PSYCHOPHYSIOLOGICAL RESPONSES TO ACUTE COLD WATER IMMERSION

by

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A THESIS

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Anecdotal reports suggest that chronic cold water immersion improves mood, immune function, and cardiovascular health. However, there are no scientific studies validating these claims. It is possible that acute cold water immersion may improve cardiovascular health and symptoms of depression. PURPOSE: To investigate the effects of acute cold water immersion on positive affect and related blood markers. METHODS: 14 participants (8 male, 6 female, age: 24 ± 4.2 years) were immersed in 10°C water for 15 minutes during one experimental session. Positive and Negative Affect Schedules (PANAS) and blood samples were obtained before and after immersion, while blood samples were also obtained during immersion. RESULTS: Participants did not experience an increase in positive affect as a result of cold water immersion but did experience a decrease in negative affect. β- endorphin concentration was elevated and cortisol concentration was reduced three hours post immersion. FGF21, TNFα , and IL-1β remained unchanged throughout the trial. Based on correlations, it appears that β- endorphin and FGF21 may drive positive affect, while rectal temperature and β- endorphin drive negative affect. CONCLUSION: These results suggest that cold water immersion does not improve positive affect, but that chronic studies may reveal other benefits to cold water exposure.

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Introduction

The knowledge that humans are homeotherms is well understood. When placed in a hot or cold environment, the average person can maintain their body temperature near 37°C through physiological mechanisms (e.g., metabolic thermogenesis to raise body temperature or perspiration to lower body temperature) and behavioral changes (e.g., taking off a jacket when it is a hot outside or going indoors if we are cold) (Morrison et al., 2008). These acute physiological responses to thermal stress are commonly researched in laboratory-controlled studies to better understand the potential mechanisms by which chronic exposure to these environments may benefit human health. The benefits of chronic exposures to thermal stress may include improved immune system function, a reduction in one's risk for cardiovascular disease, and regulation of body weight. Therefore, it is important to sufficiently research the effects of thermal stress on humans to better understand the mechanisms behind these benefits.

Thermal therapy describes the use of natural or artificially induced hot and/or cold environments to elicit beneficial health adaptations. For example, phenotypic adaptations to heat elicit enhanced skin blood flow and sweat responses (Mack et al., 2018). Adaptations to chronic cold exposure may also benefit human health, such as attenuating weight gain via non-shivering thermogenesis through activation of brown adipose tissue (Van Der Lans et al., 2013). Given these responses to thermal therapy, there is significantly more research on the benefits of heat therapy as performed by either sauna bathing or hot water immersion. Strikingly, heat therapy is associated with a 23% reduced risk of cardiovascular disease and a 66% reduced risk of neurodegenerative diseases (Heinonen et al. 2017, Laukkanen et al., 2015).

Conversely, there is less research on the potential beneficial effects of chronic exposure to cold. Many people associate cold water immersion with summer dips in alpine lakes or a winter triathlon. In the 1700s, sea bathing became a popular activity, with an emphasis on winter being the best season to jump into the ocean (Nicholas Orme, 1983). Winter swimming was thought to promote longevity for two reasons: 1) it was considered "manly", and 2) if you knew how to swim, you were less likely to drown (Nicholas Orme, 1983; Tipton et al., 2017). Since then, people have also consistently claimed that cold water exposure has a range of great health benefits such as a reduction in inflammation, increased cardiovascular health and immune response, limits to weight gain, and/or an elevated feeling of happiness and well-being (Seale & Lazar, 2009; Shevchuk, 2008). However, the physiological basis and the relationship between these responses to cold water exposure are not well understood.

Included in those that claim to experience enhanced physiological processes and improved mood from cold water immersion is Wim Hof, also known as 'The Iceman' (Wim et al., n.d.). Interestingly, scientific literature supports some of his anecdotal reports. Studies published within the last ten years have suggested that the meditation and breathing techniques practiced by Wim Hof influence the sympathetic nervous system and attenuate some of the typical physiological responses to cold water. Specifically, these techniques are associated with maintaining basal skin temperature and a reduced production of inflammatory proteins (Muzik et al., 2018; Pikkers et al., 2011). Wim Hof uses his social media platform with over 2 million followers to promote cold water immersion for its health benefits. He has implemented his own breathing techniques (in which he thoroughly inhales and exhales) to increase the

amount of oxygen in his lungs, which allows him to synthesize the necessary amounts of ATP required for proper cellular function. This allows him to combat the generalized effects of cold water, such as a reduction in skin temperature and exposure to extreme cold without hypothermia (Wim et al., n.d.). Despite Wim Hof's achievements, future research is warranted to examine the mechanistic underpinnings of how these cold water immersion techniques may benefit human health. To my knowledge, a comprehensive laboratory-controlled study has yet to be conducted to examine the cardiovascular and blood marker response to cold water immersion and how these responses may be related to improved mood. Importantly, understanding the effects of acute cold water immersion will provide foundational knowledge regarding the mechanisms of potential treatments for cardiovascular disease, obesity, depression, and arthritis.

Literature Review

The current literature suggests that the major benefit to cold water immersion lies in the acute stress response produced by the body (Tipton et al., 2017). These transient stress responses cause an increase in the immuno-protective properties of the body (e.g., efficient healing and the ability to eliminate infections) through increases in inflammatory cytokines, cortisol, and norepinephrine that may last from minutes to a few hours in duration (Dhabhar, 2014). These short bursts of elevated stress hormones acutely stimulate the immune system (Sapolsky, 1994). However, when a bout of stress persists for long enough (days or months) to fall under the category of chronic stress, the immune response begins to decline. Chronic stress negatively impacts health by leading to immuno-suppression, which is a reduction in immune system function (Dhabhar, 2014). Differentiating between acute and chronic stress is no trivial task. Life is full of psychologically stressful events (e.g., school, relationships, work, etc.) that take advantage of the innate stress response in humans (Rohleder, 2019). As a result, it is important to experience acute stressors that may reduce chronic inflammation through biological pathways (e.g., exercise). Cold water immersion is one possible form of acute stress with therapeutic potential. However, only a subset of research has focused on the chronic effects of repeated cold water exposures, making future research a necessity to understanding this stressor.

Interpreting research investigating the physiological effects of cold water immersion is difficult due to inconsistencies across studies in relation to the temperature and duration of exposure, and differences in the methodological approach. Nonetheless, important information can be gathered from several studies that focused on key

elements of cold exposure and cardiovascular elements. In one of these studies, Šrámek et al. observed fluctuations in blood pressure throughout a 60 minute exposure to various water temperatures (32°C, 20°C, and 14°C) (Šrámek, 1996). At the end of exposure, both systolic and diastolic blood pressure were decreased for water at 32°C and 20°C. For 14°C water, systolic blood pressure increased by ~8 mmHg and diastolic blood pressure increased by ~6 mmHg (Šrámek et al., 2000). Additionally, heart rate was elevated by 3 bpm during immersion in 14°C water, but was reduced during exposures to 20°C and 32°C. At the end of immersion, core temperature remained unchanged in 32°C water. For water at 20°C and 14°C, core temperature was reduced by 1.0°C and 1.7°C, respectively. Importantly, 10 minutes after immersion, the lowest core temperatures were reported for 20°C and 14°C at ~36.3°C and ~35.5°C, respectively. This result is in support of the after drop effect, which is characterized by a continued reduction in core temperature 10-40 minutes after cold water exposure (Romet, 1988). The after drop effect arises from conductive heat transfer between warm blood from the core of the body to cold tissue in the extremities. Blood that passes through the colder extremities returns to the heart at a lower temperature and therefore leads to a continued reduction in body temperature post immersion (Romet, 1988).

Šrámek et al. also found that norepinephrine increased by a factor of five within the first 40 minutes of exposure while epinephrine concentrations remained unchanged. At the end of the 60 minute exposure, dopamine ultimately increased by less than one picomole per milliliter. Cortisol concentrations were reported to decrease at all three temperatures, which was not correlated with predictions about stress hormone concentrations during cold water immersion. These important findings compel further

investigation in future studies to enhance our understanding of the physiological effects of cold water immersion and the potential subsequent cardiovascular implications.

Using a different protocol, Mourot et al. found that individuals exposed for 20 minutes to moderately cold water (26-27°C) experienced a decrease in heart rate by ~26 beats per minute and diastolic blood pressure ~9 mmHg, but experienced an increase in systolic blood pressure by ~8 mmHg (Mourot et al., 2008). The differing findings in cardiovascular responses between the studies by Šrámek et al. and Mourot et al. may be explained, at least in part, by the different temperatures and durations of exposure between protocols (Table 1). In terms of blood markers, Mourot et al. reported that epinephrine decreased in both thermoneutral water and moderately cold water compared to baseline. Meanwhile, norepinephrine decreased in thermoneutral water but increased in moderately cold water. This increase in norepinephrine was similar to the data reported by Šrámek et al., but the variability in the change from baseline is almost certainly due to the temperature differences in each study. Šrámek reported a 500% increase in norepinephrine while Mourot reported a 38% increase (Table 2). The discrepancies between these two studies further demonstrate the extent to which the effects of cold water immersion are unknown and understudied.

Author	Water Temperature (°C)	Exposure duration (min)	ΔSBP (mmHg)	ΔDBP (mmHg)	ΔHeart Rate (bpm)	ΔRectal Temperature (°C)
Mourot et al.	26-27	20	8.8	-9	-26	NA
Šrámek et al.	14	60	+8	+6	+3	-1.7
	20	60	NR	NR	NR	-1.0
	32	60	-12	-8	-9	NC

Table 1. Summary of cardiovascular responses from relevant literature

Data are reported as changes from baseline at the end of immersion. NR: not reported. NC: no change.

Author	Water Temperature (°C)	Exposure duration (min)	ΔNorepinephrine (% change)	ΔEpinephrine (%change)	ΔCortisol (%change)
Mourot et al.	26-27	20	+38	+0.09	NR
Šrámek et al.	14	60	+500	NC	-.05
	20	60	NR	NR	-20
	32	60	NR	NR	-62

Table 2. Summary of blood markers from relevant literature

Data are reported as changes from baseline at the end of immersion. NR: not reported. NC: no change.

Although heart rate may decrease in thermoneutral and moderately cold water, the ultimate increase in heart rate that occurs at colder temperatures suggests that cold water exposure may have opposite cardiovascular effects compared to warm water during long periods of immersion. Furthermore, only a fraction of critical blood markers were tested in the previous studies, all of which were related to the involuntary stress response (e.g., norepinephrine, epinephrine, and cortisol). These markers do not explain the anecdotal reports suggesting increases in immune function that occur as a result of

cold water immersion. Enhanced immune function is likely due to activation of the innate immune response, the first line of defense against physiological stressors, that induces the release of cytokines. Blood markers that would reflect such changes in immune function would be Il-1β and TNFα. Previous literature has shown that exercise effectively reduces pro-inflammatory cytokines involved in several chronic diseases (e.g., atherosclerosis, depression, arthritis) via activation of inflammatory pathways that have the capability to inhibit further release of pro-inflammatory markers (Beavers et al., 2010). These pathways ultimately lead to the release of anti-inflammatory cytokines that cause reductions in chronic low-grade inflammation. However, only recently has literature addressed these inflammatory markers in response to cold water immersion. A study by Eimonte et al. demonstrated that TNFα, a pro-inflammatory cytokine released in response to acute internal stressors, was reduced after cold water immersion in water that was 13-14°C (Eimonte et al., 2021). Furthermore, in the same study researchers found that Il-1β, another pro-inflammatory cytokine, was increased post immersion and continued to remain elevated up to four hours post immersion (Eimonte et al., 2021). These changes in Il-1β and TNFα paralleled the release of stress hormones (cortisol, norepinephrine, and epinephrine) that are known to inhibit the inflammatory pathway (Eimonte et al., 2021). These results suggest that cold water immersion may lead to possible improvements in inflammatory related diseases. Another blood marker of interest in the acute physiological response to cold water immersion is FGF21. Upon exposure to cold water immersion, FGF21 is released and induces activation of brown adipose tissue. Brown adipose tissue activation leads to heat production via the uncoupler protein 1, which dissipates the proton motive force that normally generates

ATP (Seale & Lazar, 2009). This increased heat production is thought to limit obesity by generating excess energy. The present study aims to quantify changes in FGF21 concentration as a result of cold water immersion. In addition, the previous blood markers mentioned do not support the notion that emotional state is elevated after cold water immersion. β- endorphins have been shown to elevate mood after exercise, but there is limited scientific literature that thoroughly documents improvements to mood as a result of cold water immersion (Mikkelsen et al., 2017). Several studies have used the Positive and Negative Affect Schedules (PANAS) to quantify positive and negative emotions separately in response to exercise (Watson et al., 1988). Previous literature supports increases in positive affect and decreases in negative affect post-exercise (Cox et al., 2001; LePage & Crowther, 2010). Due to the potential physiological similarities between responses to cold water immersion and exercise, it is possible that positive affect may be improved as a result of cold water immersion due to the release of β-endorphins (Vaswani et al., 1988).

In summary, inconsistencies in methodology and outcomes warrant continued study of the effects of cold water immersion. Our research on cold water immersion aims to collect data throughout a wide range of physiological responses in order to provide a comprehensive study that launches the investigation of potential health benefits associated with cold water immersion.

Specific Aims

Aim 1 – To test the hypothesis that positive affect will be increased three hours following cold water immersion compared to pre-immersion.

Aim 2 – To test the hypothesis that cortisol, β- endorphins, and FGF21 will increase upon cold water immersion compared to baseline with cortisol and β-endorphins peaking at the end of immersion. Additionally, I hypothesize that FGF21 and IL-1β concentration will be the highest 30 minutes after immersion, while TNFα concentration will decrease compared to baseline at all time points. Three hours post immersion blood marker concentrations will have returned back to baseline.

Methods

Participants

All procedures in this investigation were approved by the Institutional Review Board at the University of Oregon. Prior to enrollment, participants underwent an informed consent process (Appendix A) and a screening procedure to determine their eligibility. Twenty-three participants were screened and 14 participants (6 female), meeting all inclusion criteria and no exclusion criteria, completed the study. The characteristics of the participants who completed the study were as follows – age: 24 ± 4.2 years; height: 173 ± 10 cm; weight 65.8 ± 9.3 kg; and body mass index 21.9 ± 1.8 kg/m^2. Participants were deemed eligible for the study based on the following inclusion criteria: aged 18-40 years, non-sedentary, and had never been diagnosed with long term health issues that had the potential to impact cardiovascular and thermoregulatory processes (i.e., heart disease, fibromyalgia, hypothyroidism, multiple sclerosis, previous frostbite injury, Raynaud's, and anorexia). Participants were not taking prescription medication and were non-smokers. Two female participants reported to be normally menstruating and four female participants reported to be taking hormonal contraceptives. Hormonal contraceptive use included intrauterine devices (Mirena levonorgestrel-releasing intrauterine system, Bayer, Whippany, NJ, *n*=2), contraceptive arm implant (Nexplanon etonogestrel implant Merck & Co., Inc., Kenilworth, NJ, *n*=1), and an oral contraceptive pill (Cyred EQ, Afaxys Pharma, Charleston, SC, *n*=1).

Instrumentation and Measurements

Height was measured using a stadiometer (Seca Medical Measuring Systems and Scales, Hamburg, Germany) and weight was measured using a scale (Midrics® 2, Sartorius, Gottingen, Germany). Core temperature was continuously measured throughout the experimental trial with a rectal thermistor (Zoll YSI, MFI Medical San Diego, CA, USA). Heart rate was measured with both a Polar® Heart rate monitor (Polar; Lake Success, NY, USA) and 3-lead ECG (Cardiocap/5; GE Datex-Ohmeda®, Louisville, CO, USA). Blood pressure was measured with an arm blood pressure cuff (Tango M2; SunTech® Medical, Inc., Morrisville, NC, USA) placed on the participant's left arm. Continuous blood pressure, stroke volume, cardiac output, and total peripheral resistance were monitored using the Finometer® (Finapres Medical Systems, Enschede, Netherlands). Venous blood samples were obtained from an intravenous (IV) catheter placed in the antecubital fossa of the right arm by a trained phlebotomist.

The participants' current mood was assessed using a validated scale, the Positive and Negative Affect Schedules (PANAS) (Watson et al., 1988). The PANAS consists of 10 Positive Affect and 10 Negative Affect feelings and emotions. Participants rated each positive and negative affect feeling based on a scale ranging from 1 "not at all" to 5 "extremely" (Watson et al., 1988). Positive affect score was calculated as the sum of all positive feelings and negative affect as the sum of all negative feelings (Watson et al., 1988). Interpretation of participants perceived thermal discomfort and sensation were measured with scales that were made specifically for this study, but were modeled after ordinal scales used in previous physiology literature (West et al., 2019). Numbers

were correlated with a rating of discomfort or warmth and participants reported a single number out loud. Thermal sensation was measured using the following visual analogue scale: (20 = extremely hot, 16 = very hot, 12 = hot, 8 = warm, 4 = slightly warm, 0= neutral, -4 = slightly cool, -8 = cool, -12= cold, -16 = very cold, -20 = extremely cold). Thermal discomfort was measured using the following visual analogue scale: (9 = extremely uncomfortable, 7 = very uncomfortable, 5 = uncomfortable, 3 = slightly uncomfortable, 1 = comfortable). Participants were instructed to give a single integer response, which may be between integers on the scales.

Experimental Protocol

Participants were instructed to abstain from heavy exercise, heat therapy, and cold therapy for 24 hours; alcohol, supplements and medications (with the exception of oral contraceptives) for 12 hours; caffeine for 6 hours, and food for 2 hours prior to the study.

The day prior to the experimental session, participants were contacted by the research team to complete a coronavirus disease (COVID-19) questionnaire to ensure that they had no exposure to or symptoms of COVID-19 and that they agreed to the lab's COVID-19 policies (Appendix B). This questionnaire followed Institutional Review Board and Incident Management Team approved guidelines within the University of Oregon, which followed best practices based on guidance from the U.S. Centers for Disease Control and Prevention (Guidance for General Laboratory Safety Practices during the COVID-19 Pandemic | CDC, n.d.).

Upon arrival to the laboratory, participants confirmed that they abstained from heavy exercise, heat/cold therapy, alcohol, supplements, medications (with the exception of oral contraceptives), caffeine, and food for the proper amounts of time. Participants then completed the PANAS. Following completion of the PANAS, participants moved to a private restroom where they self-inserted a rectal thermistor to a depth of 10 cm past the anal sphincter. Then, participants were instrumented with a Polar® Heart rate monitor. Next, a trained phlebotomist placed the IV catheter.

Participants then sat in the hydraulic lift chair (S.R. Smith; Canby, OR, USA) that was used to lower participants into the cold water bath. Importantly, participants remained seated in this chair until the completion of the 30 minute recovery period following immersion. Once in a comfortable seated position, participants rested in the seated position for 20 minutes in the laboratory (temperature: 22.7±1.42°C; relative humidity 30.6±2.31%). During this time, the subject was fully instrumented as described above. Following the resting period, arm blood pressure was taken in triplicate with 60 seconds in between each measurement. Then, perceptions of thermal discomfort and thermal sensation were recorded. After this, a venous blood sample was obtained.

Participants were then lowered into the cold water (10.6±0.21°C) up to the level of approximately the xiphoid process. A venous blood sample was then immediately obtained. Arm blood pressure was obtained every two minutes during cold water immersion and during the final minute. Thermal sensation and thermal discomfort were recorded every 5 minutes. A venous blood sample was obtained during the final three minutes of immersion. After 15 minutes of cold water immersion, participants were

removed from the water by the hydraulic lift and remained seated for a 30 minute recovery period in the temperate lab environment. To increase comfort during the recovery period, participants were draped in two towels and a blanket. Arm blood pressure was recorded in 2-minute intervals for the first 10 minutes of recovery after which 5-minute intervals were used for the final 20 minutes. Thermal perception and discomfort were recorded every 5 minutes throughout recovery. After the 30 minute recovery period, a fourth venous blood sample was obtained and the second PANAS was administered. Participants were then de-instrumented from all equipment except the IV catheter. Participants left the lab for 3 hours with instructions to not exercise, eat, drink (with the exception of water), remove the IV catheter, and to keep the IV catheter dry. The return visit to the lab consisted of completion of one last PANAS scale, the fifth and final blood draw, and removal of the IV catheter. The study session was then concluded.

Data and Statistical Analyses

A data acquisition system (WinDaq, Dataq Instruments Inc. Akron, Ohio, USA) was used to continuously sample data at 185 Hz for rectal temperature, ECG, beat-to-beat blood pressure from finger photoplethymography, stroke volume, and cardiac output. Data at baseline were extracted as a 5-minute resting average. During cold water immersion and recovery, data were extracted as 1-minute averages. Stroke volume was estimated by Modelflow, an algorithm based on the blood pressure waveform (Wesseling et al., 1993). Cardiac output was calculated as a product of heart rate and stroke volume. Mean arterial pressure was calculated as the difference between one-

third pulse pressure and diastolic blood pressure. Total peripheral resistance was calculated as mean arterial pressure divided by cardiac output.

The following blood markers were tested for this study: cortisol, β-endorphins, IL-1β, TNFα, FGF21. Blood was collected at 5 time points: baseline, immediately upon immersion, at the end of immersion, at the end of a 30 minute recovery period, and three hours post immersion. Blood was collected into 7.5 mL serum separator tubes (SST) and 4 mL EDTA plasma tubes. Two serum and two plasma tubes were collected at each time point. One plasma tube was immediately centrifuged at 1.3 relative centrifugal force (rcf) for 10 minutes at 4°C, after which ~2 mL plasma was collected and frozen at -80°C. The other plasma tube was kept at room temperature for the isolation of PBMCs. These PBMCs will be used to analyze heat shock proteins. Both serum tubes were allowed to sit upright for 30 minutes before being centrifuged after which ~3 mL serum was collected and frozen. IL-1β, TNFα, and FGF21 were processed using Quantikine® ELISA kits (R&D Systems Pittsburgh, PA) following manufacturer's instructions. β-endorphins were measured using a QuickDetect® ELISA kit (BioVision Milpitas, CA) and cortisol was measured using an Invitrogen™ ELISA kit (Fisher Scientific Co. L.L.C Pittsburgh, PA) both following manufacturer's instructions.

Due to technical difficulties, core temperature is reported as n=10 and we were only able to collect data from a subset of participants (n=4) for beat-to beat-measurements. Data were analyzed with Prism software (version 9.1, GraphPad Software, La Jolla, CA).

Data were analyzed using a one-way repeated measures ANOVA. When the ANOVA revealed a significant F statistic, post hoc Dunnett's tests were used to compare changes from baseline and post hoc Dunnett's tests were used to compare changes from the end of cold water immersion. Pearson product-moment correlations were used to compare rectal temperature (baseline to end recovery), thermal perceptions, and blood markers to positive and negative affect three hours post immersion. Statistical significance was set a priori at $P \leq 0.05$. Data were reported as means $\pm$ standard deviation (SD).

Results

Core temperature and hemodynamic responses

Rectal temperature was reduced compared to baseline from minute one of cold water immersion through the end of immersion ($P \leq 0.0351$, Figure 1). Rectal temperature decreased by 0.4 ± 0.3°C at the end of cold water immersion compared to baseline (37.4 ± 0.3 °C vs. 37.0 ± 0.4°C, P=0.0161, Figure 1A). After 30 minutes of recovery, rectal temperature was reduced by 1.0 ± 0.4°C compared to baseline (37.4 ± 0.4°C vs. 36.5 ± 0.6°C, P=0.0011, Figure 1B). At the end of recovery, rectal temperature was reduced by 0.4 ± 0.3°C compared to the end of cold water immersion. (P=0.0035, Figure 1A).

Heart rate was increased at 1 minute of cold water immersion in 9 out of the 14 participants, but these increases were not statistically different compared to baseline owing to the large variability between participants ($+8 \pm 12$, P=0.3055, Figure 2B). Heart rate was reduced by 11 ± 7 bpm at the end of cold water immersion compared to baseline (76 ± 12 bpm vs. 65 ± 13 bpm, P=0.0025, Figure 2A). At the end of recovery, heart rate was reduced by 13 ± 6 bpm compared to baseline (P=0.0001) but was not different compared to the end of cold water immersion (P=0.9951, Figure 2B).

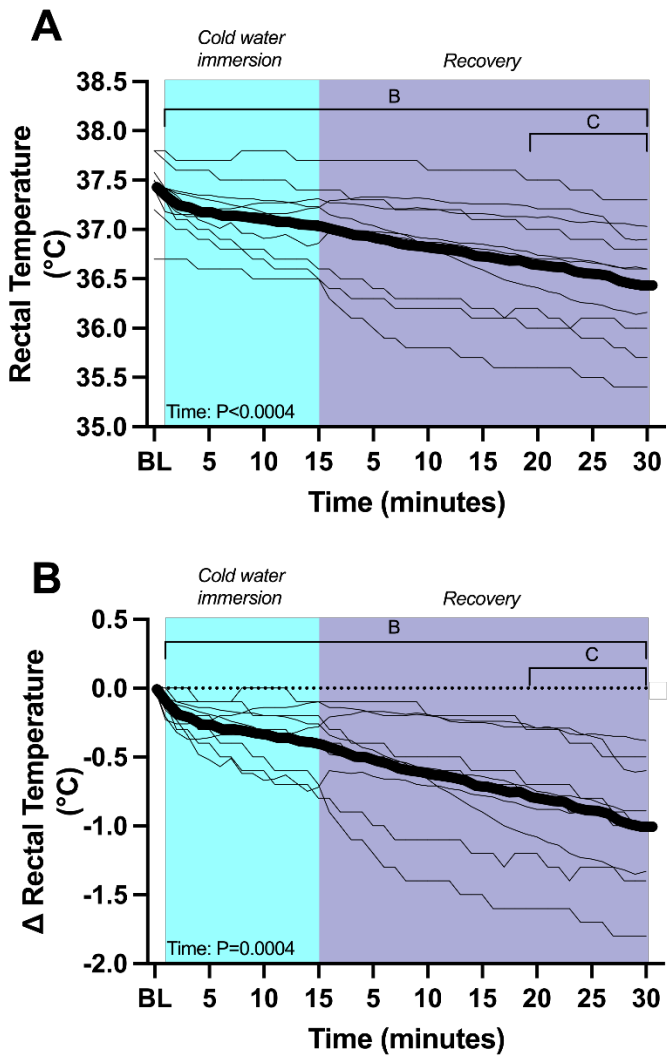

Figure 1. Rectal Temperature

The response of core temperature (rectal thermistor) to 15 minutes of cold water immersion and 30 minutes of post-immersion recovery. Data are presented as absolute values (panel A) and the change from baseline (BL, panel B). Data were analyzed using a one-way ANOVA with post hoc Dunnett's adjustments to compare changes from BL or the end of cold water immersion. The thick bold line depicts mean data and individual data are shown by thin lines. ^{B}different from baseline ($p \leq 0.0351$). ^{C}different from cold water immersion ($p \leq 0.0392$). n=10 (5 males, 5 females).

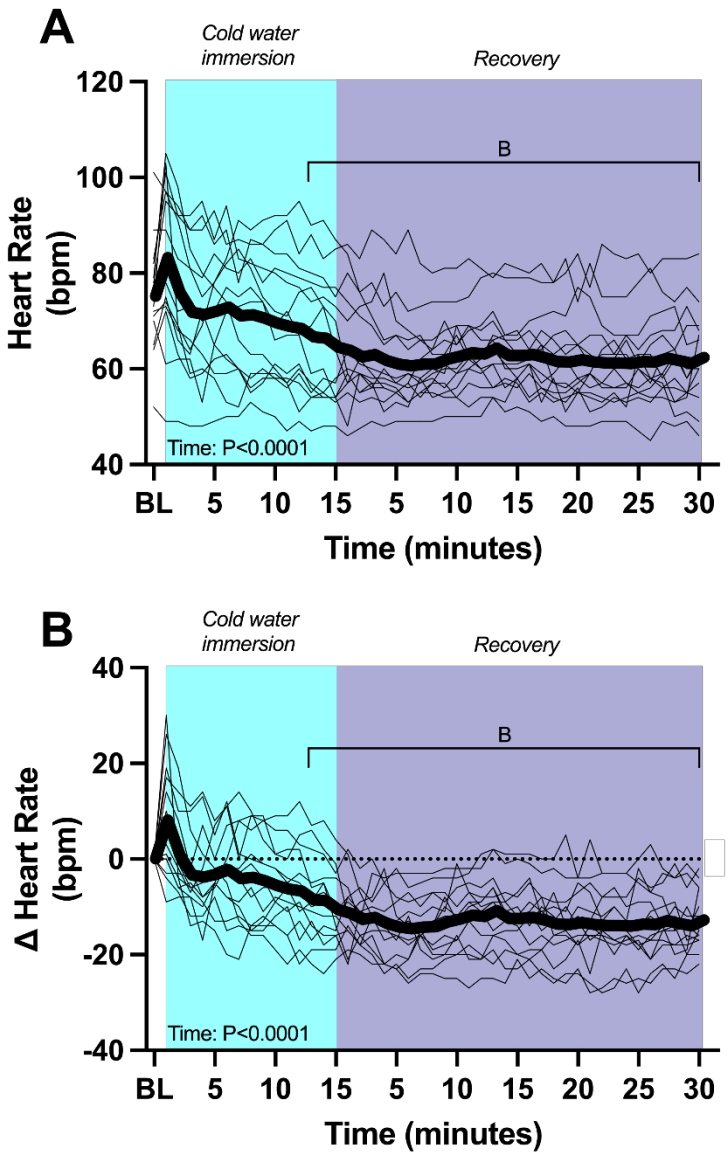

Figure 2. Heart rate

Heart rate (ECG) during 15 minutes of cold water immersion and during 30 minutes of post-immersion recovery. Data are presented as absolute values (panel A) and change from baseline (BL, panel B). Data were analyzed using a one-way ANOVA with post hoc Dunnett's adjustments to compare changes from BL or the end of cold water immersion. The thick bold line depicts mean data and individual data are shown by thin lines. [B]different from baseline ($p \leq 0.0069$). n=14 (8 males, 6 females).

Brachial artery blood pressure response

Immediately upon cold water immersion, systolic blood pressure, diastolic blood pressure, and mean arterial pressure increased (23.8±16.18 mmHg P=0.0019; 14.4±7.8 mmHg, P=0.0003; 18.2±8.8 mmHg, P<0.0001) (Figure 3). Systolic blood pressure and mean arterial pressure remained elevated compared to baseline for the remainder of the trial (P≤0.0327, Figure 3B and F). Diastolic blood pressure remained elevated compared to baseline from the beginning of cold water immersion through 14 minutes of immersion (P≤0.0370). However, diastolic blood pressure was not different compared to baseline at 15 minutes of cold water immersion (P=0.2276, Figure 3D).

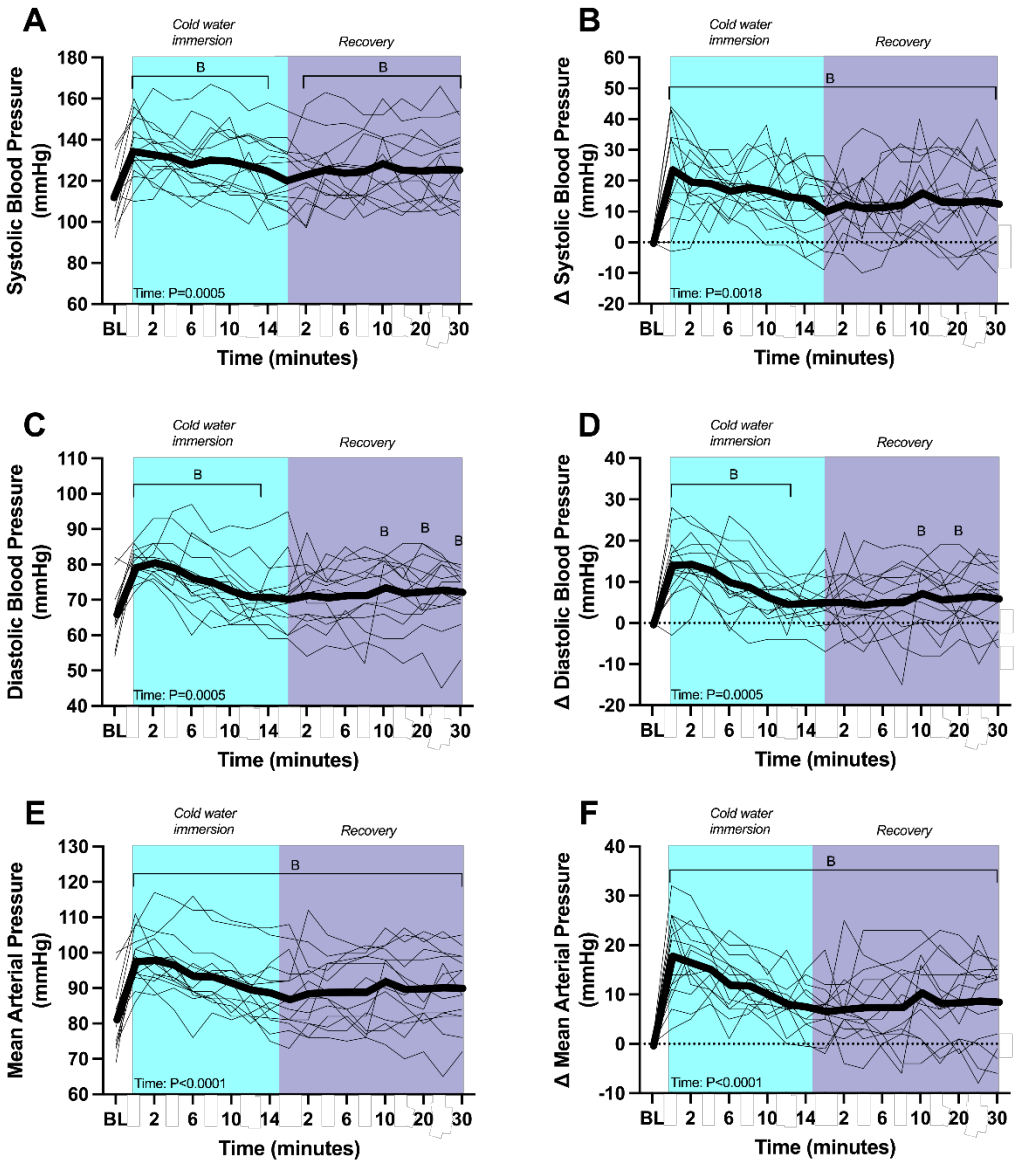

Figure 3. Arm blood pressure

Arm blood pressure (Tango M2) responses during 15 minutes of cold water immersion and during 30 minutes of post-immersion recovery. Data are presented as absolute values and change from baseline (BL). Systolic blood pressure: absolute values (panel A), change from BL (panel B). Diastolic blood pressure: absolute values (panel C), change from BL (panel D). Mean arterial pressure: absolute value (panel E), change from BL (panel F). Data were analyzed using a one-way ANOVA with post hoc Dunnett's adjustments to compare changes from BL or the end of cold water immersion. The thick bold line depicts mean data and individual data are shown by thin lines. [B]different from baseline. ($P \leq 0.0500$). *n*=14 (8 males, 6 females).

Beat-to-beat hemodynamic responses

A formal analysis was not performed for these measures due to the small sample size (n=4). Therefore, I will provide a preliminary descriptive summary of trends noted in the data collected for these measures thus far. The beat-to-beat mean arterial pressure response looks similar to that of the brachial artery blood pressure response in that there were large increases immediately upon cold water immersion that were sustained throughout the entire trial. Stroke volume and cardiac output increased immediately upon water immersion with a trend returning towards baseline by the end of cold water immersion. Total peripheral resistance appears to increase throughout cold water immersion and remained elevated during recovery.

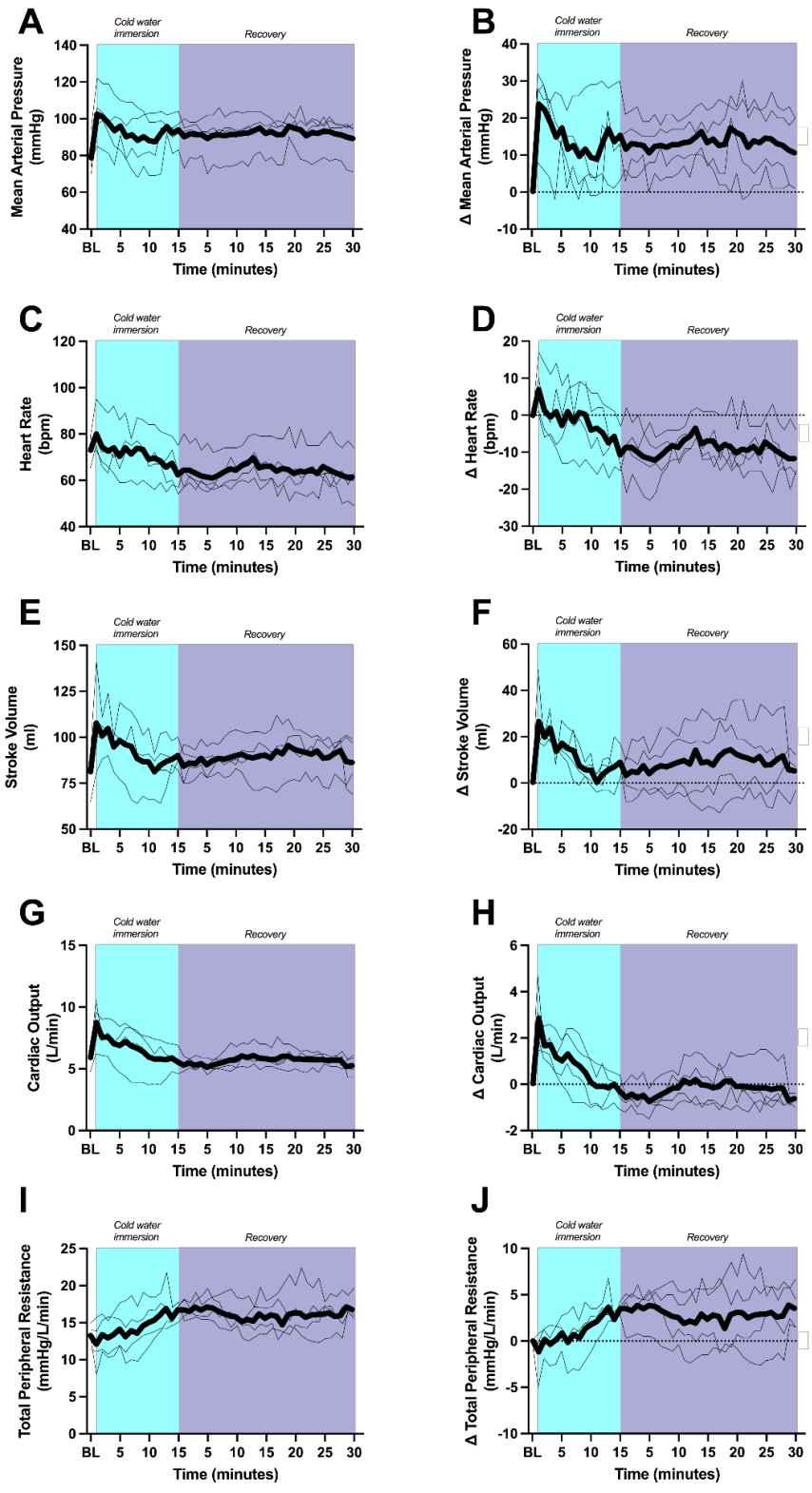
A
Cold water immersion
Recovery
Mean Arterial Pressure (mmHg)
140
120
100
80
60
40
BL 5 10 15 5 10 15 20 25 30
Time (minutes)
B
Cold water immersion
Recovery
Δ Mean Arterial Pressure (mmHg)
40
30
20
10
0
-10
BL 5 10 15 5 10 15 20 25 30
Time (minutes)
C
Cold water immersion
Recovery
Heart Rate (bpm)
120
100
80
60
40
BL 5 10 15 5 10 15 20 25 30
Time (minutes)
D
Cold water immersion
Recovery
Δ Heart Rate (bpm)
20
10
0
-10
-20
-30
BL 5 10 15 5 10 15 20 25 30
Time (minutes)
E
Cold water immersion
Recovery
Stroke Volume (ml)
150
125
100
75
50
BL 5 10 15 5 10 15 20 25 30
Time (minutes)
F
Cold water immersion
Recovery
Δ Stroke Volume (ml)
60
40
20
0
-20
BL 5 10 15 5 10 15 20 25 30
Time (minutes)
G
Cold water immersion
Recovery
Cardiac Output (L/min)
15
10
5
0
BL 5 10 15 5 10 15 20 25 30
Time (minutes)
H
Cold water immersion
Recovery
Δ Cardiac Output (L/min)
6
4
2
0
-2
BL 5 10 15 5 10 15 20 25 30
Time (minutes)
I
Cold water immersion
Recovery
Total Peripheral Resistance (mmHg/L/min)
25
20
15
10
5
0
BL 5 10 15 5 10 15 20 25 30
Time (minutes)
J
Cold water immersion
Recovery
Δ Total Peripheral Resistance (mmHg/L/min)
10
5
0
-5
-10
BL 5 10 15 5 10 15 20 25 30
Time (minutes)

Figure 4. Beat-to-beat hemodynamic responses

Beat-to-beat hemodynamic responses (Finometer) during 15 minutes of cold water immersion and 30 minutes of post-immersion recovery. Data are presented as absolute values (left panels) and change from baseline (BL, right panels). *n*=4 (2 males, 3 females).

Thermal discomfort and thermal sensation

The participants reported a perceived reduction in temperature immediately upon cold water immersion through the remainder of the trial compared to baseline ($P \leq 0.0059$, Figure 5A). Temperature was perceived to mildly increase during some portions of the recovery period compared to the end of cold water immersion (at minutes 0-5, 15, and 25-30), but not compared to baseline. Participants reported increased thermal discomfort upon cold water immersion compared to baseline (2.143 ± 1.610 a.u. vs. 5.286 ± 1.978 a.u., $P=0.0063$). Thermal discomfort was improved immediately upon exiting the cold water (4.124 ± 1.672 a.u. vs. 3.00 ± 1.301 a.u. $P=0.0844$, Figure 5C). However, at 10-15 minutes into recovery, thermal discomfort was again elevated compared to baseline ($P \leq 0.0371$, Figure 5C). After this point, thermal discomfort was no longer different from baseline for the remainder of the trial ($P \leq 0.8008$, Figure 5C).

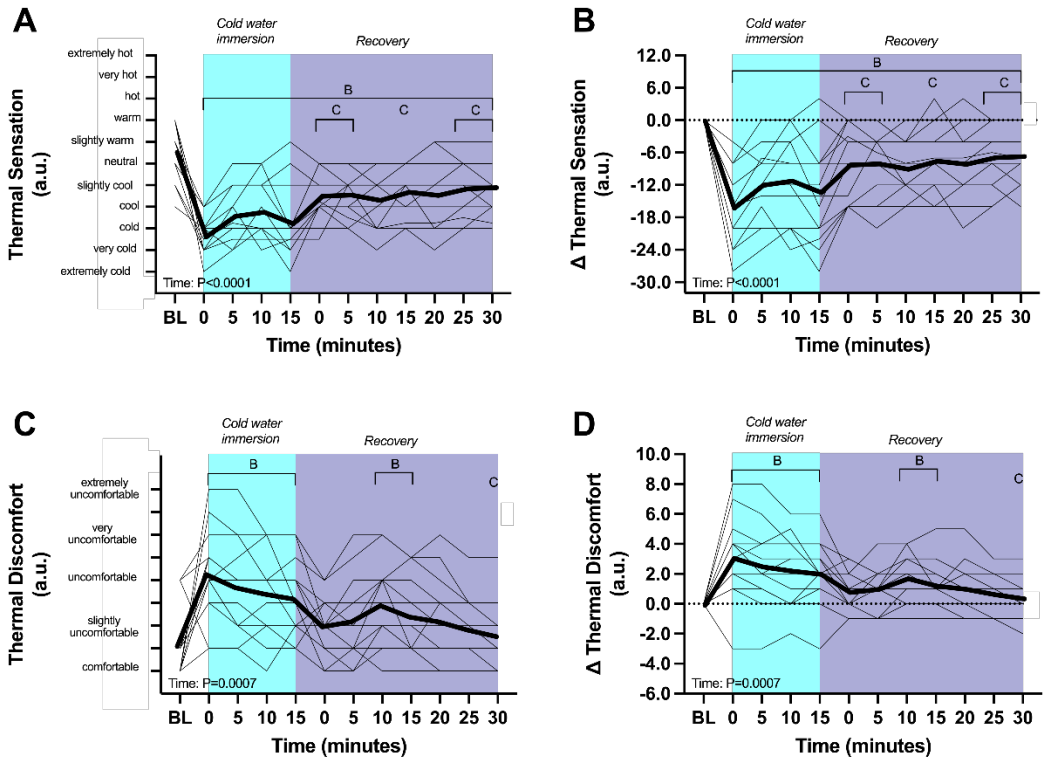

Figure 5. Thermal perceptions

Thermal sensation and discomfort responses during 15 minutes of cold water immersion and 30 minutes of post-immersion recovery. Data is reported as absolute value and change from baseline (BL). Thermal sensation: absolute values (panel A), change from BL (panel B). Thermal discomfort: absolute values (panel C), change from BL (panel D). Data were analyzed using a one-way ANOVA with post hoc Dunnett's adjustments to compare changes from BL or the end of cold water immersion. The thick bold line depicts mean data and individual data are shown by thin lines. ^{B}different from baseline ($P \leq 0.0371$). ^{C}different from cold water immersion $P \leq 0.0469$). n=14 (8 males, 6 females).

PANAS

Positive affect was not different after 30 minutes of recovery compared to baseline (P=0.0847, Figure 6A). However, further scrutiny of the individual data revealed that positive affect was reduced in 8 out of the 11 participants, not changed in 2 out of the 11 participants, and increased in 1 participant (Figure 6B). Positive affect was reduced at three hours post immersion compared to baseline (P=0.0501, Figure 6A). Negative affect followed a similar trend to positive affect showing no change after 30 minutes of recovery compared to baseline (P=0.7338, Figure 6C), and the individual data demonstrate that negative affect was reduced in 4 out of the 11 participants, not changed in 6 out of the 11 participants, and increased in 1 participant (Figure 6D).

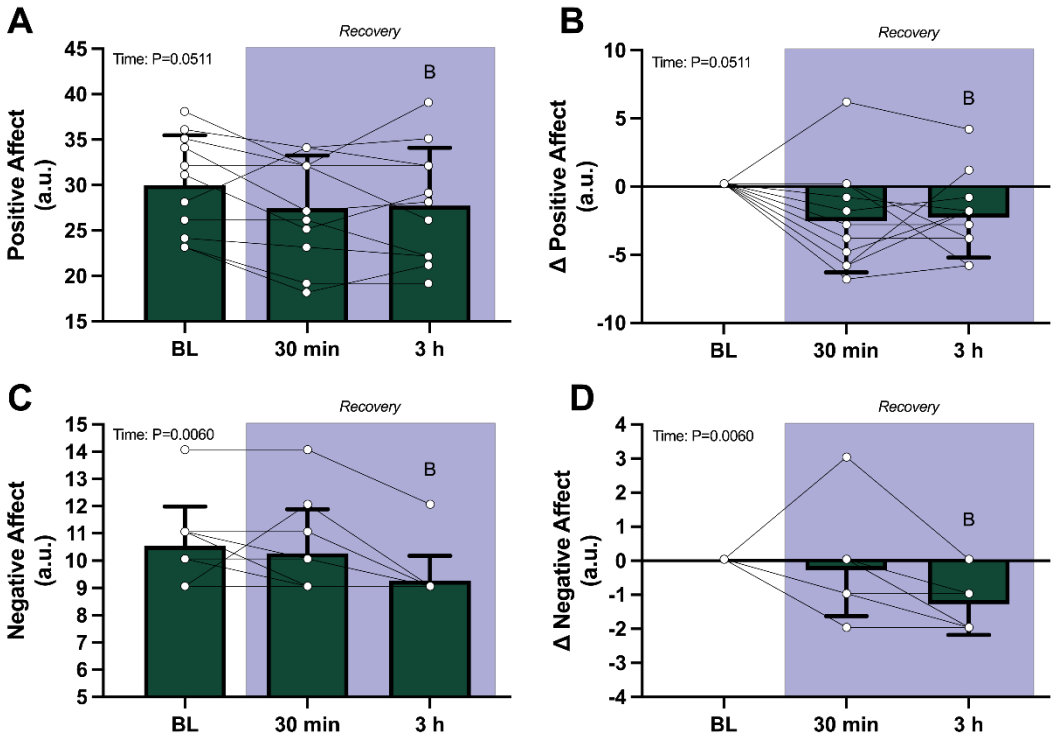

Figure 6. Positive and Negative Affect (PANAS)

PANAS responses before, during, and after cold water immersion. Data is reported as absolute value and change from baseline (BL). Positive affect: absolute values (panel A), change from BL (panel B). Negative affect: absolute values (panel C), change from BL (panel D). Data were analyzed using a one-way ANOVA with post hoc Dunnett's adjustments to compare changes from BL or the end of cold water immersion. Individual data is represented as thin lines corresponding to single values in the mean data. (Note: change from BL for negative affect has fewer individual data since several participants reported the exact same values). [B] different from baseline ($P \leq 0.0017$). n=14 (8 males, 6 females).

Blood markers

Cortisol and β-endorphins

Cortisol concentrations remained unchanged compared to baseline for most of the trial, but three hours post immersion cortisol concentrations were reduced by 680.3±595.0 pg/mL (P=0.0431, Figure 7B). There was no change in β-endorphin concentration throughout the duration of the trial (P=0.3205, Figure 7C and D). However, further scrutiny of the data revealed that three participants experienced an increased β- endorphins three hours post immersion, which potentially may implicate a responder vs. non-responder effect to cold water immersion (Figure 7D).

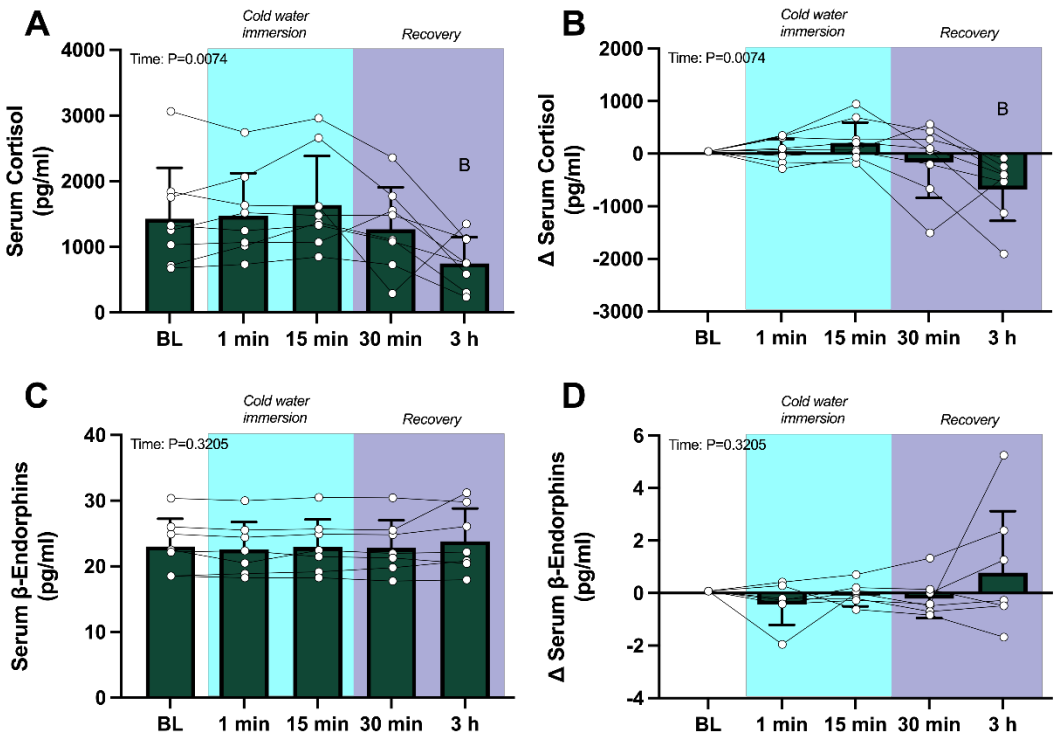

Figure 7. Serum Cortisol and β- endorphin concentration

Serum cortisol and β- endorphin concentrations taken at baseline (BL), 1 min and 15 min of immersion, and 30 min and 3 hours post-immersion. Data are presented as absolute values (left panels) and change from baseline (right panels). Data were analyzed using a one-way ANOVA with post hoc Dunnett's adjustments to compare changes from BL or the end of cold water immersion. Data is presented as mean and standard deviation. [B]different from baseline ($p \leq 0.0431$). n=8 (4 males, 4 females).

FGF21, TNFα , and IL-1β

FGF21 concentrations did not change compared to baseline for the duration of the trial ($P \geq 0.1568$, Figure 8A and B). However, four out of seven participants experienced mild increases in FGF21 (~18 pg/mL) with one subject experiencing large increases (~144 pg/mL). TNFα concentrations were not different throughout the duration of the trial (Figure 8C and D). It is unclear if the spikes seen in one individual value is representative of a physiological change or an outlier. Collecting more data would confirm this. IL-1β concentrations were below the sensitivity of detection in the ELISA kit that was used throughout water immersion (Figure 8E and F).

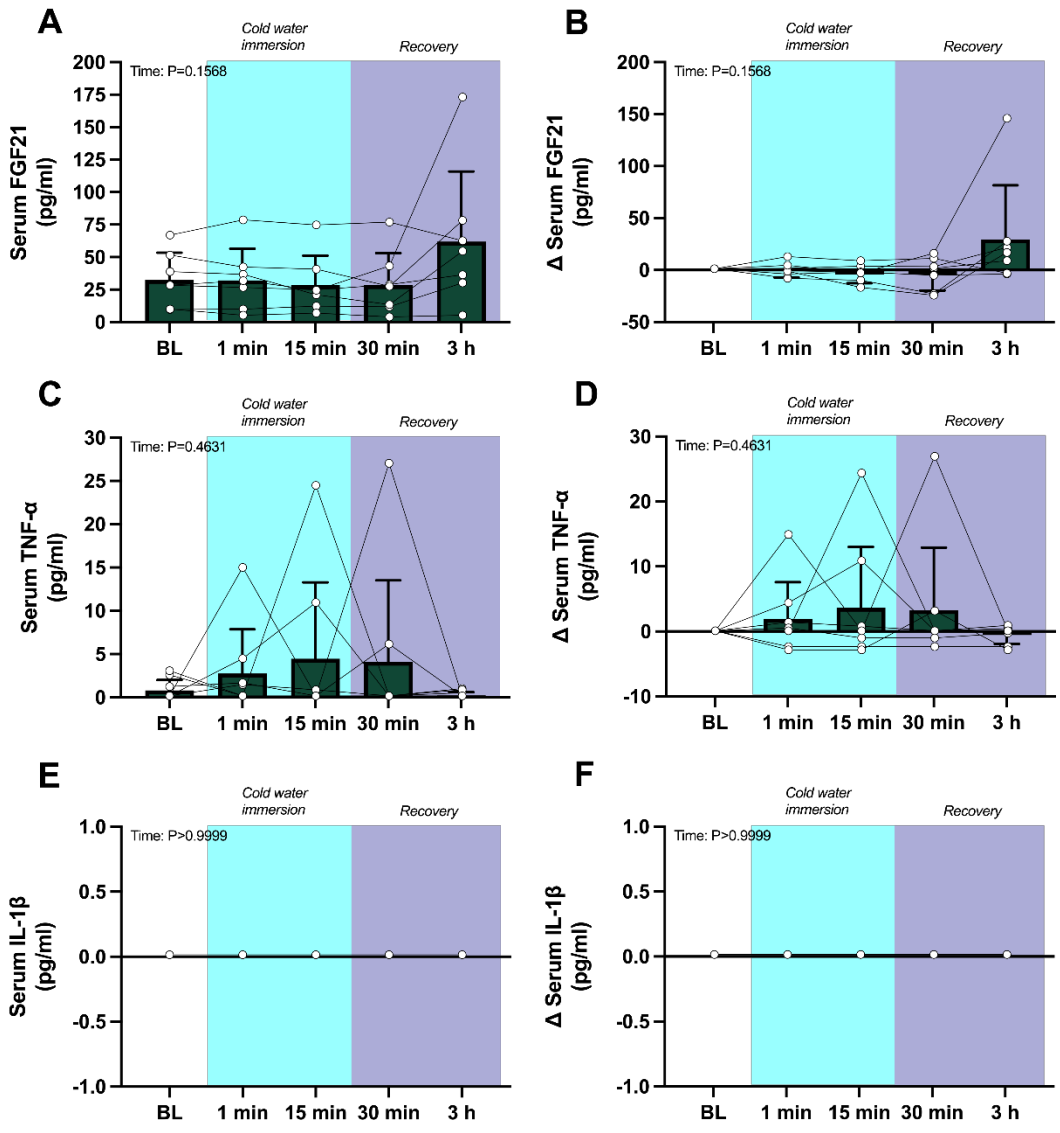

Figure 8. Serum FGF21, TNFα , and IL-1β

FGF21, TNFα , and IL-1β concentrations taken at baseline (BL), 1 min and 15 min of immersion, and 30 min and 3 hours post immersion. Data are presented as mean and standard deviation with absolute values (left panels) and change from baseline (right panels). Data were analyzed using a one-way ANOVA with post hoc Dunnett's adjustments to compare changes from BL or the end of cold water immersion. n=8 (4 males, 4 females).

Correlations

Core temperature correlations

The following rectal temperature correlations aim to quantify the relation between rectal temperature and blood markers or positive and negative affect. A trend line with 95% confidence intervals was added to correlations that may have a statistically significant correlation with future data collection (i.e., the data are currently underpowered). On the x-axis, changes in rectal temperature are the difference between the end of 30 minutes recovery and baseline. A potentially strong negative relation between reductions in rectal temperature and serum cortisol exists, where greater reductions in rectal temperature may be correlated with an attenuated reduction in serum cortisol concentrations (Figure 9A). Greater reductions in rectal temperature were potentially moderately related to less of a reduction in negative affect, meaning that greater reductions in rectal temperatures had less of an improvement on negative emotions (Figure 10E). There was no relation between the rectal temperature and FGF21, β- endorphins, and positive affect ($P \geq 0.4595$, Figure 10B, C, and D).

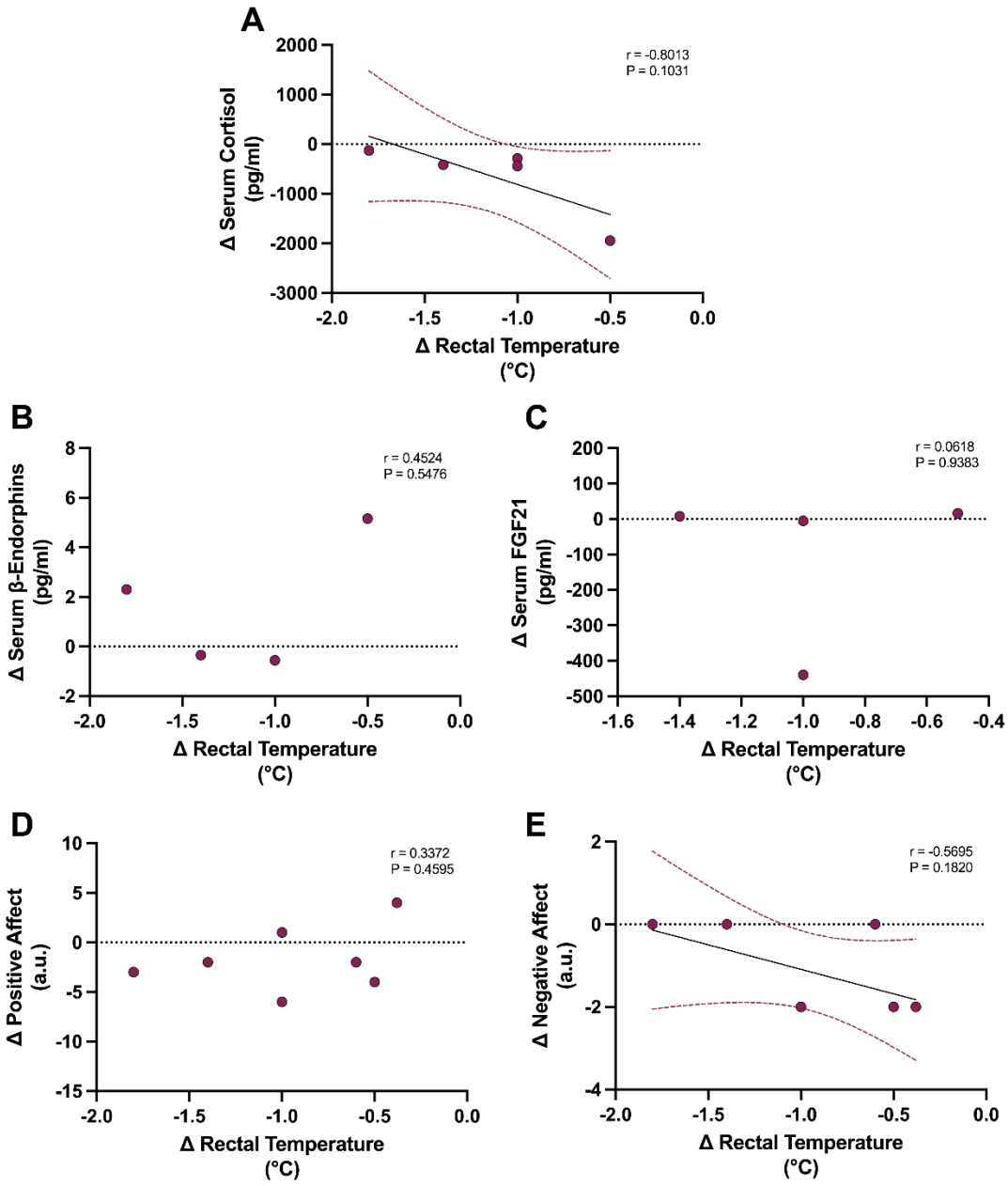

Figure 9. Preliminary analysis of the relation between rectal temperature and blood markers or positive and negative affect

Pearson-product moment correlations between the change in rectal temperature (baseline to end recovery) and the change in blood markers (panels A-C) or positive and negative affect (panels D and E) at three hours post cold water immersion. Potentially statistically significant relations with future data collection are plotted with best fit line and 95% confidence intervals. (*n*=5, panel A, *n*=4, panel B, *n*=4 panel C, *n*=6, panel D, *n*=6 panel E).

Thermal Perceptions Correlations

Data are presented as positive and negative affect three hours post immersion (y-axis) vs. thermal perceptions at the end of cold water immersion (x-axis). These correlations show that feeling colder does not relate to feeling more positive, nor does it relate to feeling less negative three hours post immersion (Figure 10A and B). These data also show that there is no relation between thermal discomfort following cold water immersion and alterations in positive or negative affect (Figure 10C and D).

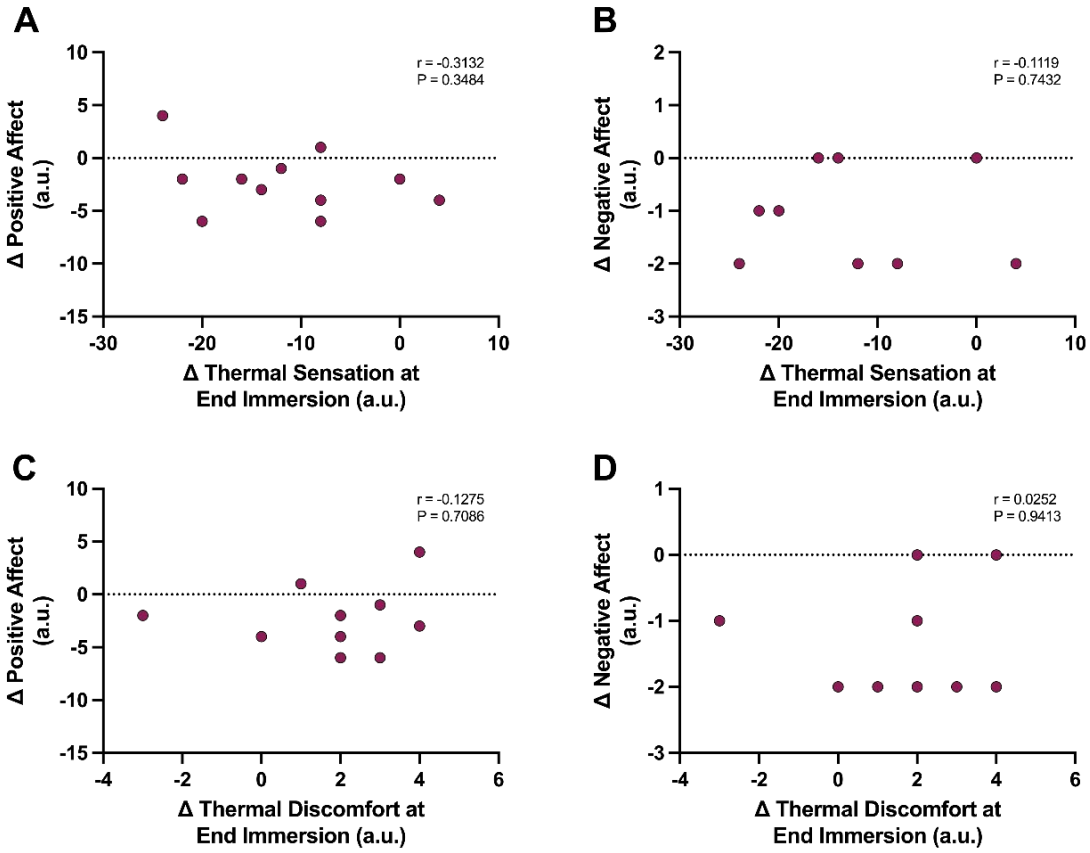

Figure 10. Preliminary analysis of the relation between thermal perceptions and positive or negative affect

Pearson-product moment correlations between the change in thermal sensation (panels A and B) and thermal discomfort (panels C and D) during cold water immersion compared to positive and negative affect three hours post immersion. (*n*=11 panel A, *n*=9 panel B, *n*=10 panel C, *n*=9 panel D).

Blood Marker Correlations

The following correlations quantify the relationship between FGF21, cortisol, and β- endorphin concentration with positive and negative affect at three hours post immersion. These data show a potential moderately to strong positive correlation between greater increases in β- endorphin concentration and decreased positive affect (Figure 11C). However, there is also a moderately positive correlation between increased β- endorphin and a decreased negative affect (Figure 11D). There is no correlation between cortisol and positive or negative affect (Figure 11A and B) and between FGF21 and negative affect (Figure 11F).

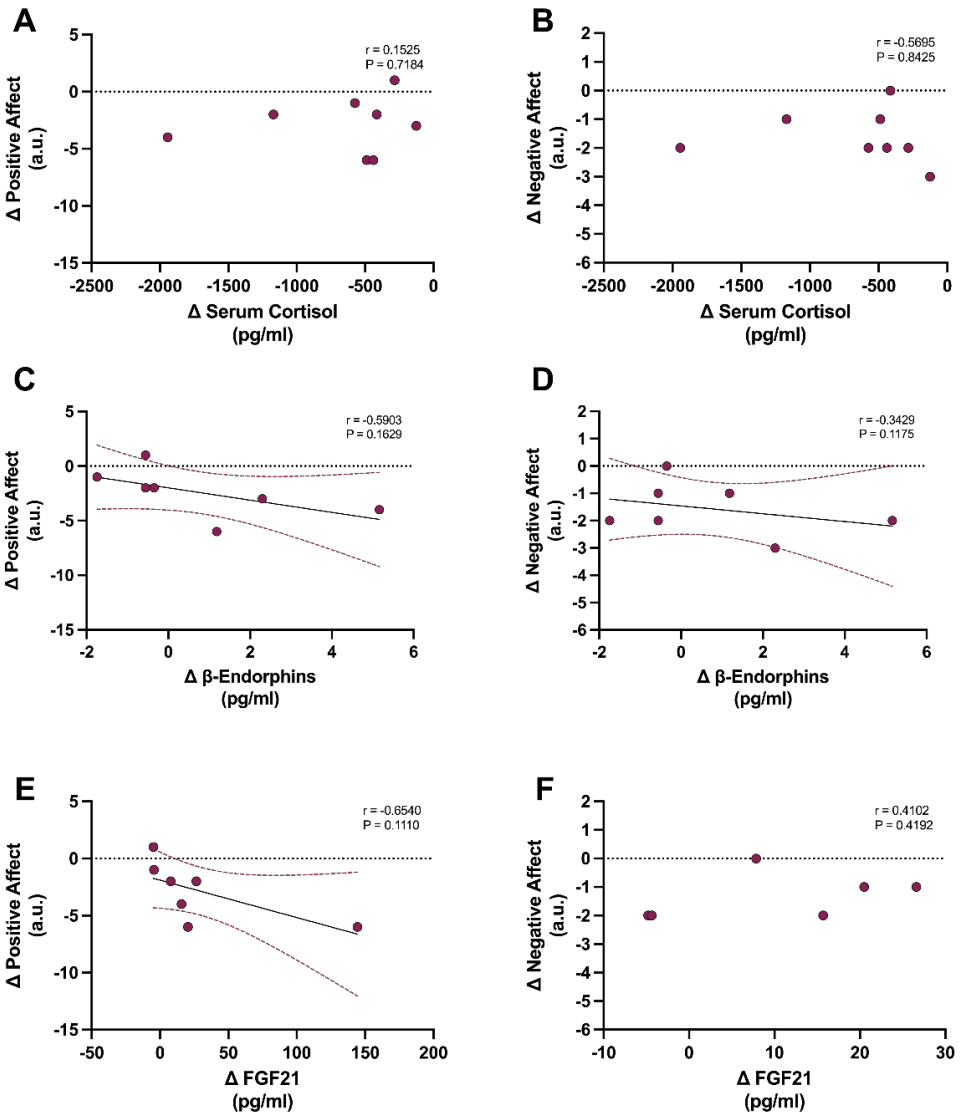

Figure 11. Preliminary analysis of the relation between blood markers and positive or negative affect

Pearson-product moment correlations between serum cortisol (panel A and B), β-endorphin (panels C and D), and FGF21 (panels E and F) compared to positive and negative affect. Blood markers are measured as changes from baseline and positive and negative affect are measured at three hours post immersion. Potentially statistically significant relations with future data collection are plotted with best fit line and 95% confidence intervals. (n=8 panel A, n=8 panel B, n=7 panel C, n=7 panel D, n=7 panel E, n=6 panel F).

Discussion

Contrary to my first hypothesis, the findings from the present study do not support that acute cold water immersion improves positive affect three hours post immersion. However, negative affect was reduced three hours after immersion, indicating that acute cold water immersion beneficially attenuates an aspect of negative emotional state. Contrary to my second hypothesis, cold water immersion did not increase the concentrations of cortisol and β- endorphins at the end of immersion. Additionally, there were no changes in FGF21 and TNFα as a result of cold water immersion.

To my knowledge, the present study was the first laboratory-controlled experiment to investigate whether positive affect is improved with acute cold water immersion and to explore the potential mechanistic underpinnings by which these perceptions may be altered. The blood markers examined in the present study were analyzed due to their implications on physiological and mental health. Chronic stress leads to reduced immune function, decreases in cardiovascular health, and often negative emotional state. However, bouts of acute stress, such as exercise, have been shown to attenuate symptoms of depression and inflammatory diseases while also enhancing cardiovascular health (Beavers et al., 2010; Mikkelsen et al., 2017). These health benefits are likely achieved via cellular mechanisms that induce changes in several critical blood markers. Therefore, the present study examined β- endorphins as a marker of elevated mood, cortisol as a marker of the involuntary stress response, TNFα, and IL-1β as indicators of inflammation pertaining to chronic stress, and FGF21 as an indicator of increased energy expenditure in relation to obesity. Based on previous

literature, we would have expected to see increases in cortisol coupled with decreases in pro-inflammatory markers (Eimonte et al., 2021). However, our results revealed no changes to the concentrations of TNFα and IL-1β, suggesting that these changes may occur under different conditions. Furthermore, there was no change to FGF21 during the period in which non-shivering thermogenesis would be expected. FGF21 is activated upon exposure to cold and induces the conversion of white adipose tissue into brown adipose tissue, which is densely populated with mitochondria that have uncoupler protein 1. The presence of moderate amounts of brown adipose tissue is considered healthy due to the heat generated by the uncoupler protein 1, which leads to non-shivering thermogenesis. This metabolic response to cold is beneficial for maintaining a stable internal temperature and is one of the physiological mechanisms by which humans are able to survive in cold environments. Since we did not see an increase in FGF21, it is possible that chronic exposures are the key to unlocking this mechanism.

This study was inspired by anecdotal reports of improved emotional state (e.g., elevated happiness) following cold water immersion (Shevchuk, 2008; Wim et al., n.d.). Contrary to these reports, the present study found that positive affect was not improved within three hours of cold water immersion. This result differs compared to literature on the effects of exercise and positive affect, suggesting different psychophysiological mechanisms between exercise and cold water immersion that have yet to be determined. Interestingly, however, cold water immersion in the present study diminished negative affect, which may potentially support some of these anecdotal findings and coincides

with previous literature on exercise and changes to affect. This observed reduction in negative affect may be enhanced for individuals with symptoms of depression.

The lack of improvement in positive affect may have been due to several logistical aspects of this experiment that warrant further investigation. Although we did not report the hunger of participants, several participants reported in passing that they were hungry three hours post immersion due to the eating constraints of the study. By three hours post immersion, participants had not eaten for seven hours. This may have contributed to lower ratings for the positive affect on the PANAS due to a reduction in glucose that is considered to be an aversive stimulus (Benton, 2002; Horman et al., 2018). Another possibility for a reduction in positive affect three hours post immersion may be attributed to the water temperature and duration of the study. Experiments on rats suggest that β- endorphin concentration increased by 337% in rats that swam in 1°C water for 5 minutes (Vaswani et al., 1988). Similarly, Wim Hof advocates for cold water immersion in which the water is near 1°C, making his exposures more akin to ice baths. These results suggest that the present study may have been too warm to induce an increase in β- endorphin concentration. Since β- endorphins are tightly linked to improved emotional state, future research testing water at colder temperatures may reveal the emotional benefits to cold water immersion.

Despite previous literature and Wim Hof's claims, our model shows that reductions in positive affect were not related to changes in rectal temperature, suggesting that the magnitude of reductions in rectal temperature do not modulate the change in positive affect. Our preliminary analysis suggests that reductions in positive affect are related to increases in β- endorphins and FGF21. It should be noted that

FGF21 has not been shown to be correlated with increases in emotional state previously, suggesting a correlation without causation effect in the present study. Our model also indicates that greater reductions in rectal temperature were correlated with less attenuation of negative affect. This suggests that slightly warmer water may actually lead to reductions in negative affect that improve emotional state. Future research is needed to determine the temperature at which cold water immersion becomes mentally and physiologically beneficial.

Limitations

The present study had several limitations that warrant discussion. First, we did not include a time control group or a cross-over experimental design. Therefore, we were unable to discern if the effects of our study were due to the duration of time spent sitting in the lab or the cold water immersion itself. However, we utilized techniques to increase scientific rigor by having the participants rest quietly in the hydraulic lift chair pre- and post- immersion. Additionally, we were constrained by current University COVID-19 restrictions placed on research which made including a time control group not feasible for this study. Second, we only recorded two PANAS following cold water immersion; one 30-minutes post immersion and one three hours post immersion. Since this period of time is quite large, it is possible we missed the window where positive affect would have been increased. Third, this study was conducted during a pandemic in which many people are feeling heightened stress as a result of social distancing, fear, and sudden life style changes that occurred in the past year (Son et al., 2020). Fourth, since we did not measure metabolic rate, we have no indication if the shivering

response attenuated core temperature and potentially modified the perceptual and blood marker responses to cold water immersion.

Perspectives and Significance

There is much interest in reducing the risk of cardiovascular disease, addressing the obesity epidemic, and attenuating negative emotional state (e.g., depression). These three long-term health issues often intersect through chronic stress, unhealthy lifestyles, or even genetics. Therefore, seeking therapies to reduce stress and promote a healthy lifestyle has the potential to alleviate three major health risks in the United States. The present study provides some support that cold water immersion reduces negative affect. Whether this occurs with chronic cold water exposures warrants further investigation.

Conclusion

Overall, the current findings indicate that acute cold water immersion for 15 minutes at 10°C did not increase positive affect, but did decrease negative affect. Our data suggests that greater reductions in rectal temperature do not lead to increases in positive affect. However, the present study showed that increases in β- endorphin concentration were correlated with a reduction in negative affect. This adds to the literature supporting that β- endorphins are responsible for elevated emotional state and opens the door for future studies on the chronic effects of cold exposure. Determining the necessary temperature and duration of cold water exposure that would lead to an increase in β- endorphins may be the key to unlocking the therapeutic effects of cold water immersion.

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:

<u>Cold Exposure</u>

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]

Bibliography

Beavers, K. M., Brinkley, T. E., & Nicklas, B. J. (2010). Effect of exercise training on chronic inflammation. *Clinica Chimica Acta*, *411*(11–12), 785–793. https://doi.org/10.1016/j.cca.2010.02.069

Benton, D. (2002). Carbohydrate ingestion, blood glucose and mood. In *Neuroscience and Biobehavioral Reviews* (Vol. 26, Issue 3, pp. 293–308). Pergamon. https://doi.org/10.1016/S0149-7634(02)00004-0

Cox, R. H., Thomas, T. R., & Davis, J. E. (2001). Positive and negative affect associated with an acute bout of aerobic exercise. *Journal of Exercise Physiology Online*, *4*(4), 13–20.

Dhabhar, F. S. (2014). Effects of stress on immune function: The good, the bad, and the beautiful. *Immunologic Research*, *58*(2–3), 193–210. https://doi.org/10.1007/s12026-014-8517-0

Eimonte, M., Eimantas, N., Daniuseviciute, L., Paulauskas, H., Vitkauskiene, A., Dauksaite, G., & Brazaitis, M. (2021). Recovering body temperature from acute cold stress is associated with delayed proinflammatory cytokine production in vivo. *Cytokine*, *March*, 155510. https://doi.org/10.1016/j.cyto.2021.155510

Guidance for General Laboratory Safety Practices during the COVID-19 Pandemic | CDC. (n.d.). Retrieved April 10, 2021, from https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-safety-practices.html

Horman, T., Fernandes, M. F., Zhou, Y., Fuller, B., Tigert, M., & Leri, F. (2018). An exploration of the aversive properties of 2-deoxy-D-glucose in rats. *Psychopharmacology*, *235*(10), 3055–3063. https://doi.org/10.1007/s00213-018-4998-1

Laukkanen, T., Khan, H., Zaccardi, F., & Laukkanen, J. A. (2015). Association between sauna bathing and fatal cardiovascular and all-cause mortality events. *JAMA Internal Medicine*, *175*(4), 542–548. https://doi.org/10.1001/jamainternmed.2014.8187

LePage, M. L., & Crowther, J. H. (2010). The effects of exercise on body satisfaction and affect. *Body Image*, *7*(2), 124–130. https://doi.org/10.1016/j.bodyim.2009.12.002

Mack, G. W., Cheung, S., Fujii, N., Heathcote sheathcote, S. L., Stevens, C. J., Heathcote, S. L., Hassmén, P., & Zhou, S. (2018). Passive Heating: Reviewing Practical Heat Acclimation Strategies for Endurance Athletes. *Frontiers in Physiology | Www.Frontiersin.Org*, *9*, 1851. https://doi.org/10.3389/fphys.2018.01851

Mikkelsen, K., Stojanovska, L., Polenakovic, M., Bosevski, M., & Apostolopoulos, V. (2017). Exercise and mental health. *Maturitas*, *106*(September), 48–56. https://doi.org/10.1016/j.maturitas.2017.09.003

Morrison, S. F., Nakamura, K., & Madden, C. J. (2008). Central control of thermogenesis in mammals. *Experimental Physiology*, *93*(7), 773–797. https://doi.org/10.1113/expphysiol.2007.041848

Mourot, L., Bouhaddi, M., Gandelin, E., Cappelle, S., Dumoulin, G., Wolf, J. P., Rouillon, J. D., & Regnard, J. (2008). Cardiovascular autonomic control during short-term thermoneutral and cool head-out immersion. *Aviation Space and Environmental Medicine*, *79*(1), 14–20. https://doi.org/10.3357/ASEM.2147.2008

Muzik, O., Reilly, K. T., & Diwadkar, V. A. (2018). "Brain over body"–A study on the willful regulation of autonomic function during cold exposure. *NeuroImage*, *172*(January), 632–641. https://doi.org/10.1016/j.neuroimage.2018.01.067

Nicholas Orme. (1983). *Early British Swimming, 55 BC-AD 1719: With the First Swimming Treatise in ... - Nicholas Orme - Google Books*. https://books.google.com/books?hl=en&lr=&id=l-iqXu2tNk8C&oi=fnd&pg=PR9&dq=nicholas+wynmann+swimming&ots=n5YsVxbS1K&sig=sNo6P9-RJ-4eIJxCSDqgixwBk2A#v=onepage&q=cold&f=false

Pikkers, P., Hopman, M., & Kox, M. (2011). *The Cholinergic Anti-Inflammatory Pathway in vitro, animal, and human studies Matthijs Kox*. www.ipskampdrukkers.nl

Rohleder, N. (2019). Stress and inflammation – The need to address the gap in the transition between acute and chronic stress effects. *Psychoneuroendocrinology*, *105*(February), 164–171. https://doi.org/10.1016/j.psyneuen.2019.02.021

Romet, T. T. (1988). Mechanism of afterdrop after cold water immersion. *Journal of Applied Physiology*, *65*(4), 1535–1538. https://doi.org/10.1152/jappl.1988.65.4.1535

Sapolsky, R. (1994). *Why Zebras Don't Get Ulcers* (Vol. 1).

Seale, P., & Lazar, M. A. (2009). Brown fat in humans: Turning up the heat on obesity. In *Diabetes* (Vol. 58, Issue 7, pp. 1482–1484). American Diabetes Association. https://doi.org/10.2337/db09-0622

Shevchuk, N. A. (2008). Adapted cold shower as a potential treatment for depression. *Medical Hypotheses*, *70*(5), 995–1001. https://doi.org/10.1016/j.mehy.2007.04.052

Son, C., Hegde, S., Smith, A., Wang, X., & Sasangohar, F. (2020). Effects of COVID-19 on college students' mental health in the United States: Interview survey study. In *Journal of Medical Internet Research* (Vol. 22, Issue 9, p. e21279). JMIR Publications Inc. https://doi.org/10.2196/21279

Šrámek, P. (1996). Change in sympathetic activity, cardiovascular functions and plasma hormone concentrations due to cold water immersion in men. *European Journal of Applied Physiology and Occupational Physiology*, *74*(1–2), 148–152. https://doi.org/10.1007/bf00376507

Šrámek, P., Šimečková, M., Janský, L., Šavlíková, J., & Vybíral, S. (2000). Human physiological responses to immersion into water of different temperatures. *European Journal of Applied Physiology*, *81*(5), 436–442. https://doi.org/10.1007/s004210050065

Tipton, M. J., Collier, N., Massey, H., Corbett, J., & Harper, M. (2017). Cold water immersion: kill or cure? *Experimental Physiology*, *102*(11), 1335–1355. https://doi.org/10.1113/EP086283

Van Der Lans, A. A. J. J., Hoeks, J., Brans, B., Vijgen, G. H. E. J., Visser, M. G. W., Vosselman, M. J., Hansen, J., Jörgensen, J. A., Wu, J., Mottaghy, F. M., Schrauwen, P., & Van Marken Lichtenbelt, W. D. (2013). Cold acclimation recruits human brown fat and increases nonshivering thermogenesis. *Journal of Clinical Investigation*, *123*(8), 3395–3403. https://doi.org/10.1172/JCI68993

Vaswani, K. K., Richard, C. W., & Tejwani, G. A. (1988). Cold swim stress-induced changes in the levels of opioid peptides in the rat CNS and peripheral tissues. *Pharmacology, Biochemistry and Behavior*, *29*(1), 163–168. https://doi.org/10.1016/0091-3057(88)90290-0

Watson, D., Clark, L. A., & Tellegen, A. (1988). Development and Validation of Brief Measures of Positive and Negative Affect: The PANAS Scales. *Journal of Personality and Social Psychology*, *54*(6), 1063–1070. https://doi.org/10.1037/0022-3514.54.6.1063

Wesseling, K. H., Jansen, J. R. C., Settels, J. J., & Schreuder, J. J. (1993). Computation of aortic flow from pressure in humans using a nonlinear, three-element model. *Journal of Applied Physiology*, *74*(5), 2566–2573. https://doi.org/10.1152/jappl.1993.74.5.2566

West, A. M., Tarrier, J., Hodder, S., & Havenith, G. (2019). Sweat distribution and perceived wetness across the human foot: the effect of shoes and exercise intensity. *Ergonomics*, *62*(11), 1450–1461. https://doi.org/10.1080/00140139.2019.1657185

Wim, T., Method, H., Hof, B. I., & Laws, F. C. (n.d.). *Colofon*.