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1	Title
2	Influence of acute beetroot juice supplementation on cold-induced vasodilation and fingertip rewarming
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#### 19 Abstract

- 20 Purpose: Vasoactive ingredients in beetroot (BR) such as nitrate are known to induce vasodilation in
- 21 temperate conditions. This study investigated the effect of BR ingestion on cold induced vasodilation
- 22 (CIVD) and rewarming of finger skin temperature ( $T_{\text{fing}}$ ) during and after hand immersion in cold water.
- 23 Methods: Twenty healthy males (mean  $\pm$  SD; age 22.2 $\pm$ 0.7 yrs, height 172.6 $\pm$ 6.0 cm, body mass 61.3 $\pm$ 11.7
- 24 kg) repeated a hand cold water immersion test twice with prior BR or water beverage ingestion (randomised
- 25 order). They rested for two hours in thermoneutral conditions (27°C, 40% relative humidity) after
- 26 consuming the beverage, then immersed their non-dominant hand in 8°C water for 30 min. They then
- 27 rewarmed their hand in the ambient air for 20 min. Skin temperature at seven body sites,  $T_{\text{fing}}$ , finger skin
- 28 blood flow ( $SkBF_{fing}$ ), and blood pressure were measured.
- 29 Results: During hand immersion parameters of CIVD (*T*<sub>fing</sub> and *SkBF*<sub>fing</sub>) were not different between BR
- 30 and water conditions although skin temperature gradient from proximal to distal body sites was significantly
- 31 smaller with BR (P<0.05). During rewarming,  $SkBF_{fing}$  and cutaneous vascular conductance were
- 32 significantly higher with BR than with water (P < 0.05). The rewarming speed in  $T_{\text{fing}}$  and  $SkBF_{\text{fing}}$  was
- 33 significantly faster with BR at 15- (BR 1.24±0.22 vs water 1.11±0.26°C/min) and 20-min rewarming
- 34 (P<0.05). Additionally, individuals with slower rewarming speed with water demonstrated accelerated
- 35 rewarming with BR supplementation.
- 36 Conclusion: BR accelerated rewarming in  $T_{\text{fing}}$  and  $SkBF_{\text{fing}}$  after local cold stimulus, whereas, CIVD
- 37 response during hand cold immersion was not affected by BR ingestion.

Nitrate, nitric oxide, rewarming speed, skin blood flow, red beet

38

# 39 Keywords

- 40
- 41

#### 42 Abbreviations

- 43 AVA Arteriovenous anastomoses
- 44 CVC Cutaneous vascular conductance
- 45 CIVD Cold-induced vasodilation
- 46 MAP Mean arterial blood pressure
- 47 NFCI Non-freezing cold injury
  48 NO<sub>3</sub><sup>-</sup> Nitrate
- 49  $NO_2^-$  Nitrite
- 50 NO Nitric oxide
- 51 NOS Nitric oxide synthase
- 52 ROS Reactive oxygen species
- 53 SkBF Skin blood flow
- 54  $T_{\rm fing}$  Finger skin temperature

55	$T_{\rm pro-dis}$	Proximal to distal skin temperature difference
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 $T_{\text{subl}}$  Sublingual temperature

 $\overline{T}_{sk}$  Mean skin temperature

#### 59 Introduction

60 In cold environments, cutaneous vasoconstriction is induced for maintaining homeostasis of core body 61 temperature. This vasomotor response for body temperature regulation results in the reduction of skin 62 temperature especially at distal extremities thereby widening the gradient between skin and core and 63 resulting in cooler extremities. Accordingly, the risk of frostbite and non-freezing cold injury in the distal 64 extremities have been reported in the workers in cold environments (e.g. fishery, military, and cold storage 65 workers), especially when they are continuously and longitudinally exposed to cold (Imray et al. 2009; 66 Makinen and Hassi 2009). Additionally, it is well known that performance of manual dexterity using fingers 67 and hands is impaired by the reduction of skin and subcutaneous tissue temperature resulting in impaired 68 motor coordination (Heus et al. 1995; Castellani and Tipton 2015; Wakabayashi et al. 2015), which could 69 be an additional injury risk factor for accidents in the workplace. Thus, wearing thermal protective clothing 70 and gloves are generally recommended for the workers in cold (Castellani et al. 2006; Holmer 2009). 71 However, wearing thick gloves can impair the manual dexterity performance especially when workers 72 conduct technical operations which need fine motor control of their fingers and hands (Brajkovic et al. 73 2001; Dianat et al. 2012). Therefore, some practically available alternative solutions for keeping warm 74 distal extremities are required for delicate manual work in cold environments in otherwise healthy 75 individuals.

76

77 Recently, supplementation with nitrate ( $NO_3$ ) rich beetroot (BR) drink has been considered as an ergogenic 78 aid for enhancing blood circulation and exercise performance, especially in athletes, with promising results 79 (Wylie et al. 2013; Hoon et al. 2013; Dominguez et al. 2017). These studies have concurrently investigated 80 the effect of BR on the blood pressure and vasodilation response but only in thermoneutral or hot 81 environments (Wylie et al. 2013; Hobbs et al. 2013; Amano et al. 2018). Wylie et al. (2013) reported a dose 82 response relationship in reducing the oxygen cost of exercise with no improvement in exercise tolerance 83 above a dose of 140 mL (~8.4 mmol NO<sub>3</sub><sup>-</sup>) in a thermoneutral environment. Hobbs et al. (2013), also in a 84 thermoneutral environment, demonstrated increase in the endothelium-independent vasodilation and 85 decreased diastolic blood pressure following beetroot infused bread ingestion. These effects were 86 concurrent with increased plasma and urinary nitrate. Amano et al. (2018) conducted their study in hot 87 conditions (30°C, 50% relative humidity) but only showed changes in mean arterial pressure but not skin 88 blood flow or cutaneous vascular conductance. Compared to studies in warm or thermoneutral 89 environments, fewer studies have investigated the effect of BR on peripheral circulation and distal skin 90 temperature during and after local cold exposure (Eglin et al. 2017; Shepherd et al. 2019; Wickham et al. 91 2021). This is surprising given that an endothelial-independent NO donor is known to increase vasodilation 92 as demonstrated following glyceryl trinitrate (GTN) ingestion in cold-sensitive individuals (Hope et al. 93 2014) and topical GTN application in patients with Raynaud's phenomenon (Anderson et al. 2002) 94 suggesting a plausible role for NO in improving peripheral re-warming. Considering the evidence of

cutaneous vasodilation with dietary ingested BR primarily in thermoneutral environments and in persons

- 96 with cold injury, this intervention could also be applied for maintaining warm finger skin temperature in a
- 97

7 cold environment which may also translate to an occupational benefit in improving manual dexterity.

98

99 Wickham et al. (2021) investigated the effect of acute BR supplementation on the cold-induced vasodilation 100 (CIVD) in finger skin temperature and blood flow *during* hand cold-water (8°C) immersion in ten healthy 101 males. They found no difference in any CIVD parameters between conditions with BR and NO3<sup>-</sup> depleted 102 placebo drink. Accordingly, they suggested only a minor contribution of nitric oxide (NO) as a mechanism 103 for the CIVD response (Wickham et al. 2021). Shephard et al. (2019) examined the effect of acute and 104 chronic BR supplementation on vasomotor regulation in a cohort of cold-sensitive elderly people 105  $(64.3\pm15.3 \text{ yrs})$  with Raynaud's syndrome (i.e., recurrent transient vasospasm of the fingers and/or toes in 106 response to a cold or stressful stimulus (Wigley 2002)). They reported that both chronic BR 107 supplementation and chronic nitrate depleted BR juice supplementation enhanced skin blood flow (SkBF) 108 during 10-min rewarming phase but in the thumb only following local transient cold stimulus (2-min cold 109 water immersion, 15°C) compared to the baseline no supplementation trial. While, noteworthy there were 110 no other differences between BR and  $NO_3^-$  depleted placebo drink. Based on the results, they suggested 111 that some of the vasoactive ingredients in BR, other than NO<sub>3</sub><sup>-</sup>, like betanin, quercetin, and chlorogenic 112 acid (Wootton-Beard et al. 2011) might be a factor for enhancing vasodilation after cold stimulus (Shepherd 113 et al. 2019). Importantly these vasoactive substances could be common to both the depleted  $NO_3^-$  test 114 supplementation conditions (i.e., the placebo controls) and the BR supplementation conditions used in most 115 studies. Hence, tests using a control condition to effectively separate and distinguish the effects of BR 116 supplementation is warranted. Consistent with this idea, Thompson et al. (2018) compared the physiological 117 effects of BR juice with potassium nitrate (KNO<sub>3</sub>) supplementation containing similar amounts of NO<sub>3</sub><sup>-</sup> 118 (Thompson et al. 2018). They found lower resting blood pressure with ingestion of BR compared to KNO<sub>3</sub>, 119 which suggested ingredients other than  $NO_3^{-}$  in BR might be responsible for improving the bioavailability 120 of NO; yet the study of Thompson et al (2018) was focussed on enhancing sprint interval training 121 performance. Whilst theoretically sound the evidence for the efficacy of BR supplementation in expediting 122 the rewarming of the extremities during and following cold exposure in a healthy population is equivocal 123 although further protocol manipulations are required to explore the putative effects.

124

With the controls used and findings revealed from previous studies, it remains possible that BR supplementation as a sole ingredient might enhance the CIVD response during local cold exposure and accelerate the subsequent rewarming, in comparison to a control absent of NO<sub>3</sub><sup>-</sup> and other vasoactive ingredients (i.e., water ingestion). Accordingly, the purpose of this study is to investigate the effect of acute BR ingestion on CIVD response during hand cold immersion and subsequent rewarming, compared to a control condition (water) in healthy young individuals. It was hypothesized that acute BR supplementation temperature rewarming after the immersion. With occupational cold exposure often far longer than the short exposure windows used in prior studies (e.g. Shephard et al. 2019 used 2-min immersion), we also sought to examine an extended period of cold exposure (matching that of Wickham et al. 2021; 30-min) coupled with an extended period of re-warming (i.e., 20-min). Lastly and from a practical perspective, the individual

would enhance the CIVD response during hand cold immersion, and finger skin blood flow and skin

- variation in the response to BR ingestion was quantified to enable targeted future intervention toward coldsensitive individuals.
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# 139 Methods

# 140 Participants

141 The experimental protocol was approved by the IRB of Hokkaido University. All participants were 142 informed of the experimental protocols and gave their written informed consent before participation. 143 Twenty healthy Japanese males living in Sapporo (mean ± standard deviation age: 22.2±0.7 yrs, height: 144 172.6±6.0 cm, body mass: 61.3±11.7 kg, % body fat: 15.2±5.0%) participated in the experiment. Their 145 percentages of body fat were estimated using bioelectrical impedance (RD-800, TANITA, Japan). All 146 experimental protocols in this study were designed according to the principle of the Helsinki Declaration. 147 Participants were asked to prohibit eating nitrate (NO<sub>3</sub><sup>-</sup>) rich foods, e.g. processed meats, green leaf 148 vegetable like Spinach, Chin gin cai, Seaweed, Sayaingen beans (Sobko et al. 2010) on the test day and the 149 day before. Additionally, they fasted for 2 hours before arriving the laboratory and were asked to refrain 150 using mouth rinse on the test day, since the oral bacteria are involved in the reduction of NO<sub>3</sub><sup>-</sup> to NO<sub>2</sub><sup>-</sup> 151 (Govoni et al. 2008).

152

# 153 Experimental Design

Participants completed a total of two test conditions separated by a minimum of 7 days to enable washing out of the BR effect (Amano et al. 2018; Shepherd et al. 2019). On each occasion hand immersion in to cold water and a rewarming test was completed with prior ingestion of beetroot (BR) or water as a representative control. The order of the test conditions was randomised using crossover design.

# 158 159 *Protocol*

# Participants arrived at the laboratory and changed their clothes to half sleeve shirts, long pants and socks (insulation ~0.6 clo). They then rested in upright sitting position on a chair with their arms on a table in a climatic chamber controlled to thermoneutral conditions (27°C and 40% relative humidity) for 30 min before drinking 140 mL of water or 140 mL of BR (Beet It Sport Pro-Elite Shot, James White Drinks, Ipswich, UK), which were maintained at room temperature (~27°C). The BR drink contains ~12.9 mmol (800 mg) NO<sub>3</sub><sup>-</sup> and 0.28 mmol (154 mg) betanin. The concentration of betanin was calculated from

 $166 \qquad absorbance \ measured \ with \ a \ UV/vis \ spectrophotometer \ (U-3310, \ Hitachi, \ Japan) \ using \ the \ molar \ extinction$ 

167 coefficient  $\varepsilon_{538}$ =60,000 M<sup>-1</sup>cm<sup>-1</sup> (Wyler and Meuer 1979; Kugler et al. 2004). After the beverage ingestion, 168 they continued resting for two hours, which has previously shown to be a sufficient time course to increase 169 plasma NO<sub>2</sub><sup>-</sup> after drinking BR containing 4.2 to 16.8 mmol NO<sub>3</sub><sup>-</sup> (Wylie et al. 2013). Then, after measuring 170 pre-immersion baseline for the measurement items described below, they immersed their non-dominant 171 hand covered by a waterproof polyethylene glove (12 µm thickness) in 8°C water up to their wrist for 30 172 min. The water in the tank was stirred and temperature controlled using a thermostat water circulation 173 device (LV-200, Advantec, Japan); water temperature was monitored at the start and end of each immersion. 174 After the 30-min hand water immersion, they removed the glove and rewarmed their hand in lateral position 175 on the table for 20 min.

176

#### 177 Measurements

178 Sublingual temperature ( $T_{subl}$ ) was measured at baseline, at the end of the hand immersion and rewarming 179 phases using a thermometer (MC-172L, Omron, Japan). Participants were asked to place the tip of the 180 thermometer below the tongue and to close their mouth for 5 minutes until the measurement stabilized. 181 Skin temperature was measured using thermistor probes (ITP082-24, Nikkiso-Therm, Japan) at seven body 182 sites (forehead, chest, forearm, thigh, foot, non-immersed hand and immersed fingertip). The skin 183 temperatures were monitored every second using data loggers (NR543R, Nikkiso-Therm, Japan), and 184 averaged every minute for subsequent data analyses. Mean skin temperature ( $\bar{T}_{sk}$ ) was estimated using a 185 modified Hardy and DuBois' equation (Hardy and Du Bois 1938), as follow:

186

188

# 187 Equation 1 $\bar{T}_{sk} = 0.07T_{head} + 0.35T_{chest} + 0.14T_{forearm} + 0.05T_{haad} + 0.32T_{thigh} + 0.07T_{foot}$

- 189 Where:  $T_{\text{chest}}$  was the selected site for trunk temperature and  $T_{\text{thigh}}$  included the additional 0.13 weighting 190 ordinarily allocated to  $T_{\text{legs}}$  (not recorded in the current study) from the original  $\bar{T}_{\text{sk}}$  formula.
- 191

192 The difference between proximal (average of forehead and chest) and distal (average of hand and foot) skin 193 temperatures (T<sub>pro-dis</sub>) were calculated as surrogate measure for assessing peripheral blood flow (Rubinstein 194 and Sessler 1990). Since vasoconstriction occurs remarkably in the distal part relative to the proximal part, 195  $T_{\rm pro-dis}$  well reflects the peripheral vasomotor tone, although there is a limitation that  $T_{\rm pro-dis}$  takes longer 196 time to reach steady state compared to the vasomotor response (Rubinstein and Sessler 1990; House and 197 Tipton 2002). This method has been verified during cooling and rewarming (House and Tipton 2002) and 198 also been used in studies on circadian rhythm as a parameter of distal heat loss (Krauchi et al. 1999). Skin 199 blood flow in the volar side of index finger (SkBF<sub>fing</sub>) was measured by laser Doppler flowmetry (ALF21, 200 ADVANCE, Japan) and sampled using an analogue to digital data converter (Powerlab/16SP, AD 201 Instruments, Australia) and recorded every 1 sec interval using a laptop computer. Arbitrary units (AU) 202 were used for the data of SkBFfing. Systolic (SBP) and diastolic blood pressure (DBP) was measured at the upper (contralateral) arm using an inflatable cuff and an automated blood pressure monitor (HEM-7430,
 Omron, Japan) every 5 min during the rewarming phase. Mean arterial blood pressure (*MAP*) was calculated

205 using the following formula:

206

207 Equation 2 
$$MAP [mmHg] = (SBP - DBP) / 3 + DBP$$

208 Cutaneous vascular conductance in finger ( $CVC_{fing}$ ) was calculated from  $SkBF_{fing}$  and MAP as follows:

209

211

210 Equation 3 
$$CVC_{\text{fing}} [AU/mmHg] = SkBF_{\text{fing}} / MAP$$

Parameters of CIVD were analysed in accordance with (Cheung 2015) where a minimum increase of  $0.5^{\circ}$ C in  $T_{\text{fing}}$  was required and the associated change in  $SkBF_{\text{fing}}$  during the hand cold water immersion was then considered. The onset time of the CIVD, the minimal ( $T_{\text{min}}$ ), the first peak ( $T_{\text{peak}}$ ), and maximal ( $T_{\text{max}}$ )  $T_{\text{fing}}$ was detected, then the amplitude from  $T_{\text{min}}$  to  $T_{\text{peak}}$  ( $T_{\text{peak}} - T_{\text{min}}$ ) and mean value of  $T_{\text{fing}}$  after 5 min to the end of the immersion (mean  $T_{\text{fing}}$ ) were calculated. Additionally, the numbers of CIVD oscillations were counted. These CIVD parameters for  $SkBF_{\text{fing}}$  were similarly analysed.

218

219 Rewarming speed in  $T_{\text{fing}}$  and  $SkBF_{\text{fing}}$  was calculated from 1 min to 5, 10, 15, and 20 min after hand 220 immersion, respectively, e.g.  $T_{\text{fing}}$  rewarming speed in 10 min was calculated as follows:

- Equation 4  $T_{\text{fing}}$  rewarming speed in 10 min [°C/min] =  $(T_{\text{fing}} \text{ at } 10 \text{ min} T_{\text{fing}} \text{ at } 1 \text{ min}) / (10 1)$
- 223

221

Thermal sensation of whole-body and immersed hand was assessed using a 7-points categorical scale (-3: cold, -2: cool, -1: slightly cool, 0: neither, +1: slightly warm, +2: warm, +3: hot) every 5 min during the experiment. Thermal comfort was assessed using a 7-points scale (-3: very uncomfortable, -2: uncomfortable, -1: slightly uncomfortable, 0: neither, +1: slightly comfortable, +2: comfortable, +3: very comfortable) every 5 min. Pain sensation of the immersed hand was assessed using 4-points scale (0: no pain, 1: slightly painful, 2: painful, 3: very painful) every 5 min.

- 230
- 231 Statistics

Comparisons of datasets from time-course measurements every 5 min were performed using repeated twoway (time × condition) analysis of variance (ANOVA) for each phase of hand cold immersion and rewarming. If Mauchly's sphericity test was not satisfied, the degrees of freedom were adjusted by Greenhouse-Geisser's  $\varepsilon$ . Partial  $\eta^2$  ( $\eta_p^2$ ) was calculated for assessing effect size for ANOVA where 0.01, 0.06 and 0.14 indicate small, medium and large effect sizes, respectively. Post-hoc test was conducted using a paired Student's *t*-test with multiple comparisons adjustment using Benjamin-Hochberg's false discovery rate (FDR) at time points between water and beetroot conditions. CIVD parameters of  $T_{\text{fing}}$  and  $SkBF_{\text{fing}}$  in

- two conditions were compared using a paired Student's *t*-test. Pearson's correlation coefficients and 95%
- 240 confidence interval (CI) for the slope of the regression line were calculated to examine the relationships
- 241 between rewarming speeds in the two conditions. Wilcoxon signed-rank test was conducted for comparing
- 242 subjective sensation between conditions. Statistical significance was set at P < 0.05. Analyses were
- 243 conducted using a statistical software (IBM SPSS Statistics version 20, IBM). All data are presented as
- 244 mean values and standard deviation (SD).
- 245
- 246 Results

#### 247 Hand cold immersion phase

Time course of  $T_{\text{fing}}$  and  $SkBF_{\text{fing}}$  in the immersed hand are shown in **Fig. 1**. A significant main effect of time was detected in  $T_{\text{fing}}$  ( $F_{3.2, 61.1}$ =3803.2,  $\eta_p^2$ =0.995, P<0.001) and  $SkBF_{\text{fing}}$  ( $F_{2.1, 40.0}$ =102.2,  $\eta_p^2$ =0.843, P<0.001). Parameters of CIVD in  $T_{\text{fing}}$  and  $SkBF_{\text{fing}}$  are presented in **Table 1**. There was no significant difference between conditions in any parameters of CIVD.

Time course of  $\overline{T}_{sk}$ ,  $T_{hand}$  on non-immersed body region and difference between proximal and distal skin temperatures ( $T_{pro-dis}$ ) are shown in **Fig. 2**. During the hand immersion phase, a significant main effect of

254 time was detected in  $\overline{T}_{sk}$  ( $F_{2.5, 47.5}$ =19.6,  $\eta_p^2$ =0.507, P<0.001),  $T_{hand}$  ( $F_{2.1, 39.6}$ =65.4,  $\eta_p^2$ =0.775, P<0.001), and  $T_{\text{pro-dis}}$  ( $F_{1.9, 36.6}$ =133.3,  $\eta_p^2$ =0.875, P<0.001). A significant main effect of condition was detected in  $T_{\text{hand}}$  ( $F_1$ , 255 256  $_{19}=6.0, \eta_p^2=0.239, P<0.05$ ) and  $T_{\text{pro-dis}}$  ( $F_{1,19}=6.4, \eta_p^2=0.253, P<0.05$ ).  $T_{\text{pro-dis}}$  in BR was significantly smaller 257 than in water condition at 20- and 25-min hand immersion (both P < 0.05) primarily due to higher 258 temperatures at the distal site. No difference in  $T_{subl}$  between water and BR conditions was observed at the 259 baseline  $(36.60\pm0.30 \text{ and } 36.66\pm0.34^{\circ}\text{C})$  and at the end of hand immersion  $(36.58\pm0.34 \text{ and } 36.66\pm0.33^{\circ}\text{C})$ . 260 Time course of thermal sensation of the whole-body and immersed hand, thermal comfort and pain 261 sensation of the immersed hand are shown in Fig. 3. No difference in these subjective sensations was 262 observed between conditions during hand immersion.

263

#### 264 *Rewarming phase*

265 Time course of  $T_{\text{fing}}$  and  $SkBF_{\text{fing}}$ ,  $CVC_{\text{fing}}$  in the immersed hand, and MAP during the rewarming phase is

- shown in **Fig. 4**. A significant main effect of time was detected in  $T_{\text{fing}}$  ( $F_{1.8, 34.0}=379.3, \eta_p^2=0.952, P<0.001$ ),
- 267  $SkBF_{\text{fing}}$  ( $F_{2.3, 43.4}$ =55.1,  $\eta_p^2$ =0.744, P<0.001),  $CVC_{\text{fing}}$  ( $F_{2.5, 47.9}$ =53.8,  $\eta_p^2$ =0.739, P<0.001), and MAP ( $F_{2.9, 47.9}$ =0.739, P<0.001), and MAP ( $F_{2.9, 47.9}$ =0.744, P<0.001),  $CVC_{\text{fing}}$  ( $F_{2.5, 47.9}$ =53.8,  $\eta_p^2$ =0.739, P<0.001), P<0.001), P
- 268 <sub>54.7</sub>=4.8,  $\eta_p^2$ =0.202, P<0.01). A significant main effect for condition was detected in CVC<sub>fing</sub> (F<sub>1, 19</sub>=4.4,
- 269  $\eta_p^2 = 0.189, P < 0.05$ ). A significant interaction between time and condition were detected in  $T_{\text{fing}}(F_{2.3, 44.6} = 5.5, 10^{-10})$
- 270  $\eta_p^2 = 0.224, P < 0.01), SkBF_{\text{fing}}(F_{2.8, 53.3} = 4.9, \eta_p^2 = 0.204, P < 0.01), CVC_{\text{fing}}(F_{2.8, 53.1} = 4.2, \eta_p^2 = 0.182, P < 0.05),$
- and MAP ( $F_{2.5, 48.1}=3.3, \eta_p^2=0.148, P<0.05$ ). SkBF fing in BR condition was significantly higher than in the
- water condition at 45- and 50-min rewarming (P<0.05). CVC<sub>fing</sub> in BR condition was significantly higher
- than in water condition at 40- to 50-min rewarming (P<0.05).

Time course of  $\bar{T}_{sk}$ ,  $T_{hand}$  and  $T_{pro-dis}$  on non-immersed body region are shown in **Fig. 2**. During the rewarming phase, a significant main effect of time was detected in  $\bar{T}_{sk}$  ( $F_{2.1, 40.1}$ =27.6,  $\eta_p^2$ =0.592, P<0.001) and  $T_{pro-dis}$  ( $F_{1.5, 28.6}$ =3.7,  $\eta_p^2$ =0.163, P<0.05). A significant main effect of condition was detected in  $T_{pro-dis}$ 

277 ( $F_{1, 19}=5.8, \eta_p^2=0.235, P<0.05$ ).  $T_{\text{pro-dis}}$  in BR condition was significantly smaller than the water condition

at 30- to 50-min rewarming (P<0.05). No difference in  $T_{subl}$  between water and BR conditions (36.52±0.33)

- and  $36.60\pm0.35^{\circ}$ C) was observed at the end of rewarming phase.
- 280

The rewarming speeds in  $T_{\text{fing}}$  from 1 min to 5, 10, 15, and 20 min after hand immersion are presented in Table 2. Significantly faster rewarming was observed in beet condition compared to water at 15- and 20min rewarming (both *P*<0.05). Similarly, the recovery speed in *SkBF*<sub>fing</sub> was significantly faster in BR condition at 15- and 20-min rewarming (both *P*<0.05).

285

The individual values of  $T_{\text{fing}}$  rewarming speeds in water and BR conditions are plotted in Fig. 5. Significant correlations were evident between conditions in 10, 15, and 20 min rewarming (r=0.69, *P*<0.01; r=0.68, *P*<0.01; r=0.58, *P*<0.01; respectively). The slope of the regression lines (95% CI) are 0.60 (0.28-0.91), 0.57 (0.27-0.88), and 0.43 (0.13-0.73) at 10, 15, and 20 min, respectively. The slopes of the regression lines (and 95% CI) were gentler compared to the y = x (reference line), which represents identical rewarming speed in water and BR conditions. Thus, the individuals with slower rewarming speed with water showed greater improvement in rewarming with BR supplementation.

293

Time course of thermal sensation of the whole-body and immersed hand, thermal comfort and pain sensation of the immersed hand are shown in **Fig. 3**. No difference in these subjective sensations was observed between conditions during the rewarming phase.

297

# 298 Discussion

This study investigated the effect of BR ingestion on CIVD response and rewarming of  $T_{\text{fing}}$  during and after hand immersion in cold water, by comparing with drinking water as a control. In contrast to our hypothesis for the immersion phase of the experiment, CIVD in  $T_{\text{fing}}$  and skin blood flow was not affected by BR. On the other hand, as a major finding of this study, rewarming of  $T_{\text{fing}}$  and skin blood flow was accelerated by drinking BR 2-hours before hand immersion. Hence, the hypothesis is only partially supported.

305

#### 306 Cold-induced vasodilation

307 We originally hypothesised that CIVD would be enhanced by ingestion of BR, since it contains vasoactive

308 ingredients such as NO<sub>3</sub><sup>-</sup>, betanin, and chlorogenic acid (Wootton-Beard et al. 2011). However, the results

309 showed no significant difference in all parameters of CIVD for  $T_{\text{fing}}$  and  $SkBF_{\text{fing}}$  between BR and water

conditions during immersion. Additionally, thermal sensation and pain sensation of the immersed hand was not different between conditions.

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313 Starting from the original observation of the hunting reaction of finger temperature to cold (Lewis 1930), 314 the CIVD response has been studied in humans (Daanen and Ducharme 1999) and animals including 315 adrenergic neural mechanism in isolated vascular smooth muscle (Rusch et al. 1981). Based on the current 316 knowledge of the CIVD, NO-dependent active vasodilation and/or sympathetic withdrawal has been 317 suggested as potential mechanisms of CIVD response (Daanen 2003; Cheung 2015). In this study, 318 considering the significantly smaller  $T_{\rm pro-dis}$  in BR condition during the latter half of the hand immersion, 319 whole-body vasodilation appears to be enhanced with BR supplementation during a prolonged (compared 320 to other studies; e.g., Eglin et al., 2017; Hope et al., 2014) cold stimulus of the extremity. However, finger 321 skin temperature and blood flow in the immersed hand did not show any effect of BR supplementation. 322 This result was in line with the previous finding of no difference in CIVD response during hand immersion 323 to 8°C water between BR and NO<sub>3</sub>-depleted placebo drink (Wickham et al. 2021). NO-mediated active 324 vasodilation probably has a minor contribution to enabling the CIVD response when cold stimulus from 325 the extremities is significant whilst also coupled with the effect of hydrostatic squeeze on the surface blood 326 vessels of the immersed hand in healthy individuals. In persons with cold injury such as Raynaud's 327 syndrome or those who are cold-sensitive with an abnormal endothelial function, NO supplementation is 328 more likely to influence the vasomotor responses during cold challenge but not by a large magnitude (e.g., 329 Shepherd et al. 2019; Hope et al. 2014). Hope et al. (2014) suggested that GTN, acting experimentally as 330 the NO donor, bypasses the endothelium dependent NO pathway to re-establish the vascular response in 331 cold-sensitive individuals. Hope et al. (2014) only observed facilitated post-immersion re-warming in their 332 cold-sensitive group but not in their healthy controls. Shepherd et al. (2019) showed some transient 333 evidence of increased CVC with chronic supplementation with BR and nitrate depleted BR but not acute 334 supplementation indicating a greater dose of BR than used here might be needed to induce CIVD during 335 cold immersion. Collectively, there is now a growing body of evidence that suggests acute BR 336 supplementation doesn't have the potency to evoke vasodilation during local cold challenge in healthy 337 (Wickham et al. 2021) and cold-sensitive/injured individuals (Eglin et al. 2013; Shepherd et al. 2019). Thus, 338 it is speculated that release of vasoconstrictor tone might be one of the major mechanisms for the CIVD 339 response rather than NO-dependent active vasodilation. On the other hand, systemic effects of BR 340 supplementation on blood pressure (Shepherd et al. 2019; Wickham et al. 2021) are plausible, as shown in 341 the observation of higher distal skin temperature during prolonged hand immersion to this body of evidence 342 (see Fig. 2). Therefore, BR could also evoke vasodilatory effects in nonglaborous regions where 343 noradrenergic vasoconstrictor nerves and cholinergic active vasodilator nerves are active (Kellogg 2006). 344 Nevertheless, our observations of accelerated rewarming following cold exposure are novel and require 345 further exploration.

#### 347 Acceleration of rewarming

348 The major finding of this study was that BR ingestion accelerated the rewarming speed of  $T_{\rm fing}$  and finger 349 skin blood flow after 30-min hand cold immersion. During the rewarming phase, after terminating the cold 350 stimulus, the vasoactive ingredients in BR enhanced the whole-body and local vasodilation but only after 351 10 ( $SkBF_{fing}$ ) to 15 minutes ( $T_{fing}$ ) of rewarming (Table 2). The duration of rewarming studied here might 352 also be another reason why prior studies have not revealed this difference having primarily measured for 353 up to 10-min of rewarming (Eglin et al. 2017; Shepherd et al. 2019). It is evident from the present data that 354 the experimental effects of BR probably extend beyond the 20-min rewarming period where these 355 significant differences remained. Although rewarming speed in SkBF fing and T fing was accelerated with BR, 356 it was not associated with perception of local thermal sensation or whole-body thermal comfort.

357

358 One of the most potent vasoactive ingredients in BR is  $NO_3^-$ , with many studies reporting  $NO_3^-$  -  $NO_2^-$ -NO 359 pathway induced vasodilation and consequently lowered blood pressure through exogenous NO mediated 360 relaxation of endothelial cells to relax vascular smooth muscle (Wylie et al. 2013; Lara et al. 2016; Richards 361 et al. 2018). Unlike the present study, previous studies reported no effect of acute BR ingestion on 362 peripheral vasodilatory response following 2-min cold immersion of extremities in comparison with NO3-363 depleted placebo (Eglin et al. 2017; Shepherd et al. 2019). The short duration (2 min) cold immersion in 364 the previous studies and prolonged (30 min) cold exposure in the present study are categorized into early-365 phase (i.e., primarily skin cooling) and late-phase local cooling (i.e., skin and superficial muscle cooling), 366 respectively (Hodges and Johnson 2009; Alba et al. 2019). At the onset of the local cold stimulus, early 367 vasoconstriction is induced mostly via adrenergic and neural mechanisms (Ekenvall et al. 1988; Stephens 368 et al. 2004), whereas later vasoconstriction during prolonged cooling is mediated via combination of 369 continued vasoconstrictor nerve excitation and inhibition of NO vasodilator pathway (Hodges et al. 2006; 370 Hodges and Johnson 2009; Alba et al. 2019). It is likely that there is a more substantial reduction in 371 bioavailable NO at the end of 30-min hand immersion in the present study due to the decreased activity of 372 endothelial NOS and downstream of NOS (Hodges et al. 2006) and hence this is the reason for the efficacy 373 of increasing NO in the present study. The likely reduction of NO bioavailability during longer cold 374 exposure enabled us to find a significant vasodilatory effect of BR, as external NO donor via NO<sub>3</sub><sup>-</sup> - NO<sub>2</sub><sup>-</sup>-375 NO pathway, accelerating  $T_{\rm fing}$  rewarming following the cold exposure. Yet, the time course of our SkBF fing 376 and  $T_{\text{fing}}$  (Table 2) data during rewarming along with the magnitude of  $T_{\text{fing}}$  change (Fig 4) suggest plausible 377 successive relief to both mechanisms (i.e., relief of vasoconstrictor nerve excitation and restoration of the 378 NO vasodilator pathway; Hodges et al. 2006; Hodges and Johnson 2009; Alba et al. 2019) that are 379 associated with prolonged cooling. SkBF fing laser doppler flowmetry data from Hodges et al. (2018) during 380 30-min hand immersion indicate the onset of the CIVD response precedes increases in  $T_{\text{fing}}$  (i.e., the same 381 as our data) but closely matched the measured neurogenic activity in the finger but not the endothelial nitric

382 oxide dependent or independent activity (Hodges et al. 2018). The changes seen in  $T_{\text{fing}}$  in the study of

383 Hodges et al. (2018) were small and transient due to the ongoing cold-water immersion. During rewarming

in the present study, we suggest the BR supplementation facilitated the earlier onset of vasoconstrictor

385 nerve relief, facilitating an increase in  $SkBF_{fing}$  at 10-min of rewarming and accelerating the increase in  $T_{fing}$ 

- 386 from 15-minutes. This increase in  $SkBF_{\text{fing}}$  raises  $T_{\text{fing}}$  towards the vasomotor range (i.e., 26°C, (Folkow
- and Neil 1971); see Fig 4) where further active vasodilation is plausible (Kellogg et al. 1998; Kellogg 2006).
- 388
- 389 The initial reduction of NO bioavailability, including the inhibition of NOS activity, is partly induced by 390 the elevation of reactive oxygen species (ROS) from vascular smooth muscle mitochondria (Bailey et al. 391 2005; Holowatz and Kenney 2007; Hodges and Johnson 2009; Johnson et al. 2014). In addition, the ROS 392 generated by local cooling also enhances Rho-kinase activity to increase vascular tone to the neural 393 noradrenalin release, which is mainly explained by the translocation of  $\alpha_{2c}$ -adrenoreceptors to the surface 394 of vascular smooth muscle cell (Bailey et al. 2004; Bailey et al. 2005; Hodges and Johnson 2009). The Rho-395 kinase pathway is relatively slow event as shown in time course of Rho activity to cooling in human cultured 396 dermal arteriolar vascular smooth muscle (Bailey et al. 2004). Thus, the enhanced vasodilatory response 397 with BR ingestion after prolonged cold exposure might also be related to slow reversal of the ROS and 398 Rho-kinase pathway which fits with the time course of responses shown in the present study. Previous 399 research investigating the vasomotor effect of BR supplementation suggested the nitrate-independent 400 vasodilatory response due to other bioactive ingredients in BR (Bahadoran et al. 2017; Thompson et al. 401 2018; Shepherd et al. 2019). Among them betanin was focused on as one of the phytochemical antioxidants 402 in BR (Esatbeyoglu et al. 2015; Hadipour et al. 2020). A study reported that local administration of 403 ascorbate antioxidant inhibited the vasoconstriction during local skin cooling (Yamazaki 2010). Thus, it 404 was suggested that quenching of ROS by the antioxidants might decrease efficacy of adrenoreceptors and 405 influence the vascular response to cooling. The antioxidant, betanin contained in BR, inhibits the diffusion-406 controlled reaction of NO with superoxide (i.e., an ROS) by scavenging super-oxide radicals that create 407 peroxynitrite thereby slowing the appearance of this ROS and improving the bio-availability of NO 408 (Sakihama et al. 2012). Hence, consumption of betanin is a plausible means to increase bioavailable NO 409 for stimulating vasodilation (Esatbeyoglu et al. 2015). In the present study, 30-min hand immersion in 8°C 410 was probably sufficient oxidative stress to decrease bioavailable NO (Christmas et al. 2016). Following this 411 cold stress, the combined effect of nitrate (NO production) and betanin (antioxidation) in BR could increase 412 bioavailable vasodilatory NO, which could plausibly enhance  $T_{\text{fing}}$  rewarming compared to the water 413 ingestion condition following initial relief to vasoconstrictor nerve activity. It is a limitation of the present 414 study that we cannot discern the separate contribution of the bioactive ingredients (e.g., nitrate and betanin) 415 in BR that contribute to the responses we report and that we could not blind the treatment conditions; 416 although it did not evoke significant change in thermal sensation and comfort, both scenarios require further 417 research. Lastly, our observations are restricted to males only.

#### 419 Individual variation in the effect of beetroot supplementation

420 There are individual variations in vasomotor response to local cooling. Cold sensitive individuals such as 421 with non-freezing cold injury (NFCI) and Raynaud's syndrome present with colder hands and foot skin 422 temperature, greater vasoconstriction to cold and slower rewarming rate following cold exposure compared 423 to normal individuals (Eglin et al. 2013; O'Reilly et al. 1992). Patients with Raynaud's phenomenon show 424 deficiency of NO in response to cold stimulus (Tanaka et al. 2012), which could be due to their greater 425 oxidative damage resulting from higher serum ROS level (Biondi et al. 2008). It was also reported that 426 increased ROS in response to cooling might be one of the mediators for tissue damage in NFCI (Geng et 427 al. 2015). Moreover, even in healthy people with cold constitution ("hi-e-sho" in Japanese), greater 428 vasoconstriction was provoked to local skin cooling and iontophoretic noradrenaline application (Yamazaki 429 2015). These findings indicated that cold sensitive individuals would have an increased sensitivity of 430 adrenoreceptor on vascular smooth muscle and/or decreased bioavailability of NO, that has been shown to 431 evoke oxidative stress (Biondi et al. 2008).

432

433 In the present study, individual variation in healthy participants was observed in the rewarming speed after 434 30-min cold exposure. The slopes of the regression lines between the rewarming speed in water and BR 435 conditions were gentler compared to the y = x, which represents identical speed in both conditions. This 436 result indicated that cold sensitive individuals with slower rewarming in the water condition showed more 437 remarkable improvement in the rewarming speed with BR ingestion. We speculate that cold sensitive 438 individuals, who potentially have more oxidative stress and less bioavailable NO, could benefit more so 439 following BR supplementation containing antioxidants like betanin and NO3<sup>-</sup> as NO donor; a focus of future 440 research.

441

# 442 Conclusions

This study investigated the effect of beetroot supplementation on vasomotor responses during and after hand cold immersion in human. CIVD in finger temperature and skin blood flow was not affected by BR ingestion, but a significantly smaller  $T_{pro-dis}$  in BR condition, primarily due to higher distal temperature, would indicate that whole-body vasodilation was enhanced during the local cold stimulus. The major finding of this study was that BR accelerated rewarming in finger skin temperature and skin blood flow after the local cold stimulus. Additionally, cold sensitive individuals with a slower rewarming rate with water demonstrated more remarkable acceleration in rewarming with BR supplementation.

450

# 451 Declarations

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454	
455	Conflicts of interest None declared.
456	
457	Ethics approval All experimental protocols in this study were approved by the IRB of Hokkaido
458	University.
459	
460	Author contributions HW and MB conceived and designed research. KS and SS conducted the
461	experiments. KS and HW analyzed data. HW and MB wrote the draft of manuscript. All authors read and
462	approved the manuscript.
463	
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465	
466	References
467	Alba BK, Castellani JW, Charkoudian N (2019) Cold-induced cutaneous vasoconstriction in humans:
468	Function, dysfunction and the distinctly counterproductive. Exp Physiol 104 (8):1202-1214.
469	doi:10.1113/EP087718
470	Amano T, Okushima D, Breese BC, Bailey SJ, Koga S, Kondo N (2018) Influence of dietary nitrate
471	supplementation on local sweating and cutaneous vascular responses during exercise in a hot
472	environment. Eur J Appl Physiol 118 (8):1579-1588. doi:10.1007/s00421-018-3889-9
473	Anderson ME, Moore TL, Hollis S, Jayson MIV, King TA, Herrick AL (2002) Digital vascular response to
474	topical glyceryl trinitrate, as measured by laser Doppler imaging, in primary Raynauds
475	phenomenon and systemic sclerosis. Rheumatology 41 (3):324-328. doi:DOI
476	10.1093/rheumatology/41.3.324
477	Bahadoran Z, Mirmiran P, Kabir A, Azizi F, Ghasemi A (2017) The Nitrate-Independent Blood Pressure-
478	Lowering Effect of Beetroot Juice: A Systematic Review and Meta-Analysis. Adv Nutr 8 (6):830-
479	838. doi:10.3945/an.117.016717
480	Bailey SR, Eid AH, Mitra S, Flavahan S, Flavahan NA (2004) Rho kinase mediates cold-induced
481	constriction of cutaneous arteries: role of alpha2C-adrenoceptor translocation. Circ Res 94
482	(10):1367-1374. doi:10.1161/01.RES.0000128407.45014.58
483	Bailey SR, Mitra S, Flavahan S, Flavahan NA (2005) Reactive oxygen species from smooth muscle
484	mitochondria initiate cold-induced constriction of cutaneous arteries. Am J Physiol Heart Circ
485	Physiol 289 (1):H243-250. doi:10.1152/ajpheart.01305.2004
486	Biondi R, Coaccioli S, Lattanzi S, Puxeddu A, Papini M (2008) Oxidant/antioxidant status in patients with
487	Raynauds disease. Clin Ter 159 (2):77 -81
488	Brajkovic D, Ducharme MB, Frim J (2001) Relationship between body heat content and finger temperature
489	during cold exposure. J Appl Physiol 90 (6):2445-2452

- 490 Castellani JW, Tipton MJ (2015) Cold stress effects on exposure tolerance and exercise performance.
   491 Compr Physiol 6 (1):443-469. doi:10.1002/cphy.c140081
- 492 Castellani JW, Young AJ, Ducharme MB, Giesbrecht GG, Glickman E, Sallis RE, American College of
  493 Sports M (2006) American College of Sports Medicine position stand: prevention of cold injuries
  494 during exercise. Med Sci Sports Exerc 38 (11):2012-2029.
  495 doi:10.1249/01.mss.0000241641.75101.64
- 496 Cheung SS (2015) Responses of the hands and feet to cold exposure. Temperature (Austin) 2 (1):105-120.
  497 doi:10.1080/23328940.2015.1008890
- 498 Christmas KM, Patik JC, Khoshnevis S, Diller KR, Brothers RM (2016) Sustained cutaneous
  499 vasoconstriction during and following cyrotherapy treatment: Role of oxidative stress and Rho
  500 kinase. Microvasc Res 106:96-100. doi:10.1016/j.mvr.2016.04.005
- Daanen HA (2003) Finger cold-induced vasodilation: a review. Eur J Appl Physiol 89 (5):411-426.
   doi:10.1007/s00421-003-0818-2
- Daanen HAM, Ducharme MB (1999) Finger cold-induced vasodilation during mild hypothermia,
   hyperthermia and at thermoneutrality. Aviat Space Environ Med 70 (12):1206-1210
- 505 Dianat I, Haslegrave CM, Stedmon AW (2012) Methodology for evaluating gloves in relation to the effects
   506 on hand performance capabilities: a literature review. Ergonomics 55 (11):1429-1451.
   507 doi:10.1080/00140139.2012.708058
- Dominguez R, Cuenca E, Mate-Munoz JL, Garcia-Fernandez P, Serra-Paya N, Estevan MC, Herreros PV,
   Garnacho-Castano MV (2017) Effects of Beetroot Juice Supplementation on Cardiorespiratory
   Endurance in Athletes. A Systematic Review. Nutrients 9 (1). doi:10.3390/nu9010043
- Eglin CM, Costello JT, Bailey SJ, Gilchrist M, Massey H, Shepherd AI (2017) Effects of dietary nitrate
  supplementation on the response to extremity cooling and endothelial function in individuals with
  cold sensitivity. A double blind, placebo controlled, crossover, randomised control trial. Nitric
  Oxide 70:76-85. doi:10.1016/j.niox.2017.09.005
- 515 Eglin CM, Golden FS, Tipton MJ (2013) Cold sensitivity test for individuals with non-freezing cold injury:
  516 the effect of prior exercise. Extrem Physiol Med 2 (1):16. doi:10.1186/2046-7648-2-16
- 517 Ekenvall L, Lindblad LE, Norbeck O, Etzell BM (1988) alpha-Adrenoceptors and cold-induced
  518 vasoconstriction in human finger skin. Am J Physiol 255 (5 Pt 2):H1000-1003.
  519 doi:10.1152/ajpheart.1988.255.5.H1000
- 520 Esatbeyoglu T, Wagner AE, Schini-Kerth VB, Rimbach G (2015) Betanin--a food colorant with biological
   521 activity. Mol Nutr Food Res 59 (1):36-47. doi:10.1002/mnfr.201400484
- 522 Folkow B, Neil E (1971) Circulation. Oxford University Press, London-Toronto.
- 523 Geng Z, Tong X, Jia H (2015) Reactive oxygen species (ROS) mediates non-freezing cold injury of rat 524 sciatic nerve. Int J Clin Exp Med 8 (9):15700-15707
- 525 Govoni M, Jansson EA, Weitzberg E, Lundberg JO (2008) The increase in plasma nitrite after a dietary

526	nitrate load is markedly attenuated by an antibacterial mouthwash. Nitric Oxide-Biol Ch 19				
527	(4):333-337. doi:10.1016/j.niox.2008.08.003				
528	Hadipour E, Taleghani A, Tayarani-Najaran N, Tayarani-Najaran Z (2020) Biological effects of red beetroot				
529	and betalains: A review. Phytother Res 34 (8):1847-1867. doi:10.1002/ptr.6653				
530	Hardy JD, Du Bois EF (1938) The technic of measuring radiation and convection. J Nutr 15 (5):461-475				
531	Heus R, Daanen HA, Havenith G (1995) Physiological criteria for functioning of hands in the cold: a review.				
532	Appl Ergon 26 (1):5-13				
533	Hobbs DA, Goulding MG, Nguyen A, Malaver T, Walker CF, George TW, Methven L, Lovegrove JA (2013)				
534	Acute ingestion of beetroot bread increases endothelium-independent vasodilation and lowers				
535	diastolic blood pressure in healthy men: a randomized controlled trial. J Nutr 143 (9):1399-1405.				
536	doi:10.3945/jn.113.175778				
537	Hodges GJ, Johnson JM (2009) Adrenergic control of the human cutaneous circulation. Appl Physiol Nutr				
538	Metab 34 (5):829-839. doi:10.1139/H09-076				
539	Hodges GJ, Mallette MM, Cheung SS (2018) Cutaneous neural activity and endothelial involvement in				
540	cold-induced vasodilatation. Eur J Appl Physiol 118 (5):971-978. doi:10.1007/s00421-018-3832-				
541	0				
542	Hodges GJ, Zhao K, Kosiba WA, Johnson JM (2006) The involvement of nitric oxide in the cutaneous				
543	vasoconstrictor response to local cooling in humans. J Physiol 574 (Pt 3):849-857.				
544	doi:10.1113/jphysiol.2006.109884				
545	Holmer I (2009) Evaluation of cold workplaces: an overview of standards for assessment of cold stress. Ind				
546	Health 47 (3):228-234. doi:10.2486/indhealth.47.228				
547	Holowatz LA, Kenney WL (2007) Local ascorbate administration augments NO- and non-NO-dependent				
548	reflex cutaneous vasodilation in hypertensive humans. Am J Physiol-Heart C 293 (2):H1090-				
549	H1096. doi:10.1152/ajpheart.00295.2007				
550	Hoon MW, Johnson NA, Chapman PG, Burke LM (2013) The effect of nitrate supplementation on exercise				
551	performance in healthy individuals: a systematic review and meta-analysis. Int J Sport Nutr Exerc				
552	Metab 23 (5):522-532. doi:10.1123/ijsnem.23.5.522				
553	Hope K, Eglin C, Golden F, Tipton M (2014) Sublingual glyceryl trinitrate and the peripheral thermal				
554	responses in normal and cold-sensitive individuals. Microvasc Res 91:84-89.				
555	doi:10.1016/j.mvr.2013.11.002				
556	House JR, Tipton MJ (2002) Using skin temperature gradients or skin heat flux measurements to determine				
557	thresholds of vasoconstriction and vasodilatation. Eur J Appl Physiol 88 (1-2):141-145.				
558	doi:10.1007/s00421-002-0692-3				
559	Imray C, Grieve A, Dhillon S, Caudwell Xtreme Everest Research G (2009) Cold damage to the extremities:				
560	frostbite and non-freezing cold injuries. Postgrad Med J 85 (1007):481-488.				
561	doi:10.1136/pgmj.2008.068635				

- Johnson JM, Minson CT, Kellogg DL (2014) Cutaneous Vasodilator and Vasoconstrictor Mechanisms in
   Temperature Regulation. Comprehensive Physiology 4 (1):33-89. doi:10.1002/cphy.c130015
- Kellogg DL, Jr. (2006) In vivo mechanisms of cutaneous vasodilation and vasoconstriction in humans
  during thermoregulatory challenges. J Appl Physiol (1985) 100 (5):1709-1718.
  doi:10.1152/japplphysiol.01071.2005
- Kellogg DL, Jr., Crandall CG, Liu Y, Charkoudian N, Johnson JM (1998) Nitric oxide and cutaneous active
  vasodilation during heat stress in humans. J Appl Physiol (1985) 85 (3):824-829.
  doi:10.1152/jappl.1998.85.3.824
- 570 Krauchi K, Cajochen C, Werth E, Wirz-Justice A (1999) Warm feet promote the rapid onset of sleep. Nature
  571 401 (6748):36-37. doi:10.1038/43366
- Kugler F, Stintzing FC, Carle R (2004) Identification of betalains from petioles of differently colored Swiss
  chard (Beta vulgaris L. ssp. cicla [L.] Alef. Cv. Bright Lights) by high-performance liquid
  chromatography-electrospray ionization mass spectrometry. J Agric Food Chem 52 (10):29752981. doi:10.1021/jf035491w
- Lara J, Ashor AW, Oggioni C, Ahluwalia A, Mathers JC, Siervo M (2016) Effects of inorganic nitrate and
  beetroot supplementation on endothelial function: a systematic review and meta-analysis. Eur J
  Nutr 55 (2):451-459. doi:10.1007/s00394-015-0872-7
- 579 Lewis T (1930) Observations upon the reactions of the vessels of the human skin to cold. Heart-J Stud Circ
   580 15 (2):177-208
- 581 Makinen TM, Hassi J (2009) Health problems in cold work. Ind Health 47 (3):207-220.
  582 doi:10.2486/indhealth.47.207
- OReill y D, Taylor L, el-Hadidy K, Jayson MI (1992) Measurement of cold challenge responses in primary
   Raynauds phenomenon and Raynauds phenomenon associated with systemic sclerosis. Ann
   Rheum Dis 51 (11):1193-1196. doi:10.1136/ard.51.11.1193
- 586 Richards JC, Racine ML, Hearon CM, Jr., Kunkel M, Luckasen GJ, Larson DG, Allen JD, Dinenno FA
  587 (2018) Acute ingestion of dietary nitrate increases muscle blood flow via local vasodilation during
  588 handgrip exercise in young adults. Physiol Rep 6 (2). doi:10.14814/phy2.13572
- Rubinstein EH, Sessler DI (1990) Skin-Surface Temperature-Gradients Correlate with Fingertip Blood Flow in Humans. Anesthesiology 73 (3):541-545. doi:Doi 10.1097/00000542-199009000-00027
- Rusch NJ, Shepherd JT, Vanhoutte PM (1981) The effect of profound cooling on adrenergic
  neurotransmission in canine cutaneous veins. J Physiol 311:57-65.
  doi:10.1113/jphysiol.1981.sp013572
- Sakihama Y, Maeda M, Hashimoto M, Tahara S, Hashidoko Y (2012) Beetroot betalain inhibits
   peroxynitrite-mediated tyrosine nitration and DNA strand cleavage. Free Radic Res 46 (1):93-99.
   doi:10.3109/10715762.2011.641157
- 597 Shepherd AI, Costello JT, Bailey SJ, Bishop N, Wadley AJ, Young-Min S, Gilchrist M, Mayes H, White D,

- 598 Gorczynski P, Saynor ZL, Massey H, Eglin CM (2019) "Beet" the cold: beetroot juice 599 supplementation improves peripheral blood flow, endothelial function, and anti-inflammatory 600 status in individuals with Raynauds phenomenon. J Appl Physiol (1985) 127 (5):1478-1490. 601 doi:10.1152/japplphysiol.00292.2019
- Sobko T, Marcus C, Govoni M, Kamiya S (2010) Dietary nitrate in Japanese traditional foods lowers
  diastolic blood pressure in healthy volunteers. Nitric Oxide 22 (2):136-140.
  doi:10.1016/j.niox.2009.10.007
- Stephens DP, Saad AR, Bennett LA, Kosiba WA, Johnson JM (2004) Neuropeptide Y antagonism reduces
  reflex cutaneous vasoconstriction in humans. Am J Physiol Heart Circ Physiol 287 (3):H1404doi:10.1152/ajpheart.00061.2004
- Tanaka A, Yamazaki M, Saito M, Oh IT, Watanabe Y, Tsuboi R (2012) Highly sensitive high-pressure liquid
  chromatography with ultraviolet light method detected the reduction of serum nitrite/nitrate levels
  after cold exposure in patients with Raynauds phenomenon. J Dermatol 39 (10):889-890.
  doi:10.1111/j.1346-8138.2011.01433.x
- Thompson C, Vanhatalo A, Kadach S, Wylie LJ, Fulford J, Ferguson SK, Blackwell JR, Bailey SJ, Jones
  AM (2018) Discrete physiological effects of beetroot juice and potassium nitrate supplementation
  following 4-wk sprint interval training. J Appl Physiol (1985) 124 (6):1519-1528.
  doi:10.1152/japplphysiol.00047.2018
- Wakabayashi H, Oksa J, Tipton MJ (2015) Exercise performance in acute and chronic cold exposure. The
  Journal of Physical Fitness and Sports Medicine 4 (2):177-185. doi:10.7600/jpfsm.4.177
- Wickham KA, Steele SW, Cheung SS (2021) Effects of acute dietary nitrate supplementation on coldinduced vasodilation in healthy males. Eur J Appl Physiol 121 (5):1431-1439.
  doi:10.1007/s00421-021-04621-8
- Wigley FM (2002) Clinical practice. Raynauds Phenomenon. N Engl J Med 347 (13):1001-1008.
  doi:10.1056/NEJMcp013013
- Wootton-Beard PC, Moran A, Ryan L (2011) Stability of the total antioxidant capacity and total polyphenol
  content of 23 commercially available vegetable juices before and after in vitro digestion measured
  by FRAP, DPPH, ABTS and Folin-Ciocalteu methods. Food Research International 44 (1):217224. doi:10.1016/j.foodres.2010.10.033
- Wyler H, Meuer U (1979) Zur Biogenese der Betacyane: Versuche mit [2-14C]-Dopaxanthin. Helv Chim
  Acta 62 (4):1330-1339. doi:10.1002/hlca.19790620447
- Wylie LJ, Kelly J, Bailey SJ, Blackwell JR, Skiba PF, Winyard PG, Jeukendrup AE, Vanhatalo A, Jones
   AM (2013) Beetroot juice and exercise: pharmacodynamic and dose-response relationships. J
- 631 Appl Physiol (1985) 115 (3):325-336. doi:10.1152/japplphysiol.00372.2013
- G32 Yamazaki F (2010) Local ascorbate administration inhibits the adrenergic vasoconstrictor response to local
  G33 cooling in the human skin. J Appl Physiol (1985) 108 (2):328-333.

- 634 doi:10.1152/japplphysiol.00814.2009
- 635 Yamazaki F (2015) The cutaneous vasoconstrictor response in lower extremities during whole-body and
  636 local skin cooling in young women with a cold constitution. J Physiol Sci 65 (5):397-405.
- 637 doi:10.1007/s12576-015-0378-3

	Water		Beet		T-test
	mean	(SD)	mean	(SD)	p value
$T_{\mathrm{fing}}$					
Baseline (°C)	32.6	(1.6)	32.8	(1.5)	0.41
Onset time (sec)	521	(78)	535	(145)	0.68
$T_{\min}$ (°C)	9.3	(0.7)	9.4	(0.7)	0.36
$T_{\text{peak}}$ (°C)	10.6	(1.1)	11.0	(1.3)	0.12
$T_{\max}$ (°C)	11.0	(1.2)	11.2	(1.5)	0.48
$T_{\text{peak}}$ - $T_{\min}$ (°C)	1.2	(0.9)	1.6	(1.1)	0.21
Mean $T_{\text{fing}}$ (°C)	10.1	(0.9)	10.3	(0.9)	0.27
Number of CIVD	1.6	(0.7)	1.9	(1.0)	0.21
$SkBF_{\rm fing}$					
Baseline (AU)	26.8	(5.7)	30.2	(10.6)	0.14
Onset time (sec)	83	(58)	78	(64)	0.80
$SkBF_{\min}$ (AU)	2.7	(1.9)	3.3	(2.1)	0.30
SkBF <sub>peak</sub> (AU)	15.6	(4.7)	15.8	(5.2)	0.90
$SkBF_{max}$ (AU)	17.4	(6.3)	17.5	(6.1)	0.98
$SkBF_{peak}$ - $SkBF_{min}$ (AU)	12.9	(3.8)	12.5	(3.9)	0.66
Mean $SkBF_{\text{fing}}$ (AU)	11.8	(4.7)	12.2	(5.0)	0.74
Number of CIVD	2.1	(0.8)	2.4	(1.0)	0.18

Table 1 Parameters of cold induced vasodilation during hand cold immersion (n = 20).

 $T_{\text{fing}}$ : finger skin temperature,  $T_{\text{min}}$ : minimal  $T_{\text{fing}}$ ,  $T_{\text{peak}}$ : the first peak  $T_{\text{fing}}$ ,  $T_{\text{max}}$ : maximal  $T_{\text{fing}}$  during hand cold immersion, Mean  $T_{\text{fing}}$ : mean value of  $T_{\text{fing}}$  after 5 min to the end of immersion, CIVD: cold induced vasodilation, *SkBF*: skin blood flow, AU: arbitrary unit.

	Water		Beet		T-test	FDR
Rewarming speed	mean	SD	mean	SD	p value	p value
T <sub>fing</sub> (°C/min)						
in 5 min (31-35 min)	1.92	(0.63)	1.86	(0.57)	0.74	0.74
in 10 min (31-40 min)	1.47	(0.43)	1.61	(0.38)	0.09	0.11
in 15 min (31-45 min)	1.11	(0.26)	1.24	(0.22)	0.01	0.02
in 20 min (31-50 min)	0.86	(0.19)	0.97	(0.14)	0.01	0.02
SkBF <sub>fing</sub> (AU/min)						
in 5 min (31-35 min)	2.33	(1.74)	2.75	(1.98)	0.40	0.40
in 10 min (31-40 min)	1.50	(0.91)	1.88	(0.91)	0.04	0.05
in 15 min (31-45 min)	0.88	(0.59)	1.29	(0.61)	0.01	0.02
in 20 min (31-50 min)	0.67	(0.45)	0.99	(0.44)	0.01	0.02

Table 2 Rewarming speed in finger skin temperature and blood flow after hand cold immersion (n = 20)

Rewarming speed is calculated from 1 min to 5, 10, 15, and 20 min after hand immersion.

 $T_{\text{fing}}$ : finger skin temperature,  $SkBF_{\text{fing}}$ : finger skin blood flow. P values of multiple paired T-tests are corrected with false discovery rate (FDR).





Figure 2





