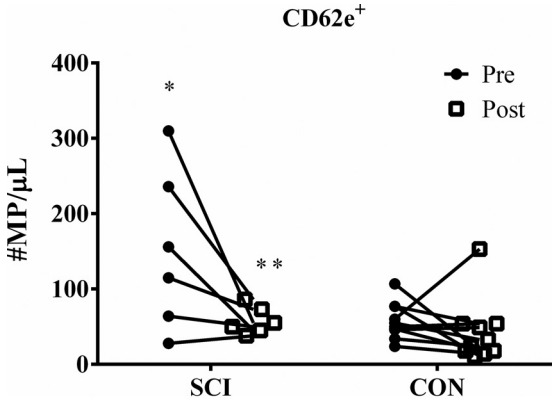

Fig. 5. Individual responses of circulating endothelial microparticles (MPs) before and after heat exposure. Shown are results for $CD62e^+$ (i.e., cellular activation; *left*) and $CD31^+/42b^-$ (i.e., cellular apoptosis; *right*). Spinal cord injury (SCI) group: $n = 6$ and uninjured control (CON) group: $n = 10$. *Different from the CON group ($P < 0.05$); **different from preheating ($P < 0.05$).

um-independent (i.e., responsiveness to sodium nitroprusside infusion) dilation of the femoral artery were not different in SCI compared with CON groups (67). These findings indicate that both endothelial function and smooth muscle cell sensitivity to nitric oxide were normal after thoracic SCI. Other studies have similarly reported that FMD is maintained after SCI (34, 35). However, when corrected for the potentially confounding influence of baseline diameter, FMD was lower in the SFA but not BA in the thoracic SCI group compared with the uninjured CON group (71). This observation contrasts with the present study, where we found that FMD was only lower in the BA in SCI (Fig. 3), but similar findings have previously been reported in low-level SCI as well (35). It is unclear why differences exist between studies; however, to the best of our knowledge, the present study is the first to measure both brachial and femoral FMD in cervical SCI participants. It is noteworthy that despite the propensity of the SFA to develop atherosclerosis compared with the BA, the BA has been demonstrated to provide predictive value of future CVD events (32, 40), whereas this link has not yet been tested in the SFA. Additionally, our study participants included chronic SCI (i.e., >2 yr, range: 2–44 yr), which might allow sufficient time for vascular remodeling to reverse any physiological impairments from the acute phase of injury despite complete inactivity of the lower limbs.

Microvascular function. In addition to macrovascular FMD, we measured reactive hyperemia after 5-min artery occlusion as an index of microvascular function (50). Similar to FMD, reactive hyperemia has been reported to predict cardiovascular events in both healthy (2) and at-risk groups (39, 48). We found that peak vascular conductance in both the SFA and BA after cuff release was ~40% lower in the SCI group compared with the CON group (Fig. 4), whereas the 5-min conductance area under the curve was not different between groups. The differences in peak but not total flow (i.e., area under the curve) suggest that microvascular function is impaired primarily because of the structure of resistance vessels (62) rather than an impaired production of vasodilating substances or resistance vessel responses to those substances (23). In support of our findings, several studies have reported impairments of microvascular function in SCI measured via both Doppler ultrasound of the femoral artery (53) and laser-Doppler flowmetry of the cutaneous microvessels (55, 56, 73). Importantly, Van Duijnhoven et al. (73) reported that microvascular function did not change after 8 wk of electrically stimulated cycling exercise in SCI (thoracic injuries). However, Nash et al. (53) observed that reductions in reactive hyperemia were offset in participants with cervical SCI who had participated in weekly sessions of electrically stimulated cycling for a more prolonged period (at least 5 mo and up to 7 years). These data demonstrate the potential for long-term improvement in vascular function in SCI. In the context of SCI, where macrovascular dysfunction is likely unchanged because of vascular remodeling and exposure to high shear stress, microvascular function might provide an important early insight into continued CVD risk in this population.

Influence of heat on shear patterns and vascular function. In the present study, lower limb heating resulted in beneficial changes in shear patterns in both the SCI and CON groups (Fig. 2). As a result of increased femoral blood flow and conductance, antegrade shear rates in the femoral artery were greater in the SCI versus CON group. Heating also reduced retrograde shear rates to nearly zero in both groups. Previous research has demonstrated a detrimental impact of retrograde shear rates in uninjured individuals (41, 64) and SCI (71) as well as beneficial effects of increases in antegrade blood flow patterns (61, 68, 70). Inducing changes in shear patterns, at least in uninjured CON individuals, via local heating has been demonstrated to improve FMD acutely when measured immediately postheating (33, 70); however, the data are equivocal when allowing a recovery period postheating (61, 69). Romero et al. (61) observed improvements of FMD in their older group only but in both young and older participants for microvascular function. The ages of participants in our study were younger compared with the older group studied by Romero et al. (61), so it follows that acute heating in our study may not have altered FMD. It is unclear, however, why we did not also observe similar improvements in microvascular function after heating. Despite a lack of improvement in macro- or microvascular functions following heating, we observed beneficial increases in antegrade and decreases in retrograde shear rates, which are considered to be important stimuli for maintaining vascular function (31, 49). Indeed, despite inconsistent findings between acute heating studies, repeated hemodynamic stimuli via chronic heating protocols have proved effective at improving both macrovascular endothelial (11, 54) and microvascular (14) function in non-SCI groups. Thus, to explain when—and if— the favorable changes in shear patterns can improve overall vascular function, study of the chronic effects of heating in SCI are clearly warranted.

Microparticles. Experimental models that reduce mean and increase retrograde shear stress via cuff inflation have demonstrated impairments to endothelial function (64) and increased MP release indicating activation and apoptosis of the endothelium (41). Conversely, passive heat stress, which is generally associated with beneficial flow and shear patterns, has been demonstrated to reduce circulating MPs of both endothelial activation and apoptosis in healthy young men (5). In our study, concentrations of endothelial $CD62e^+$ (activation) but not $CD31^+/42b^-$ (apoptosis) were elevated in SCI versus CON at baseline, but $CD62e^+$ was reduced to levels similar to CON

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after heating (Fig. 5). The unchanged MP response in CON and the fact that $CD31^+/42b^-$ concentration did not change in SCI suggest that the reduction in $CD62e^+$ is not only a function of greater clearance via increased blood flow but is likely a reduction in the presence of these specific MPs. Endothelium-derived MPs have been reported to be elevated in several disease states, including hypertension, coronary artery disease, and metabolic syndrome, and have been implicated in the development of endothelial dysfunction and atherosclerosis by promoting inflammation and thrombosis and reducing nitric oxide bioavailability (1, 7, 15). These data indicate that SCI is associated with chronic endothelial cell activation resulting in elevated concentrations of activation-derived endothelial MPs. However, SCI does not appear to confer a proapoptotic influence on the endothelium as circulating apoptosis-derived endothelial MPs were not significantly different compared with the uninjured adults. Endothelial activation and the subsequent release of activation-derived MPs may contribute to the observed impairments of vascular function in the SCI group. Importantly, the effects of passive heating appear to be a promising strategy to counteract endothelial activation and its detrimental sequelae, including vascular inflammation and endothelial dysfunction.

Changes in metabolic markers. Although glucose, insulin, and HOMA-IR were all within the normal range and similar between SCI and CON groups in our study, glucose and insulin resistance are reported to be highly prevalent in SCI populations (6, 16). Thus, the clear reductions in these values, particularly in the SCI group for glucose, after heat stress potentially highlight clinically important metabolic responses. Indeed, at least in animal models, increases in temperature reduce insulin resistance and inflammation by augmenting the cell stress response and heat shock proteins (17, 38). The acute effects of heat on glucose control in humans, however, are less conclusive. For example, although one study (24) has reported that postprandial glucose levels were attenuated after heat stress (+1°C core temperature) compared with the same core temperature change after exercise, several other studies (22, 25, 52) have reported greater glucose and insulin levels at higher ambient temperatures. It is noteworthy that increases in core temperature in the latter studies were limited to <0.5°C. An important consideration when measuring glucose/insulin responses during whole body heating is the increased forearm blood flow via cutaneous vasodilation, which might lead to an arterialization of venous blood samples (27). This could explain, in part, the different responses of glucose concentrations between SCI and CON groups in our study given the slightly greater increases in brachial blood flow in the CON group. Although there are little data on the chronic effects of heating and glucose/insulin control, Hooper et al. (37) reported that HbA1c was reduced by one percentage point after 3 wk of hot tub therapy in patients with type II diabetes. The effects of long-term heating interventions on glucose and insulin resistance in SCI and prediabetic populations are therefore of great interest.

Experimental limitations. Although the sample size in the present study was larger than the majority of previous studies of peripheral vascular function in SCI and the first study of cervical SCI, the sample was still relatively small. The nature of this SCI population meant that many of the participants were on indicated medications (Table 1). On one hand, inclusion of SCI participants on medications is highly appropriate, and the majority of the findings persistent; however, some of the findings (e.g., endothelial MPs) became clearer when isolated to those patients with SCI not on medications. Although our study would benefit from extension into a larger cohort, the current results may provide some insights on knowledge gaps underlying CVD risk in SCI. Furthermore, we provide strong evidence for continued research on the therapeutic effects of passive heating. Although a 60-min bout of heating could be considered long, it might be a necessary stimulus to detect acute responses. However, evidence exists to suggest that shorter bouts of 30-min hot water immersions (4, 68) and 10- to 15-min sauna exposures (45), over long periods of time, confer vascular and health benefits. The implementation of longer-term heating interventions, as used in young healthy volunteers (12), are especially warranted in the cervical SCI population who have a compromised capacity to exercise.

Another important consideration is the extent of thermoregulatory impairment in SCI. The lack of sweating and diminished cutaneous vasodilatory responses below the level of injury result in rapid increases in core temperature. Similar to previous reports (59), the observed core temperature increases were twofold greater with the same heat stimulus in SCI compared with CON (Fig. 1). The differential changes in core temperature responses are important for the parsimonious interpretation of the physiological stimulus of vascular benefits from heating. The relatively small changes in core temperature in our CON group (+0.35°C) might explain the lack of changes in vascular function compared with other studies where increases in core temperature were generally >0.5°C. Repeated bouts of hemodynamic stimuli (e.g., heat-induced shear stress) have been widely reported to underlie improvements in vascular function (30, 70), but increases in temperature may also be an important stimulus. In particular, increases in core temperature may lead to improvements of metabolic function (i.e., glucose/insulin control) via heat-induced cellular stress. However, our intention with this experimental design was to test a practical and safe method of heating (i.e., lower limb water immersion), which would be feasibly implemented as a therapy for the SCI population.

In our participants with cervical SCI, we observed selectively impaired macrovascular function in the BA and impaired microvascular function in both the BA and SFA. Circulating biomarkers of endothelial activation were also elevated in SCI. Lower limb heating, however, induced beneficial changes to arterial shear patterns, which were associated with reductions in endothelial MPs. This study provides evidence that, at least acutely, passive heating can be used as a safe and practical therapy for improving vascular function in a SCI population. This type of therapy might be of particular benefit, especially in individuals with high-level SCI who experience greater difficulties exercising.

ACKNOWLEDGMENTS

The authors thank the participants for their time and Josh Tremblay for critical feedback on the manuscript.

GRANTS

This work was supported, at least in part, by a Seed Grant from the International Collaboration on Repair Discoveries (ICORD) supported by the Blusson Integrated Cures Partnership (to P. Ainslie), Canada Research Chairs program (to P. Ainslie), University of Calgary startup funds to (to A. Phillips), and National Heart, Lung, and Blood Institute Grants HL-077450 and HL-107715 (to C. DeSouza).

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

G.B.C. and P.N.A. conceived and designed research; G.B.C., O.F.B., A.A.P., T.M., Z.K.S., A.H.X.L., J.W.S., A.V.K., and P.N.A. performed experiments; G.B.C., T.D.B., and N.M.D. analyzed data; G.B.C., O.F.B., A.A.P., T.M., Z.K.S., A.H.X.L., J.W.S., T.D.B., N.M.D., D.G., A.V.K., Z.D., C.A.D., and P.N.A. interpreted results of experiments; G.B.C. prepared figures; G.B.C. drafted manuscript; G.B.C., O.F.B., A.A.P., T.M., Z.K.S., A.H.X.L., J.W.S., T.D.B., N.M.D., D.G., A.V.K., Z.D., C.A.D., and P.N.A. edited and revised manuscript; G.B.C., O.F.B., A.A.P., T.M., Z.K.S., A.H.X.L., J.W.S., T.D.B., N.M.D., D.G., A.V.K., Z.D., C.A.D., and P.N.A. approved final version of manuscript.

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