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Remote Ischemic Preconditioning Attenuates Oxidative Stress during Cardiopulmonary Bypass

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ABSTRACT

Background: Deep hypothermic circulatory arrest (DHCA) is used to overcome the threat of cerebral ischemia during complex surgical operations of the heart and the aortic arch. Remote ischemic preconditioning (RIPC) has been shown to mitigate neurological damage.

Methods: We analyzed blood samples in a consecutive series of 52 piglets that underwent a 60-min period of DHCA with RIPC (the RIPC group) or without (the control group), to reveal whether the protective effect to oxidative stress could be seen by measuring serum 8-hydroxydeoxyguanosine (8-OHdG). The piglets were cannulated and cooled to 18°C using a heart-lung machine, for the DHCA. The piglets were then rewarmed to normothermic temperature. Blood sampling was taken at baseline, after 30 minutes of cooling, 2 hours postoperatively, and 8 hours postoperatively, and analyzed. 8-hydroxydeoxyguanosine (8-OHdG) from blood samples was analyzed by using Enzyme Linked Immunosorbent Assay (ELISA).

Results: The serum 8-OHdG concentration was lower in the RIPC group after the cooling phase, 1.84 (1.44-2.17) ng/mL, and at 8 hours after HCA 1.48 (1.39-1.69) ng/mL, when compared with the control group, where the values were 2.14 (1.81-2.56) and 1.84 (1.62-2.44) ng/mL, respectively (P = .025) and (P = .004).

Conclusion: Remote ischemic preconditioning lowers oxidative stress during cardiopulmonary bypass.

INTRODUCTION

Deep hypothermic circulatory arrest (DHCA) provides a bloodless operating field during complex cardiac and aortic operations. Regarding of the technical issues, lesser cannulas are required while protecting metabolically active tissues, such as the central nervous system. However, it is well known that in exceeding the safe time of DHCA, neurological complications are not uncommon [McCullough 1999; Griepp 2001].

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Remote ischemic preconditioning (RIPC) is a concept of brief intermittent episodes of non-lethal ischemia, followed by reperfusion at a distant organ to protect another organ from subsequent ischemic insult. Neuronal and humoral [Birkelund 2015] pathways are studied, as well as attenuation of ischemia/reperfusion injury in brain, lungs, liver, kidney, and other tissues [Heusch 2015]. In animal studies, positive neurological outcomes have often been reported [Byrne 2012; Jensen 2011]. On the other hand, in trials with patients undergoing coronary artery bypass graft surgery (with or without valve replacement), RIPC did not improve neurological outcome [Meybohm 2015; Hausenloy 2015]. However, the weaknesses of human studies include the heterogeneity of the study population, which might cover the real beneficial effect of RIPC.

Both ischemia and reperfusion result in the creation of reactive oxygen species (ROS). The hydroxyl radical (•OH) is the most unstable ROS, and it is associated as a mediator of ischemia/reperfusion (I/R) injury [Granger 2015]. It reacts rapidly, and interaction with DNA leaves a specific and stable adduct, 8-hydroxydeoxyguanosine (8-OHdG). The formed 8-OHdG in DNA causes G to T transversion mutations [Shibutani 1991]. The cells endeavor to maintain genomic integrity, and the ROS-mediated 8-OHdG is recognized, cut, and secreted out of the cell and ultimately to the urine [Mazurek 2002]. The expression of 8-OHdG can be measured reliably with specific antibodies [Chiou 2003]. We hypothesized that RIPC reduces the expression of 8-OHdG.

We recently demonstrated that the remote ischemic preconditioning before deep HCA seemed to lower ischemia reperfusion-related cerebral oxidative stress [Arvola 2016]. In this study, we analyzed retrospectively 52 piglets' blood samples from consecutive series with the same surgical protocol and intervention with RIPC, to reveal whether the protective effect of RIPC can be seen in oxidative stress marker 8-OHdG after DHCA with sufficient statistical power.

MATERIALS AND METHODS

Preoperative Care

All animals received humane care following the instructions of the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources, National

Resource Council (published by National Academy Press, revised 1996). All of the individual studies were approved by the Research Animal Care and Use Committee of the University of Oulu.

Experimental Setup

Fifty-two native stock piglets we randomly assigned to undergo 60-minute deep hypothermic circulatory arrest at 18°C with or without preconditioning. In this consecutive series, there were 28 animals that were randomized using sealed envelopes for remote ischemic preconditioning as a protective strategy, and 24 control animals. Characteristics of the series included cerebral microdialysis and EEG analysis (20 piglets) [Yannopoulos 2010]; cerebral oxygen tension measurements (12 piglets) [Yannopoulos 2012]; cerebrocortical vessel visualization via intravital microscopy (12 piglets) [Yannopoulos 2014]; and microdialysis combined with magnetic resonance imaging (8 piglets) (not yet published). Twenty-eight piglets were given an intervention of remote ischemic preconditioning 60 minutes prior to cannulation (RIPC group). Twenty-four piglets were given a sham treatment and no preconditioning (control group).

Anesthesia Protocol

Preoperatively, all piglets were sedated with intramuscular injection of midazolam (35 mg), ketamine hydrochloride (350 mg), and medetomidine (1.5 mg). A peripheral catheter was inserted into a vein in the right ear for administration of drugs and to maintain fluid balance, with Ringer acetate solution. Electrocardiographic monitoring was carried out throughout the operation. The anesthesia induction consisted of an intravenous injection of thiopental (5-7 mg/kg) and fentanyl (50 µg/kg) prior to endotracheal intubation with 6.5 mm cuffed tube. Cefuroxime 750 mg was administered intravenously after anesthesia induction. Anesthesia was maintained with continuous infusion of fentanyl (25 µg/ [kg·h]), midazolam (0.25 µg/ [kg·h]), and rocuronium (1.5 mg/ [kg·h]) or pancuronium (0.2 mg/ [kg·h]), in combination with inhalation anesthesia of 1.0% sevoflurane or 0.5% isoflurane in expirated air, using End Tidal Control of the GE Aisys Carestation (GE Healthcare, Madison, WI, USA) throughout the surgical protocol and follow-up up to 8 h, excluding hypothermic circulatory arrest (HCA). All piglets were maintained on 5 cmH2O positive-pressure ventilation with 50% oxygen, ventilated 20 times per minute, and end-tidal carbon dioxide in expirated air (EtCO2) was kept at 5.0 kPa. Venous blood sampling was collected, and invasive hemodynamic monitoring was performed using a pulmonary artery thermodilution catheter (CritiCath 7F; Ohmeda, Erlangen, Germany) inserted through the left femoral vein into the pulmonary artery. Invasive arterial blood pressure measurements and arterial blood samples were gathered via an arterial line inserted into the left femoral artery.

Preoperatively, a membrane oxygenator (D905 Eos, Dideco, Mirandola, Italy) was primed using 500-800 mL of Ringer acetate solution and heparin (15,000 IU) as well as 3-4 units of donor blood to keep hematocrit and hemoglobin levels adequate. The donor piglets were sedated and anesthetized as previously described, and euthanized after the collection using pentobarbital (60 mg/kg) while anesthetized.

Remote Ischemic Preconditioning

Remote ischemic preconditioning was performed after thoracotomy and cranial procedures were finished. Preconditioning was induced by inflating 250 mmHg pressure to a 9-cm-wide blood pressure cuff placed around the right hind leg of the RIPC group. The control group had the blood pressure cuff wrapped around the right hind leg, but not pressurized for the same period of time. Four cycles of 5-minute ischemia, followed by 5-minute reperfusion, were performed. Systemic arterial pressure was monitored continuously to confirm reactive hypertension and hypotension, respectively, of the right femoral artery. Cardiopulmonary bypass (CPB) was initiated 60 minutes after the last reperfusion phase in all individual studies.

Cardiopulmonary Bypass

A right anterolateral thoracotomy was performed through the 3rd or 4th intercostal space and the right atrium was exposed, prior to the preconditioning phase. Sixty minutes after preconditioning or sham treatment, the piglets were heparinized 500 IU/kg and the right atrial appendage was cannulated with a 24F venous cannula. Aortic cannulation was performed using an 18F arterial straight tip cannula, and CPB was initiated. The nonpulsatile flow was adjusted to maintain mean arterial pressure or 55-60 mmHg, and cooling was started immediately using a heat exchanger. A pH-stat strategy was selected to maintain adequate cerebral blood flow during the cooling phase to the target temperature of 18°C in 30 minutes. To adjust CO_2 gas tension with extra care, blood sampling was performed at least every 15 minutes during CPB.

After the cooling period, a 60-minute period of hypothermic circulatory arrest (HCA) was initiated at 18 degrees centigrade, and potassium chloride (40 mmol) mixed with 450 mL of blood was administered to the heart via CPB arterial cannula. Arterial and rectal temperatures were monitored throughout the HCA. Core cooling was maintained using topical ice packs, and cardiac cooling was ensured using topical ice slush. After the 60-minute HCA, the piglets were rewarmed to normothermic temperature during a 45-minute rewarming period, before weaning from CPB. After 5 minutes of rewarming, lidocaine (40 mg), methylprednisolone (80 mg), mannitol (150 g), calcium glubionate (90 mg), and furosemide (40 mg) were administered into the pump flow. The pH-stat strategy was used for the 45 minutes of rewarming. The heart was defibrillated, if necessary, at 30°C. Ventilation with 50% oxygen was started 10 minutes before weaning from CPB, with positive air pressure of 5 cmH2O. The metabolic and biochemical follow-up lasted for 8 hours.

Biochemical and Hemodynamic Data

Hemodynamic data and blood samples from the femoral artery and pulmonary artery were gathered for arterial and mixed venous samples, respectively, at baseline, end of cooling, and 2 hours and 8 hours after the beginning of rewarming.

Arterial gases, pH, electrolytes, serum ionized calcium, glucose, hemoglobin, and hematocrit were measured (i-STAT Analyzer, i-STAT Corp, East Windsor, NJ). At these time points, complete blood count, troponin level I, creatinine kinase isoenzyme MB (CKMB), and 8-OHdG concentration were also collected.

Enzyme-Linked Immunosorbent Assay (ELISA)

The serum values of 8-OHdG were measured from stored pulmonary artery blood samples. The serum samples were stored in polypropylene tubes at -80-88°C until analyzed. Highly sensitive 8-OHdG Check ELISA kits were used to analyze the samples (from the Japan Institute for the Control of Aging, Fukuroi, Japan), following the manufacturer's instructions.

Statistical Analysis

Summary measurements are expressed as mean and standard deviation (SD) or as median and 25th-75th percentiles. Either the Student t test or the Mann-Whitney U test was used for between group comparisons of continuous and ordinal variables. The repeatedly measured data were analyzed using a linear mixed model (LMM) with subjects fitted as random, and the covariance pattern was chosen. *P* values reported for the LMM are as follows: p_t indicates change over time, p_g indicates a level of difference between groups, and p_{t*g} indicates interaction between groups and time. Between-groups comparisons for categorical variables were made using the Pearson chi-square test or Fisher exact test, when appropriate. Two-sided *P* values are reported.

Experimental and Metabolic Data

Variable	Baseline	End of Cooling	Warming 2h	Warming 8h	p_g	p_{t*g}
MAP						
RIPC	102 (85-114)	58 (56-65)	82 (68-90)	69 (63-83)		
Control	92 (81-101)	58 (55-64)	78 (72-95)	76 (62-88)	0.245	0.171
Arterial pH						
RIPC	7.44 (7.42-7.47)	7.29 (7.19-7.34)	7.41 (7.35-7.43)	7.46 (7.43-7.50)		
Control	7.43 (7.41-7.46)	7.28 (7.21-7.34)	7.34 (7.26-7.42)*	7.44 (7.38-7.49)	0.018	0.559
PaCO2, kPa						
RIPC	5.20 (4.81-5.45)	6.31 (5.47-7.59)a	5.98 (5.61-6.36)	5.45 (5.1-5.71)		
Control	5.35 (4.83-5.57)	6.28 (5.38-7.60)a	6.40 (5.86-7.02)*	5.28 (5.04-5.81)	0.583	0.175
PaO2, kPa						
RIPC	31.7 (28.6-35.0)	106.6 (106.6-106.6)	28.1 (20.8-37.4)	30.7 (24.0-34.2)		
Control	32.7 (30.7-35.0)	106.6 (106.6-106.6)	30.0 (22.8-37.2)	28.2 (26.7-30.4)	0.836	0.706
SvO2, %						
RIPC	77 (73-80)	100 (100-100)	66 (60.5-77)	61 (51-69)		
Control	73 (71-78)	100 (100-100)	71 (65-77.5)	60.5 (55-63)	0.908	0.525
Hemoglobin (g/L)						
RIPC	82 (77-88)	78 (70-86)	95 (85-106)	88 (82-99)		
Control	78 (73-80) *	75 (61-75)	85 (74-95)*	90 (78-105)	0.064	0.445
Hematocrit %						
RIPC	24 (22-26)	24 (20.5-25.5)	27 (25-30.5)	26 (24-29)		
Control	23 (21-24)*	22 (18-22.5)	26 (22-28.5)	27 (23-31)	0.063	0.753
Rectal Temperature (°C)						
RIPC	38.6 (38.1-39.3)	17.6 (16.5-19.1)	36.2 (34.6-37.4)	38.5 (37.6-39.4)		
Control	38.7 (38.0-39.2)	17.3 (15.9-18.6)	34.8 (33.9-35.5)	39.5 (38.1-39.9)	0.576	0.330
Blood Temperature (°C)						
RIPC	38.6 (37.9-39.5)	13.4 (8.8-15.5)	36.1 (34.7-37.3)	38.9 (37.8-39.5)		
Control	38.8 (38.0-39.2)	14.1 (11.2-16.1)	35.0 (34.5-35.8)	39.3 (38.3-39.6)	0.721	0.179

RIPC, n = 28; Control, n = 24. Values are shown as medians and 25th and 75th percentiles; aTemperature-corrected arterial carbon dioxide; p_g = *P* value between the groups (level of difference between the groups); p_{t*g} = value time * group (behavior between groups over time); $PaCO_2$ indicates arterial CO_2 partial pressure; SvO_2, mixed venous oxygen saturation, *$P < .05$ at a single time point.

Analyses were performed using SPSS (IBM, Released 2012, IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY, USA) and SAS (version 9.3, SAS Institute, Gary, NC, USA).

RESULTS

Comparison of Study Groups

There were no statistically significant differences with respect to the weight or the amount of donated blood between the groups. The median weight of the piglets in the RIPC group was 21.9 kg (20.4-25.0) and 22.1 kg (20.0-30.7) in the control group (P = .545). The volume of transfused blood was 53.5 mL/kg (40.2-57.0) in the RIPC group and 48.3 mL/kg (41.4-59.4) in the control group (P = .756).

Metabolic and Hemodynamic Data

Baseline hemoglobin and hematocrit differed statistically significantly between the groups. The hemoglobin in the RIPC group was 82 g/L (77-88) and 78 g/L (73-80) in the control group (P = .011). The baseline hematocrit was 24 (22-26) in the RIPC group and 23 (21-24) in the control group (P = .017). However, the differences were rectified at subsequent time points, respectively. Experimental and metabolic data are shown in the Table. There were no statistically significant differences with respect to blood gas analysis or electrolyte content between the groups at baseline. Additionally, cardiac index, 8-OHdG concentration, troponin isoenzyme I levels, and creatine kinase isoenzyme MB levels were similar between the groups at baseline. Blood and rectal temperatures were comparable and had no statistically significant differences throughout the experiment.

8-OHdG Concentration

The level of 8-OHdG increased in the control group after cardiopulmonary bypass, but decreased in the RIPC group during that time. The difference was most notable at 8 hours after rewarming, when the RIPC group 8-OHdG concentration was 1.48 (1.39-1.69) ng/mL, whereas the control group remained at 1.84 (1.62-2.44) ng/mL (P = .004). The 8-OHdG measurements are shown in Figure 1.

Systemic Complete Blood Count

The total leukocyte count in blood was 16.2 × 10^9/L (14.0 × 10^9/L-22.5 × 10^9/L) in the RIPC group and 18.9 × 10^9/L (16.12 × 10^9/L-21.7 × 10^9/L) in the control group at the baseline, with no statistically significant difference between groups. There were no differences between the groups with respect to white blood cell count and differential white cell count at any time point throughout the follow-up period.

Cardiac Parameters

Cardiac troponin I, creatine kinase isoenzyme MB, and cardiac index did not reveal any statistically significant differences between the groups (Figure 2, 3 , and 4).

DISCUSSION

The protective effects of RIPC have been under intensive discussion during the past decades. We have shown RIPC has beneficial effects on neuron viability, as NAD+/NADH autofluorescence indicated better respiratory chain function [Yannopoulos 2014]. The key channel for mitochondrial viability, the mitochondrial permeability transition pore (MPTP) opening, and subsequent burst in ROS production leading eventually to inevitable cell death plays a key role in

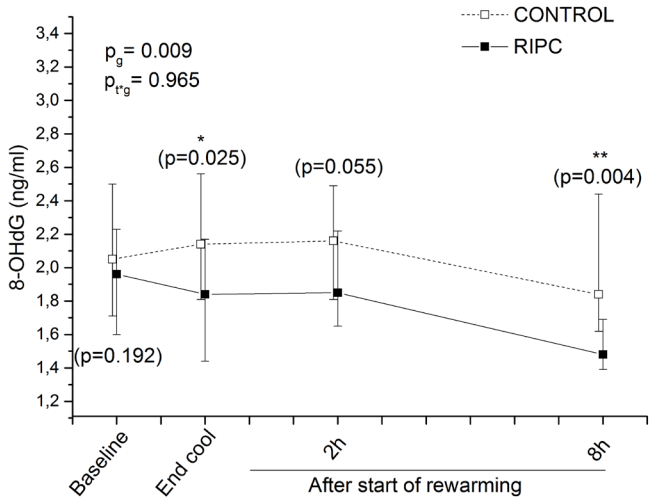

Figure 1. The measured level of 8-OHdG in ng/mL; values represent medians. Error bars show the interquartile range (25%-75%); p_g = P value between groups (level of difference between groups); p_{t*g} = P = value time * group (behavior between groups over time), * = P < .05, ** P < .01; p = P value at a single time point. RIPC = study group; control = sham group; h = hour.

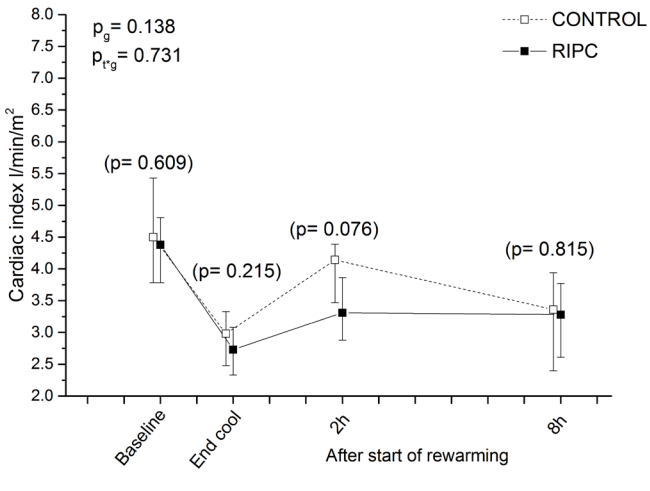

Figure 2. The measured cardiac index, L/min/m^2; values represent medians. Error bars show the interquartile range (25%-75%); p_g = P value between groups (level of difference between groups); p_{t*g} = p = value time * group (behavior between groups over time), * = P < .05, ** P < .01; p = P value at a single time point. RIPC = study group; control = sham group; h = hour.

ischemia-reperfusion injury [Halestrap 2006]. It has been shown that preconditioning plays a significant role in inhibiting MPTP-mediated cell death [Garcia-Dorado 2006]. When it comes to measuring the damage caused by ROS, 8-OHdG is one of the most-used markers. It is a rapidly induced acute phase DNA adduct [Hamilton 2001] that is a reliable marker of oxidative DNA damage, and can be measured from blood samples using ELISA [Chiou 2003]. In the present study, we have demonstrated a significant reduction in systemic oxidative stress in preconditioned piglets after prolonged deep hypothermic circulatory arrest.

Strikingly, the significant reduction in ROS activity was found prior to major ischemia, and, moreover, the difference became more obvious during the follow-up period. Providing more value to our finding, a significant inflammatory response and subsequent I/R injury is known to be triggered already by surgery and the CPB per se [Franke 2005]. Several studies have proposed strategies attenuating reperfusion injury during open heart surgery to improve clinical outcome [Suleiman 2008]. We have demonstrated in this experimental retrospective study the neuroprotective pathways triggered by RIPC are functional more rapidly than previously thought.

Additionally, we have recently demonstrated the beneficial effect of RIPC to the spinal cord function prior to the major ischemia measured by motor evoke potentials (MEP) after occlusion of segmental arteries [Haapanen 2016]. Worthy of emphasizing, the MEP findings already occurred before the induction of spinal cord ischemia, after the RIPC. Our findings in this study are in line with the previous findings, as 8-OHdG differ also prior to prolonged DHCA.

During hypoxia-reperfusion phases, ROS formation and oxidative stress are notably increased [Raedschelders 2012]. Yildirim et al recently showed elevated superoxide dismutase (SOD) levels in a clinical study designed to investigate the role of ROS during on-pump coronary artery bypass surgery with RIPC. Also, malondialdehyde (MDA) concentration was non-significantly lower in the RIPC group, indicating less oxidative damage to lipids. The SOD measurements were reported at baseline and at 1 minute and 15 minutes after the release of the cross-clamp [Yildirim 2016].

The source of 8-OHdG is metabolically active tissues, since it is one of the end products of hydroxyl radical production. In this study, there were no differences between the study groups in cardiac index, cardiac troponin I, or creatinine kinase isoenzyme MB. However, this was expected, because there was no ischemic cardiac insult in the protocol. Walsh et al could not demonstrate a difference in cardiac enzymes in high-risk patients undergoing cardiac surgery in a randomized controlled trial [Walsh 2016].

In other human studies, a relevant benefit to end-points could not be demonstrated with adults undergoing complex heart surgery [Meybohm 2015; Hausenloy 2015]. Li et al showed statistically significant differences in MDA concentrations with RIPC-treated patients undergoing pulmonary resection in a prospective, randomized, and controlled trial, indicating a lower production of free oxygen radicals, and also showing protection from pulmonary injury [Li 2014].

Conclusion

The effects of RIPC take place rapidly after the precondition stimulus and most conspicuously, the attenuation of oxidative stress can be seen already during CPB. However, additional prospective studies are required to confirm the outcome.

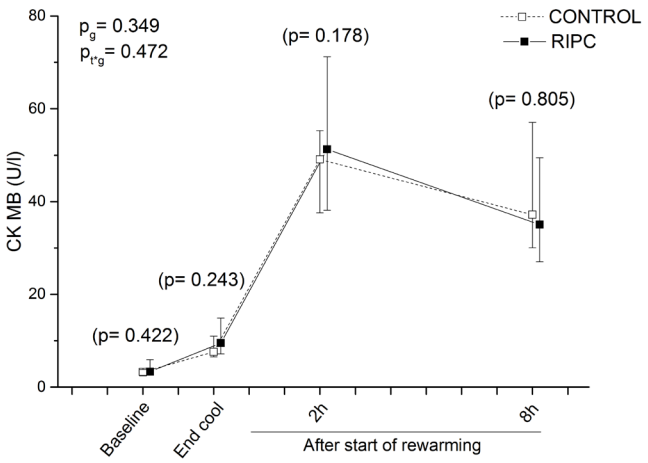

Figure 3. The measured level of creatine kinase isoenzyme MB; U/l (units per liter); values represent medians. Error bars shows the interquartile range (25%-75%); p_g = *P* value between groups (level of difference between groups); p_{t*g} = p = value time * group (behavior between groups over time), * = *P* < .05, ** *P* < .01; p = *P* value at a single time point. RIPC = study group; control = sham group, h = hour.

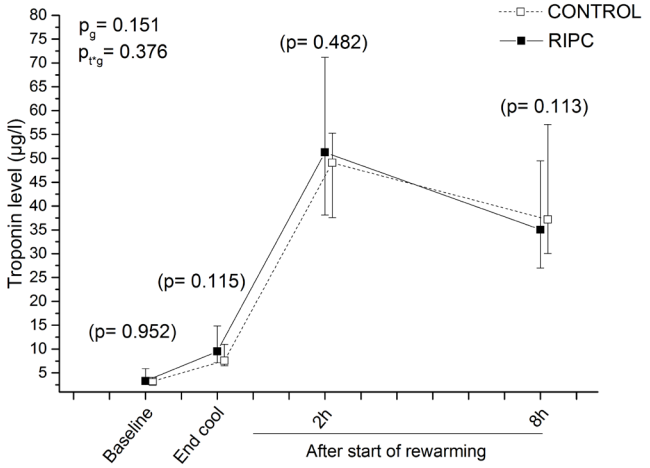

Figure 4. The measured troponin I level µg/L; values represent medians. Error bars show the interquartile range (25%-75%); p_g = *P* value between groups (level of difference between groups); p_{t*g} = p = value time * group (behavior between groups over time), * = *P* < .05, ** *P* < .01; p = *P* value at a single time point. RIPC = study group, control = sham group, h = hour.

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