Endothelial function in response to exercise in the cold in patients with coronary artery disease

Rasmus I. P. Valtonen1,2 | Tiina M. Ikäheimo1,2 | Heidi E. Hintsala1,2,3 | Niilo R. I. Ryti1,2 | Arto Hautala4 | Juha S. Perkiömäki5 | C. G. Crandall6 | Matti Mäntysaari7 | Jouni J. K. Jaakkola1,2 | Antti M. Kiviniemi5

1Center for Environmental and Respiratory Health Research (CERH), University of Oulu, Oulu, Finland
2Medical Research Center, University of Oulu and Oulu University Hospital, Oulu, Finland
3Centria University of Applied Sciences, Kokkola, Finland
4Cardiovascular Research Group, Division of Cardiology, Oulu University Hospital, University of Oulu, Oulu, Finland
5Research Unit of Internal Medicine, Medical Research Center Oulu, University of Oulu and Oulu University Hospital, Oulu, Finland
6Department of Internal Medicine, University of Texas Southwestern Medical Center and the Institute for Exercise and Environmental Medicine, Texas Health Presbyterian Hospital, Dallas, TX, USA
7Aeromedical Centre, Finnish Defence Forces, Helsinki, Finland

Abstract

Background: Regular long-term physical exercise has favourable effects on endothelial function in patients with coronary artery disease (CAD). However, the effects of an acute exercise bout in the cold on endothelial function are not known.

Methods: At first, the effects of moderate-intensity aerobic lower-body exercise were assessed in CAD patients (n = 16) in a neutral [+22°C] and cold [−15°C] environment. Secondly, responses to static and dynamic upper-body exercise in a neutral [+22°C] and cold [−15°C] environment were investigated in CAD patients (n = 15). All experiments were performed in a random order. Endothelial function was measured by flow-mediated dilation (FMD) of the brachial artery in response to reactive hyperaemia, before and after the exposures in a neutral environment.

Results: No significant temperature*exercise*condition (pre–post) interaction was observed in FMD% when comparing rest versus aerobic exercise or static versus dynamic upper-body exercise. Relative reactive hyperaemia during FMD protocol, measured by changes in shear rate, was elevated after rest compared to aerobic exercise (p = .001) and after static compared to dynamic upper-body exercise (p < .001). However, no significant temperature*exercise*condition interaction was observed when FMD% was normalized for shear rate.

Conclusions: Endothelial function to an acute bout of exercise among CAD patients was not modified by the environmental temperature where the exercise was performed. The present findings argue against the hypothesis that exercise in cold environmental conditions impairs endothelial function in patients with CAD.

Keywords: aerobic exercise, atherosclerosis, cold temperature, static, upper-body, vascular

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
1 | INTRODUCTION

Short-term exposure to cold measured as daily mean temperature has been shown to increase both morbidity and mortality (Fares, 2013; Gasparrini et al., 2015), particularly from cardiovascular causes (Fares, 2013; Liu, Yavar, & Sun, 2015). Long-term cold exposure may also exert adverse health effects suggested by increased wintertime occurrence of cardiac events, such as atrial fibrillation, ventricular arrhythmias, angina pectoris and myocardial infarctions (Fares, 2013). Cold exposure increases cardiovascular strain as a result of reflex and/or local cooling-induced cutaneous vasoconstriction and related higher peripheral resistance elevating blood pressure and increasing cardiac workload (Castellani & Young, 2016; Johnson, Minson, & Kellogg, 2014). Cold-related cardiovascular events may be more common among persons with coronary artery disease (CAD) due to their reduced vascular responsiveness and lesser ability for coronary vasodilation in response to higher myocardial oxygen demand (Ikäheimo, 2018; Manou-Stathopoulou et al., 2015). Regular exercise is effective in the treatment of coronary artery disease (CAD) and in preventing its progress, alleviating its symptoms, as well as reducing the risk of myocardial infarction or fatal cardiac event (Anderson et al., 2016). However, as both exercise and cold exposure increase cardiac workload, it is not well established whether their combination would increase the risk for adverse cardiovascular events (Ikäheimo, 2018; Manou-Stathopoulou et al., 2015; Valtonen et al., 2018). For example, sudden heavy exercise, such as snow shovelling, triggers cardiac events in patients with ischaemic heart disease (Janardhanan et al., 2010; Toukola et al., 2015).

The inner lining of the vascular wall, the endothelium, has an important role in regulating vascular tone through the production and release of several endothelium-derived vasoconstrictors (endothelin-1 and thromboxane) and vasodilators (particularly nitric oxide (NO)) (Furchgott & Zawadzki, 1980; Veerasamy et al., 2015). Laminar shear stress has probably a key role in the NO production in the endothelium (Gimbrone, 1999). An imbalance between factors related to vaso dilatation and vasoconstriction leads to impaired endothelium-dependent vasodilation, that is endothelial dysfunction (Hadi, Carr, & Al, 2005). It is considered to be the earliest marker of atherosclerosis (Veerasamy et al., 2015) and is a characteristic feature of CAD (Grover-Páez & Zavalza-Gómez, 2009). Endothelial dysfunction also independently predicts cardiovascular events (Matsuzawa, Kwon, Lennon, Lerman, & Lerman, 2015). Therefore, flow-mediated dilation (FMD) serves as a non-invasive measure of vascular health (Harris, Nishiyama, Wray, & Richardson, 2010).

Regular exercise is known to improve endothelial-dependent dilation (Early et al., 2017). Even a sudden, short-term episode of aerobic exercise augments shear stress and has often (Currie, McKelvie, & Macdonald, 2012; Dawson, Green, Cable, & Thijsen, 2013), but not always (Currie, McKelvie, & Macdonald, 2014), resulted in transiently increased FMD. Such divergent findings are likely related to varying exercise regimes (intensity, type and duration of exercise), as well as study protocols (e.g. subjects and timing of the measurements). In addition, a sudden, short-term episode of dynamic and static exercise each exerts different types of haemodynamic effects, with brachial measures of endothelial function improved following dynamic (Dawson et al., 2013) and impaired after static (Jurva et al., 2006) exercise. In contrast to exercise, exposure to low temperatures during the cold season reduces FMD, possibly as a result of the vasoconstriction and related elevated peripheral resistance, (Iwata, Miyashita, & Kumagai, 2012; Widlansky et al., 2007). To the best of our knowledge, there are no previous controlled studies assessing the effects of short-term whole-body cold exposure on endothelial function.

As exercise predominantly improves and while cold exposure may worsen endothelial function, it would be important to understand their joint effects. Secondly, short-term cold exposure may affect endothelial function differently during static compared with dynamic exercise. To that end, we tested two unique hypotheses: (1) cold exposure blunts the beneficial effects of aerobic lower-body exercise on endothelial function and (2) cold exposure together with static upper-body exercise worsens endothelial function to a greater extent than dynamic upper-body exercise. This response could be due to cold-induced vasoconstriction coupled with the effect caused by the static exercise itself (i.e. reduced blood flow as a result of mechanical compression of blood vessels) and reducing postexercise FMD responses further. Such a response during static exercise could be diminished during dynamic upper-body exercise, because of the maintenance of the circulation (and shear rate) in the arms. These questions are important from the perspective of enabling safe year-round exercise among patients with CAD in regions with cold climate.

2 | METHODS

2.1 | Patients

The study consisted of two groups of male patients with CAD treated at the Oulu University Hospital and participating in two study protocols: the first evaluated the effects of lower-body aerobic and the second dynamic and static upper-body exercise in cold (Table 1). The inclusion criteria consisted of diagnosed CAD (Canadian Cardiovascular Society [CCS] class I–II) and a non-ST-elevation myocardial infarction occurring at least 3 months prior to initiation of the study. The exclusion criteria were as follows: CCS class III–IV, chronic atrial fibrillation, claudication, unstable angina pectoris, left ventricular ejection fraction <40%, a history of coronary artery bypass grafting, pacemaker, complex anomalies in resting electrocardiogram (ECG), asthma, diabetes and current smoking. Clinical exercise tests were performed to assess maximal exercise capacity and to detect possible ECG abnormalities, with these findings described elsewhere (Ikäheimo et al., 2019;
Prior to the experiments, body composition was assessed by bioimpedance measurements (InBody720 Biospace). The subjects also completed a questionnaire inquiring about their perceived health, physical activity at work and during leisure time, physical fitness and use of alcohol. The study was approved by the Ethics Committee of Oulu University Hospital District (EETTMK: 97/2012, 267 §) and registered at ClinicalTrials.gov (NCT02855905). Concerning with the lower-body aerobic exercise protocol, one patient was excluded due to changed antihypertensive medication and one withdrew from the study (n = 16). In the upper-body study protocol, two patients were excluded due to poor quality of FMD measurements and three withdrew from the study before completing all the experiments (n = 15).

| Variables                              | Lower-body aerobic exercise (n = 16) | Upper-body dynamic and static exercise (n = 15) |
|----------------------------------------|--------------------------------------|-----------------------------------------------|
| Age, years                             | 59 (7)                               | 60 (8)                                        |
| Body mass index, kg/m²                 | 29 (5)                               | 28 (4)                                        |
| Body fat, %                            | 26 (8)                               | 23 (6)                                        |
| Exercise capacity, METs                | 8.5 (1.7)                            | 9.0 (1.7)                                     |
| Systolic blood pressure, mmHg          | 126 (19)                             | 121 (8)                                       |
| Diastolic blood pressure, mmHg         | 81 (10)                              | 77 (6)                                        |
| Hypertension, n                        | 14 (87%)                             | 14 (93%)                                      |
| Time from MI, months                   | 15 (5)                               | 27 (10)                                       |
| Single vessel disease                  | 11 (69%)                             | 8 (53%)                                       |
| Double vessel disease                  | 4 (25%)                              | 5 (33%)                                       |
| Triple vessel disease                  | 1 (6%)                               | 2 (13%)                                       |
| Number of stents                       | 2 (varied 1 to 5)                    | 2 (varied 0 to 5)                             |
| Left ventricular ejection fraction     | 61 (10)%                             | 61 (8)%                                       |
| Medication                             |                                      |                                               |
| Acetylsalicylic acid, n                | 14 (88%)                             | 15 (100%)                                     |
| Beta-blockers, n                       | 9 (56%)                              | 11 (73%)                                      |
| Statins, n                             | 12 (75%)                             | 13 (87%)                                      |
| Angiotensin-converting enzyme inhibitors, n | 10 (62%)                        | 6 (40%)                                       |
| Angiotensin receptor blockers, n       | 3 (19%)                              | 4 (27%)                                       |
| Calcium channel blockers, n            | 2 (13%)                              | 2 (13%)                                       |
| Self-perceived health, n               |                                      |                                               |
| Excellent                              | 3 (19%)                              | 4 (27%)                                       |
| Quite good                             | 5 (31%)                              | 6 (40%)                                       |
| Average                                | 8 (50%)                              | 5 (33%)                                       |
| Quite poor                             | 0 (0%)                               | 0 (0%)                                        |
| Use any alcoholic drinks (even occasionally), n | 14 (88%)                        | 14 (93%)                                      |
| Physical demands at work, n           |                                      |                                               |
| Mainly sitting                         | 7 (44%)                              | 11 (73%)                                      |
| Much walking                           | 4 (25%)                              | 1 (7%)                                        |
| Much waking and lifting                | 4 (25%)                              | 3 (25%)                                       |
| Heavy manual labour                    | 1 (6%)                               | 0 (0%)                                        |
| Leisure-time physical activity, n      |                                      |                                               |
| Never                                  | 2 (13%)                              | 0 (0%)                                        |
| Rarely                                 | 10 (63%)                             | 9 (60%)                                       |
| Often                                  | 3 (19%)                              | 5 (33%)                                       |
| Very often                             | 1 (6%)                               | 1 (7%)                                        |

Note: Values are the number of the patients or means (standard deviation) of n (proportion).
Abbreviations: MET, metabolic equivalent; MI, myocardial infarction.
2.2 | Study design

2.2.1 | Aerobic lower-body exercise in cold

Each patient participated to the following four experimental conditions, administered in random order: (a) exercise in a cold (−15°C) and (b) neutral (+22°C) environment, as well as (c) rest in a cold (−15°C) and (d) neutral (+22°C) environment. The level of exercise was adjusted to correspond to the recommended intensity and duration of health-enhancing aerobic exercise (European Association of Cardiovascular Prevention and Rehabilitation Committee for Science Guidelines, et al., 2010; Fletcher et al., 2013). It consisted of moderate-intensity walking for 30 min on a treadmill. Walking speed was adjusted based on target HR based on the following formula: resting HR + 0.45*(peak HR – resting HR) corresponding to moderate exercise intensity, the HR being approximately 65%–70% of peak HR.

2.2.2 | Static and dynamic upper-body exercise in cold

Each patient participated to four different experimental conditions in random order: (a) dynamic upper-body exercise in a cold (−15°C) and (b) neutral (+22°C) environment, as well as (c) static upper-body exercise in a cold (−15°C) or (d) neutral (+22°C) environment. The dynamic upper-body exercise consisted of 5-min pre-exposure, three 5-min work cycles via an arm crank (Monark 881E) each with different intensities and two 4-min resting postexercise periods. The intensity of exercise was adjusted based on subjective judgements of perceived exertion to mild (11–12 fairly light), moderate (13–14 somewhat hard) and high (15–16 hard) and kept constant between the different environmental conditions. The level of static upper-body exercise was adjusted based on maximal voluntary contraction (MVC) (Newtest Leg Force [bench press mode], Newtest). MVC was measured in the beginning of the first visit to the laboratory and at least 1 hr before the baseline measurements. The exercise itself consisted of 5-min pre-exposure and five 1.5-min isometric contractions at the following workloads: 10%, 15%, 20%, 25% and 30% of MVC. Patients had a 4-min break following each work cycle.

The employed cold temperature (−15°C, 1.0 m/s) was selected to simulate conditions often encountered in the northern hemisphere during the winter. Similarly, the administered clothing represented that typically worn during the winter where cold exposure is largely targeted to the face. For the upper-body exercise, the insulation value of the clothing ensemble was 2.13 clo and consisted of underwear, insulated trousers and jacket, overtrousers and jacket, socks and shoes. Clothing insulation was reduced for lower-body exercise (1.88 clo) in insulated trousers and jacket, overtrousers and jacket, socks and shoes. The assessed region of interest was kept constant for each patient throughout the acquired Doppler signal. Diameter and blood velocity were used to calculate shear rate (8 × blood velocity/diameter) at baseline and at peak blood velocity during first 20 s of postocclusion. For peak shear rate, the baseline diameter was used due to missing corresponding B-mode image of artery. It is recommended that mean blood velocity, instead of peak blood velocity, acquired by outer envelope of Doppler spectrum, should be used in calculation of shear rate (Harris et al., 2010). However, peak blood velocity may still be used if kept consistent within a study (Harris et al., 2010). FMD% was calculated by $100 \times \frac{\text{Diameter}_{\text{Baseline}} - \text{Diameter}_{\text{Postocclusion}}}{\text{Diameter}_{\text{Baseline}}}$. FMD% was normalized to relative change of experiments. They were also asked to continue their normal use of medication.

2.3 | Measured parameters

2.3.1 | Endothelial function

FMD, as a measure of endothelial function, was assessed from the brachial artery by standard ultrasound technique (Harris et al., 2010) (Vivid S5, 9L RS transducer, GE Medical Systems) once before each intervention, twice after the lower-body exercise (10 min and 30 min postexercise) and once after the upper-body exercise trials (20 min postexercise). All FMD trials were performed in a neutral environment before and after the exposures. The measurement protocol involved a 1-min baseline, 5-min forearm occlusion and 3-min postocclusion period, while the patient was in a seated position, the right forearm being supported at the level of the heart. The employed FMD protocol has provided comparable results between seated and more widely used supine position (Soga et al., 2007). During baseline, recording B-mode images of the brachial artery were acquired for 30 s before switching to Doppler mode for the recording of blood velocity. The occlusion was induced by inflating supra-systolic pressure (220 mmHg) into a cuff placed on the forearm just distal to the cubital fossa for 5 min. The Doppler signal was recorded 10 s prior to 20 s following the release of occlusion, followed by a switch to B-mode and subsequent recording for 160 s. Instead of the use of duplex mode, this switching method was used to ensure image quality.

Video with B-mode images and Doppler signal were captured from the ultrasound unit using DVI-to-RGBS scalar (Gefen) and video capture device (720p, 50 frames/s; Intensity Shuttle, Blackmagic Design) for further analysis (Vascular Research Tools 6, Medical Imaging Applications LLC). The frame rate of the video was reduced to 10 frames/s. The type and order of intervention recordings were blinded. Brachial artery diameter was analysed from media to media. The assessed region of interest was kept constant for each patient within the four trials. Peak envelope of Doppler spectrum was identified throughout the acquired Doppler signal. Diameter and blood velocity were averaged over baseline recording. During the postocclusion period, the 5-s moving average was calculated for diameter (from 20 to 180 s after release of occlusion) and blood velocity (from 10 s before to 20 s after the release of occlusion). Peak 5-s diameter and blood velocity were identified.

Diameter and blood velocity were used to calculate shear rate (8 × blood velocity/diameter) at baseline and at peak blood velocity during first 20 s of postocclusion. For peak shear rate, the baseline diameter was used due to missing corresponding B-mode image of artery. It is recommended that mean blood velocity, instead of peak blood velocity, acquired by outer envelope of Doppler spectrum, should be used in calculation of shear rate (Harris et al., 2010). However, peak blood velocity may still be used if kept consistent within a study (Harris et al., 2010). FMD% was calculated by $100 \times \frac{\text{Diameter}_{\text{Baseline}} - \text{Diameter}_{\text{Postocclusion}}}{\text{Diameter}_{\text{Baseline}}}$. FMD% was normalized to relative change of
shear rate from baseline to peak, due to the expected differences in basal shear rate after different interventions. Mean arterial pressure (MAP) was calculated from brachial blood pressure recording performed just before FMD baseline measurement. Vascular resistance at baseline was further determined by dividing mean arterial pressure with brachial blood flow. In the lower-body aerobic exercise protocol, the two postintervention FMD measurements were averaged due to lack of significant differences. In case of failure in either postintervention measurement ($n = 4$), the other successful measurement was used.

Intra- and inter-observer reproducibility were assessed by blinded analysis of FMD% for random 30 recordings from the first data set. Intra-observer coefficient of variation (CV) was 15.4%, being absolute of 0.5% in FMD% (mean FMD% = 3.1%). Inter-observer CV was 19.0%, being absolute of 0.6% in FMD% (mean FMD% = 3.0%). Test–retest reliability between 2 consequent assessments from the first data set ($n = 18$) showed minor variation between the baseline measurements for artery diameter. CV was 7.1% for brachial artery diameter at pre-occlusion condition, 6.2% for peak diameter after occlusion and 25.1% for FMD%.

### 2.3.2 Thermal responses and ratings of physical strain

Skin temperature was measured continuously during the experiments using thermistors (NTC DC95, Digi-Key) attached to the right scapula, left cheek, forehead, left calf, right anterior thigh, dorsal side of left index finger (middle phalanx), left hand, left forearm, right shoulder and left upper chest. Data were recorded at 20-s intervals with two temperature data loggers (SmartReaderPlus; Acr Systems Inc.). Mean skin temperature ($T_{sk}$) was calculated as follows: $T_{sk} = \sum ki*tski = [0.07*forehead + 0.175*right scapula + 0.175*left upper chest + 0.07*right arm + 0.07*left arm + 0.05*left hand + 0.19*right anterior thigh + 0.2*left calf] \text{ (ISO 9886, 2004).}$

Thermal sensations were inquired using scales of perceptual judgments on personal thermal state (ISO 10551, 2019). Ratings of perceived of exertion were obtained throughout exercise (Borg, 1998).

### 2.4 Statistical analysis

The Gaussian distribution of these responses was assessed by Shapiro–Wilk tests. In case of non-Gaussian distribution, the values were transformed into natural logarithm followed by confirmation of Gaussian distribution by Shapiro–Wilk. An ANOVA (analysis of variance) for repeated measurements was applied using three within-subject factors that were condition (pre versus post), exercise (exercise versus rest or dynamic versus static) and temperature (neutral versus cold). The main effects of condition, temperature and exercise as well as their interactions are reported. Pearson correlation analysis was performed to pooled data to verify relationship between FMD% and shear rate. Statistical analyses were performed with IBM SPSS version 21 (IBM SPSS Statistics 21, IBM Corp.). Statistical significance was set at $p < .05$.

### 3 RESULTS

#### 3.1 Hypothesis 1—Cold exposure blunts the beneficial effects of aerobic lower-body exercise on endothelial function

Exposure to cold temperature during exercise decreased $T_{sk}$ by 6.3°C ($p < .001$) and by 0.9°C when exposed to the neutral temperature ($p < .001$), both compared with pre-exposure baseline. For the resting trial in the cold, $T_{sk}$ decreased by 3.8°C. Facial skin temperature decreased considerably from 31 to 12°C ($p < .001$) both during rest and exercise in a cold environment. At the end of exercise, the average whole-body thermal sensation of the patients was −3/cold (cold rest), −1/so slightly cool (cold exercise), 0/neutral (neutral rest) and +2/warm (neutral exercise). The achieved exercise intensity represented 69% and 66% of maximum HR at cold and neutral temperature, respectively. The rate of perceived exertion (RPE) varied from light to somewhat hard (11–14), both while exercising in a neutral and cold environment. No significant differences were observed in any pre-intervention baseline values of brachial artery variables within study protocol (Table 2).

#### 3.1.1 Pre-occlusion condition

Brachial artery diameter decreased after exposures without significant interaction with exercise or temperature when measured before the pre-occlusion period (Table 2). Brachial blood flow decreased after exposures to cold compared to neutral temperature and also after rest compared to exercise. Cold temperature increased and exercise decreased MAP and vascular resistance compared to neutral temperature and rest, respectively. No significant interaction of temperature*exercise interactions were observed (Table 2).

#### 3.1.2 Hyperaemic response

Exercise increased peak shear rate compared to rest and tended so also after exposure to cold compared to neutral temperature (Figure 1a). In contrast, the relative change in shear rate from pre- to postocclusion condition increased after rest compared to exercise and after exposure to cold compared to neutral temperature (Figure 1b). No significant condition*temperature*exercise interactions were observed.

#### 3.1.3 Flow-mediated dilation

FMD% decreased after exposures without significant interaction with exercise or temperature (Figure 1c). Results were the same when FMD% was normalized to the relative change in shear rate from pre- to postocclusion condition with tendency towards lesser decrease in normalized FMD% after exercise than rest (Figure 1d).
Table 2. Pre-occlusion brachial artery variables from pre-exposure baseline to condition after lower-body aerobic exercise (Hypothesis 1) and rest in neutral and cold environmental temperatures

| Variables                          | Lower-body aerobic exercise (n = 16) | Main effects | Interactions |
|------------------------------------|--------------------------------------|--------------|--------------|
|                                    | Neutral | Cold |              | Temp | Exe | Cond | Temp*Exe | Temp *Cond | Exe * Cond | Temp*Exe *Cond |
| Pre-occlusion condition            |         |      |              |      |     |      |          |            |            |               |
| Diameter (mm)                      |         |      |              |      |     |      |          |            |            |               |
| Pre                                | 4.84 (0.51) | 4.87 (0.51) | 4.88 (0.55) | 4.81 (0.60) | 0.67 | 0.82 | 0.005 | 0.08 | 0.50 | 0.38 | 0.83 |
| Post                               | 4.75 (0.53) | 4.82 (0.47) | 4.78 (0.57) | 4.73 (0.61) |      |     |      |      |      |      |      |
| Shear rate (1/s)                   |         |      |              |      |     |      |          |            |            |               |
| Pre                                | 289 (112) | 259 (66) | 309 (87) | 311 (99) | 0.16 | <0.001 | 0.018 | 0.43 | 0.07 | <0.001 | 0.403 |
| Post                               | 198 (43) | 317 (87) | 192 (50) | 321 (109) |      |     |      |      |      |      |      |
| Blood flow (ml/min)                |         |      |              |      |     |      |          |            |            |               |
| Pre                                | 194 (89) | 184 (79) | 212 (76) | 205 (79) | 0.49 | <0.001 | 0.002 | 0.95 | 0.04 | <0.001 | 0.80 |
| Post                               | 125 (36) | 213 (84) | 120 (23) | 206 (87) |      |     |      |      |      |      |      |
| Mean arterial pressure (mmHg)      |         |      |              |      |     |      |          |            |            |               |
| Pre                                | 98 (9) | 98 (12) | 97 (13) | 97 (14) | 0.56 | 0.005 | 0.44 | 0.82 | 0.02 | <0.001 | 0.59 |
| Post                               | 101 (10) | 92 (11) | 103 (10) | 96 (13) |      |     |      |      |      |      |      |
| Vascular resistance (mmHg ml⁻¹ min⁻¹) |         |      |              |      |     |      |          |            |            |               |
| Pre                                | 0.60 (0.29) | 0.64 (0.31) | 0.51 (0.18) | 0.56 (0.28) | 0.60 | <0.001 | <0.001 | 0.76 | 0.02 | <0.001 | 0.60 |
| Post                               | 0.88 (0.28) | 0.51 (0.25) | 0.89 (0.15) | 0.59 (0.36) |      |     |      |      |      |      |      |
| Hyperaemic response and FMD        |         |      |              |      |     |      |          |            |            |               |
| Time to peak blood velocity (s)    |         |      |              |      |     |      |          |            |            |               |
| Pre                                | 11 (2) | 11 (3) | 11 (2) | 11 (2) | 0.87 | 0.54 | 0.32 | 0.93 | 0.78 | 0.41 | 0.32 |
| Post                               | 11 (2) | 11 (2) | 11 (1) | 12 (2) |      |     |      |      |      |      |      |
| Time to peak diameter (s)          |         |      |              |      |     |      |          |            |            |               |
| Pre                                | 61 (19) | 62 (23) | 64 (18) | 69 (24) | 0.97 | 0.06 | 0.53 | 0.32 | 0.02 | 0.55 | 0.49 |
| Post                               | 68 (24) | 70 (21) | 57 (17) | 70 (22) |      |     |      |      |      |      |      |
| Peak diameter (mm)                 |         |      |              |      |     |      |          |            |            |               |
| Pre                                | 5.01 (0.47) | 5.04 (0.49) | 5.12 (0.53) | 5 (0.61) | 0.67 | 0.37 | <0.001 | 0.01 | 0.45 | 0.33 | 0.66 |
| Post                               | 4.88 (0.51) | 4.94 (0.43) | 4.95 (0.57) | 4.89 (0.60) |      |     |      |      |      |      |      |

Note: Values are mean (standard deviation).
Abbreviations: Cond, condition; Exe, exercise; FMD, flow-mediated dilation; Temp, temperature.
Hypothesis 2—Cold exposure together with static upper-body exercise worsens FMD more than the corresponding dynamic upper-body exercise

Exposure to cold temperature decreased $T_{sk}$ by 3.7°C during dynamic ($p < .001$) and by 4.0°C ($p < .001$) during static exercise, both when compared with pre-exposure baseline. Facial skin temperature decreased considerably from 31 to 15°C ($p < .001$) both during dynamic and static exercise in the cold environment. At the end of the intervention, the average whole-body thermal sensation of patients was $-1$/slightly cool (cold dynamic), $-2$/cold (cold static), $+2$/warm (neutral dynamic) and $+1$/slightly warm (neutral static). The achieved exercise intensity represented 56%, 62% and 73% of HRmax during dynamic exercise in a neutral and 59%, 66% and 80% of HRmax in a cold environment. The corresponding %HRmax during static exercise was 46%, 47%, 48%, 52% and 56% at neutral and 42%, 43%, 44%, 47% and 50% at the cold temperatures. The RPE during dynamic exercise varied from fairly light to hard (11–15) at the neutral temperature and from somewhat hard to very hard (12–16) in the cold environment. During static exercise, RPE varied from fairly light to very hard (10–16) both while exercising in the neutral and cold environment. No significant differences were observed in any pre-intervention baseline values of brachial artery variables within study protocol (Table 3).

3.2.1 | Pre-occlusion condition

Brachial artery diameter, blood flow and shear rate were increased after dynamic upper-body exercise compared to static exercise regardless of temperature, when measured before the occlusion (Table 3). Dynamic exercise also decreased MAP and vascular resistance compared to static exercise, particularly following exercise in a cold environment as observed as significant condition*exercise*temperature interaction in MAP and tendency in vascular resistance. No other significant interactions were found.

3.2.2 | Hyperaemic response

No significant interactions were observed in postocclusion peak shear rate between the interventions (Figure 2a). The relative change in shear rate from pre- to postocclusion condition increased after static exercise compared to dynamic exercise (Figure 2b). No other significant interactions were observed in shear rate responses.

3.2.3 | Flow-mediated dilation

No significant interactions were observed in FMD% (Figure 2c) or FMD% normalized to the relative change in shear rate from pre- to postocclusion condition (Figure 2d).

3.2.4 | Relationship between flow-mediated dilation and shear rate

Pooled data from all observations ($n = 248$) showed that FMD% correlated strongly with peak shear rate ($r = .583$) and relative shear rate change from baseline to peak shear rate ($r = .378$, $p < .001$ for both).
Table 3: Pre-occlusion brachial artery variables from pre-exposure baseline to condition after static and dynamic upper-body exercise (Hypothesis 2) and rest in neutral and cold environmental temperatures

| Variables                  | Upper-body aerobic exercise (n = 15) | Main effects | Interactions |
|----------------------------|-------------------------------------|--------------|--------------|
|                            | Neutral                             | Main effects | Interactions |
|                            | Static     | Dynamic | Static     | Dynamic | Temp Exe | Exe | Cond | Temp*Exe | Temp*Cond | Exe*Cond | Temp*Exe *Cond |
| Pre-occlusion condition    |                                      |              |              |
| Diameter (mm)              | Pre       | 4.95 (0.55) | 4.99 (0.47) | 4.92 (0.53) | 4.89 (0.44) | 0.51 | <0.001 | <0.001 | 0.95 | 0.16 | <0.001 | 0.07 |
|                           | Post      | 4.91 (0.52) | 5.24 (0.50) | 4.88 (0.56) | 5.29 (0.51) | 0.16 | <0.001 | <0.001 | 0.51 | 0.28 | 0.001 | 0.81 |
| Shear rate (1/s)           | Pre       | 288 (108)  | 259 (104)  | 274 (80)   | 270 (100)  | 0.42 | 0.06  | 0.80  | 0.28 | 0.36 | 0.001 | 0.25 |
|                           | Post      | 220 (75)   | 312 (126)  | 228 (144)  | 353 (87)   | 0.36 | 0.001 | 0.81  | 0.21 | 0.24 | <0.001 | 0.25 |
| Blood flow (ml/min)        | Pre       | 203 (78)   | 189 (85)   | 192 (66)   | 192 (93)   | 0.50 | 0.002 | 0.09  | 0.21 | 0.24 | <0.001 | 0.25 |
|                           | Post      | 148 (43)   | 263 (106)  | 146 (68)   | 301 (66)   | 0.58 | 0.07  | 0.76  | 0.48 | 0.09 | 0.002 | 0.03 |
| Mean arterial pressure (mmHg) | Pre        | 92 (11)    | 90 (12)    | 92 (8)     | 91 (10)    | 0.58 | 0.07  | 0.76  | 0.48 | 0.09 | 0.002 | 0.03 |
|                           | Post      | 93 (10)    | 90 (11)    | 94 (10)    | 86 (10)    | 0.58 | 0.07  | 0.76  | 0.48 | 0.09 | 0.002 | 0.03 |
| Vascular resistance (mmHg ml⁻¹ min⁻¹) | Pre | 0.52 (0.21) | 0.56 (0.23) | 0.54 (0.20) | 0.56 (0.22) | 0.87 | 0.002 | 0.67  | 0.06 | 0.45 | <0.001 | 0.06 |
|                           | Post      | 0.67 (0.18) | 0.40 (0.17) | 0.74 (0.23) | 0.30 (0.06) | 0.87 | 0.002 | 0.67  | 0.06 | 0.45 | <0.001 | 0.06 |

Note: Values are mean (standard deviation).

Abbreviations: Cond, condition; Exe, exercise; FMD, flow-mediated dilation; Temp, temperature.
To our knowledge, this is the first study that examines the combined effects of whole-body cold exposure and exercise on endothelial function. In addition, the study involved CAD patients who may be susceptible to impaired acute response of endothelial function to exercise in cold due to their limited capacity for endothelium-related vasodilation (Grover-Páez & Zavalza-Gómez, 2009). A few previous studies investigating this patient population suggest a higher cardiac workload and earlier occurrence of myocardial ischaemia during exercise in the cold compared with a neutral environment (Ikäheimo, 2018; Manou-Stathopoulou et al., 2015; Valtonen et al., 2018). The main finding of the present study was that acute responses of endothelial function to exercise among CAD patients were independent of the temperature of the environment where the exercise was performed.

### 4.1 | Aerobic lower-body exercise in cold and endothelial function

Contrary to our hypothesis, the effects of lower-body aerobic exercise were independent of the temperature the exercise bout was performed in. A slight tendency towards lesser decrease in normalized FMD was observed after lower-body aerobic exercise. Previous studies have demonstrated beneficial effects of regular exercise on endothelial function and reflected as improved FMD in both healthy persons (Early et al., 2017) and those with CAD (Hambrecht et al., 2000; Kingwell, Sherrard, Jennings, & Dart, 1997; Vona et al., 2004). On the other hand, studies assessing the acute effects of exercise have shown either an improved or worsened FMD response (Dawson et al., 2013). The divergent findings probably reflect differences in the applied exercise regimes (intensity, type and duration of exercise) and study protocols (e.g. subjects and timing of the measurements). The likely mechanisms explaining why exercise training improves FMD include an increase in blood flow and higher shear rate affecting the dilation response (Laughlin, Newcomer, & Bender, 2008).

We assumed that cold exposure could impair FMD as a response of exercise. This was based on a few previous studies where a cool or cold season lowers FMD at rest (IWata et al., 2012; Widlansky et al., 2007). Even a short-term reduction (prior 24 hr) in ambient temperature reduced FMD significantly (Ejike et al., 2017). However, some other studies have not observed such an effect (Klein-Weigel et al., 2003; Patel, Han, Lteif, Wallace, & Mather, 2011). We are not aware of any previous controlled studies examining the acute effects of whole-body cold exposure on FMD at rest or during exercise in human. Cold exposure may itself reduce the FMD response due to cold-induced sympathetic activation and resulting vasoconstriction and increase in peripheral resistance (Castellani & Young, 2016; Dyson, Shoemaker, & Hughson, 2006). On the other hand, Dyson et al. did not observe impaired FMD in response to sympathetic stimulation by muscle ischaemia or a cognitive task (Dyson et al., 2006). According to our previous report based on the same data, such a response may persist during moderate exercise in the cold, as judged by sustained higher systolic BP (Valtonen et al., 2018) and where the superficial cooling is considerable (e.g. Tsk decreases ~ 6°C). Consequently, blood flow and shear rate could be reduced, and the beneficial effect of exercise on FMD diminished. Although speculative, the reason we did not detect an effect of cold exposure postexercise on FMD responses may be due to exercise-related warming of muscles and maintenance of adequate blood flow and shear rate.
despite a higher systolic BP and lower skin temperatures during exercise in the cold (Valtonen et al., 2018). Although present findings cannot be extrapolated to exercise training adaptations, they may suggest that training responses of FMD may not be compromised even if the exercise is performed in cold temperature. Also, while brachial endothelial function is only modestly correlated with coronary endothelial function (Anderson et al., 1995), our results may suggest that untoward response of endothelial function may not be a mechanism to trigger myocardial ischaemia after exercise in cold temperature.

### 4.2 Static and dynamic upper-body exercise in cold and endothelial function

Similarly, as with lower-body aerobic exercise, we did not observe any effects of temperature on FMD responses to upper-body exercises. Some previous studies (Gonzales, Thompson, Thistlethwaite, & Scheuermann, 2011;Jurva et al., 2006;Phillips, Das, Wang, Pritchard, & Gutterman, 2011) have detected impaired FMD in response to static exercise, presumably due to increased sympathetic effects via muscle chemoreflex in response to lesser perfusion compared to dynamic exercise. However, such mechanism has not been confirmed (Dyson et al., 2006). To our knowledge, there are no studies on the effects of dynamic upper body exercise on FMD responses. The hypothesized differences in FMD responses between static and dynamic upper-body exercise would have been likely due to varying haemodynamic effects of the two exercise modes where static/resistance exercise primarily causes a pulsatile pressure load, whereas dynamic exercise increases blood flow and shear rate gradually (volume load) (Heffernan et al., 2017), however the differences being absent in the present study.

The reason why we did not detect any effect of cold exposure on postexercise FMD may be related to the exercise-related heat production, which would reduce the need for vasoconstriction in the cold. For the incremental upper-body exercise, the exercise intensities ranged from moderate to heavy exercise (42%–90% of HR max) at the end of the intervention. Furthermore, the stationary posture reduced convective heat loss in the cold, as judged by a lesser amount of superficial cooling during the incremental upper-body exercise (decrease in T sk 3–4°C) compared with the moderate lower-body exercise (6°C). More severe superficial cooling, with increased sympathetic activity, could have resulted in greater reduction in FMD (Dyson et al., 2006). Finally, it is possible that despite the comprehensive protocol, involving even more CAD patients would have improved precision and reduced the risk of type II error (false-negative conclusion).

### 4.3 Implications

Information on the effect of exercise in the cold on endothelial function is relevant for a number of reasons, particularly given that low environmental temperatures increase cardiovascular morbidity and mortality (Fares, 2013; Liu et al., 2015). Moreover, exercise in the cold may trigger cardiovascular events (Iwata et al., 2012; Tanasescu et al., 2002) and which may be especially prominent among persons with cardiovascular diseases (Heffernan et al., 2017; Liu et al., 2015). The present results among patients with stable CAD suggest that the potentially higher acute exercise-induced health risk could occur irrespective of endothelium-dependent factors given the lack of an effect of cold exposure on subsequent FMD responses. Other known cold-related pathophysiological mechanisms, such as the substantial increase in BP, arrhythmias triggered by altered autonomic nervous system function or increase in blood coagulation factors, can contribute to the higher cardiovascular risk of exercise in the cold (Heffernan et al., 2017; Liu et al., 2015). However, more data on the combined effects of exercise and cold on endothelial function from CAD patients involving varying disease severities, as well as different exercise types and intensities, are needed to confirm our findings. Such information is important for supporting year-round outdoor exercise in patients with CAD, as exercise in its various forms (Tanasescu et al., 2002) effectively prevents the progression of the disease, alleviates its symptoms and reduces the occurrence of myocardial infarctions or fatal cardiac events (L. Anderson et al., 2016).

### 4.4 Limitations and strengths

With regard to reliability, reproducibility of the present analyses was suboptimal but satisfactory, being considerably better than that reported with manual analysis (Woodman et al., 2001), particularly taking into account the low mean FMD in the assessed CAD patients. The test–retest assessment demonstrated relatively consistent measures of artery diameter and FMD. Any differences between measurements can be due to unknown technical issues (operator or instrument-related), as well as biological or physiological variation of the patients. It is possible that measuring postexercise FMD later could have provided further insight to the responses. This is due to a biphasic response of FMD, with an immediate decrease, followed by a gradual increase after exercise (Dawson et al., 2013; Gonzales et al., 2011). In addition, while the employed brachial FMD protocol is widely used (Harris et al., 2010), recording (duplex versus switching between B- and Doppler modes) and analysis (e.g. normalization to shear rate) techniques may vary (Atkinson & Batterham, 2013; McLay, Nederveen, Koval, Paterson, & Murius, 2018). The employed FMD procedure was not optimal, as duplex mode was not used to acquire image and blood flow data simultaneously throughout the protocol. However, the procedure was kept constant throughout the measurements. Ideally, hyperaemic responses are measured from cuff release to peak arterial diameter and include the mean blood velocity in the calculations (Harris et al., 2010). Instead, only immediate postocclusion peak shear rate was captured. However, this approach yielded significant relationship between FMD and the measured hyperaemic response. Also, it is suggested that the
observed FMD responses are, in majority, endothelial-dependent (Dawson et al., 2013). However, the current study does not enable us to separate endothelium-dependent and endothelium-independent mechanisms. It should also be remembered that FMD of the brachial artery being a conduit artery does not represent the entire vascular tree. Finally, the FMD measurements were restricted to the recovery period obtained under neutral temperature conditions. The assessment of FMD responses during exercise in thermo-neutral and cold temperature may have provided further insight for the interpretation of the FMD findings. For safety reasons, the medication of the patients was kept constant during the experiments, for which their role on cardiovascular responses cannot be distinguished.

A major strength of this study is our comprehensive a priori study design, where both the level of exposure and exercise were strictly controlled. Each patient served as his own control through participating in each of the experimental conditions of the two protocols, which eliminates confounding related to inter-individual variation. Confounding due to circadian rhythms was reduced by performing the experiments at the same time of the day for each individual. Each experiment was performed meticulously and within a relatively narrow time period. In addition, randomization of the trials reduces any possible order effect. Finally, the strict selection of patients reduces confounding from other causes than those related to the cardiovascular disease (CAD combined with hypertension).

5 | CONCLUSIONS

Acute endothelial function responses obtained postexercise among CAD patients were not modified by the temperature of the environment where exercise was performed. The present findings argue against the hypothesis that cold environmental conditions impair exercise responses of endothelial function in patients with CAD.

ACKNOWLEDGMENTS

The study was funded through grants from the Finnish Ministry of Education and Culture (TI), Yrjö Jahnsson Foundation (TI), Juho Vainio Foundation (RV), Paulo Foundation (AK) and Finnish Foundation for Cardiovascular Research (AK). The authors wish to thank research nurse Mia Länsitie, research assistant Daniel Rodríguez Yanez, Marjukka Alaperä and Elina Salla for their help with the data collection.

CONFLICT OF INTEREST

The authors have no conflicts of interest.

ORCID

Rasmus I. P. Valtonen https://orcid.org/0000-0002-1395-0916
Tiina M. Ikäheimo https://orcid.org/0000-0002-2763-6004
Antti M. Kiviniemi https://orcid.org/0000-0002-1160-493X

REFERENCES

Anderson, L., Thompson, D. R., Oldridge, N., Zwisler, A., Rees, K., Martin, N., & Taylor, R. S. (2016). Exercise-based cardiac rehabilitation for coronary heart disease. Cochrane Database Systematic Review, 1, CD001800.

Anderson, T. J., Uehata, A., Gerhard, M. D., Meredith, I. T., Knab, S., Delagrange, D., ... Selwyn, A. P. (1995). Close relation of endothelial function in the human coronary and peripheral circulations. Journal of the American College of Cardiology, 26(5), 1235–1241.

Atkinson, G., & Batterham, A. M. (2013). Allometric scaling of diameter change in the original flow-mediated dilation protocol. Atherosclerosis, 226(2), 425–427.

Borg, G. (1998). Borg's perceived exertion and pain scales (pp. 29–31). Champaign, IL: Human Kinetics.

Castellani, J. W., & Young, A. J. (2016). Human physiological responses to cold exposure: Acute responses and acclimatization to prolonged exposure. Autonomic Neuroscience, 196, 63–74.

Currie, K. D., Mckelvie, R. S., & Macdonald, M. J. (2012). Flow-mediated dilation is acutely improved after high-intensity interval exercise. Medicine and Science in Sports and Exercise, 44(11), 2057–2064.

Currie, K. D., Mckelvie, R. S., & Macdonald, M. J. (2014). Brachial artery endothelial responses during early recovery from an exercise bout in patients with coronary artery disease. BioMed Research International, 2014, 591918.

Dawson, E. A., Green, D. J., Cable, N. T., & Thijssen, D. H. J. (2013). Effects of acute exercise on flow-mediated dilation in healthy humans. Journal of Applied Physiology, 115(1), 1589–1598.

Dyson, K. S., Shoemaker, J. K., & Hughson, R. L. (2006). Effect of acute sympathetic nervous system activation on flow-mediated dilation of brachial artery. American Journal of Physiology. Heart and Circulatory Physiology, 290(4), H1446–H1453.

Early, K. S., Stewart, A., Johannsen, N., Lovie, C. J., Thomas, J. R., & Welsch, M. (2017). The effects of exercise training on brachial artery flow-mediated dilation: A meta-analysis. Journal of Cardiopulmonary Rehabilitation and Prevention, 37(2), 77–89.

Eijke, C., Wang, L., Liu, M., Wang, W., Morishita, M., Bard, R. L., ... Brook, R. D. (2017). Personal-level exposure to environmental temperature is a superior predictor of endothelial-dependent vasodilatation than outdoor-ambient level. Journal of the American Society of Hypertension, 11(11), 746–753.e1.

European Association of Cardiovascular Prevention and Rehabilitation Committee for Science Guidelines, EACPR, Corra, U., Piepoli, M. F., Carre, F., ... Schmid, J. P. (2010). Secondary prevention through cardiac rehabilitation: Physical activity counselling and exercise training: Key components of the position paper from the Cardiac Rehabilitation Section of the European Association of Cardiovascular Prevention and Rehabilitation. European Heart Journal, 31(16), 1967–1974.

Fares, A. (2013). Winter cardiovascular diseases phenomenon. North American Journal of Medical Sciences, 5(4), 266–279.

Fletcher, G. F., Ades, P. A., Klipfield, P., Arena, R., Balady, G. J., Bittner, V. A., American Heart Association Exercise, Cardiac Rehabilitation, and Prevention Committee of the Council on Clinical Cardiology, Council on Nutrition, Physical Activity and Metabolism, Council on Cardiovascular and Stroke Nursing, and Council on Epidemiology and Prevention (2013). Exercise standards for testing and training: A scientific statement from the American Heart Association. Circulation, 128, 873–934.

Furchgott, R. F., & Zawadzki, J. V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature, 288(5878), 373–376.

Gasparini, A., Guo, Y., Hashizume, M., Lavigne, E., Zanobetti, A., Schwartz, J., ... Armstrong, B. (2015). Mortality risk attributable to high and low ambient temperature: A multicountry observational study. Lancet, 386(9991), 369–375.
Gimbrone, M. A. (1999). Endothelial dysfunction, hemodynamic forces, and atherosclerosis. *Thrombosis and Haemostasis*, 82(2), 722–726.

Gonzales, J. U., Thompson, B. C., Thistlethwaite, J. R., & Scheuermann, B. W. (2011). Association between exercise hemodynamics and changes in local vascular function following acute exercise. *Applied Physiology, Nutrition and Metabolism*, 36(1), 137–144.

Grover-Páez, F., & Zavalza-Gómez, A. B. (2009). Endothelial dysfunction and cardiovascular risk factors. *Diabetes Research and Clinical Practice*, 84(1), 1–10.

Hadi, H. A. R., Carr, C. S., & Al Suwaidi, J. (2005). Endothelial dysfunction: Cardiovascular risk factors, therapy, and outcome. *Vascular Health and Risk Management*, 1(3), 183–198.

Hambrecht, R., Wolf, A., Gielen, S., Linke, A., Hofer, J., Erbs, S., ... Schuler, G. (2000). Effect of exercise on coronary endothelial function in patients with coronary artery disease. *New England Journal of Medicine*, 342(7), 454–460.

Harris, R., Nishiyama, S., Wray, D., & Richardson, R. (2010). Ultrasound assessment of flow-mediated dilation. *Hypertension*, 55(5), 1075–1085.

Heffernan, K. S., Lefferts, W. K., Yoon, E. S., Park, S. H., Lee, Y. H., & Jae, S. Y. (2017). Carotid artery reactivity during sympathetic activation following acute resistance exercise. *Clinical Autonomic Research*, 27(6), 417–421.

Ikäheimo, T. M. (2018). Cardiovascular diseases, cold exposure and exercise. Temperature, 5(2), 123–146.

Ikäheimo, T. M., Länsitie, M., Valtonen, R., Hintsala, H. E., Ryti, N., Perkiömäki, J., ... Jaakola, J. J. K. (2019). Good safety practice in a randomized controlled trial (CadColdEx) involving increased cardiac workload in patients with coronary artery disease. *BMC Cardiovascular Disorders*, 19(1), 69.

ISO 10551 (2019). *Ergonomics of the physical environment — Subjective judgement scales for assessing physical environments*. Geneva, Switzerland: The International Organization for Standardization. 28 pages.

ISO 9886 (2004). *Ergonomics — Evaluation of thermal strain by physiological measurements*. Geneva, Switzerland: The International Organization for Standardization. 21 pages.

Iwata, M., Miyashita, Y., & Kumagai, H. (2012). Seasonal variation of endothelium-dependent flow-mediated vasodilation measured in the same subjects. *American Journal of Cardiovascular Diseases*, 2(2), 111–115.

Janardhanan, R., Henry, Z., Hur, D. J., Lin, C. M., Lopez, D., Reagan, P. M., ... Keeley, E. C. (2010). The snow-shoveler's ST elevation myocardial infarction. *American Journal of Cardiology*, 106(4), 596–600.

Johnson, J. M., Minson, C. T., & Kellogg, D. L. (2014). Cutaneous vasodilator and vasoconstrictor mechanisms in temperature regulation. *Comprehensive Physiology*, 4(1), 33–89.

Juvra, J. W., Phillips, S. A., Syed, A. Q., Syed, A. Y., Pitt, S., Weaver, A., & Gutterman, D. D. (2006). The effect of exertional hypertension evoked by weight lifting on vascular endothelial function. *Journal of the American College of Cardiology*, 48(3), 588–589.

Kingwell, B. A., Sherrard, B., Jennings, G. L., & Dart, A. M. (1997). Four weeks of cycle training increases basal production of nitric oxide from the forearm. *American Journal of Physiology-Heart and Circulatory Physiology*, 272(3), H1070–H1077.

Klein-Weigel, P., Kral, K., Falkensammer, J., Heinz-Erian, P., Ulmer, H., & Fraedrich, G. (2003). Lack of seasonal variation in flow-mediated dilation of the brachial artery in women with primary Raynaud's phenomenon and healthy controls. Vasa, 32(2), 69–73.

Laughlin, M. H., Newcomer, S. C., & Bender, S. B. (2008). Importance of hemodynamic forces as signals for exercise-induced changes in endothelial cell phenotype. *Journal of Applied Physiology*, 104(3), 588–600.

Liu, C., Yavar, Z., & Sun, Q. (2015). Cardiovascular response to thermoregulatory challenges. *American Journal of Physiology-Heart and Circulatory Physiology*, 309(11), H1793–H1812.

Manou-Stathopoulos, V., Goodwin, C. D., Patterson, T., Redwood, S. R., Marber, M. S., & Williams, R. P. (2015). The effects of cold and exercise on the cardiovascular system. Heart, 101(10), 808–820.

Matsuzawa, Y., Kwon, T., Lennon, R. J., Lerman, L. O., & Lerman, A. (2015). Prognostic value of flow-mediated vasodilation in brachial artery and fingertip artery for cardiovascular events: A systematic review and meta-analysis. *Journal of the American Heart Association*, 4(11), e002270.

McLay, K. M., Nederveen, J. P., Koval, J. J., Paterson, D. H., & Murias, J. M. (2018). Allometric scaling of flow-mediated dilation: Is it always helpful? *Clinical Physiology and Functional Imaging*, 38(4), 663–669.

Patel, Y. R., Han, K. A., Lteif, A. A., Wallace, J. P., & Mather, K. J. (2011). A cross-sectional evaluation of seasonality as a determinant of endothelial function. *Nitric Oxide*, 25(3), 282–287.

Phillips, S. A., Das, E., Wang, J., Pritchard, K., & Gutterman, D. D. (2011). Resistance and aerobic exercise protects against acute endothelial impairment induced by a single exposure to hypertension during exertion. *Journal of Applied Physiology*, 110(4), 1013–1020.

Soga, J., Nishioka, K., Nakamura, S., Umemura, T., Itsuiki, D., Hidaka, T., ... Higashi, Y. (2007). Measurement of flow-mediated vasodilation of the brachial artery: A comparison of measurements in the seated and supine positions. *Circulation Journal*, 71(5), 736–740.

Tanasescu, M., Leitzmann, M. F., Rimm, E. B., Willett, W. C., Stampfer, M. J., & Hu, F. B. (2002). Exercise type and intensity in relation to coronary heart disease in men. JAMA, 288(16), 1994–2000.

Toukola, T., Hookana, E., Junntila, J., Kalkkinen, K., Tikkanen, J., Perkiomäki, J., ... Hukkuri, H. V. (2015). Sudden cardiac death during physical exercise: Characteristics of victims and autopsy findings. *Annals of Medicine*, 47(3), 263–268.

Valtonen, R. I. P., Kiviniemi, A., Hintsala, H. E., Ryti, N. R. I., Kenttä, T., Hukkuri, H. V., & Ikäheimo, T. M. (2018). Cardiovascular responses to cold and submaximal exercise in patients with coronary artery disease. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology*, 315(4), R768–R776.

Veerasamy, M., Bagnall, A., Neely, D., Allen, J., Sinclair, H., & Kunadian, V. (2015). Endothelial dysfunction and coronary artery disease: A state of the art review. *Cardiology in Review*, 23(3), 119–129.

Vona, M., Rossi, A., Capodaglio, P., Rizzo, S., Servi, P., De Marchi, M., & Cobelli, F. (2004). Impact of physical training and detraining on endothelium-dependent vasodilation in patients with recent acute myocardial infarction. *American Heart Journal*, 147(6), 1039–1046.

Widansky, M. E., Vita, J. A., Keyes, M. J., Larson, M. G., Hamburg, N. M., Levy, D., ... Benjamin, E. J. (2007). Relation of season and temperature to endothelium-dependent flow-mediated vasodilation in subjects without clinical evidence of cardiovascular disease (from the Framingham Heart Study). *American Journal of Cardiology*, 100(3), 518–523.

Woodman, R. J., Playford, D. A., Watts, G. F., Cheetham, C., Reed, C., Taylor, R. R., ... Green, D. (2001). Improved analysis of brachial artery ultrasound using a novel edge-detection software system. *Journal of Applied Physiology*, 91(2), 929–937.

How to cite this article: Valtonen RIP, Ikäheimo TM, Hintsala HE, et al. Endothelial function in response to exercise in the cold in patients with coronary artery disease. *Clin Physiol Funct Imaging*. 2020;40:245–256. https://doi.org/10.1111/cpf.12631