PERIPHERAL VASCULAR RESPONSES TO ACUTE COLD-WATER IMMERSION

by

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A THESIS

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Exercise is effective in improving cardiovascular health, specifically, as a result of the increase shear stress on peripheral vasculature. Similarly, heat therapy (i.e., sauna bathing and hot water immersion) has indicated similar responses to exercise on endothelial function and vascular remodeling. However, less is understood about the peripheral vascular responses of a single bout of cold-water immersion and if it could be used as an alternative to exercise to improve cardiovascular health. Therefore, the purpose of this study was to test the hypothesis that cold-water immersion results in peripheral vascular changes that may be beneficial to overall cardiovascular health (i.e., increase in shear stress through increased blood flow). Eight young, healthy adults (4 self-reporting male and 4 self-reporting females: age: 24±7 years; height: 172±39 cm; weight: 65±24 kg; and BMI: 22.2±7.9 kg/m^2) completed one study visit where they were immersed to the sternum in ~10°C water for 15 minutes. Ultrasound imaging of the brachial artery was taken prior to, during, and after the cold-water immersion. Our results indicate there was a decrease in brachial artery diameter during and after cold-water immersion, and blood flow and shear decreased during the post-immersion

recovery period in comparison to pre-immersion. Therefore, this may indicate a negative effect on peripheral vascular function; future research should focus on these peripheral vascular changes with repeated bouts of cold-water immersion.

Acknowledgements

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Introduction

Background

According to the Centers for Disease Control, cardiovascular disease (CVD) is the leading cause of death in the United States; this includes coronary artery disease, strokes, hypertension, and congestive heart failure[1]. The American Heart Association predicts that around 82.6 million people in the United States have at least one form of CVD [2]. Exercise is widely used as a means to improve cardiovascular health by improving endothelial function via increases in shear stress on vessel walls[3], reducing arterial stiffness, and decreasing the risk of developing hypertension and heart failure[4]. Although exercise offers the most accepted method for reducing the risk of CVD and mortality, many populations (i.e., elderly, obese, coronary heart disease, etc.) experience barriers to exercise participation. Further, those who do participate may have exercise intolerance, defined as a decreased ability to meet the intensity and/or duration of exercise necessary to provide a sufficient stimulus for the beneficial cardiovascular adaptations[5]. Therefore, there is a need to explore the alternatives to exercise for populations, unable to or with limitations, to participate in exercise.

Heat therapy, in the form of sauna bathing and hot water immersion, has shown improvements in peripheral vascular function, blood pressure, and all-cause mortality that are comparable and/or superior to exercise[3,6–8]. These improvements to cardiovascular health show potential in this alternative to exercise as a promising method to reduce the risks associated with the development of CVD[7]. The thermoregulatory responses to heat stress result in a cascade of physiological responses, such as increases in cardiac output and peripheral blood flow, that ultimately lead to

improvements in peripheral vascular function (i.e., increase in shear stress), thus, reducing the risk of CVD[3]. However, less is known about if the physiological responses to the opposite temperature extreme, cold, could also result in these same peripheral vascular adaptions that could be beneficial to overall cardiovascular health, and therefore act as another alternative to exercise to combat CVD.

Cold-water immersion is often considered to result in unfavorable outcomes (i.e., cardiac arrythmias, drowning), specifically in accidental and non-controlled settings[9]. Cold-water immersion at low temperatures (<15°C) and/or for long durations (> 30 minutes), has been attributed to hyperventilation, drowning, and hypothermia[9]. However, despite these elevated risks, cold therapy, such as cryotherapy and ice baths, are common practices used across disciplines (i.e. athletic training, physical therapy, sports medicine) to reduce inflammation[10], and improve muscle recovery and endurance[9,11]. Further, cold-water immersion is marked as the most effective treatment for heat-related illness (i.e., exertional heat stroke)[10,12]. These uses of cold-water immersion for clinical and recreational (i.e., Wim Hof) settings have become common practice despite the lack of understanding and research surrounding the physiological responses.

In addition to the clinical and recreational uses of cold-water immersion, evidence shows benefits to using cold therapy as a potential therapeutic modality for improved health. For example, 2-3 minute cold showers at 20°C once or twice a day over prolonged periods of weeks to months have reduced depressive symptoms through increased endorphins[13]. Similarly, acute bouts of cold-water immersion and cold pressor tests have shown to increase dopamine, endorphins, and have increased mood once

immersion is over[9]. One report indicated that contrast bath therapy, alternating immersion between hot and cold water, reduces pain and increases relaxation[14]. Along with these psychophysiological improvements, there are potential cellular and molecular responses that could indicate therapeutic benefit of cold-water immersion. These include the activation of the cold-inducible RNA-binding protein (CIRBP)[15], RNA Binding Motif Protein 3 (RBM3)[16], and heat shock proteins (HSPs)[17]. CIRBP specifically responds to cold and hypoxic stress in cells and could act to treat myocardial ischemia and other cardiovascular diseases through reducing oxidative stress and cell death[15]. Additionally, RBM3 may be upregulated during different levels of cold stress and decrease risk of neurodegenerative diseases such as Alzheimer's and Dementia[16], and HSPs are upregulated as a result of cold stress in human keratinocytes and act to protect cells from subsequent stress[18]. Additionally, brown adipose tissue (BAT) activation is another known long-term adaptation to cold exposure, which can lead to beneficial changes in metabolic capacity (i.e., fatty acid uptake, insulin sensitivity, and energy expenditure[19,20]) and cardiovascular health. However, despite these beneficial cases and potential chronic adaptations, little has been done to discern the cardiovascular and peripheral vascular effects and adaptations of cold-water immersion.

Physiological Responses

Cold Exposure

The acute response to cold-water immersion includes an increase in heart rate, blood pressure via peripheral vasoconstriction, and ventilation (i.e., hyperventilation)

due to the increased sympathetic activation in response to the afferent signals from peripheral thermoreceptors marked as the "cold shock" response[11,21,22]. Peripheral vasoconstriction occurs in order to reduce heat loss from the peripheral limbs and to maintain flow of warm blood to vital organs. This occurs through sympathetic nervous system activation seen by a significant increase in plasma norepinephrine concentrations after cold exposures[23–25]. However, after the initial response to cold and with continued exposure, cold-induced vasodilation (CIVD) often follows as a method to protect limbs from cold induced injury such as frost bite, through phases of rewarming and decreased sensitivity of adrenergic stimulation over time in cold water[26,27]. Some literature suggests that during cold pressor tests, CIVD occurs thus increasing temperature of the skin and causing bradycardia at the end of limb immersion sessions[27]. Although CIVD has been mainly investigated during single limb immersion, less has been determined about CIVD during whole body water immersion and how peripheral vascular responses may be affected in result. Furthermore, although not fully understood, the cyclical CIVD that occurs after local cooling has been hypothesized by other literature to change eNOS activation and alter shear stress on endothelial cells[28] which might contribute to beneficial adaptations during and/or after cold-water immersion.

Cardiovascular Responses

Cardiac output (Q) and stroke volume (SV) are indicators of cardiovascular capacity and function. SV is defined as amount of blood pumped through the ventricles of the heart in a given beat, whereas Q is described as the product of HR and SV, and

indicates the volume of blood pumped by the heart each minute[29,30]. Factors that can lead to a change in SV include an increase in sympathetic activity which result in cardiac contractility, increased venous return, the amount of blood returning to the heart, and increased preload[31]. During water immersion in thermoneutral water (32-35°C) the increase in hydrostatic pressure on the peripheral circulation results in a 500-700mL increase in central blood volume[11]. This hydrostatic pressure results in a greater venous return, preload, and stroke volume, ultimately increasing Q[11]. These increases may be further enhanced during cold-water immersion due to the heightened vasoconstrictor response[25] from the elevated sympathetic output shunting blood from the periphery to the core in order to protect core body temperature against the cold temperature of the water[32,33].

Peripheral Vascular Responses

Increases in systolic antegrade blood flow, that is, blood flow in the forward direction away from the heart, has been correlated with increased vascular function, whereas increases in retrograde blood flow, blood flowing in the reverse direction towards the heart, has been correlated with impaired vascular function[34]. Retrograde flow can result from increased peripheral vascular resistance, due to activation of the sympathetic nervous system which increases peripheral vascular tone, the degree of constriction in vascular blood vessels[35], or can result from pathological conditions such as obesity and hypertension thus contributing to compromised endothelial function. Exercise is known to increase vascular health by increasing shear stress and as a result, increasing antegrade blood flow, promoting vasodilation and remodeling of vessels[3].

Increasing blood flow through an artery produces shear stress, a frictional force from red blood cells on the surface of the endothelium, which is the single cell layer lining the innermost surface of blood vessels[36,37]. Further, during exercise, the increase in antegrade shear may outweigh the negative consequences of a parallel increase in retrograde shear[35]. Other studies have shown significant increase in antegrade shear blood flow after sauna bathing in the popliteal artery, mimicking the response to exercise[3].

This change in blood flow has been shown to be beneficial to cardiovascular health[3]. This increased blood flow and shear stress leads to structural remodeling of walls of the blood vessels and promotes cardiovascular and immune health[37]. The increase in shear stress promotes the dilation of blood vessels by the release of endothelial nitric oxide synthase (eNOS) which then promotes the release of nitric oxide (NO). NO is a potent vasodilator, antioxidant, and anti-inflammatory substance[37,38] and is an important biomarker of endothelial function. A reduction in NO bioavailability is responsible for increased oxidative stress and endothelial dysfunction which can be attributed to the development of hypertension and atherosclerosis[39]. Furthermore, oscillatory shear patterns as a result of changes in blood flow reduce production of NO[40], and influence vascular health by inducing atherosclerosis[40,41]. However, the effect of cold stress on blood flow and shear during cold water immersion has not been determined; our research intends to examine this potential therapy and its effect on vascular function.

Purpose and Relevance

The purpose of this study was to quantify the effects of an acute bout of cold-water immersion on cardiovascular parameters, specifically peripheral vascular function marked by the blood flow and shear stress patterns within the brachial artery in healthy, young adults. We hypothesized that during the exposure of cold-water immersion, there would be an increase in hemodynamics and peripheral vasoconstriction followed by a return to pre-immersion values after a post-immersion recovery period. Further, we hypothesized that these fluctuations in the hemodynamic and peripheral vascular responses will result in changes in blood flow and shear stress patterns that may be beneficial to overall cardiovascular health.

If research on acute cold-water immersion elicits similar beneficial responses to other stressors such as exercise and heat, this could indicate potential therapeutic benefit of acute or prolonged cold-water immersion as a nonpharmaceutical therapy, and exercise alternative to reduce CVD and mortality. As CVD remains the leading cause of death in the United States, determining the physiological effects of cold water on cardiovascular health is advantageous for determining it as an alternative treatment and prevention measure for those who experience exercise intolerance. Vascular function (blood flow, vessel diameter, and dilatory functionality) is an important component of cardiovascular health. Depending on the pattern of blood flow and vascular responses, cold-water immersion could dictate similar physiological patterns to other forms of environmental and physical stress. This study could show physiological effects that may shape future long-term intervention studies to discern the implications of cold-water adaptations on overall health.

Methods

The research protocol took place in Drs. Christopher Minson and John Halliwill's Human Cardiovascular Control and Exercise & Environmental Physiology Laboratories while COVID-19 restrictions were in place. Due to COVID-19, all experimental procedures were required to be completed within 2 hours total in one experimental day. This study was approved by the Institutional Review Board of Human Subjects by the University of Oregon in agreement with the Declaration of Helsinki to ensure the study is ethical and safe for all potential participants.

Participants

Sixteen young, healthy adults (9 self -reported male and 7 self-reported females; age: 25±6 years; height: 172±10 cm; weight: 62.1±19.7 kg; and body mass index: 21.0±6.3 kg/m^2) volunteered to participate in this study. A subset of these participants, including 8 young, healthy adults (4 self-reporting male and 4 self-reporting females: age: 24±7 years; height: 172±39 cm; weight: 65±24 kg; and BMI: 22.2±7.9 kg/m^2) participated specifically in the ultrasound portion of the protocol. The remaining participated in a parallel protocol investigating blood biomarkers. Volunteers were recruited by members of the research team by word of mouth, newspaper advertisements placed in local newspapers, website, by email, and by flyers distributed around the University of Oregon and surrounding community.

Participants were between the ages of 18-40, and self-reported to be inactive to highly active, healthy, nonsmokers without diagnosed or history of cardiovascular conditions, and were not pregnant, trying to conceive, or undergoing treatments to

increase sperm count. Participants were excluded if they were taking prescription medications other than birth control, participating in cold or heat therapy such as cold or hot showers or baths, or had conditions that reduce cold tolerance including anemia, anorexia, hypothyroidism, Raynaud's, disorders of the hypothalamus, fibromyalgia or previous frostbite injuries. Of the total 16 participants, three female participants reported to be regularly menstruating and four female participants reported hormonal contraceptive use. Hormonal contraceptive use included intrauterine devices (Mirena levonorgestrel-releasing intrauteric system. Bayer, Whippany, NY *n*=1), arm implant (Nexplanon etonogestrel implant Merck & Co., Inc., Kenilworth, NJ, *n*=1), and oral contraceptive pill (Cyred EQ, Afaxys Pharma, Charleston, SC, *n*=1 and Yaz drospirenone and ethinyl estradiol, Bayer, AG, Leverkusen, Germany (*n*=1).

Screening Session

A pre-screening session was conducted to ensure that volunteers meet the study inclusion criteria and did not meet exclusion criteria. They were asked a series of questions regarding their smoking, exercise habits, medications, and a brief health history before scheduling a screening session in the laboratory.

The screening sessions consisted of a tour of the laboratory space, reviewing the informed consent form with the lead research team member, and completing a brief health history questionnaire to ensure they did not meet exclusion criteria of the study for their safety. Once providing verbal and written informed consent, participants' height, body weight with a stadiometer and an electronic scale (Sartorius, Bohemia, NY), arm circumference, and seated resting blood pressure were measured and

recorded. Female participants with child-bearing potential were asked to provide a urine sample for a pregnancy test to confirm they were not pregnant.

Experimental Session

Upon arrival into the laboratory, participants verbally confirmed they had abstained from heavy exercise, heat therapy (such as sauna or hot tub), cold therapy (such as cold showers or baths) for 24 hours; medications or supplements with the exception of oral contraceptives for 12 hours; alcohol for 12 hours; caffeine for 6 hours; and food for 2 hours prior to the start of the experimental session. Participants were instrumented with a heart rate telemetry band (Polar®). In a private room, participants changed into a bathing suit and self-inserted a rectal thermistor 10 cm past the anal sphincter. They were then seated into a hydraulic lift chair (S.R. Smith, Canby, OR) for 20 minutes. During this, participants were instrumented with an arm blood pressure cuff, 3-lead electrocardiogram (ECG), and a finger blood pressure cuff. This allowed the research team to closely monitor the participants cardiac and respiratory responses to the cold-water immersion. The conditions of the laboratory space were 23.0±1.5°C, 31±3% relative humidity.

After instrumentation and the 20-minute resting period, participants completed a 5-minute seated, resting baseline and measurements (heart rate, rectal temperature, and arm blood pressure) were taken in triplicate. The participants' right arm was elevated and supported at the level of the heart for the ultrasound measurement. At the end of baseline, a trained sonographer located and marked the location of the right brachial artery and recorded a one-minute video of the brachial artery. Participants were then

lifted and lowered in the cold-water bath (10.5±0.2°C) up to their sternum for 15 minutes. Within the first minute of immersion, a second ultrasound of the brachial artery was recorded. Arm blood pressure, heart rate, and rectal temperature were manually recorded every two minutes and perceptual scales every 5 minutes. A third ultrasound video was taken at the end of the 15-minute immersion period. Participants were removed from the water and remained seated in the chair for a 30-minute recovery period. During this time, blood pressure, heart rate, and rectal temperature were taken every 2 minutes for the first 10 minutes of recovery, and then every 5 minutes thereafter until the end of recovery period. Perceptual scales were recorded every 5 minutes during the recovery period. A fourth ultrasound video of the brachial artery was taken at the end of the 30-minute recovery period. Upon completion of the experimental sessions, participants were de-instrumented and existed the laboratory.

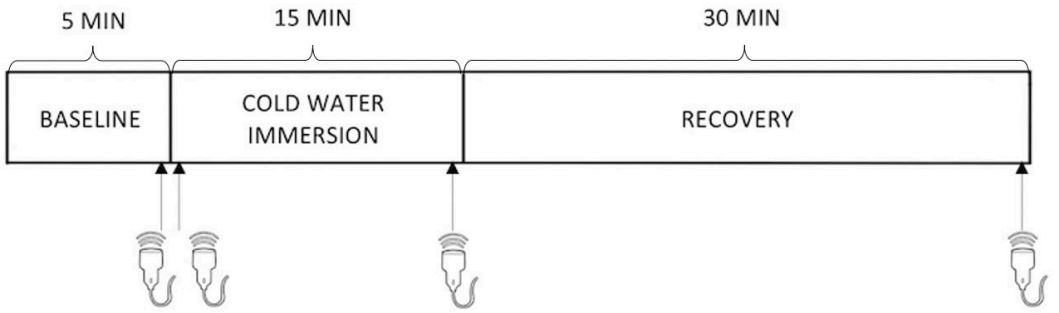

Figure 1: Schematic of protocol

Dependent Variables

Rectal temperature was measured with a self-inserted rectal probe (YSI Incorporated, Yellow Springs, OH). A 3-Lead Electrocardiogram (CardioCap5, Datex

Ohmeda, Tewksbury, MA) was used to calculate heart rate from the R-R interval. Our intent was to have beat-to-beat blood pressure via finger photoplethysmography (Finometer Pro, FMS Amsterdam, The Netherlands) for all participants. Due to technical complications with excessive movement (i.e., transition periods and/or shivering and/or peripheral vasoconstriction), only 6 of the total 16 participants had adequate continuous photoplethysmography waveforms. In the participants with beat-to-beat blood pressure, we also derived stroke volume and cardiac output via ModelFlow[42]. Arm blood pressure was periodically (i.e., in triplicate during baseline, every two minutes during cold water immersion, every two minutes during the first ten minutes, and every five minutes during recovery) measured via automatic electrosphygmomanometry (Tango M2, SunTech Medical, Raleigh, SC) was used as the main measure for hemodynamics. The right brachial artery was imaged with an insonation angle of 60 degrees using a 10.0 MHz linear array ultrasound probe. At each time point (i.e., baseline, first and last minute of cold-water immersion, and the last minute of recovery) both the image (artery diameter) and blood velocity tracing were recorded for one minute at 20 frames per second (Camtasia Studio, TechSmith, Okemos, MI) to be analyzed offline. Two sonographers (EL and BK) obtained the images, but only one sonographer completed all ultrasound scans within the same visit. The recorded ultrasound video clip was analyzed (Brachial Analyzer for Research, Medical Imaging Applications, LLC, Coralville, IA) for diameter, and mean and peak blood velocity during each cardiac cycle. We further calculated blood flow (antegrade, retrograde and mean) as $Blood\ Flow = \pi(\frac{diameter}{2})^2 \times velocity$ and shear stress (antegrade, retrograde, and mean) as $Shear\ Stress = 4 \times (\frac{velocity}{diameter})$, and oscillatory

shear index as $\frac{retrograde\ shear}{retrograde\ shear+antegrade\ shear}$ [43,44]. Oscillatory shear index is the axial direction of flow of wall shear stress where a value of 0 implies unidirectional flow and a value >0.35 implies multidirectional flow flow[45]. Brachial artery vascular conductance was calculated as $Conductance = \frac{blood\ flow}{mean\ arterial\ pressure}$. Perceptual scales were thermal sensation (-20= "extremely cold" to +20= "extremely hot") and thermal comfort (1= "comfortable" to 9= "extremely uncomfortable").

Data Analysis

A signal processing software (WinDaq, dataq instruments, Akron, OH) digitized and continuously recorded ECG, rectal temperature, beat-to-beat blood pressure and derived variables (all 180 Hz). A one-way analysis of variance (ANOVA) was conducted to examine the effect of time. If the ANOVA revealed a significant F statistic, post hoc Dunnett's tests were used to make comparisons from baseline, during cold water immersion, and recovery. Further comparisons were also made to recovery from the last minute of cold-water immersion. A priori significance was set at $P \leq 0.05$. Statistical analyses were performed using Prism software (GraphPad software, version 9.1, La Jolla, CA). Data reported as mean ±standard deviation. However, due to this being a preliminary analysis at the time of submission, we speculated a trend towards significance with $P \leq 0.10$.

Results

Cardiovascular & Hemodynamics Variables	
Heart Rate (bpm)	77±12
Mean Arterial Pressure (mmHg)	81±7
Systolic Blood Pressure (mmHg)	111±13
Diastolic Blood Pressure (mmHg)	66±7
Stroke Volume (mL/min)	80.3±11.0
Cardiac Output (L/min)	6.1±0.8
Total Peripheral Resistance (mmHg/L/min)	12.9±1.4
Rectal Temperature (°C)	37.5±0.3

Brachial Artery Variables	
Diameter (mm)	3.70±0.68
Mean Blood Velocity (cm/s)	15.2±5.4
Mean Blood Flow (mL/min)	41±23
Antegrade Blood Flow (mL/min)	59±39
Retrograde Blood Flow (mL/min)	11±10
Mean Shear (s^{-1})	156±53
Antegrade Shear (s^{-1})	184±54
Retrograde Shear (s^{-1})	28±14
Oscillatory Shear Index (a.u.)	0.14±0.6
Vascular Conductance (mL/min/mmHg)	0.60-±0.38

Table 1: Pre-immersion baseline values reported in mean ± standard deviation for measured variables.

Rectal temperature

There was a significant effect of time for rectal temperature (P=0.0003). Rectal temperature was reduced from baseline at minute one and through minute 15 of cold-water immersion (P≤0.0142). Rectal temperature decreased by 0.4±0.3°C (Figure 2B) from baseline to minute 15 minutes of cold-water immersion (BL 37.5±0.3°C vs Min 15 CWI 37.1±0.4°C, P=0.0106, Figure 2A). Compared to baseline, rectal temperature was reduced at minute one and through minute 30 of recovery (P≤0.0142). Rectal temperature decreased by 0.9±0.5°C (Figure 1B) from baseline to minute 30 of recovery

(BL 37.5±0.3°C vs Min 15 CWI 36.5±0.7°C, P=0.001, Figure 2A). Compared to minute 15 of cold-water immersion, rectal temperature was reduced at minute 14 and remained reduced through minute 30 of recovery (P≤ 0.0556). Compared to minute 15 of cold-water immersion, rectal temperature was reduced by 0.6±0.3°C (Figure 2B) at minute 30 of recovery (Min 15 CWI 37.1±0.4°C vs Min 30 REC 36.5±0.7°C, P=0.003, Figure 2A).

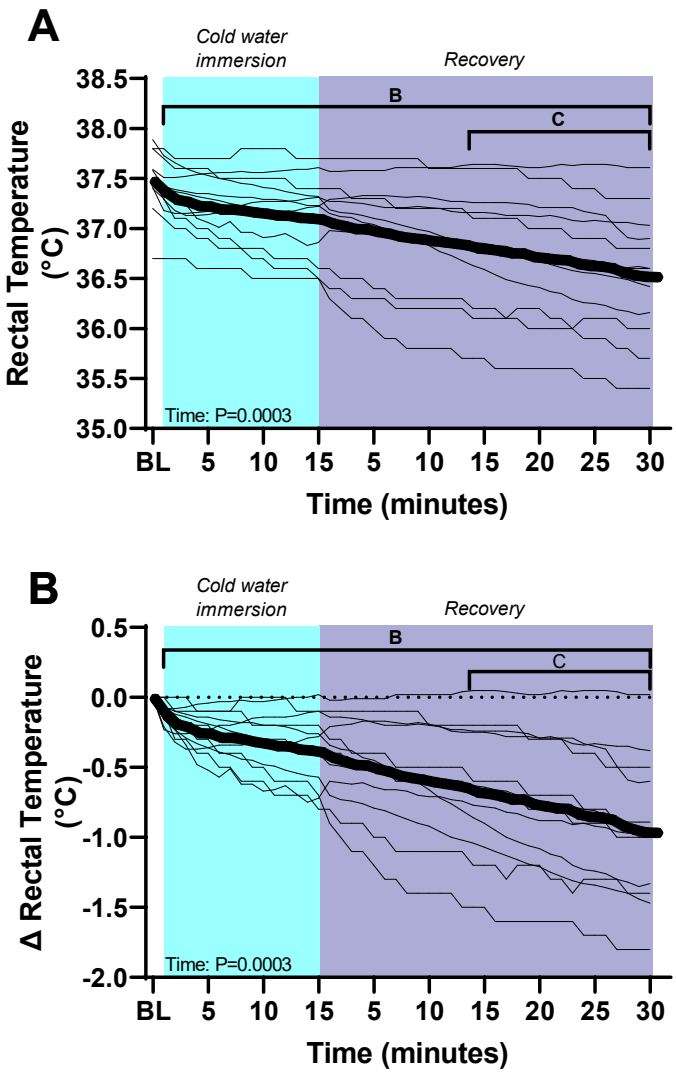

Figure 2: Rectal temperature

The rectal temperature response shown as absolute (Panel A) and change (Panel B) values from pre-immersion baseline (BL) during cold-water immersion (15 Minutes) and post-immersion recovery (30 Minutes). The thick bold line illustrates the mean data, and the thin lines illustrate individual responses. Data was analyzed using a one-way ANOVA with post-hoc Dunnett's test for multiple comparisons. [B]different from baseline (P≤0.05); [C]different from minute 15 of cold-water immersion (P≤0.05); *n*=12 (6 females).

Heart Rate

There was a significant effect of time on heart rate ($P < 0.0001$). Heart rate did not change compared to baseline from minute one to minute 12 of cold-water immersion ($P \geq 0.0789$) but was reduced from baseline at minute 13 of cold-water immersion until the end of cold-water immersion (BL 77±12 bpm vs. CWI Min 15 65±12 bpm, $P=0.0006$; Figure 3A) and remained reduced through minute 30 of recovery ($P \leq 0.016$). Compared to baseline, heart rate decreased by 11 ±8 bpm (Figure 3B) by the minute 15 of cold-water immersion (BL 77±12 bpm vs Min 15 CWI 65±12 bpm; $P=0.0006$; Figure 3A) and decreased by 13±7 bpm (Figure 3B) by minute 30 of recovery (BL 77±12 bpm vs. Min 30 REC 63±9 bpm, $P<0.0001$; Figure 3A). There was no change in heart rate from minute 15 of cold-water immersion to minute 30 of recovery ($P=0.9948$).

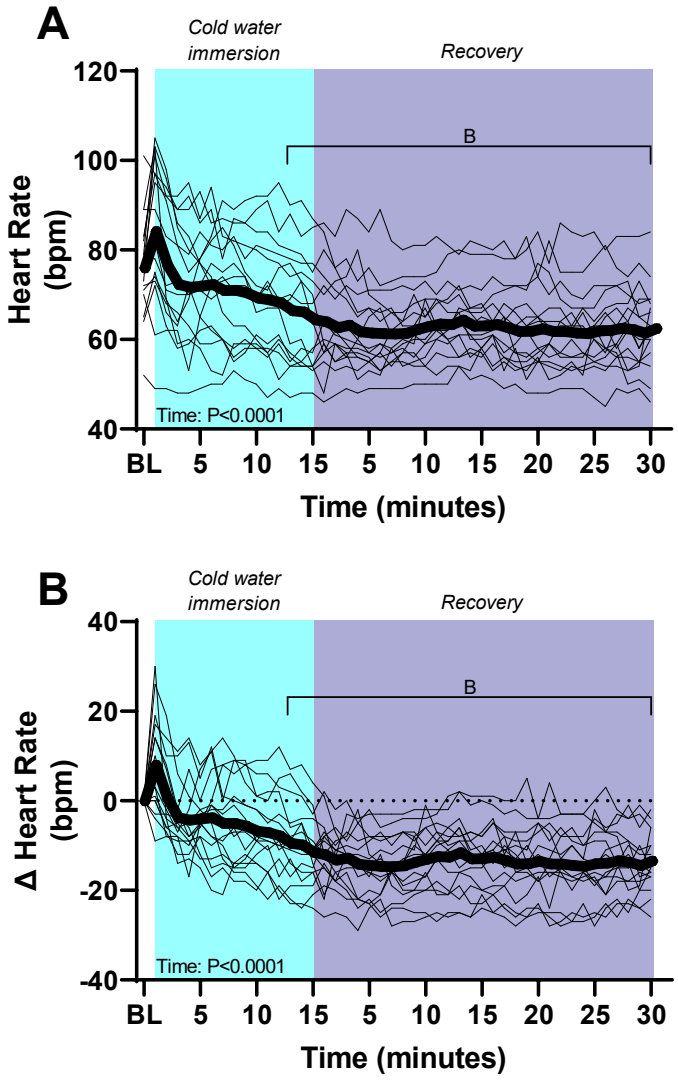

Figure 3: Heart rate

The heart rate response shown as absolute (Panel A) and change (Panel B) values from pre-immersion baseline (BL) during cold-water immersion (15 Minutes) and recovery (30 Minutes). The thick bold line illustrates the mean data, and the thin lines illustrate individual responses. Data was analyzed using a one-way ANOVA with post-hoc Dunnett's test for multiple comparisons. [B]different from baseline ($P \leq 0.05$); [C]different from minute 15 of cold-water immersion ($P \leq 0.05$); n=16 (7 females).

Brachial Hemodynamics

There was a significant effect of time on brachial mean arterial pressure ($P<0.0001$). Mean arterial pressure was elevated compared to baseline at minute 0 of cold-water immersion and remained elevated through minute 30 of recovery ($P\leq0.0136$). Mean arterial pressure increased by 6.93±5.33 mmHg (Figure 4F) compared to baseline after 15 minutes of cold-water immersion (BL 81±9 mmHg vs Min 15 CWI 87±10 mmHg, $P=0.0108$; Figure 4E) with the greatest increase at the initial onset of immersion (+20±10 mmHg) (Figure 4F). Compared to baseline, mean arterial pressure was elevated by 9±7mmHg (Figure 4F) at the end of the 30-minute recovery (BL 81±9 mmHg vs Min 30 REC 90±10 mmHg, $P=0.0018$; Figure 4E). Compared to minute 15 of cold-water immersion, there was no change in mean arterial pressure after 30 minutes of recovery ($P=0.6054$).

There was a significant effect of time on brachial systolic blood pressure ($P<0.0001$). Systolic blood pressure was elevated compared to baseline after minute 0 of cold-water immersion and remained elevated through minute 15 ($P\leq0.0529$). Our results indicates a trend towards increased systolic blood pressure by 10±10 mmHg compared to baseline after 15 minutes of cold-water immersion (BL 111±13 vs Min 15 CWI 119±13, $P=0.0529$; Figure 4A) with the greatest increase at the initial onset of immersion (26±17 mmHg) (Figure 4B). Compared to baseline, mean arterial pressure was elevated by 13±11 mmHg at the end of the 30-minute recovery (BL 111±13 vs. Min 30 REC 125±17 mmHg, $P=0.0030$; Figure 4A). Compared to minute 15 of cold-water immersion, there was no change in mean arterial pressure after 30 minutes of recovery ($P=0.5442$).

There was a significant effect of time on brachial diastolic blood pressure ($P<0.0001$). Diastolic blood pressure was elevated compared to baseline after minute 0 of cold-water immersion and remained elevated through minute 14 ($P\leq0.0097$). Systolic blood pressure increased by 6±5 mmHg compared to baseline after 14 minutes of cold-water immersion (BL 66±7 mmHg vs Min 14 CWI 71± mmHg, $P=0.0097$; Figure 4C) with the greatest increase at the initial onset of immersion (+16±9 mmHg) (Figure 4D). Compared to baseline, diastolic blood pressure was elevated by 6.5±6.623 mmHg at the end of the 30-minute recovery (BL 66±7 mmHg vs Min 30 REC 73±7 mmHg, $P=0.0152$; Figure 4C). Compared to minute 15 of cold-water immersion, there was no change in mean arterial pressure after 30 minutes of recovery ($P=0.9993$).

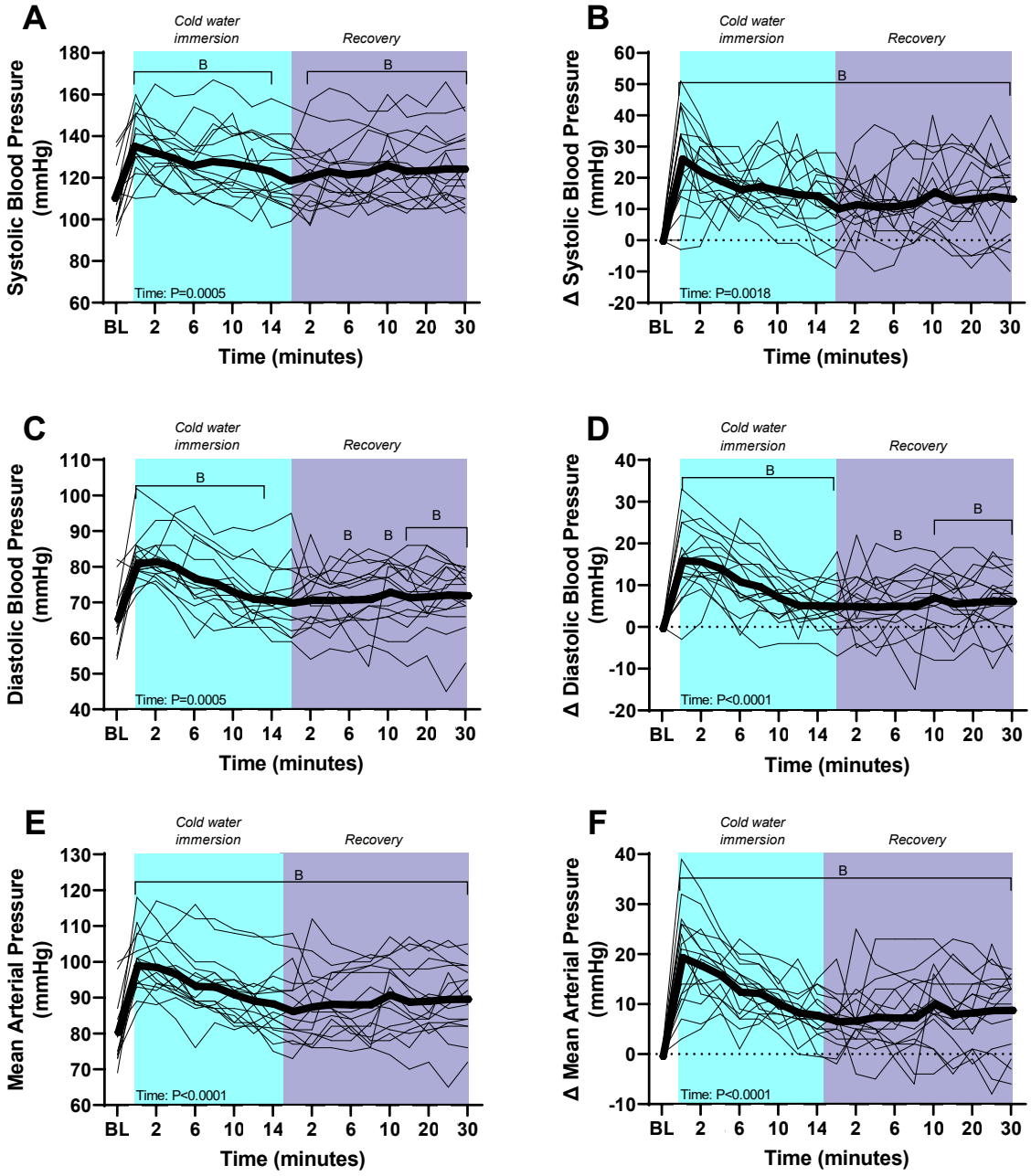

Figure 4: Arm blood pressure

The systolic blood pressure (Panels A & B), diastolic blood pressure (Panels C & D) and mean arterial blood pressure (Panels E & F) responses shown as absolute (Panel A, C & E) and change (Panel B, D & F) values from pre-immersion baseline (BL) during cold-water immersion (15 Minutes) and recovery (30 Minutes). The thick bold line illustrates the mean data, and the thin lines illustrate individual responses. Data was analyzed using a one-way ANOVA with post-hoc Dunnett's test for multiple comparisons. [B]different from baseline (P≤0.05); [C]different from minute 15 of cold-water immersion (P≤0.05); *n*=16 (7 females).

Beat-to-beat Blood Pressure Subset Analysis (*n*=6)

Our preliminary analysis of this subset of data revealed a trend towards a significant effect of time on mean arterial pressure (P=0.0935). Compared to baseline (80±9 mmHg), there was a near significant increase in mean arterial pressure within the first five minutes (range: 95-105 mmHg) of immersion (P≤0.0597), excluding minute 4. Mean arterial pressure was also trending to indicate elevation during recovery between minutes 10 and 14 (range: 89-93 mmHg, P≤0.097; Figure 5A) compared to baseline. There was no change in mean arterial pressure from the end of cold-water immersion to the end of recovery (P>0.597). There was no significant effect of time on stroke volume (P=0.3097) or total peripheral resistance (P=0.2714). There was a significant effect of time on cardiac output (P=0.0497), but multiple comparisons did not reveal significance during cold-water immersion (P≥0.1049). Q was reduced between minutes 29 (5.4±0.8 L/min) and 30 (5.4±0.9 L/min) of recovery (P≤ 0.054) (Figure 5H). Compared to baseline, there was no difference in Q after 15 minutes of cold-water immersion (P=0.9992). Compared to minute 15 of cold-water immersion, there was no difference in Q after 30 minutes of recovery (P=0.5537).

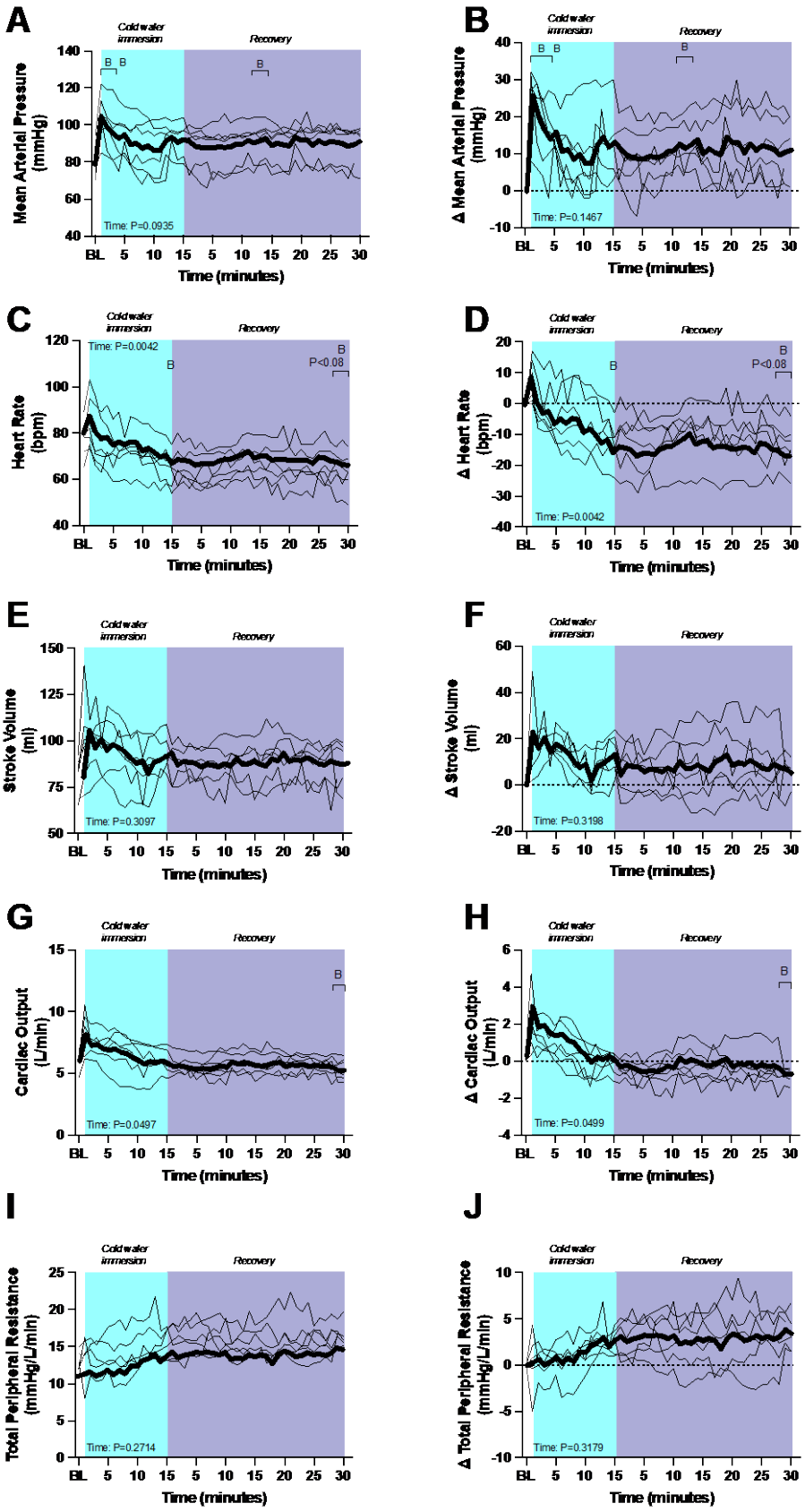
A
Cold water immersion
Recovery
Mean Arterial Pressure (mmHg)
Time: P=0.0935
Time (minutes)
B
Cold water immersion
Recovery
Δ Mean Arterial Pressure (mmHg)
Time: P=0.1467
Time (minutes)
C
Cold water immersion
Recovery
Heart Rate (bpm)
Time: P=0.0042
P<0.08
Time (minutes)
D
Cold water immersion
Recovery
Δ Heart Rate (bpm)
P<0.08
Time: P=0.0042
Time (minutes)
E
Cold water immersion
Recovery
Stroke Volume (ml)
Time: P=0.3097
Time (minutes)
F
Cold water immersion
Recovery
Δ Stroke Volume (ml)
Time: P=0.3198
Time (minutes)
G
Cold water immersion
Recovery
Cardiac Output (L/min)
Time: P=0.0497
Time (minutes)
H
Cold water immersion
Recovery
Δ Cardiac Output (L/min)
Time: P=0.0499
Time (minutes)
I
Cold water immersion
Recovery
Total Peripheral Resistance (mmHg/L/min)
Time: P=0.2714
Time (minutes)
J
Cold water immersion
Recovery
Δ Total Peripheral Resistance (mmHg/L/min)
Time: P=0.3179
Time (minutes)

Figure 5: Beat-to-beat hemodynamic subset analysis

The mean arterial pressure (Panels A & B), heart rate (Panels C & D), stroke volume (Panels E & F), cardiac output (Panels G & H) and total peripheral resistance (Panels I & J) responses shown as absolute (Panel A, C, E, G & I) and change (Panel B, D, F, H & J) values from pre-immersion baseline (BL) during cold-water immersion (15 Minutes) and recovery (30 Minutes). The thick bold line illustrates the mean data, and the thin lines illustrate individual responses. Data was analyzed using a one-way ANOVA with post-hoc Dunnett's test for multiple comparisons. [B]different from baseline (P≤0.05); [C]different from minute 15 of cold-water immersion (P≤0.05); *n*=6 (4 females).

Ultrasound

Brachial Diameter & Mean Blood Velocity

There was a significant effect of time on brachial artery diameter (P=0.0436). Compared to baseline, brachial artery diameter was reduced by 0.23±0.34 (Figure 6B) mm after 15 minutes of cold-water immersion (BL 3.70±0.68 mm vs. Min 15 CWI 3.24±0.66 mm; P=0.0507; Figure 6A) and by 0.35±0.23 mm after 30 minutes of recovery (BL 3.70±0.68 mm vs. Min 30 REC 3.35±0.68 mm; P=0.0099; Figure 6A). Compared to 15 minutes of cold-water immersion, there was no change in diameter after 30 minutes of recovery (P=0.738). There was a trend for a significant effect of time on mean blood velocity (P=0.0536). Preliminary analysis of the multiple comparisons revealed a trend for decreased mean blood velocity by 5±6 cm/s from baseline to minute 30 of recovery (BL 15±5 vs. Min 30 REC 10±4, P=0.0988; Figure 6C) and trend towards being decreased by 3±3 cm/s from minute 15 of cold-water

immersion to minute 30 of recovery (BL 13±4 vs. Min 30 REC 10±4 cm/s, P=0.0902; Figure 6C).

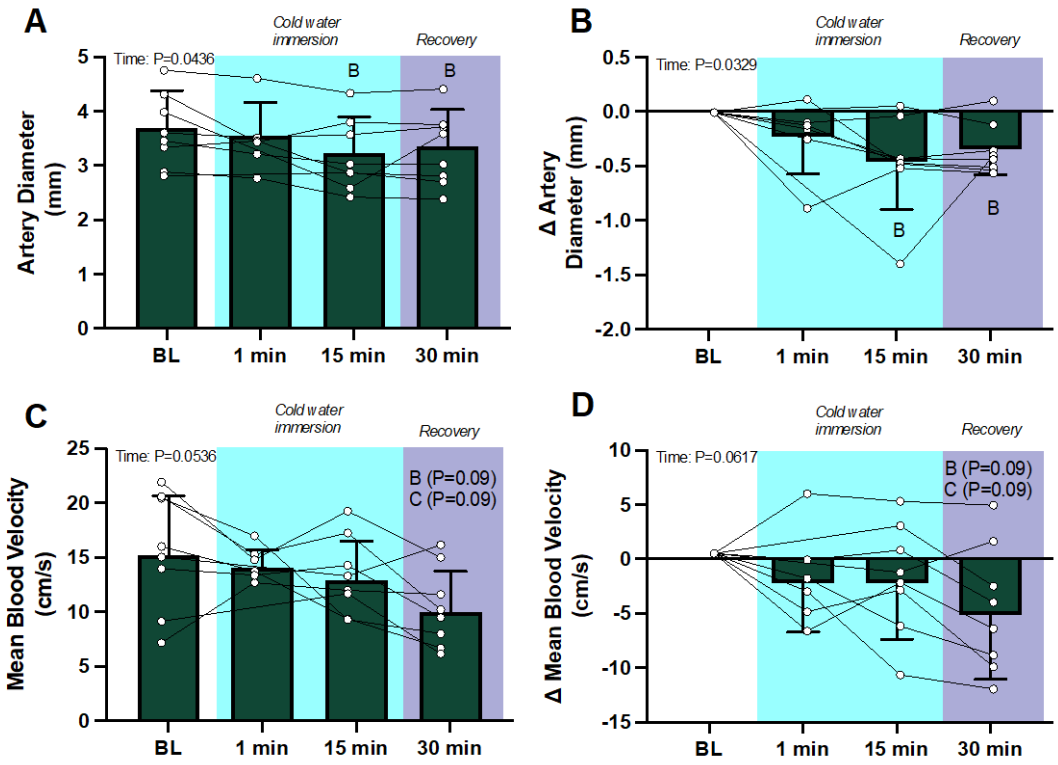

Figure 6: Brachial artery diameter and mean blood velocity

The brachial diameter (Panels A & B) and mean blood velocity (Panels C & D) responses shown as absolute (Panel A & C) and change (Panel B & D) values from pre-immersion baseline (BL) at BL, minute 1 of cold-water immersion, minute 15 of cold-water immersion and at minute 30 of recovery. The thick bold line illustrates the mean data, and the thin lines illustrate individual responses. Data was analyzed using a one-way ANOVA with post-hoc Dunnett's test for multiple comparisons. [B]different from baseline (P≤0.05); [C]different from minute 15 of cold-water immersion (P≤0.05); at BL, 15 Min and 30 Min *n*=8 (5 females), at 1 Min *n*=6 (4 females).

Brachial Artery Blood Flow Patterns

Our preliminary analysis indicates a trend towards significant effect of time on mean blood flow (P=0.0602). There was no change in mean blood flow from baseline to

minute 15 of cold-water immersion (P=0.1589). We observed a trend towards significant decrease in brachial mean blood flow by 25±28mL/min (Figure 7B) from baseline to minute 30 of recovery (BL 49±30 mL/min vs Min 30 REC 23±10mL/min, P=0.0857; Figure 7A). There was no change in mean blood flow from minute 15 of cold-water immersion to minute 30 of recovery (P=0.1666).

Our preliminary analysis showed a trend towards a significant effect of time on antegrade blood flow (P=0.0604). Compared to baseline, there was a trend towards antegrade blood flow being decreased by 18±20 mL/min (Figure 7D) at minute 15 of cold-water immersion (BL 59±39 vs Min 15 CWI 42±24, P=0.0966; Figure 7C) and a trend towards being decreased by 26±29 mL/min (Figure 7D) in antegrade blood flow after minute 30 of recovery (59±39 mL/min vs 33±16 mL/min; P=0.0932; Figure 7C). There was no change in antegrade blood flow from minute 15 of cold-water immersion to minute 30 of recovery (P=0.1388). There was no effect of time on retrograde blood flow (P=0.4697).

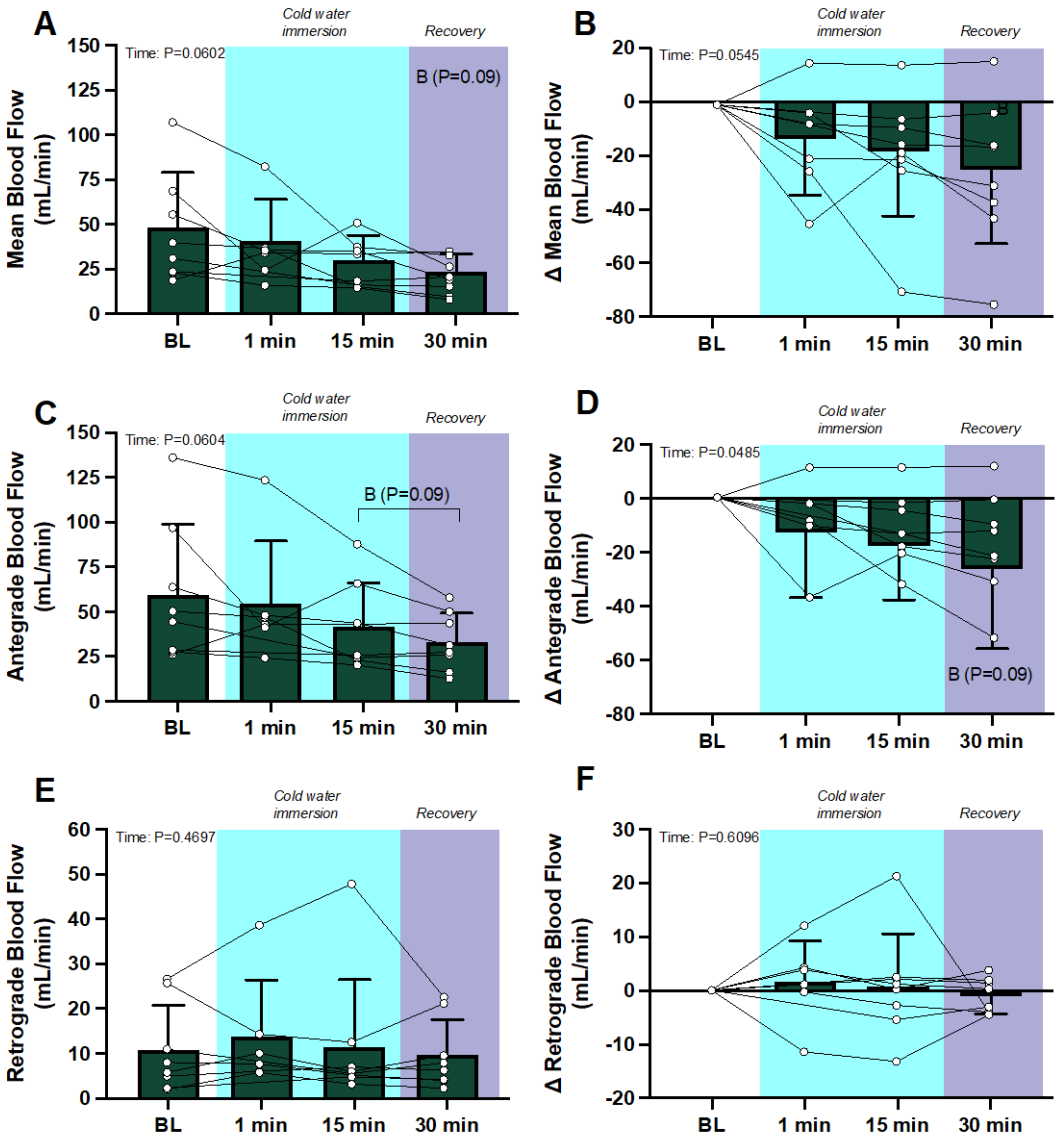

Figure 7: Brachial artery blood flow

The mean blood flow (Panels A & B), antegrade blood flow (Panels C & D) and retrograde blood flow (Panels E & F) responses shown as absolute (Panel A, C & E) and change (Panel B, D & F) values from pre-immersion baseline (BL) at BL, minute 1 of cold-water immersion, minute 15 of cold-water immersion and at minute 30 of recovery. The thick bold line illustrates the mean data, and the thin lines illustrate individual responses. Data was analyzed using a one-way ANOVA with post-hoc Dunnett's test for multiple comparisons. [B]different from baseline ($P \leq 0.05$); [C]different from minute 15 of cold-water immersion ($P \leq 0.05$); at BL, 15 Min and 30 Min $n=8$ (5 females), at 1 Min $n=6$ (4 females).

Brachial Artery Shear Patterns

There was a significant effect of time on mean shear (P=0.0353). Compared to baseline, there was no difference in mean shear after 15 minutes of cold-water immersion (P=0.9966) but there was decrease in mean shear by 52±50s^{-1} (Figure 8B) after 30 minutes of recovery (BL 156±53s^{-1} vs Min 30 REC 104±32s^{-1}, P=0.0511; Figure 8A). Compared to minute 15 of cold-water immersion, there was a decrease in mean shear by 48±44 s^{-1} (Figure 8B) after 30 minutes of recovery (BL 152±44s^{-1} vs Min 30 REC 104±32s^{-1}, P=0.0404; Figure 8A).

There was a significant effect of time on antegrade shear (P=0.0282). There was no difference in antegrade shear after 15 minutes of cold-water immersion (P=0.8991). Compared to baseline, our preliminary analysis of antegrade shear revealed a trend towards being decreased by 44±48s^{-1} (Figure 8D) after 30 minutes of recovery (BL 184±54s^{-1} vs Min 30 REC 140±33s^{-1}, P=0.0880; Figure 8C). Compared to minute 15 of cold-water immersion, there was a decrease in antegrade shear by 55±43s^{-1} (Figure 8D) after 30 minutes of recovery (Min 15 CWI 195±37s^{-1} vs Min 30 REC 140±33s^{-1}, P=0.0218; Figure 8C). There was no significant effect of time on brachial retrograde shear (P=0.1586).

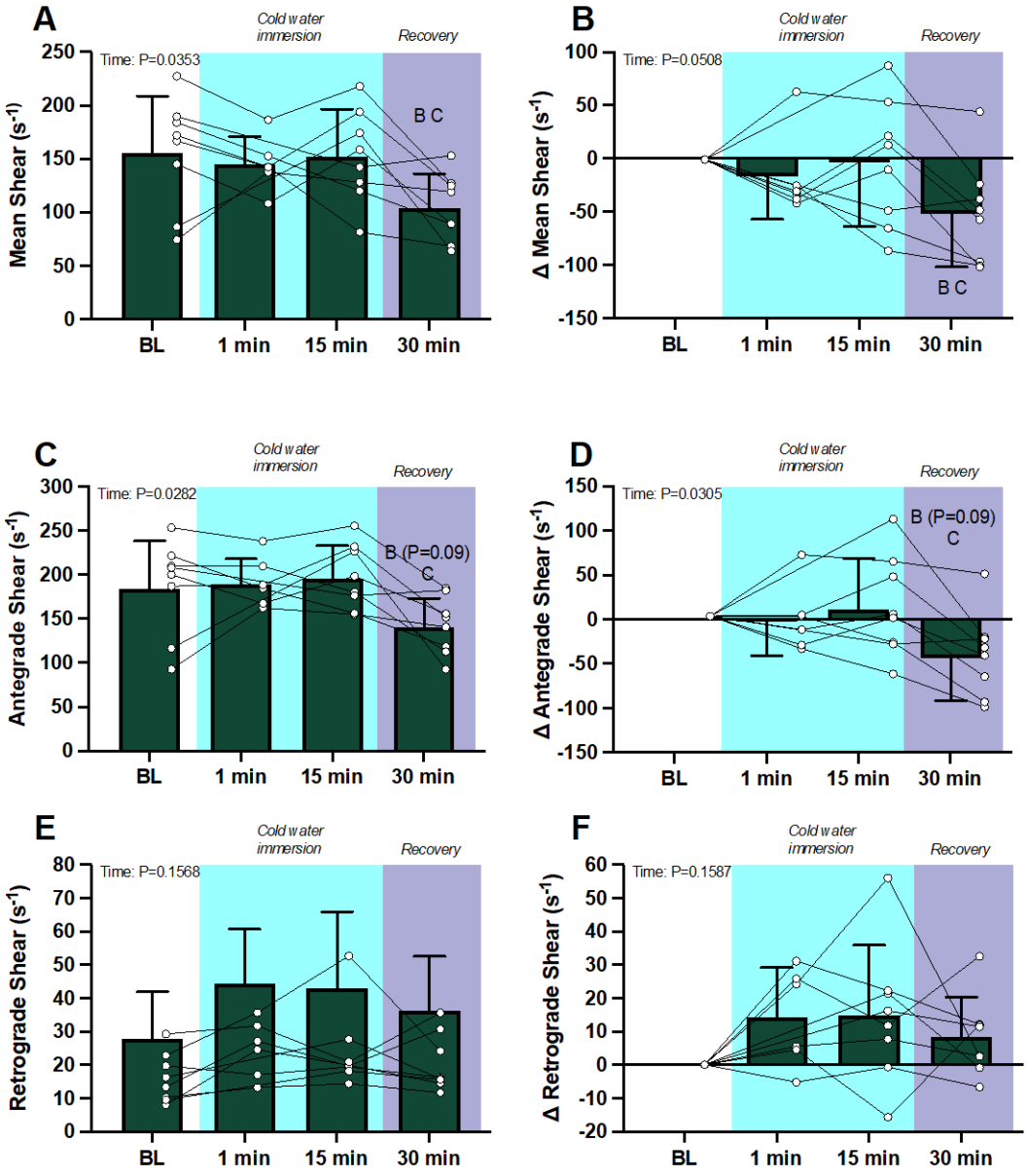

Figure 8: Brachial artery shear patterns

The mean shear (Panels A & B), antegrade shear (Panels C & D) and retrograde shear (Panels E & F) responses shown as absolute (Panel A, C & E) and change (Panel B, D & F) values from pre-immersion baseline (BL) at BL, minute 1 of cold-water immersion, minute 15 of cold-water immersion and at minute 30 of recovery. The thick bold line illustrates the mean data, and the thin lines illustrate individual responses. Data was analyzed using a one-way ANOVA with post-hoc Dunnett's test for multiple comparisons. [B]different from baseline ($P \leq 0.05$); [C]different from minute 15 of cold-water immersion ($P \leq 0.05$); at BL, 15 Min and 30 Min $n=8$ (5 females), at 1 Min $n=6$ (4 females).

Oscillatory Shear Index

Our preliminary analysis indicates a trend towards a significant effect of time on oscillatory shear index (P=0.0643). Compared to baseline, there was no difference in oscillatory shear index after 15 minutes of cold-water immersion (P=0.3350). Oscillatory shear index increased from baseline by 0.07±0.05 (Figure 9B) after 30 minutes of recovery (BL 0.14±0.19 vs Min 30 REC 0.21±0.07, P=0.0213; Figure 9A)

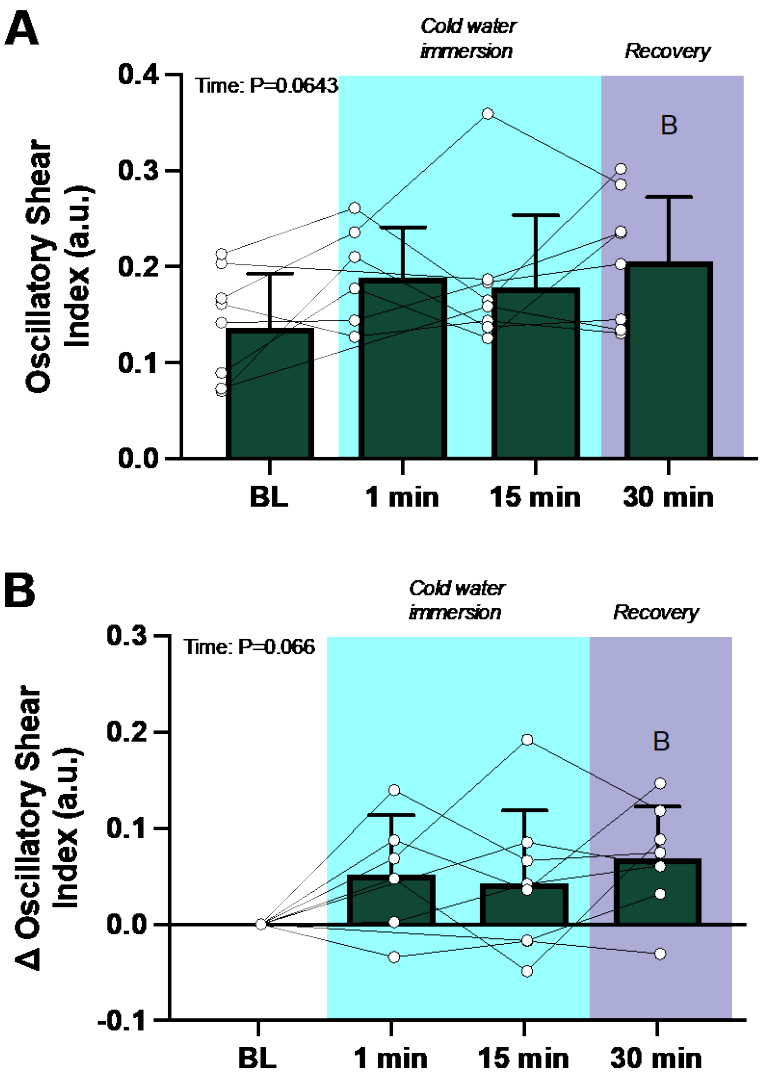

Figure 9: Brachial artery oscillatory shear index

The oscillatory shear index responses shown as absolute (Panel A) and change (Panel B) values from pre-immersion baseline (BL) at BL, minute 1 of cold-water immersion, minute 15 of cold-water immersion and at minute 30 of recovery. The thick bold line illustrates the mean data, and the thin lines illustrate individual responses. Data was analyzed using a one-way ANOVA with post-hoc Dunnett's test for multiple comparisons. [B]different from baseline (P≤0.05); [C]different from minute 15 of cold-water immersion (P≤0.05); at BL, 15 Min and 30 Min *n*=8 (5 females), at 1 Min *n*=6 (4 females).

Brachial Vascular Conductance

There was a significant effect of time on brachial vascular conductance (P=0.0360). Compared to baseline, our preliminary analysis revealed a trend towards a significant decrease in vascular conductance by 0.25±0.26 mL/min/mmHg (Figure 10B) after 15 minutes of cold-water immersion (BL 0.60±0.38 vs. Min 15 CWI 0.44±0.28 mL/min/mmHg, P=0.0875; Figure 10A) and a trend for being decreased by 0.35±0.35 mL/min/mmHg (Figure 10B) after 30 minutes of recovery (BL 0.60±0.38 vs. Min 30 REC 0.33±0.13 mL/min/mmHg P=0.0667; Figure 10A). There was no change in vascular conductance from minute 15 of cold-water immersion to minute 30 of recovery (P=0.1180).

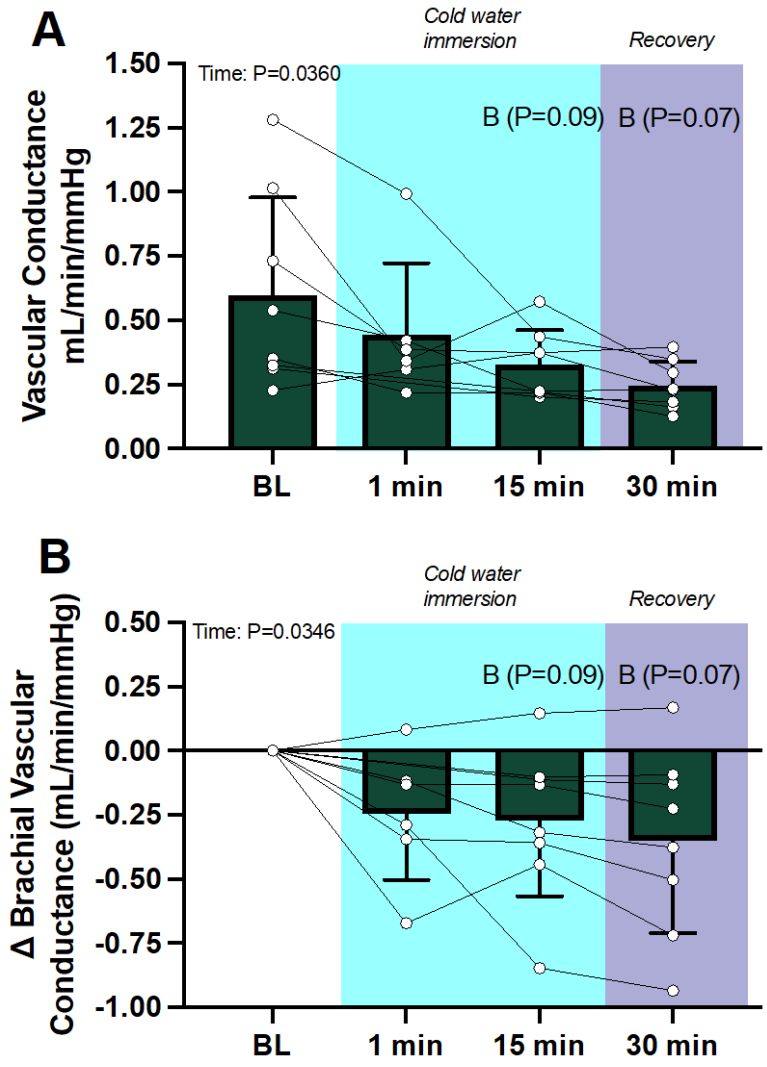

Figure 10: Brachial artery vascular conductance

The brachial vascular conductance responses shown as absolute (Panel A) and change (Panel B) values from pre-immersion baseline (BL) at BL, minute 1 of cold-water immersion, minute 15 of cold-water immersion and at minute 30 of recovery. The thick bold line illustrates the mean data, and the thin lines illustrate individual responses. Data was analyzed using a one-way ANOVA with post-hoc Dunnett's test for multiple comparisons. [B]different from baseline (P≤0.05); [C]different from minute 15 of cold-water immersion (P≤0.05); at BL, 15 Min and 30 Min *n*=8 (5 females), at 1 Min *n*=6 (4 females).

Thermal Perceptions

Thermal sensation and thermal comfort

There was a significant effect of time on thermal sensation ($P<0.0001$). Compared to baseline, participants reported that thermal sensation was reduced (i.e., felt more cold) from the start of cold-water immersion through the end of the 30-minute recovery ($P\leq0.0225$), with the largest reported reduction in thermal sensation at minute 0 and 5 of cold-water immersion ($P<0.0001$). Compared to baseline, thermal sensation was reduced by 12 ±9 (Figure 11B) by the end of cold-water immersion (BL 2±5 vs Min 15 CWI -11±6, P=0.0007; Figure 11A). Compared to baseline, thermal sensation was reduced by 5±6 (Figure 11B) by the end of minute 30 of recovery (BL 2±5 vs Min 30 of REC -4±5, P=0.0225; Figure 11A). Compared to minute 15 of cold-water immersion, thermal sensation was reported to be improved at minute 0 of recovery, and minute 25 and 30 of recovery ($P\leq0.0417$). Compared to minute 15 of cold-water immersion, thermal sensation was improved by 7±7 (Figure 11B) by the end of minute 30 of recovery (Min 15 CWI -11±6 vs Min 30 REC -4±5, P=0.0179; Figure 11A).

There was a significant effect of time on thermal comfort ($P<0.0001$). Compared to baseline, thermal discomfort increased by 2±2 (Figure 11D) by the end of cold-water immersion (BL 2±2 vs Min 15 CWI 4±2, P=0.0124; Figure 11C) with the largest reported reduction in comfort at minute 0 of cold-water immersion (P=0.001). During minute 0, 20, 25, and 30 of recovery participants did not report an increase in thermal discomfort compared to baseline ($P\geq0.0919$), however, during minutes 5, 10, and 15 of recovery participants reported an increase in thermal discomfort compared to baseline ($P\leq0.043$), with the largest increase in thermal discomfort at minute 10 of recovery (BL

2±2 vs Min 10 REC 4±2, P=0.0026; Figure 11C). Compared to minute 15 of cold-water immersion, participants reported an increase in thermal comfort at minute 30 of recovery. Participants reported a decrease in thermal discomfort by 2±2 (Figure 11C) by the end of recovery (Min 15 CWI 4±2 vs Min 30 REC 2±1, P=0.0318; Figure 11D).

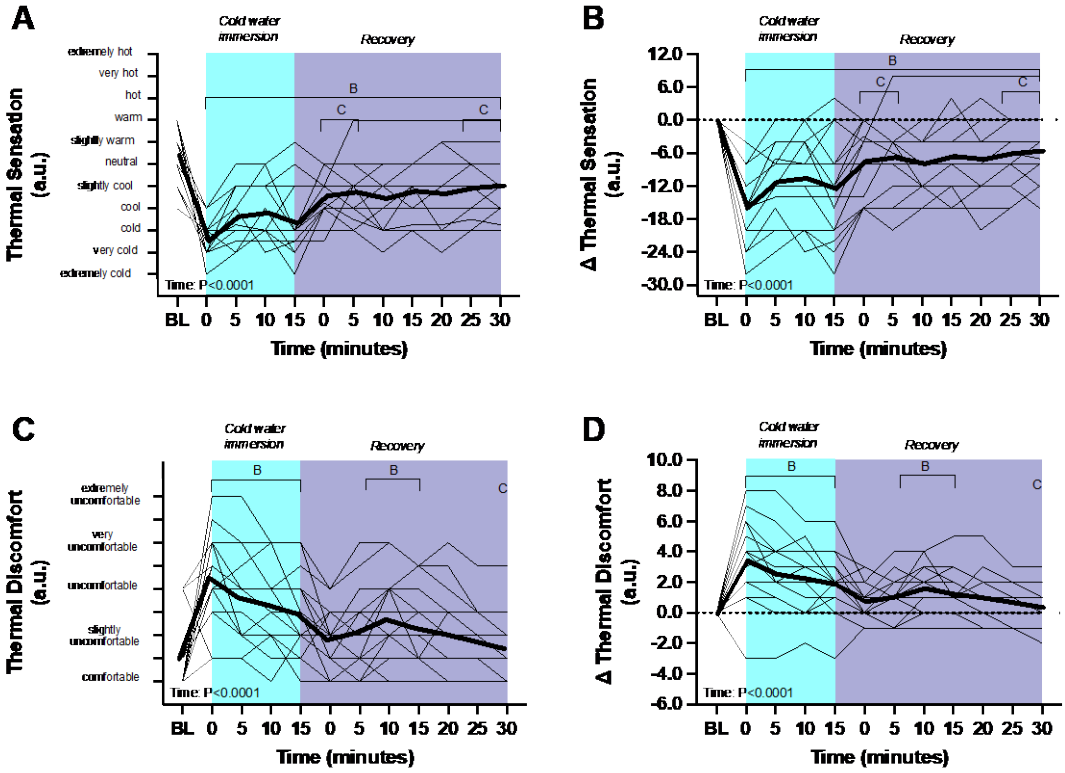

Figure 11: Thermal sensation and discomfort

Thermal sensation (Panels A & B), thermal comfort (Panels C & D) shown as absolute (Panel A, C & E) and change (Panel B, D & F) values from pre-immersion baseline (BL) at BL, minute 1 of cold-water immersion, minute 15 of cold-water immersion and at minute 30 of recovery. The thick bold line illustrates the mean data, and the thin lines illustrate individual responses. Data was analyzed using a one-way ANOVA with post-hoc Dunnett's test for multiple comparisons. [B]different from baseline (P≤0.05); [C]different from minute 15 of cold-water immersion (P≤0.05); *n*=16 (7 females)

Discussion

The purpose of this study was to determine the effects of an acute bout of cold-water immersion on cardiovascular parameters including peripheral vascular responses of brachial artery diameter, blood flow, and blood vessel shear patterns in healthy, young adults. Our main findings do not fully support our original hypotheses. We hypothesized there would be an increase in hemodynamics and peripheral vasoconstriction during cold-water immersion followed by a return to pre-immersion values after the post-immersion recovery period. Further, we hypothesized that these fluctuations in these responses would suggest potential cardiovascular benefits through increased blood flow and shear stress patterns. Our results showed an increase in hemodynamics during immersion and throughout the post-immersion recovery period. Additionally, peripheral vasoconstriction of the brachial artery occurred at the end of cold-water immersion and throughout post-immersion recovery instead of returning to pre-immersion values. Further, due to the trending decrease in antegrade blood flow and shear patterns and increase in oscillatory shear index after post-immersion recovery, our original hypothesis is likely not supported as these may indicate a potential negative effect on peripheral vascular function.

There was decrease in rectal temperature during both cold-water immersion and recovery compared to baseline, and a continued decrease throughout recovery in comparison to end of immersion. This change in rectal temperature during post-immersion recovery has been discussed as the "afterdrop" phenomenon and is the transient continuous drop in body temperature[45,46]. Webb reported a ~30 minute duration of "afterdrop" following either 22, 15, and 5°C cooling paired with 43°C

rewarming in perfused suits[45]. One possible mechanism for afterdrop phenomenon has been attributed to the return of cold blood to the periphery therefore promoting convective and conductive heat loss mechanisms[45]. During cold-water immersion, blood is shunted to the core to maintain core body temperature[10,47], and the hydrostatic pressure of the water increases central blood volume[48]. The result of afterdrop in our current study was a potential driver for the peripheral vascular and hemodynamic responses that occurred during the recovery period.

There was a significant decrease in brachial artery diameter at the end of immersion and recovery thus indicating vasoconstriction of the brachial artery did not return to pre-immersion values within 30 minutes of seated recovery. The greatest difference in brachial artery diameter from baseline occurred at the end of recovery indicating there was a continued vasoconstrictor response. This decrease in diameter aligns with previous literature surrounding cold exposure and the initial acute responses (i.e., vasoconstriction) as a result of sympathetic activation[23–25]. Mean blood flow did not change during cold-water immersion and was decreased after 30 minutes of recovery compared to baseline. Although we saw no change in flow during cold-water immersion, previous research has shown decreases in peripheral blood flow in water temperatures below 33°C[11]. We may have been statistically underpowered and/or methodology (i.e., time in water) to detect these changes. Additionally, there was no change in mean shear during cold-water immersion but there was a decrease in mean shear at the end of recovery compared to baseline as well as compared to the end of cold-water immersion. Antegrade shear and antegrade flow both were reduced at the end of recovery compared to baseline as well as recovery compared to the end of cold-

water immersion. This was likely due to redistribution of blood flow due to peripheral vasoconstriction in order to maintain core body temperature[10,47]. There was no significant change in retrograde shear or blood flow during this study which differs from previous reports which indicate that vasoconstriction causes increases in retrograde flow and shear[41,49]. However, the continuous decrease of mean and antegrade flow was a possible result of decreasing core temperature, re-stimulating the vasoconstrictor response, thus resulting less peripheral blood flow. Similarly, afterdrop likely caused a further decrease in the diameter of the brachial artery, flow, shear, and increasing blood pressure as the body continues to respond to dropping core temperature. This decrease in peripheral blood flow has been shown to continue until afterdrop has subsided and core temperature returns to normal[45]. Although we saw no change in retrograde flow after cold-water immersion, the decrease in antegrade flow indicates that a larger proportion of retrograde flow made up the mean blood flow at the end of the post-immersion recovery period compared to baseline. Further, this could explain the increase in oscillatory shear index during recovery, indicating a more oscillatory or multidirectional flow. An increase in oscillatory shear index may indicate a negative influence on endothelial function[40,41]. A lower antegrade shear, induced by forearm compression, paired with an unchanged retrograde shear, during recumbent cycling attenuated flow mediated dilation in comparison to the contralateral limb that did not have forearm compression and thus had an increase in antegrade shear[51]. From this, we conclude that our results may show a negative effect of cold-water immersion on endothelial function. However, without a longer recovery period to follow transient changes to these parameters (i.e., antegrade and retrograde shear, oscillatory shear

index, etc.) and how these may respond differently to repeated exposures, we are unable to fully support this conclusion.

Our results showed an increase in brachial mean arterial pressure, systolic blood pressure, and diastolic blood pressure during cold-water immersion, with mean arterial pressure remaining increased throughout recovery, and diastolic blood pressure increasing during the second half of recovery. This increase in systolic blood pressure and diastolic blood pressure in recovery could be attributed to a further decrease in brachial artery diameter as a result of afterdrop and vasoconstriction. Previous reports have concluded that initially there is an increase in systolic blood pressure and diastolic blood pressure after beginning cold-water immersion, as well as an initial increase in heart rate followed by a return to pre-immersion values[11,24,27]. Although our heart rate data did not reveal a significant initial increase in heart rate, 11 of the 16 participants did have an increase. Few studies have shown that following ~10-20 minutes of cold water immersion, heart rate returned to baseline values and decreased further beyond baseline after a longer post-immersion period[24,27]. Other studies noted a decrease in heart rate in cold water (30°C) compared to thermoneutral water (34.5°C)[32]; White and Wells attributed this decrease in heart rate to a restoring of vagal influence[10]. Our results indicate that upon initial immersion, systolic blood pressure and diastolic blood pressure increase and remained elevated through the majority of immersion. Similar to previous literature, heart rate decreased after an initial increase, and remained reduced through recovery. Full body cold water immersion, including the face, stimulates the mammalian diving reflex due to the stimulation of the trigeminal nerve. The diving reflex is represented by bradycardia and increases in blood pressure due to the dual

activation of both the sympathetic and parasympathetic nervous systems. This can result in autonomic conflict and increase the risk of cardiac arrythmias[9,50]. Despite our participants completing head out water immersion, we found similar cardiovascular and hemodynamic patterns to this reflex.

Although total peripheral resistance did not change from baseline during cold-water immersion or recovery in our study, previous literature shows total peripheral resistance increases as a result of peripheral vasoconstriction and peripheral vascular resistance when comparing colder water to thermoneutral immersion[51,52]. Due to total peripheral resistance being derived from the beat-to-beat blood pressure, we were potentially underpowered to detect a change. There was no change in cardiac output during cold-water immersion, despite the significant decrease in heart rate by the end of immersion. Although we did not see an increase in stroke volume, previous data shows that during cold-water immersion, stoke volume increases for two main reasons, one, as a result of peripheral vasoconstriction increasing preload[32] likely maintaining or increasing cardiac output[10,11,25], and two, due the hydrostatic pressure of the water increases venous return and preload[10,48]. These changes in cardiac preload have been shown to change as a result of both head out and waist up cold-water immersion[10]. Although we saw no change in cardiac output during cold-water immersion, a study by Keatinge et al. showed an increase in cardiac output during ice cold showers in two different participants[25]. In contrast to this, other literature suggests that because of an increase in total peripheral resistance, cardiac output may not increase in cold-water immersion due to an increase in afterload[53]. However, cardiac output was decreased by

the end of recovery, which could potentially be due to the decrease in preload after a reduction in hydrostatic pressure once removed from the water.

Limitations

Although this study has contributed to the understanding of cold-water immersion, our study is not without limitations that should be addressed in future research. First, we are likely statistically under powered for the results for beat-to-beat data as well as some ultrasound measures; with only 6 participants for beat-to-beat parameters (due to the poor quality of waveforms from vasoconstriction and/or shivering causing excessive movement), and 8 participants for ultrasound. We are continuing to collect and analyze additional data sets from this protocol at the time of submission. This study only included young, healthy individuals therefore we may not have a full representation of the general public and if these responses may differ between different populations. Furthermore, this study was measuring the acute response to cold-water immersion, rather than a chronic and repeated exposure study which could be more telling of the cardiovascular adaptations, and if these adaptions are beneficial to cardiovascular health, as well as cold-water immersion in comparison to exercise.

Due to COVID-19, we were limited by the amount of time we were able to spend with the participants in a given study day as well as the amount of study days we were able to have each week. Given the protocol guidelines, we were limited to two hours with less than 15 minutes close contact within each hour. Due to this, our protocol was restricted to measurements that could be taken both quickly and accurately, and our

participants could only participate in one of the two subgroups (ultrasound or blood biomarkers). Therefore, we do not have blood markers in the same individuals who were in the ultrasound arm. We also could not include a more comprehensive measure of peripheral vascular function, flow mediated dilation, due to time and close contact-restraints. Further, these restrictions created the inability to use a control study session (i.e., thermoneutral water and/or time control) and future studies could benefit from a more comprehensive protocol.

Future Directions

Future studies may benefit from including a control thermoneutral water immersion group in which subjects underwent two testing days, a thermoneutral control as well as the cold-water immersion. Similarly, it would be valuable for all participants to have both ultrasound as well as blood measurements taken to have a comprehensive understanding for the physiological effects of acute cold-water immersion. Investigating the effect of multiple and repetitive bouts of cold-water immersion would also be useful to determine whether or not cold-water immersion could be used as a therapeutic modality and whether or not cold-water immersion has similar long-term benefits as exercise or heat therapy. Furthermore, future studies could benefit from both increasing recovery time to determine if there is change in flow, shear, and artery diameter once after drop ends and core temperature returns to normal, as well as passively and slowly rewarming participants after cold-water immersion is over to determine the effect of core temperature and peripheral vascular responses.

Conclusion

Our results indicate that an acute bout (15 minutes) of cold-water immersion in 10°C water caused significant changes to cardiovascular, specifically the peripheral vasculature, and hemodynamic variables. Specifically, brachial artery diameter decreased during water immersion and further decreased from pre-immersion values. Further, there were minimal changes to brachial artery blood flow and shear during cold water immersion, but both were reduced after a post-immersion recovery period. Overall, the changes in peripheral blood flow and shear acutely indicate there was not an increase in shear, an important response for beneficial vascular adaptations. However, it remains unknown if blood flow and shear return, or even overshoot, pre-immersion values or if there are cyclic changes in peripheral blood flow and shear that would result in improved vascular function and cardiovascular health.

Appendix A: Informed Consent Documents

Consent for Research Participation

Title: Cardiovascular Responses to Acute Cold-Water Immersion

Researcher(s): Dr. Christopher T Minson and colleagues, University of Oregon

Researcher Contact Info: (541) 632-4151
minson@uoregon.edu

You are being asked to participate in a research study. The box below highlights key information about this research for you to consider when making a decision whether or not to participate. Carefully consider this information and the more detailed information provided below the box. Please ask questions about any of the information you do not understand before you decide whether to participate.

Key Information for You to Consider
• **Voluntary Consent**. You are being asked to volunteer for a research study. It is up to you whether you choose to participate or not. There will be no penalty or loss of benefits to which you are otherwise entitled if you choose not to participate or discontinue participation. • **Purpose**. The purpose of this research is to test whether cold water baths improve cardiovascular health. • **Duration.** It is expected that your participation will last a total of 2.5-5.5 hours, including a 30-minute screening session and a 2 or 5-hour experimental session on a separate day. • **Procedures and Activities.** You will be asked to lay in a cold bath 50°F (10°C) for 15 minutes. We will perform tests while you are in the bath and during a short recovery period. • **Risks.** Some of the foreseeable risks or discomforts of your participation include physical risks including cold-related pain or illness during cold exposure. • **Benefits**. No direct benefits expected. • **Alternatives.** Participation is voluntary and the only alternative is to not participate.

Why is this research being done?

The purpose of this research is to determine the cardiovascular effects of acute cold-water immersion. Determining the cardiovascular effects of acute cold-water immersion will provide a better understanding of the mechanisms that lead to long term adaptations and potential cardiovascular benefits. Additionally, we will make measurements to address safety issues, determining whether cold water immersion is safe and beneficial for young, healthy individuals. You are being

asked to participate because you are an inactive to highly active nonsmoker between the ages of 18 and 40 with no underlying cardiovascular or cold intolerance limitations. About 20 people will take part in this research.

What happens if I agree to participate in this research?
If you agree to be in this research, your participation will include:

Screening Visit

You will arrive at Dr. Minson's laboratory at the University of Oregon for a screening visit. This visit will take approximately 30 minutes. You will meet with one of the investigators of the study to discuss the project, see the laboratory, read this form, and get all your questions answered. Your height, weight, arm circumference, and blood pressure will be measured, and you will fill out a health history form. If you are a woman who can still become pregnant, you will be asked to undergo a pregnancy test. For the pregnancy test, you will be asked to collect a sample of urine in a private restroom in the lab. If the test is positive, indicating that you are pregnant, you will not be allowed to participate, as the study procedures could be harmful to an unborn child, and you will be advised to see your physician or the University of Oregon Health Center. If you meet all of the inclusion criteria, do not meet any of the exclusion criteria, and decide to participate in this study, you will give your consent by signing this form. You will then be assigned to either **Group 1** or **Group 2** (described below) and we will schedule you for an experimental visit.

Experimental Visit

1) Your cumulative time spent in the lab for the experimental visit will last no more than 2 hours. Prior to arrival, you will be asked to abstain from heavy exercise, heat therapy (e.g. hot tub, sauna, hot yoga), or cold therapy (e.g. cold shower/bath) for 24 hours; medications and supplements (except oral contraceptives) for 12 hours; alcohol for 12 hours, caffeine for 6 hours, and food for 2 hours (water is fine). Additionally, you will be asked to bring a swimsuit for the water immersion.

2) You will fill out the PANAS form. The PANAS consists of 20 words that describe different feelings and emotions. You will read each word and then indicate to what extent you feel this way at the present moment using a scale of 1 (very slightly or not at all) to 5 (extremely).

3) You will change into your swimsuit and be given a Polar® heart rate monitor chest strap, which you will wear around your torso.

4) You will be given a rectal thermistor and instructions on how to self-insert, as well as how to remove it and clean it. It is made of a thin flexible rubber material that is inserted 10 cm (approximately 4 inches) past the anal sphincter. The thermistor will remain in place throughout the entire study session. The thermistor has a "tail" that will be connected to an external apparatus. The procedure may be a little uncomfortable at first (during insertion) but it should not be painful at any time. Once in place, you may not feel the probe at all. This technique is widely used and it's considered the gold standard procedure for measuring body (core) temperature.

Group 1:

5) A trained phlebotomist will place a needle with a flexible intravenous (IV) catheter in a vein near your elbow, the needle will be removed, and the flexible catheter will be left in your vein for the remainder of the study. Once the catheter is in place, you may not feel it at all. This allows us to draw blood at **5** timepoints throughout the study without using another needle.

6) A blood pressure cuff will be put on your upper arm and you will wear this throughout the study.

7) We will also place a finger blood pressure cuff around the middle finger of one of your hands to continuously measure beat-by-beat changes in blood pressure throughout the study. This cuff will inflate and deflate around your finger throughout the study. An associated wrist unit that accompanies the finger cuff will be secured around your wrist and will remain in place throughout the study. If the finger cuff becomes uncomfortable at any time, we will turn the finger blood pressure cuff off for as long as needed.

8) We will place 3 sticky electrodes on your skin and attach a small wire or lead to each electrode. These leads will be attached to a monitor that will allow us to measure your heart rate and heart rhythm. These electrodes will be placed on your body in the following locations: 2 electrodes are placed on your upper chest close to your shoulder (one on the left and one on the right) and 1 will be placed just above your hip bone (just above where your pants line is) on the left side. These electrodes will be taped in place until the end of the study.

9) You will rest quietly for 15-20 min while sitting in a lift that will be used to place you in the cold-water bath. At the end of the rest period, a small sample of blood will be drawn.

10) You will be placed in a $50°F\ (10°C)$ bath up to your breastbone (mid-ribs) for 15 minutes. We will take a small blood sample during the first 2 and last 2 minutes of the bath. We will monitor your heart rate, blood pressure, and core temperature throughout the bath and will ask you questions about your perception of thermal sensation and thermal comfort every 5 min.

11) After the bath, you will be given towels and blankets and will rest for another 15-30 minutes. During this rest period, we will continue to monitor your heart rate, blood pressure, and core temperature and ask you questions about your perception of thermal sensation and thermal comfort. After this rest period, another small blood sample will be taken. The blood pressure cuffs will be removed and you will move to the restroom where you will take off the Polar® heart rate monitor chest strap, and remove and clean the rectal thermistor. The IV catheter will remain in place near your elbow. You will fill out another PANAS form and then be instructed to leave the lab and return for one more small blood sample after 3 hours. After the last blood sample, the IV catheter will be removed and a bandage will be applied. While you are away from the lab for the 3-hour post-bath interval, you will be asked not to eat or drink anything other than water.

12) Before leaving the lab, you will fill out the PANAS form one more time.

Group 2:

5) Ultrasound: A small amount of gel will be placed on an ultrasound probe and the probe will be placed on your inner upper arm. A trained sonographer will search for an ultrasound image of your brachial artery. Once a good image is found (this may take a few minutes), a 1-2-minute video will be recorded. The sonographer will mark your arm to indicate where the probe was placed (this mark will be removed at the end of the study). The probe and gel will then be removed.

6) A blood pressure cuff will be put on your upper arm and you will wear this throughout the study. We will inflate this cuff every 5 min while you are in the bath and throughout a 15-30 min recovery period.

7) We will also place a finger blood pressure cuff around the middle finger of one of your hands to continuously measure beat-by-beat changes in blood pressure throughout the study. This cuff will inflate and deflate around your finger throughout the study. An associated wrist unit that accompanies the finger cuff will be secured around your wrist and will remain in place throughout the study. If the finger cuff becomes uncomfortable at any time, we will turn the finger blood pressure cuff off for as long as needed.

8) We will place 3 sticky electrodes on your skin and attach a small wire or lead to each electrode. These leads will be attached to a monitor that will allow us to measure your heart rate and heart rhythm. These electrodes will be placed on your body in the following locations: 2 electrodes are placed on your upper chest close to your shoulder (one on the left and one on the right) and 1 will be placed just above your hip bone (just above where your pants line is) on the left side. These electrodes will be taped in place until the end of the study.

9) You will rest quietly for 15-20 min while sitting in a lift that will be used to place you in the cold-water bath. At the end of the rest period, a small sample of blood will be drawn.

10) You will be placed in a $50°F\ (10°C)$ bath up to your breastbone (mid-ribs) for 15 minutes. We will take 2 more ultrasound videos during the bath; once immediately upon entering the bath, and once at the end of the bath. We will monitor your heart rate, blood pressure, and core temperature throughout the bath and will ask you questions about your perception of thermal sensation and thermal comfort every 5 min.

11) After the bath, you will be given towels and blankets and will rest for another 15-30 minutes. During this rest period, we will continue to monitor your heart rate, blood pressure, and core temperature and ask you questions about your perception of thermal sensation and thermal comfort. After this rest period, one last ultrasound video will be taken.

12) The blood pressure cuff will be removed and you will move to the restroom where you will take off the Polar® heart rate monitor chest strap, and remove and clean the rectal thermistor.

13) Before leaving the lab, you will fill out another PANAS form.

14) 3 hours after leaving the lab, you will call the lab at **541-600-4095** to verbally complete a final PANAS survey.

We will tell you about any new information that may affect your willingness to continue participation in this research.

What happens to the information collected for this research?
Information and specimens collected for this research will be used to better understand the physiology of how the human body responds to cold water baths, and may be used in published reports and conference presentations. Your name will not be used in any published reports or conference presentations about this study. Identifiers will be removed from identifiable private information or identifiable biospecimens collected in this research, which may be used for future research without additional informed consent.

How will my privacy and data confidentiality be protected?
We will take measures to protect your privacy including conducting research in a private setting and using secure data collection platforms. Despite taking steps to protect your privacy, we can never fully guarantee your privacy will be protected. We will take measures to protect the security of all your personal information including coding all data collected in connection with this study by assigning a subject identification number. The document that links your identity with your subject number will be kept in a locked file cabinet within a locked office separated from all data. The coded list of names will be destroyed when study results are published or 24 months after your participation, whichever comes first. All blood samples will be destroyed when study results are published or 5 years after your participation, whichever comes first. Any information that can be identified with you will remain confidential and will be disclosed only with your permission. Other information may be stored by the researchers indefinitely. Despite these precautions to protect the confidentiality of your information, we can never fully guarantee confidentiality of all study information.
Individuals and organizations that conduct or monitor this research may be permitted access to and inspect the research records. This may include access to your private information and medical results. These individuals and organizations include:

- The Institutional Review Board (IRB) that reviewed this research
- Government regulatory agencies
- The Food and Drug Administration

If data is shared with researchers outside of the University of Oregon physiology lab for the purpose of statistical analysis, all personally identifiable information will be removed.

What are the risks if I participate in this research?

The risks or discomforts of participating in this research include:

Cold Exposure

You may be at a slight risk of feeling numbness or tingling as a result of the cold-water exposure. This risk will be mitigated by only having you bathe for 15 minutes in 50°F (10°C) water, which is very unlikely to cause severe cold induced injuries. Cold water immersion can lead to an initial cold shock and could cause rapid and uncontrolled breathing, or hyperventilation. Hyperventilation can lead to the feeling of dizziness, fainting, ringing in ears, and numbness in limbs. You could feel a loss of feeling or control in your limbs or experience muscle cramping. You will be seated during the procedure and recovery to reduce risk of injury. Researchers will check in with you regularly to determine if you are experiencing any of these symptoms. Alert researchers if you feel lightheaded, are in pain, or do not want to continue, and you will immediately be removed from the water and warmed under blankets or may elect to take a warm shower if deemed safe by investigators. Hypothermia is unlikely due to limited time duration. For healthy adults, it takes at least 30 minutes of ice-cold water immersion before onset of hypothermia. In the unlikely event that your body temperature drops to 95°F (35°C), or if you show signs of hypothermia including lack of coordination, slurred speech, or confusion, you will immediately be removed from the water and warmed. Additionally, cold exposure may have detrimental effects on a developing fetus in females and on sperm count in males. Thus, if you are pregnant, trying to conceive, breast-feeding, and/or undergoing treatment to increase sperm count, you will be excluded from this study.

Blood Draw

Four blood draws will be performed, which is a total of up to 160 mL (about 11 tablespoons) of blood. The amount drawn is far less than the standard donation, which is 450-500 mL. There is a possibility of bruising, bleeding, or infection at the puncture site. Bruising is temporary and does not pose any long-term risks, aside from mild discomfort. Pressure will be applied to the site after the removal of the needle to assure the bleeding is quickly ceased. A bandage will be applied to the puncture site to keep the site protected. The risk of infection at the site is low. Your skin will be cleaned with alcohol swabs prior to intravenous access and all equipment used is sterile. Occasionally, subjects may experience lightheadedness or fainting. You will be seated during the procedure and recovery to reduce risk of injury.

Ultrasonography

Ultrasound uses sound waves to image structures within your body. You will not hear these sound waves and you will not feel anything except the probe touching your skin. There are no major risks associated with this procedure.

Finger Blood Pressure

In some people, this blood pressure cuff becomes uncomfortable after a long period. If your finger becomes uncomfortable during the time the cuff is inflated,

let the investigator know and they will turn it off for a few minutes. There are no major risks associated with this device.

Rectal Thermistors

The use of rectal thermistors to measure core temperature carries minimal risk. The primary risk is damage to the lining of the rectum; however, this risk is very slight as we use a flexible thermistor that is designed for this purpose. There is also the risk of infection. The risk of infection is similar to that of having a bowel movement and is considered minimal.

Emergencies

In the event of a life-threatening emergency, investigators will follow the established Human Physiology Emergency procedures, which include an investigator providing basic first aid as appropriate (including high quality CPR and the use of an Automated External Defibrillator (AED) if needed) and calling 911 to activate an emergency response. After activation of emergency response, the emergency personnel will determine if transport is necessary. If transport is necessary, subject will be transported by ambulance to a local emergency facility.

Privacy

Risk of invasion of privacy is minimal due to the privacy and protocols in place to ensure that all data will only include subject number, and all information will be kept in a locked cabinet in a locked room.

COVID-19

To participate in this study, you must agree to comply with federal regulations, state law, and University policy on physical distancing and use of personal protective equipment. It is University policy for face masks to be worn on campus including in outdoor spaces. If you do not have a mask when you arrive on campus, we will provide you with one. When you enter our lab, you will be asked to wash your hands and/or use hand sanitizer.

During the experimental session, you will also be required to wear a face shield. Researchers will be required to wear a face mask at all times and will also be required to wear a face shield, lab coat, and gloves during the experimental session. Physical distance of at least 6 feet must be maintained between individuals at all times except for brief periods of close contact required by study procedures. During these periods of close contact, individuals should speak as little as possible and turn their heads away from each other. When possible, hand signs will be used to communicate to reduce talking.

Researchers and subjects must eliminate close contacts outside of their immediate household for 7 days prior to research activities and 7 days following. Close contact is defined as being within 6 feet of an individual outside your immediate household for 15 minutes or more. Subject visits will be scheduled outside of any planned close contacts such as doctor's appointments or family gatherings. No more than 1 subject visit will be scheduled in the lab during a 24-hour period. All

lab equipment and touched surfaces are disinfected by researchers before and after each visit to the lab. The morning before each lab visit, a researcher will call you to inquire about any symptoms you may be experiencing. You will not be able to participate if you are experiencing any symptoms of COVID-19. Despite these precautions, there remains a risk of exposure to COVID-19. By signing this form, you acknowledge this risk and agree to comply with federal regulations, state law, and University policy on physical distancing and use of personal protective equipment.

In addition to these risks, taking part in this research may have risks that are unknown or currently unforeseeable including potential unforeseeable risks due to COVID-19.

Taking part in this study may hurt a pregnancy or fetus in unknown ways. These may be minor or so severe as to cause death.

What are the benefits of participating in this research?
You may or may not benefit from participating in this research. Measurements are not being conducted for diagnostic purposes. The results will not be reviewed by a physician. The purpose of this study is to provide more information on how healthy humans respond to cold water baths. Our hope is that by better understanding the physiology of how the human body responds, we will be better able to understand the safety and possible applications of cold therapy.

What are my responsibilities if I choose to participate in this research?
If you take part in this research, you will be responsible for:

- Adhering to scheduled sessions and communicating with the researchers in the event that you need to reschedule any sessions.
- Adhering to instructions from the researchers regarding when you need to fast, refrain from consuming caffeine or medications, abstain from alcohol, exercise, or heat therapy for specific testing days.

What other choices do I have besides participation in this research?
It is your choice to participate or not to participate in this research.

What if I want to stop participating in this research?
Taking part in this research study is your decision. Your participation in this study is voluntary. You do not have to take part in this study, but if you do, you can stop at any time. You have the right to choose not to participate in any study activity or completely withdraw from continued participation at any point in this study without penalty or loss of benefits to which you are otherwise entitled. Your decision whether or not to participate will not affect your relationship with the researchers or the University of Oregon.
The investigators may stop you from taking part in this study. Reasons for withdrawal might include:

- It is in your best interest
- You have a side effect that requires stopping the research
- You need a treatment not allowed in this research
- You become pregnant
- You are unable to keep your scheduled appointments
- You are unable to adhere to instructions from researchers

Will it cost me money to take part in this research?
There are no costs associated with participation in this research study.

What if I am injured because of participating in this research?
If you are injured or get sick because of being in this research, call the researchers immediately.

In the event you suffer a research-related injury your medical expenses will be your responsibility or that of your insurance company (or other third-party payer), although you are not precluded from seeking to collect compensation for injury related to malpractice, fault, or blame on the part of those involved in the research. If you are a UO student or employee and are covered by a UO medical plan, that plan might have terms that apply to your injury.
If you experience harm because of the project, you can ask the State of Oregon to pay you. If you have been harmed, there are two University representatives you need to contact. Here are their addresses and phone numbers:

General Counsel/ Office of the President	**Research Compliance Services**
1226 University of Oregon	5237 University of Oregon
Eugene, OR 97403-1226	Eugene, OR 97403-5237
(541) 346-3082	(541) 346-2510

A law called the Oregon Tort Claims Act may limit the amount of money you can receive from the State of Oregon if you are harmed.

Will I be paid for participating in this research?

You will receive $15 per hour for participating in this study. This money is for the inconvenience and time you spent in this study. If you start the study but stop before the study has ended, you will get part of this money. The partial amount will be calculated as $15 per hour participated in the study. With full participation, we anticipate those in Group 1 will receive $75 total (5-hour commitment) and those in Group 2 will receive $30 total(2-hour commitment).

Who can answer my questions about this research?
If you have questions, concerns, or have experienced a research related injury, contact the research team at:

Research Coordinator	Dr. Minson
(541) 600-4095	(541) 346-4105

exercise@haywardfield.net minson@uoregon.edu

An Institutional Review Board ("IRB") is overseeing this research. An IRB is a group of people who perform independent review of research studies to ensure the rights and welfare of participants are protected. UO Research Compliance Services is the office that supports the IRB. If you have questions about your rights or wish to speak with someone other than the research team, you may contact:

Research Compliance Services
5237 University of Oregon
Eugene, OR 97403-5237
(541) 346-2510

STATEMENT OF CONSENT

I have had the opportunity to read and consider the information in this form. I have asked any questions necessary to make a decision about my participation. I understand that I can ask additional questions throughout my participation.
I understand that by signing below, I volunteer to participate in this research. I understand that I am not waiving any legal rights. I have been provided with a copy of this consent form. I understand that if my ability to consent or assent for myself changes, either I or my legal representative may be asked to re-consent prior to my continued participation in this study.
I consent to participate in this study.

_____ _____ __

Name of Adult Participant Signature of Adult Participant Date

Researcher Signature (to be completed at time of informed consent)
I have explained the research to the participant and answered all of his/her questions. I believe that he/she understands the information described in this consent form and freely consents to participate.

_____ _____ __

Name of Research Team Member Signature of Research Team Member Date

Appendix B: COVID-19 Pre-Visit Questionnaire

COVID-19 PRE-VISIT QUESTIONNAIRE

1. In the past 2 weeks, have you had a cough, shortness of breath, difficulty breathing, fever >100°F, chills, loss of smell or taste, nausea, vomiting, diarrhea, runny nose, sore throat, muscle pain, or nasal congestion? [No] (If yes) Are these symptoms unusual for you? [No]
2. In the past 2 weeks, have you come in contact with someone with these symptoms? [No]
3. Are you currently feeling healthy and well? [Yes]
4. In the past 2 weeks, have you been diagnosed with or come in contact with someone diagnosed with COVID-19? [No]
5. Have you received both doses of a two-dose COVID-19 vaccine or one dose of a single-dose vaccine? [Yes]
    a. Has it been at least 14 days since your final dose of COVID-19 vaccine? [Yes]
6. In the past 2 weeks, have you traveled outside of Oregon? [No]
    a. *Can say [Yes] if they have received both doses of a two-dose COVID-19 vaccine or one dose of a single-dose vaccine.
7. Do you agree to contact the lab team if you begin to experience symptoms of COVID-19 in the next 2 weeks? [Yes]
8. Do you agree to follow all Federal, State, University, and Lab COVID-19 policies while on campus? [Yes]

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