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Effects of ischaemic conditioning on tissue oxygen saturation and heart rate variability: an observational study

Youn Joung Cho[1], Hyung-Chul Lee[1], Eue-Keun Choi[2], Seoyeong Park[1], Je Hyuk Yu[1], Karam Nam[1], Tae Kyong Kim[3] and Yunseok Jeon[1]

Abstract

Objective: Ischaemic conditioning (IC) has organ-protective effects, but its clinical results have been inconsistent. Tissue oxygen saturation (StO_2) and heart rate variability (HRV) reflect peripheral microcirculation and autonomic nervous system activity, but their changes during IC have not been well documented. We assessed StO_2 and HRV during IC in patients undergoing cardiac surgery and healthy volunteers.

Methods: Ten patients undergoing cardiac surgery and 10 healthy male volunteers underwent remote IC (four 5-minute cycles of ischaemia/reperfusion) applied to the upper arm. Changes in StO_2 at the thenar eminence and HRV according to the R-R intervals were recorded during IC.

Results: The lowest StO_2 during ischaemia significantly decreased in patients and significantly increased in volunteers. Among the HRV parameters, the low-frequency domain, which corresponds to sympathetic activity, significantly increased after IC in volunteers but not in patients. Other variables were similar between the groups.

Conclusions: These results suggest that the minimum tissue oxygen content is depleted during ischaemia in patients and preserved in healthy volunteers. Sympathetic nervous activity seems to

[1]Department of Anaesthesiology and Pain Medicine, Seoul National University Hospital, Seoul National University College of Medicine, Seoul, Republic of Korea
[2]Department of Internal Medicine, Seoul National University Hospital, Seoul National University College of Medicine, Seoul, Republic of Korea
[3]Department of Anaesthesiology and Pain Medicine, SMG-SNU Boramae Medical Center, Seoul, Republic of Korea

Corresponding author:
Yunseok Jeon, Department of Anaesthesiology and Pain Medicine, Seoul National University Hospital, Seoul National University College of Medicine, 101 Daehak-ro, Seoul 03080, Republic of Korea.
Email: jeonyunseok@gmail.com

increase after IC in healthy volunteers but remains unaffected in patients. Thus, IC may act differently between patients undergoing cardiac surgery and healthy subjects.

Keywords
Ischaemic conditioning, cardiac surgery, tissue oxygen saturation, heart rate variability, sympathetic nervous activity, ischaemia/reperfusion

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Introduction

In ischaemic conditioning (IC), brief ischaemic episodes are applied to elicit an organ-protective effect against a subsequent prolonged ischaemic insult.[1] Remote IC (RIC) consists of repeated non-lethal cycles of ischaemia/reperfusion applied to remote organs, such as the limbs, to protect a target organ, such as the myocardium, from subsequent sustained lethal ischaemia/reperfusion injury.[2,3]

Tissue oxygen saturation (StO_2) reflects the peripheral microperfusion, and its dynamic derivatives, such as the occlusion and recovery slopes during vascular occlusion and recirculation, have been shown to represent local tissue oxygen consumption and microvascular reactivity in critically ill patients.[4,5] StO_2 and its parameters can also be obtained during IC by repeated ischaemia/reperfusion of the extremities; however, they have not been well documented in patients or healthy volunteers undergoing IC or RIC.

Although the detailed mechanisms of RIC have not yet been elucidated, they are believed to include both neural and humoral signalling pathways.[6] In one study, vagus nerve sectioning or stimulation abolished or mimicked the effects of RIC by altering infarct sizes in animal myocardium.[7] These autonomic modifications can be reflected in heart rate variability (HRV), but the changes in HRV during IC have not been compared between patients and healthy subjects.

IC and RIC have been shown to confer protective effects in several clinical situations.[3,8,9] However, in recent large clinical trials of patients undergoing cardiac surgery, RIC did not exert strong protective effects.[10–12] Although several hypotheses pertaining to this discrepancy have been proposed, the causes of the negative results during cardiac surgery remain unclear. We hypothesised that IC may modulate tissue oxygenation and the balance of autonomic nervous system activity and that patients undergoing cardiac surgery may respond differently to IC than healthy volunteers. To evaluate our hypothesis, we measured and compared the changes in StO_2 and HRV during IC in patients undergoing cardiac surgery and healthy volunteers.

Methods

This observational study protocol was approved by the Institutional Review Board of Seoul National University Hospital (#1702-055-832 on 3 March 2017) and registered at clinicaltrials.gov (NCT03089814; Principal investigator, Yunseok Jeon; 14 March 2017) before patient enrolment. The study was performed according to Good Clinical Practice guidelines and the principles of

the Declaration of Helsinki. All participants provided written informed consent.

Study population

Equal numbers of patients undergoing cardiac surgery and healthy volunteers were enrolled in this pilot study (Figure 1). Patients aged 20 to 80 years and scheduled for cardiac surgery at Seoul National University Hospital were screened. The exclusion criteria were as follows: baseline systolic blood pressure (SBP) of >150 mmHg or diastolic blood pressure (DBP) of >100 mmHg, body mass index of <18 or >30 kg/m^2, uncontrolled hypertension or diabetes mellitus, use of beta-blockers, severe renal dysfunction requiring any type of dialysis, the presence of an anatomical anomaly in or arteriovenous fistula on the upper extremities, peripheral vasculopathy or neuropathy, coagulopathy, refusal to participate, and pregnancy.

Healthy male volunteers aged 20 to 45 years were recruited by local advertising. The exclusion criteria were as follows: any previously diagnosed disease, regular medication, recent (within 2 weeks) exposure to herbal medication, baseline SBP of >150 mmHg or DBP of >100 mmHg, body mass index of <18 or >30 kg/m^2, anomaly in the upper extremities, and refusal to participate.

Subjects who had atrial fibrillation or any rhythm disturbance, including a pacemaker, were excluded to avoid interference with the accuracy of the HRV analyses in both groups.

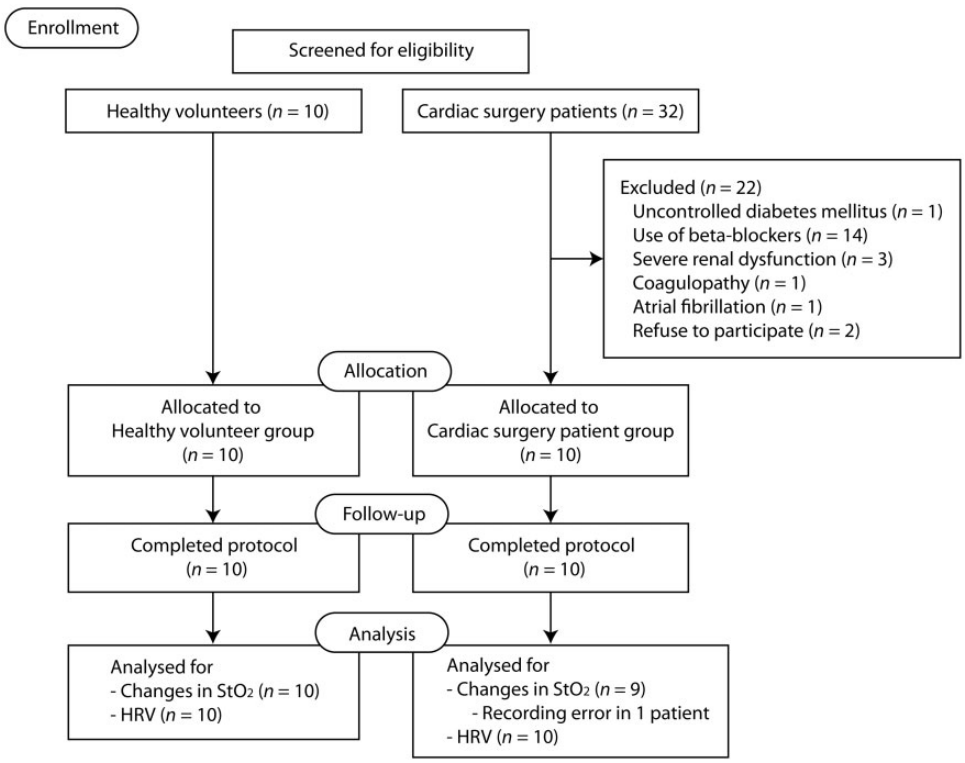

Figure 1. CONSORT flow diagram. StO_2, tissue oxygen saturation; HRV, heart rate variability.

Study protocol

Investigations of the patients were performed in the operating room before anaesthetic induction, and investigations of the healthy volunteers were performed in a quiet room. All subjects were placed in the supine position without supplemental oxygen. The investigations were conducted in a calm circumstance with minimal noise and external stimuli during the study procedure and data measurement to avoid any influence of autonomic and emotional or psychological reactions that may also affect each other.[13] The subjects were asked to refrain from excessive alcohol and caffeine intake, smoking, and strenuous exercise for 24 h prior to the study. Preoperatively, the patients and volunteers maintained a *nil per os* status for >8 and >4 h, respectively, prior to the investigations. All participants were monitored with invasive (for cardiac patients) or non-invasive (for volunteers) blood pressure, standard five-lead (for cardiac patients) or three-lead (for volunteers) electrocardiography (ECG), and pulse oximetry.

The IC was performed by applying four 5-minute cycles of ischaemia induced by blood pressure cuff inflation to 200 mmHg and subsequent 5-minute reperfusion by removing the cuff pressure at the upper arm (Figure 2(a)). During the IC process, StO_2 and its changes were recorded using an InSpectra™ StO_2 tissue oxygenation monitor (model 650; Hutchinson Technology Inc., Hutchinson, MN, USA) and sensor (model 1615; Hutchinson Technology Inc.,) attached to the thenar eminence of the hand of the same side (Figure 2(a)).

HRV was analysed according to the R-R intervals of ECG lead II obtained from the patient monitor (Solar 8000M; GE Medical Systems, Milwaukee, WI, USA). The raw ECG waveform was recorded at a sampling frequency of 500 Hz using a DATAQ analogue-to-digital converter (DI-155, 13-bit resolution; DATAQ Instruments, Inc., Akron, OH, USA) on a Vital Recorder (ver. 1.8.0.3; VitalDB Team, Seoul National University, Seoul, Korea). The R-R interval series were manually inspected, and segments showing excessive noise and artefacts were excluded. In cases of atrial or ventricular premature complexes, the preceding and succeeding R-R intervals were excluded from the analyses. The pre- and post-IC HRV was measured for 2 minutes before and after IC, respectively (Figure 2(a)). During IC, each 5-minute period of HRV data was obtained and analysed.

StO_2 parameters

The StO_2 and its changes during IC were analysed using the InSpectra Analysis Program (ver. 4.03; Hutchinson Technology Inc.) by an experienced researcher (Y.J.C.) blinded to the group assignment. The baseline and lowest StO_2 values during each ischaemia/reperfusion cycle were identified. The occlusion slope was defined as the slope calculated during the first 1 minute after initiation of desaturation during cuff inflation, and the recovery slope was calculated from deflation of the cuff until restoration of 85% of the baseline StO_2 value (Figure 2(b)).

HRV indices

Time and frequency domain variables for HRV were analysed. Time domain parameters reflect overall variability and included the standard deviation of each normal-to-normal R-R interval (SDNN), the root mean square of successive differences (RMSSD), and the proportion of R-R intervals with a >50-ms variation (pNN50). The frequency domain variables, which were calculated using fast Fourier transform, were the total power

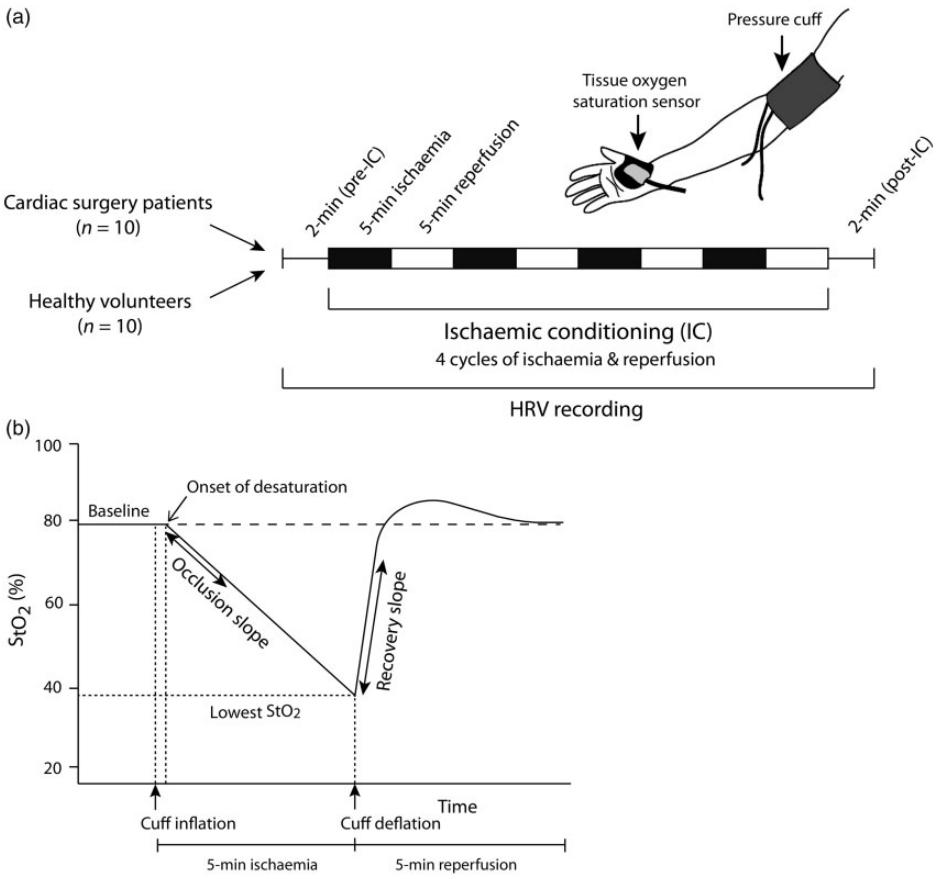

Figure 2. (a) Schematic study protocol. (b) Measurement of changes in tissue oxygen saturation during ischaemia and reperfusion. IC, ischaemic conditioning; HRV, heart rate variability; StO_2, tissue oxygen saturation.

(0.0–0.4 Hz), low-frequency power (LF) (0.04–0.15 Hz), high-frequency power (HF) (0.15–0.4 Hz), and LF/HF ratio.

Statistical analysis

The normality of the data was tested using the Kolmogorov–Smirnov test and Shapiro–Wilk test. For repeated-measures data, plots of residuals versus fitted values were used to check that the error terms (residuals) had a mean of zero and constant variance. The plots did not reveal any violation of the equal variance assumption. The normality assumption for the model residuals was checked using histograms and normal quantile–quantile plots of residuals, and the data appeared to be normally distributed.

Depending on the distributions, continuous variables are expressed as mean ± SD, median (interquartile range [range]), or mean difference with 95% confidence intervals and were compared using the independent *t*-test or Mann–Whitney U test. Categorical variables are expressed as number (proportion) and were compared using Pearson's chi squared test or Fisher's exact test. Repeated-measures

values were analysed using linear mixed models with Bonferroni correction. In the mixed model, group, time, and the interaction between group and time were treated as fixed effects, and the subject was treated as a random effect. In the comparison of pre- and post-IC values, the paired *t*-test or Wilcoxon signed rank test was used. We calculated the statistical power of the study to detect clinically significant changes in StO_2 and HRV in the study population after data collection. Analyses were performed using SPSS version 21.0 for Windows (IBM Corp., Armonk, NY, USA) and SAS software version 9.3 (SAS Institute, Cary, NC, USA). A p value of <0.05 was taken to indicate statistical significance.

Results

From 23 March to 10 July 2017, 10 patients undergoing cardiac surgery and 10 healthy volunteers were enrolled in the study (Figure 1). The baseline characteristics of the participants are shown in Table 1. The median age and pre-IC SBP were higher in the patient group than healthy volunteer group (Tables 1 and 2). In one patient, a technical error occurred during recording of the StO_2 parameters; therefore, the remaining nine patients were analysed for changes in StO_2. No patients developed any complications related to the IC process or HRV recordings.

The initial values of the baseline StO_2, occlusion slope, lowest StO_2, and recovery slope were similar between the groups (Table 3). During IC, there was no difference in the changes in baseline StO_2, occlusion slope, or recovery slope between the groups (Table 3). Interactions between time and group were not significant for the baseline StO_2, occlusion slope, or recovery slope. There was a significant interaction between time and group for the lowest StO_2 ($p < 0.05$), with no difference at any time point between the groups. However, during IC, the lowest StO_2 decreased in the patient group but increased in the healthy volunteer group (adjusted $p = 0.042$ and $p = 0.006$, respectively) (Figure 3(a)). The percent change in the lowest StO_2 from the initial value differed significantly between the groups (mean difference, 20%; 95% confidence interval, 9%–32%; $p = 0.002$) (Figure 3(a)).

Among the haemodynamic variables, the SBP and mean blood pressure (MBP) decreased after IC in both groups ($p = 0.007$ and $p = 0.008$ in cardiac patients, $p = 0.014$ and $p = 0.012$ in healthy volunteers, respectively) (Table 2). DBP also decreased after IC in healthy volunteers ($p = 0.038$) (Table 2). There was no difference in the changes in other haemodynamics between the groups.

Among the HRV parameters, all baseline (pre-IC) values were significantly lower in patients undergoing cardiac surgery than in healthy volunteers (all $p < 0.05$) except for the LF/HF ratio (Table 2). Throughout the procedure (before, during, and after RIC), all HRV values were significantly lower in the patient group than healthy volunteer group. However, the LF/HF ratio was higher in the patient group (Table 2). In the comparison of the pre- and post-IC values, the LF domain increased after IC in the healthy volunteer group ($p = 0.028$) but not in the patient group (Table 2 and Figure 3(b)). There were no differences in the changes in other HRV parameters following IC between the groups.

Discussion

We investigated changes in StO_2 and HRV during IC in patients undergoing cardiac surgery and healthy volunteers. During IC, the lowest StO_2 value decreased in the patient group and increased in healthy volunteers. All HRV indices except the LF/HF ratio were lower in the patient group than

Table 1. Baseline characteristics of patients undergoing cardiac surgery and healthy volunteers.

	Patients ($n = 10$)	Healthy volunteers ($n = 10$)	p value*
Male	5 (50%)	10 (100%)	0.033
Age, years	66 (57–77 [21–78])	27 (26–29 [26–32])	0.002
Height, cm	158 (151–174 [141–182])	176 (171–182 [168–185])	0.013
Weight, kg	64.5 (59.9–72.3 [57.9–85.0])	69.1 (64.7–81.4 [60.0–88.0])	0.226
Body mass index, kg/m^2	25.5 (23.4–28.2 [22.0–29.7])	23.9 (21.8–24.4 [20.8–26.0])	0.059
Current smoker	1 (10%)	0 (0%)	>0.999
Preoperative LVEF, %	61 (56–67 [54–73])	–	–
Current medications			
Beta blocker	0 (0%)	0 (0%)	–
ACE inhibitor	0 (0%)	0 (0%)	–
ARB	1 (10%)	0 (0%)	>0.999
Dihydropyridine CCB	5 (50%)	0 (0%)	0.033
Aspirin	0 (0%)	0 (0%)	–
Platelet inhibitor	0 (0%)	0 (0%)	–
Digoxin	0 (0%)	0 (0%)	–
Diuretics	2 (20%)	0 (0%)	0.474
Nitroglycerin	1 (10%)	0 (0%)	>0.999
Heparin or warfarin	0 (0%)	0 (0%)	–
OHA	0 (0%)	0 (0%)	–
Statins	5 (50%)	0 (0%)	0.033
Comorbidities			–
Hypertension	6 (60%)	0 (0%)	0.011
Diabetes	0 (0%)	0 (0%)	–
Coronary artery disease	3 (30%)	0 (0%)	0.211
Cerebrovascular disease	0 (0%)	0 (0%)	–
Chronic liver dysfunction	0 (0%)	0 (0%)	–
Chronic renal dysfunction	0 (0%)	0 (0%)	–
Operations			
AVR	4 (40%)	–	–
AVR + MVR	1 (10%)	–	–
AVR + CABG	1 (10%)	–	–
AVR + aorta surgery	4 (40%)	–	–

Data are presented as n (%) or median (interquartile range [range]).

*p values were assessed by Pearson's chi square test or Fisher's exact test for non-continuous variables and by the Mann–Whitney U test for continuous variables.

LVEF, left ventricular ejection fraction; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; CCB, calcium channel blocker; OHA, oral hypoglycaemic agent; AVR, aortic valve replacement; MVR, mitral valve replacement; CABG, coronary artery bypass graft.

in healthy volunteers. After IC, the LF domain increased in healthy volunteers but not in the patient group. This is the first observational study to investigate the changes in StO_2 and HRV during IC in patients undergoing cardiac surgery compared with healthy subjects.

In the first report regarding preconditioning by Murry et al.,[1] the authors initially expected that repeated ischaemic episodes might cause cumulative adenosine triphosphate (ATP) depletion and harmful effects. However, the ATP level did not decrease; rather, the episodes conferred

Table 2. Haemodynamics and heart rate variability of patients undergoing cardiac surgery and healthy volunteers receiving remote ischaemic conditioning.

	Patients ($n = 10$)			Healthy volunteers ($n = 10$)			
	Before IC	During IC	After IC	Before IC	During IC	After IC	p value*
HR, beats/minute	71 ± 17	–	71 ± 15	68 ± 10	–	66 ± 8	0.493
SBP, mmHg	155 ± 17**	–	141 ± 17**	130 ± 14**	–	120 ± 10**	0.001
DBP, mmHg	60 ± 15	–	57 ± 13	69 ± 8**	–	64 ± 6**	0.095
MBP, mmHg	96 ± 10**	–	87 ± 10**	89 ± 10**	–	81 ± 6**	0.134
SpO_2, %	98 ± 2	–	98 ± 2	99 ± 1	–	99 ± 2	0.100
SDNN, ms	29.5 (17.4–31.8 [9.3–38.8])	28.4 (24.0–48.0 [11.9–55.8])	31.9 (18.1–52.2 [10.4–100.5])	66.3 (42.0–77.6 [26.1–88.8])	72.0 (44.5–77.1 [43.8–94.7])	74.2 (51.6–85.7 [41.6–100.1])	<0.001
RMSSD, ms	14.9 (8.3–22.8 [4.6–32.0])	15.3 (11.0–23.3 [8.1–54.2])	14.3 (8.4–25.6 [8.2–39.2])	54.5 (39.5–74.0 [11.2–89.2])	54.2 (43.1–74.4 [25.3–89.0])	48.9 (34.1–62.5 [19.4–73.6])	<0.001
pNN50, %	0 (0–1.5 [0–11.3])	0.7 (0.1–2.1 [0.1–10.7])	0.8 (0–4.3 [0–15.6])	33.7 (18.8–46.2 [0–63.6])	28.8 (13.6–42.0 [5.8–57.3])	28.4 (13.0–41.1 [1.9–51.2])	<0.001
TP, ms^2	828.2 (254.0–892.6 [61.2–1414.4])	584.5 (376.7–1615.7 [94.7–2481.7])	936.5 (297.6–2980.9 [93.5–9084.9])	4000.5 (1279.0–5202.1 [630.1–6000.7])	3870.1 (1599.9–4618.0 [945.2–6389.1])	4575.8 (2402.3–6184.4 [1651.1–9802.9])	0.001
LF, ms^2	176.9 (21.3–312.7 [7.9–609.1])	150.9 (80.0–343.6 [17.7–506.4])	252.8 (21.5–516.4 [12.3–1379.3])	952.3 (299.4–1876.8 [186.2–3397.8])**	1336.8 (588.1–1811.7 [316.3–2456.5])	1296.6 (716.3–2389.0 [504.9–4411.9])**	0.001
HF, ms^2	33.6 (14.0–125.7 [9.1–219.7])	47.3 (26.7–105.3 [13.5–529.9])	50.2 (15.5–127.9 [11.5–165.5])	726.6 (355.7–1136.5 [43.6–3143.7])	756.5 (363.8–1167.7 [209.3–2768.1])	718.1 (297.1–1166.9 [165.6–1351.3])	0.001
LF/HF ratio	2.7 (1.3–6.3 [0.3–13.6])	4.9 (2.1–7.4 [0.7–11.1])	4.5 (1.8–6.8 [0.8–9.7])	1.7 (0.7–2.8 [0.4–4.3])	1.6 (1.3–1.9 [0.9–2.2])	2.0 (1.1–4.4 [0.9–9.0])	0.020

Data are presented as mean ± standard deviation or median (interquartile range [range]).

*p value for repeatedly measured haemodynamic and heart rate variability parameters throughout the RIC process: between-group comparison (mixed model). Interactions between time and group were insignificant for HR ($p = 0.511$), SBP ($p = 0.436$), DBP ($p = 0.383$), MBP ($p = 0.807$), SpO_2 ($p = 0.070$), SDNN ($p = 0.844$), RMSSD ($p = 0.417$), pNN50 ($p = 0.070$), TP ($p = 0.918$), LF ($p = 0.387$), HF ($p = 0.645$), and LF/HF ratio ($p = 0.441$).

**$p < 0.05$ between the pre- and post-RIC value within a group (Wilcoxon signed rank test).

HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; SpO_2, peripheral pulse oxygen saturation; IC, ischaemic conditioning; HR, heart rate; SDNN, standard deviation of each normal-to-normal R-R interval; RMSSD, root mean square of successive differences; pNN50, proportion of R-R intervals with >50-ms variation; TP, total power; LF, low-frequency power; HF, high-frequency power.

Table 3. Changes in dynamic microcirculatory parameters during remote ischaemic conditioning in patients undergoing cardiac surgery and healthy volunteers.

	Patients ($n = 9$)				Healthy volunteers ($n = 10$)			
	IC #1	IC #2	IC #3	IC #4	IC #1	IC #2	IC #3	IC #4
Baseline StO_2, %	83 ± 6	83 ± 5	81 ± 5	80 ± 6	84 ± 5	84 ± 4	83 ± 4	83 ± 4
Occlusion slope, %/minute	−7.6 ± 1.9	−8.4 ± 2.9	−7.9 ± 2.0	−8.9 ± 2.4	−7.9 ± 1.6	−7.5 ± 0.9	−7.4 ± 1.0	−7.4 ± 0.8
Recovery slope, %/s	4.8 ± 1.2	4.8 ± 1.5	4.9 ± 1.7	5.6 ± 1.0	4.9 ± 0.7	5.0 ± 0.9	4.9 ± 1.0	5.0 ± 1.1
Lowest StO_2, %	41 ± 9	37 ± 11	37 ± 10	34 ± 11	39 ± 6	41 ± 7	42 ± 7	42 ± 7

Data are presented as mean ± standard deviation.
IC, ischaemic conditioning; StO_2, tissue oxygen saturation.

myocardial protective effects. In subsequent studies, the initial ATP levels were lower in the preconditioned heart, and the sluggish utilisation of ATP resulted in preservation of the ATP levels.[14] If the initial depletion of ATP was excessive, the beneficial effects of preconditioning may have not been present or the ischaemic insult may have been aggravated. This is partly consistent with the results of the present study, in which the lowest StO_2 value increased in healthy volunteers but decreased in the patient group following repeated ischaemia/reperfusion, which corresponds to preservation of tissue oxygen contents in healthy volunteers but progressive tissue hypoxia in patients undergoing cardiac surgery during the repeated short ischaemic insult.

IC produces transient ischaemia and reperfusion, consisting of two to four cycles of 5-minute ischaemia followed by 5-minute reperfusion, applied to the upper or lower extremities.[8,15,16] IC was demonstrated to reduce the myocardial infarct size after sustained ischaemia in animal models, and the effects of RIC on myocardial protection were subsequently demonstrated in some clinical settings, including primary coronary intervention or cardiac bypass surgery with reduced release of cardiac biomarkers.[3,8,9] More recently, however, several multicentre trials and meta-analyses generated inconsistent results regarding the effects of RIC, raising concerns with respect to potential confounders such as propofol or beta-blockers.[10–12,17]

During vascular occlusion tests (VOTs), changes in StO_2 and its derivatives, such as the occlusion slope (during ischaemia) and recovery slope (during reperfusion), have been shown to correlate well with tissue oxygen consumption and microvascular reactivity and are related to outcomes in critically ill patients or patients undergoing cardiac surgery.[18,19] These parameters are impaired during cardiopulmonary bypass in cardiac surgery and have been suggested

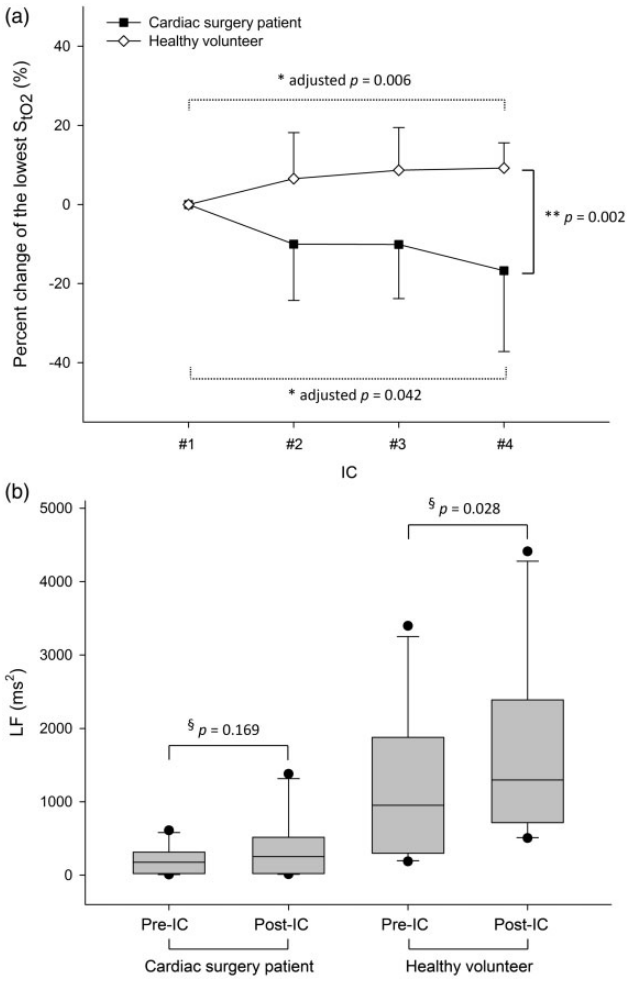

Figure 3. (a) Changes in the lowest tissue oxygen saturation during remote ischaemic conditioning. Data points are means and error bars are standard deviations. (b) Box-and-whisker plots of low-frequency-domain heart rate variability. Horizontal line within the box indicates the median value; lower and upper boundaries of the box indicate the 25th and 75th percentiles, respectively; horizontal lines outside the box indicate the 10th and 90th percentiles, respectively; dots indicate outliers. StO_2, tissue oxygen saturation; IC, ischaemic conditioning; LF, low frequency.

*Adjusted *p* value for changes within a group (mixed model).

***p* value for percent change from the initial value: comparison between groups (mixed model). The interaction between time and group was not significant for changes in the lowest StO_2 value from the initial value ($p = 0.121$).

§*p* value for comparison between pre- and post-IC values within a group (Wilcoxon signed rank test).

to indicate significant worsening of microcirculatory function during extracorporeal circulation and recovery after separation from cardiopulmonary bypass.[20] Moreover, patients with hypertension are known to exhibit a blunted StO_2 response during arterial occlusion and reperfusion, suggesting reduced tissue oxidative capacity

and reduced microvascular reactivity in relation to arterial stiffness.[21] The occlusion and recovery slopes have also been shown to be impaired in non-survivors compared with survivors among patients with sepsis or undergoing cardiac surgery.[22,23] Therefore, although StO_2 may not be a sufficiently sensitive marker of poor tissue perfusion in all patients, its changes imply microcirculatory function and peripheral microvascular reactivity during alteration in tissue perfusion.

IC and VOTs have similar technical aspects. Although they are not the same procedure, both methods include inflation of the pneumatic cuff around the arm, inducing transient ischaemia, and subsequent deflation of the cuff, allowing reperfusion. The major difference between the two methods is the ischaemic time. The ischaemic time in IC is usually 5 minutes, while that in VOTs extends until the StO_2 reaches 40%. Nevertheless, several previous studies have analysed the changes in StO_2 during IC or repeated VOTs mimicking IC protocols.[19,24] Among those studies, Orebgozo Cortes et al.[24] assessed the effects of local ischaemic preconditioning in the muscle by measuring StO_2 parameters during repeated VOTs. In that study on healthy volunteers and haemodynamically stable patients with shock, repeated VOTs (four VOTs at 30-minute intervals or two VOTs for 3 days) caused a decrease in the occlusion slope in healthy volunteers.[24] Moreover, patients who showed a decrease in the occlusion slope after two VOTs had less organ dysfunction at admission, required less inotrope support, and had a lower mortality rate than patients who did not show such a decrease in the occlusion slope.[24] In the present study, however, the occlusion and recovery slopes did not change during IC in either group.

HRV has been used in various clinical and experimental settings to assess autonomic nervous system activity and modeling.[25,26] SDNN reflects global autonomic regulation of the heart, whereas RMSSD and pNN50 reflect the parasympathetic outflow.[27] HF reflects the activity of the parasympathetic system, while LF predominantly corresponds to the activity of the sympathetic system.[27] The LF/HF ratio measures the balance of sympatho-vagal activity.[28]

Higher HRV is generally considered to be cardioprotective and is regarded as an indicator of health and well-being,[13] whereas decreased HRV has been associated with elevated cardiovascular risk and end organ damage.[29] We observed greater HRV in healthy volunteers than in patients undergoing cardiac surgery; the only exception was the LF/HF ratio, which was lower in healthy subjects. Moreover, we identified an increase in the LF domain following IC in healthy subjects but found no changes in HRV in the patient group. In contrast, Zagidullin et al.[30] reported that the total power of HRV was enhanced by RIC in patients with coronary heart disease compared with healthy volunteers. These differential responses of HRV according to IC may be related to the inconsistent results of RIC in clinical trials on patients undergoing cardiac surgery.[10–12]

Although the mechanism underlying the organ-protective role of IC has not yet been fully elucidated, this mechanism is thought to involve neuronal signalling pathways. Following vagus nerve sectioning, the effect of IC on the myocardial infarct size was abolished in experimental animals, suggesting the existence of cardioprotective signals delivered to the heart through a parasympathetic efferent pathway.[7,31] In the present study, the LF domain increased after IC in the healthy volunteer group (952.3 to 1,296.6 ms^2, $p = 0.028$), indicating increased sympathetic activation in healthy subjects. Similarly, in a preclinical study, intermittent hind limb ischaemia/reperfusion produced an increase in the LF/HF ratio combined with an increasing HR and vasoconstriction

in limb vessels, suggesting enhancement of sympathetic activity.[32] In another study, however, RIC (two 5-minute cycles of ischaemia/reperfusion) attenuated ischaemia-induced sympathetic activation.[33]

We observed significant drops in SBP and MBP in both groups after IC. These findings are consistent with previous results on vasodilatory responses to IC in healthy subjects.[33,34] However, in a study by Enko et al.,[34] the HF domain increased significantly (425 ± 69 vs. 617 ± 108 ms^2) in 20 healthy volunteers after three cycles of IC on the upper arm. This was accompanied by a decrease in the LF/HF ratio, suggesting that IC enhanced parasympathetic activity.[34] Moreover, in our patient group, 60% of subjects had hypertension and 50% had been taking a dihydropyridine calcium channel blocker, which may act as a vasodilator, at the time of study enrolment. Because calcium antagonists can decrease MBP and increase the HR by stimulating sympathetic activity,[35] the influence of co-medications in the patient group should also be considered when interpreting our results. Therefore, these inconsistent results may be attributed to different IC techniques, confounders affecting the autonomic status (such as co-medications or the fasting time), or unknown factors not yet proven.

The present study had several limitations. First, most of the patients enrolled in this study underwent cardiac valvular surgery (cardiac valve and/or aorta surgery). Thus, the results of the present study may not be applied to patients undergoing coronary revascularisation surgery. Second, we did not match the age or sex of the two groups in this study. The median age of the patient group was greater than that of the healthy volunteer group (66 vs. 27 years). Global measures of HRV generally decrease with increasing age, and this may reflect the reduced autonomic response to external stimuli with aging.[36] Moreover, the protective effect of ischaemic preconditioning is altered according to aging among healthy volunteers.[37] Men have been shown to have a lower HR and higher SDNN and LF/HF ratio than women.[38,39] Thus, we cannot completely exclude the possibility of any influence of age or sex differences in this study, and this should be considered when interpreting or extending our data. However, in this study we intended to identify any factors associated with the inconsistent results of clinical trials regarding RIC or any different aspects associated with the discrepancy of the results between pre-clinical and clinical studies with respect to the effects of IC. In most pre-clinical studies, including animal studies, experiments are performed using pathogen-free sex-specified young healthy animals in a well-controlled environment, while most clinical studies involve patients with ischaemic heart disease or undergoing cardiac surgery, who also have higher ages and multiple comorbidities. Therefore, based on our results, the organ-protective effects of IC may manifest differently between healthy young male subjects and mixed populations of patients of both sexes with comorbidities and taking multiple medications. Third, this was not a randomised controlled trial, and the sample size calculation may not have been properly powered to detect differences in StO_2 or HRV between the groups. The study's relatively small sample size provided statistical power of $<20\%$ for the StO_2 and HRV parameters; that is, this study has insufficient power to detect differences in the study groups despite a clinically meaningful difference possibly having been present. However, the results of this study may provide a basis to design further studies investigating changes in StO_2 or HRV responses following IC with an appropriate number of subjects. Fourth, the ischaemia/reperfusion technique using IC is not identical to the original VOT methods. As aforementioned, the

IC technique uses fixed time intervals, whereas VOTs generally produce ischaemia until the StO_2 reaches the pre-defined level. Thus, these differences should be taken into account when interpreting our results.

In conclusion, the lowest StO_2 during the ischaemic period of IC decreased in patients undergoing cardiac surgery and increased in healthy volunteers. This suggests reduced tissue oxygen reserve in the patients, but not in the healthy subjects, with repeated ischaemia/reperfusion stimuli. The overall HRV parameters were lower in patients undergoing cardiac surgery than in young healthy volunteers. The LF domain increased after IC compared with baseline in the healthy volunteers, suggesting increased sympathetic activity after intermittent ischaemia/reperfusion in healthy subjects. Our results suggest that the effects of IC may act differently in patients undergoing cardiac surgery and healthy subjects.

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Author contributions

YJC and H-CL conceived and designed the study, performed the statistical analyses, and drafted the manuscript. SP and JHY participated in the data acquisition, performed the statistical analyses, and participated in the discussion. KN and TKK performed the statistical analyses, prepared the figures, and revised the manuscript. E-KC made substantial contributions to the conception and design of the study, participated in the discussion, and revised the manuscript. YJ participated as the corresponding author, designed the study, revised the manuscript, and supervised the overall study. All authors contributed to the manuscript and read and approved the final version of the manuscript.

Declaration of conflicting interest

Hippo Medical Company (Seoul, Korea) and Hutchinson Technology Inc. (MN, USA) provided the InSpectra^{TM} StO_2 Tissue Oxygenation monitor during the study. The authors declare that they have no competing interests.

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ORCID iD

Youn Joung Cho https://orcid.org/0000-0001-6478-8616

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