



# Catheter-Based Transcoronary Myocardial Hypothermia Attenuates Arrhythmia and Myocardial Necrosis in Pigs With Acute Myocardial Infarction

Otake, Hiromasa ; Shite, Junya ; Oscar Luis Paredes ; Shinke, Toshiro ; Yoshikawa, Ryohei ; Tanino, Yusuke ; Watanabe, Satoshi ; Ozawa, Toru ; ...

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**Catheter-based Transcoronary Myocardial Hypothermia Attenuates Arrhythmia and Myocardial Necrosis in Pigs with Acute Myocardial Infarction**

Brief title: Catheter-based Transcoronary Hypothermia

Hiromasa Otake M.D., Junya Shite M.D., Toshiro Shinke M.D., Ryohei Yoshikawa M.D., Oscar Luis Paredes M.D., Yusuke Tanino M.D., Satoshi Watanabe M.D., Toru Ozawa M.D., Daisuke Matsumoto M.D., Daisuke Ogasawara M.D., Mitsuhiro Yokoyama M.D.

Kobe University Graduate School of Medicine Division of Cardiovascular and Respiratory Medicine  
Department of Internal Medicine.

Name and address for correspondence:

Junya Shite M.D.

Kobe University Graduate School of Medicine, Department of Cardiology

7-5-1 Kusunoki-cho, Chuo-ku, Kobe, Hyogo, 650-0017, Japan

Tel: 81-78-382-5846

Fax: 81-78-382-5859

E-mail: shite@med.kobe-u.ac.jp

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### **Structured Abstract**

**OBJECTIVES** This study evaluated the efficacy of catheter-based transcatheter myocardial hypothermia (CTMH) in pigs with acute myocardial ischemia.

**BACKGROUND** Although it has been suggested hypothermia therapy can attenuate myocardial necrosis, few applications have been accepted for clinical use.

**METHODS** This study comprises two sub-studies. In both studies, pigs underwent 60 min of coronary occlusion and 180 min of reperfusion. In study 1, after 15 min of coronary occlusion with over-the-wire-type balloon (OTWB), pigs in the Hypothermia group (H; n=13) were directly infused with 4°C saline into the coronary artery through the OTWB wire lumen (2.5 ml/min) for 60 min. Pigs in the Normothermia group (N; n=15) received the same amount of 36.5°C saline. In study 2, pigs in the Hypothermia-reperfusion group (HR; n=5) were infused with 4°C saline through the infusion catheter (8 ml/min) for 30min with a later start (60min after coronary occlusion), while

simple reperfusion was used for the Reperfusion group (R; n=6) .

**RESULTS** CTMH was successful in both studies. In study 1, CTMH significantly decreased ventricular arrhythmia and the ratio of necrosis to ischemic risk area (H:  $9\pm 2\%$ , N:  $36\pm 4\%$ ;  $P < 0.0001$ ) with significant reduction of enzyme leaks. In study 2, CTMH tended to reduce the ratio of necrosis (HR:  $33\pm 2\%$ , R:  $45\pm 5\%$ ;  $P = 0.08$ ). In both studies, CTMH significantly suppressed the increase of 8-iso-prostaglandin  $F_{2\alpha}$  while preserving the coronary flow reserve (CFR).

**CONCLUSION** CTMH reduced myocardial necrosis while preserving CFR, due in part to attenuation of oxidative stress.

**Key words:** hypothermia, oxidative stress, arrhythmia,

### **Condensed Abstract**

We evaluated the efficacy of a new method of catheter-based transcatheter myocardial hypothermia (CTMH) for attenuating myocardial necrosis and arrhythmia in 39 pigs with acute myocardial ischemia. CTMH reduced myocardial temperature by 3-4°C while rectal temperature remained stable. CTMH reduced myocardial necrosis and the frequency of ventricular arrhythmia in pigs with on-going ischemia. Furthermore, CTMH preserved coronary flow reserve while attenuating oxidative stress expressed by 8-iso-prostaglandin F<sub>2α</sub>. Our findings demonstrate that CTMH may be a useful method for treating acute myocardial ischemia.

## Introduction

While coronary reperfusion therapy is widely used for patients with acute myocardial infarction (MI), its cardio-protective effect remains unsatisfactory. Reperfusion injury induces persistent myocardial necrosis in conjunction with increased oxidative stress (1-3) and activity of [?] cytokines (4, 5), which are believed to be major factors contributing to the deterioration of cardiac function after coronary reperfusion therapy. Although several agents, such as antioxidants (6), genes (7), and hormones (8), have been administered as adjuncts to coronary reperfusion, their efficacy for preventing ischemic damage has been found lacking. Findings from preliminary animal studies, however, have shown that mild hypothermia markedly ameliorates tissue damage after the onset of ischemia in many organs (9-12). As for the heart, several experimental studies have demonstrated that mild hypothermia can minimize myocardial necrosis resulting from acute MI (13-16). In addition, ongoing research into systemic core cooling with an endovascular cooling system for patients with acute MI has shown its safety (17). With this method, however, sufficient cooling of the ischemic myocardium cannot be attained due to severe shivering caused by lowering the whole body temperature, so that the apparent myocardial protective effect is negated.

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We therefore developed a new method involving cold saline infusion into an infarct-related coronary artery by means of a catheter. With this method, the cooling effect was restricted to the ischemic myocardium, thus resulting in a substantial reduction in systemic complications. Furthermore, this technique is simple, so that it may be suitable for widespread clinical application. The purpose of the study presented here was to determine whether this method could effectively induce regional hypothermia as well as attenuate arrhythmia and myocardial injury in pigs with acute MI.

## **Methods**

This study comprises two sub-studies. In study 1, we evaluated whether direct infusion of cold saline into the coronary artery could induce regional hypothermia and attenuate myocardial injury in pigs with on-going ischemia. In study 2, to examine the clinical feasibility and efficacy of this procedure for acute MI, hypothermal-reperfusion therapy was initiated after a longer period of coronary occlusion and the result compared to that obtained with simple immediate reperfusion-therapy in pigs with established MI.

## **Subjects**

Thirty-nine Yorkshire pigs (28 for study 1, 11 for study 2) were used and the study procedure conformed to 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 published by US National Institutes of Health.

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## **Surgical Preparation**

The pigs were sedated with intramuscular ketamine hydrochloride (20 mg/kg) and atropine sulfate



(0.05 mg/kg). After tracheal intubation, deep anesthesia was induced with mechanical ventilation of oxygen and sevoflurane. Through a median sternotomy and systemic heparinization (100 U/kg IV per hour), the pericardium was incised and a deep body thermister (Coretemp<sup>TM</sup> CM-210; Terumo Co., Tokyo, Japan) to monitor the myocardial temperature at 5-6 mm depth was placed directly onto the area at risk of ischemia. A 6Fr Swan-Ganz catheter (CCOM catheter; Terumo Co.) was advanced via the left internal jugular vein into the pulmonary artery to monitor cardiac output (CO). A 5Fr catheter was then inserted through the right internal jugular vein into the coronary sinus for blood sampling, while a 2Fr micromanometer-tipped catheter (Millar Instruments, Houston, TX) was advanced into the left ventricular (LV) cavity through a 5Fr pigtail catheter via the right femoral artery for measuring peak positive first derivative of LV (LVdP/dt<sub>max</sub>), and time constant of LV relaxation ( $\tau$ ). Finally, a 7Fr angioplasty-guiding catheter (Heartrail<sup>TM</sup>; Terumo Co.) was used for entry into the left coronary from the right carotid artery.

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### **Experimental procedure**

Figure 1 provides an overview of the study protocols. First, baseline hemodynamics, myocardial and rectal temperature, and blood samples were obtained. Following coronary angiography, an over-the-wire type percutaneous transluminal coronary angiography balloon (OTWB) mounted on a 0.014-inch wire was advanced into the left anterior descending coronary artery (LAD), positioned at

approximately one-third of the distance from the apex and inflated to occlude the LAD for 60 min.

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After 15 min of coronary occlusion, the pigs in study 1 were randomly assigned to the Hypothermia or the Normothermia group. For animals in the Hypothermia group, cooled saline (4 °C) was infused into the ischemic myocardium through the wire lumen of the OTWB at 2.5 ml/min (the maximum flow rate possible for this wire lumen). Pigs in the Normothermia group were administered the same amount saline, but at 36.5 °C. in the same manner. After 60 min of coronary occlusion, reperfusion was achieved by complete deflation of the OTWB, and intra-coronary saline infusion was continued for 15 min after reperfusion in both groups.

In study 2, the LAD was occluded at the same position by using an infusion balloon (Helios™; Avantec Vascular Corporation, Sunnyvale, CA), which has a larger lumen than conventional OTWB so that a higher volume of saline could be infused. After 60 min of coronary occlusion, pigs were assigned to the Hypothermia-reperfusion group or the Reperfusion group. For pigs in the Hypothermia-reperfusion group, cooled saline (4 °C) was infused through the infusion balloon at 8

ml/min for 30 min followed by complete balloon deflation. For pigs in the Reperfusion group, simple reperfusion was used after 60 min of coronary occlusion. In both studies, reperfusion was observed for 180min.

### **Incidence of arrhythmia**

Twenty-four-hour Holter recordings (Holtrec<sup>TM</sup>; Terumo Co.) were obtained and reviewed with Holtrec Analysis System software to determine the total number of ventricular premature beats (VPBs) and sustained ventricular tachycardia (sVT). VPBs were defined as the presence of at least two out of three criteria: (1) atypical QRS configuration with alteration or inversion of the T wave; (2) post-extrasystolic pause; (3) atrioventricular dissociation. sVT was defined as a fast ventricular rhythm of 15 or more beats in accordance with the Lambeth Conventions (18).

### **Coronary flow velocity measurements**

Intra-coronary Doppler flow measurements were performed with a 0.014-inch Doppler-tipped guidewire (FloWire<sup>TM</sup>; Volcano Therapeutics, Inc., Rancho Cordova, CA) and a velocimeter (FloMap<sup>TM</sup>; Volcano Therapeutics Inc.) at baseline, 60 min and 180 min after reperfusion. Doppler flow velocity spectra were analyzed on-line to determine time-averaged peak velocity (APV) during two cardiac cycles. After measurement of the baseline APV, the hyperemic APV for intra-coronary papaverine (10 mg) injection was recorded and the coronary flow reserve (CFR) was obtained as the ratio of hyperemic APV to baseline APV.

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### **Blood sample analysis**

Blood samples were obtained from CS at baseline and 180 min after reperfusion and stored at  $-80^{\circ}\text{C}$  until analysis. To assess myocardial damage, Creatinin kinase MB isozyme (CKMB) was measured with the aid of an Automated Chemiluminescence System (ADVIA Centaur; Bayer HealthCare, Leverkusen, Germany) with a detection limit of 5 ng/ml and the levels of cardiac troponin T (cTnT) were determined by means of a third-generation assay on an Elecsys 2010 (Roche Diagnostics, Indianapolis, IN), with a detection limit of 0.10 ng/ml. Production of 8-iso-prostaglandin  $\text{F}_{2\alpha}$  ( $\text{PGF}_{2\alpha}$ ) was quantified for assessment of the oxidative stress by using an 8-Isoprostane EIA KIT (Cayman Chemical Company, Ann Arbor, MI).

### **Measurement of myocardial area at risk and necrosis**

At the end of the experiment, LAD was reoccluded and 2% Evans blue was injected into the LV. Following euthanasia, the heart was rapidly excised, and cut transversely into 0.5 cm thick slices, which were then photographed to identify the ischemic risk regions (not stained blue) and incubated in 2% triphenyl tetrazolium chloride (TTC) for 20 min to delineate the area of necrosis. The ischemic and necrotic areas were traced and planimetered on each slice and the results summed to calculate the ratio of total necrotic area to ischemic area.

### **Histopathological assessment**

LV sections from the ischemic area from both groups were collected at the end of study 2 and fixed with 10% formalin for 48 hours, frozen in OCT compound, sliced into 5- $\mu$ m sections, and stained with hematoxylin-eosin for light microscopic examinations.

### **Determination of myocardial water content**

One aim of study 2 was to evaluate whether intra-coronary saline infusion may cause myocardial edema. Post-experimental myocardium samples of 0.3 g were obtained from both the subendocardial and epicardial side of the ischemic area. The tissue was weighed and desiccated for 48 hours at 80°C and the percentage of water content was calculated as  $(\text{wet weight} - \text{dry weight}) / (\text{wet weight}) \times 100$ .

### **Statistical analysis**

Statistical analysis was conducted with a commercially available software package (StatView ver.5.0, SAS Institute Inc., Cary, NC). The differences between variables of the two groups were compared by using Student's unpaired *t* test. Comparison of proportions [Meaning?] of pigs with sVT was performed with Fisher's exact test and two-way analysis of variance (ANOVA) was used to compare serial parameters. The differences at specific stages between the two groups were analyzed by one-way ANOVA, followed by Bonferroni's multiple-comparison *t* test. Results were expressed as the mean  $\pm$  SEM and a P value  $<0.05$  was considered significant.

## **Results**

## **Study 1**

### **Ameliorative effects of hypothermia on on-going myocardial ischemia**

One animal in the Hypothermia and three in the Normothermia group died of ventricular fibrillation (VF) and their data were excluded from analysis. The body weight and LV weight of the remaining animals (Hypothermia: 12, Normothermia: 12) showed no significant differences between the two groups.

### **Changes in myocardial temperature associated with regional hypothermia**

Figure 2A shows the changes in ischemic myocardial temperature. The baseline myocardial temperature of the two groups did not differ, but myocardial and rectal temperature of the Normothermia group did not change throughout the study, while myocardial temperature of the Hypothermia group started to decrease immediately after the start of cold saline infusion and reached a minimum ( $33.1 \pm 0.5$  °C; 3.2 °C absolute reduction) just before reperfusion. The temperature then increased gradually after reperfusion. Rectal temperature, on the other hand, remained constant for the Hypothermia group as well (data are not shown).

### **Incidence of arrhythmia**

The total number of VPBs was significantly less for the Hypothermia group than for the Normothermia group ( $246 \pm 66$  vs.  $476 \pm 30$ ,  $P=0.02$ ), as was the ratio of pigs with at least one episode of sVT during the study (33% vs. 73%,  $P<0.05$ , Fisher's exact test).

### **Hemodynamics and LV function**

Figure 3 shows the changes in hemodynamic parameters. Mean arterial pressure, heart rate and CO were similar for the two groups. Systolic function assessed by LVdP/dt<sub>max</sub> for the Hypothermia group was markedly preserved at 60min after reperfusion (103±10.4% vs. 73.8±6.7%, P<0.05). Diastolic performance assessed in terms of  $\tau$  did not show any statistical difference between the two groups.

### **Doppler flow wire assessment of coronary blood flow**

While baseline CFR of the two groups showed no difference either, the Hypothermia group maintained its baseline level throughout the study, but that of the Normothermia group had decreased significantly 60 min and 180 min after reperfusion (60min: 2.44±0.19 vs. 1.81±0.09, P<0.05; 180min: 2.58±0.22 vs. 1.99±0.14, P<0.05) (Fig. 4A).

### **Blood sample analysis for myocardial necrosis and oxidative stress**

Baseline concentrations of CKMB, cTnT and 8-iso-PGF<sub>2 $\alpha$</sub>  in coronary sinus blood showed no significant differences between the two groups. However, the increases in these indices 180 min after reperfusion were significantly less for the Hypothermia group ( $\Delta$ CKMB: 19.7±3.7 ng/ml vs. 38.5±7.7 ng/ml, P<0.05;  $\Delta$ cTnT: 0.84±0.19 ng/ml vs. 2.83±0.83 ng/ml, P<0.05;  $\Delta$ 8-iso-PGF<sub>2 $\alpha$</sub> : -2.09±1.98 pg/ml vs. 5.29±1.97 pg/ml, P=0.02) (Fig. 5A).

### **LV necrotic area in relation to ischemic risk area**



Figure 6A shows that, although the ischemic risk area was the same for both groups ( $12\pm 2\%$  vs.  $13\pm 1\%$ ,  $P=0.65$ ), the ratio of total necrotic area to risk area for the Hypothermia group was significantly lower than that for the Normothermia group ( $9\pm 2\%$  vs.  $36\pm 4\%$ ,  $P<0.0001$ ). Figure 7 is a scattergram of the relationship between the necrotic area and the risk area, which clearly demonstrates that regional hypothermia dramatically reduced the necrotic area regardless of the ischemic risk area. Figure 8 shows representative photographs of LV sections from both groups.

## **Study 2**

### **Hypothermia for extended myocardial infarction**

In study 2, one animal in the Reperfusion group died of VF and its data were excluded from analysis. Five animals each of the Hypothermia-reperfusion and Reperfusion group were thus evaluated.

Figure 2B shows the changes in myocardial temperature. In the Reperfusion group, myocardial temperature did not change during the study, while in the Hypothermia-reperfusion group, the ischemic myocardial temperature began to decrease after the start of cooling and reached a similar level to that of the Hypothermia group in study 1 ( $33.1\pm 0.4\text{ }^{\circ}\text{C}$ :  $3.4\text{ }^{\circ}\text{C}$  absolute reduction) even after complete deflation of the balloon. As for CFR levels, the Hypothermia-reperfusion group showed a higher CFR than that for the Reperfusion group 60 min after reperfusion (Fig. 4B). Blood sample analysis, demonstrated that  $\Delta 8\text{-iso-PGF}_{2\alpha}$  for the Hypothermia-reperfusion group was significantly

smaller than that for the Reperfusion group ( $-1.20 \pm 1.24 \text{ pg/ml}$  vs.  $3.50 \pm 0.87$ ,  $P=0.02$ ), while  $\Delta\text{CKMB}$  and  $\Delta\text{cTnT}$  showed no significant differences between the two groups ( $\Delta\text{CKMB}$ :  $40.7 \pm 9.9 \text{ ng/ml}$  vs.  $46.7 \pm 11.1 \text{ ng/ml}$ ,  $P=0.71$ ;  $\Delta\text{cTnT}$ :  $1.78 \pm 0.64 \text{ ng/ml}$  vs.  $2.26 \pm 0.27 \text{ ng/ml}$ ,  $P=0.56$ .) (Fig. 5B).

Although the ischemic risk area was nearly the same for both groups ( $12 \pm 1\%$  vs.  $13 \pm 3\%$ ;  $P=0.72$ ), the necrotic area for the Hypothermia-reperfusion group tended to be smaller than that for the Reperfusion group but without statistical significance ( $33 \pm 2\%$  vs.  $45 \pm 5\%$ ,  $P=0.08$ ) (Fig. 6B). Myocardial water content showed no difference between the two groups, indicating that saline infusion did not cause myocardial edema (Fig. 9). Histological examination with hematoxylin-eosin staining disclosed no significant differences in gross hemorrhage, microscopic hemorrhagic infarction or inflammatory cell infiltration between the two groups.

## Discussion

In this study, we achieved regional myocardial hypothermia by means of cold saline infusion via an OTWB catheter without accompanying hemodynamic deterioration or other adverse effects. Furthermore, hypothermia preserved CFR and dramatically reduced ongoing myocardial ischemia-related injury together with a reduction in ventricular arrhythmias and the extent of myocardial necrosis and attenuation of oxidative stress. Moreover, use of the infusion catheter, which makes it possible to infuse a larger amount of cold saline, enabled us to attain regional

hypothermia without coronary artery occlusion. Once MI was established, however, hypothermia could not provide the same beneficial effects for ischemic necrosis as in the ongoing ischemia model. On the other hand, considering that this method suppressed the increase in 8-iso-PGF<sub>2α</sub> and maintained the CFR level, regional hypothermia may have some cardioprotective effect even in the case of established MI.

### **Previous studies of hypothermia for the prevention of ischemic injury**

Hypothermia is currently an established method used not only for surgical procedures such as cardio-pulmonary bypass surgery and organ transplantation, but also in several high-risk clinical settings, including acute ischemic stroke, traumatic brain injury, and cardiac arrest (19-21). Although hypothermia has gained much attention primarily for its neuroprotective effects (19-21), recent researches have provided evidence of its efficacy for myocardial protection as well (13-16). Dave et al. succeeded in reducing the extent of myocardial infarcts by 49% with cold saline perfusion of the pericardiac cavity (16). They also demonstrated that hypothermia by direct application of an ice-filled bag to the risk zone initiated after 10 min of occlusion reduced infarct size by 50% (14). Although these studies clearly demonstrate the effectiveness of hypothermia for MI, the methods used to achieve hypothermia are too invasive to be implemented in clinical settings. Whereas systemic hypothermia attained with an endovascular cooling device for human acute MI has been shown to be safe, cooling of the ischemic myocardium is too slow, so that this method has

not yet proven itself to be effective (17). Additionally, many patients undergoing systemic hypothermia suffered from episodic shivering due to reduced core body temperature.

### **Advantages of catheter-based transc coronary myocardial hypothermia**

This study demonstrated that cold saline infusion into the MI-related coronary artery successfully lowered myocardial temperature. One advantage of our method is that the localized effect within the ischemic myocardium is enhanced while systemic effects are reduced. Moreover, this method entailed few complications such as hemodynamic instability, coronary vasoconstriction, and bradycardia. On the contrary, we found that hypothermia-treated pigs were electrically more stable than control-treated pigs. Although the mechanisms of the anti-arrhythmic effect of hypothermia remain uncertain, regional hypothermia may help suppress myocardial electrical irritability during ischemia and reperfusion.

In study 1, regional myocardial hypothermia effectively reduced the elevation of CKMB and cTnT levels after reperfusion. Histochemical studies showed that hypothermia led to a 75% reduction in infarct size compared with that for the Normothermia group. In spite of a modest decline in myocardial temperature, this cardioprotective effect was relatively pronounced compared to previously results reported for cooling of the epicardium. We suggest that the success of our method may be primarily attributable to the route used for cooling, since transc coronary hypothermia may lower myocardial temperature more homogeneously, and thus more effectively, than cooling

from the epicardium.

Transcoronary hypothermia may also improve microcirculation. The pigs treated with hypothermia in both study 1 and study 2 showed higher CFR in the infarct-related coronary artery than the normothermia-treated pigs after reperfusion. It has been suggested that CFR can be used as an indirect parameter for evaluating the function of coronary circulation (22), which involves both the epicardial coronary arteries (23) and microcirculation (24). Since the epicardial arteries showed no organic stenosis in our study, preservation of CFR in the hypothermia-treated pigs reflects better microcirculation. Dae et al. used sestamibi autoradiography to demonstrate that hypothermia preserves microvasculature functions (15), while Hale et al. showed that hypothermia significantly improves coronary reflow and reduces the no-reflow area in a rabbit MI model (25). Our finding is thus in agreement with that of previous studies, namely, that hypothermia appears to improve coronary flow in MI.

One possible mechanism for the myocardial protection provided by hypothermia involves diminished metabolic demand on the ischemic myocardium. Previous animal studies have shown that hypothermia increases myocardial ATP preservation during both ischemia and reperfusion (26). From the fact that a significant reduction in infarct size was seen only in study 1, the reduction in metabolic demand within the ischemic myocardium may be one of the major mechanisms of the cardioprotection provided by hypothermia. Using an isolated rat liver model of ischemia and

reperfusion, Zar et al. showed that hypothermic -perfusion reduced the formation of reactive oxygen species as well as post-ischemic vascular resistance compared with findings for normothermic-perfusion (27). It is further known that oxidative stress plays an important role in the deterioration of cardiac function (28), and that excessive stress induces tissue necrosis. Moreover, a previous study has shown that Vitamin C restores microcirculatory function in patients with cardiovascular risk factors (29). The findings of only our study therefore do not clearly rule out the possibility that the reduction in 8-iso PGF<sub>2α</sub> may be a result rather than mechanisms. It was demonstrated, however, that isoprostans themselves possess biological activities such as vasoconstriction (30), and free radicals are thought to mediate reperfusion injury. These findings thus seem to support the hypothesis that the cardioprotective effect of hypothermia is in part due to reduced oxidative stress.

### **Limitations**

In this study, the duration of coronary occlusion was 60 min, which is unrealistic for acute human MI. Because pigs tend to be much more frail than humans when it comes to ischemia, nearly all cells in the ischemic area in pig hearts become necrosed after only 75min of coronary occlusion (31). We therefore reduced the occlusion time to avoid death from heart failure and arrhythmia. In human, however, the myocardial necrosis generally develops more slowly due to the greater collateral blood

flow. Hypothermia may thus be beneficial for humans even when initiated later in the ischemic period. As for clinical application, a tool for monitoring the temperature, such as a thermo wire, would be helpful to keep the myocardial temperature within therapeutic levels.

On the basis of our preliminary findings, we believe that further experimental and clinical trials are warranted to determine whether adjunctive hypothermia therapy can limit infarct size during reperfusion therapy for MI.

### **Conclusion**

We successfully achieved catheter-based regional hypothermia within the ischemic myocardium. This method preserved CFR and attenuated oxidative stress, which may be beneficial for the recovery of cardiac function in acute MI. We speculate that transc coronary myocardial hypothermia may be an effective therapy especially for patients with acute MI who are susceptible to ischemia-reperfusion injury.

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## Figure Legends

**Figure 1.** Study protocol over time. A: Coronary artery was occluded for 60 min. In study 1, intra-coronary saline infusion (4°C or 36.5°C, 2.5 ml/min) with balloon inflation was started 15 min after coronary occlusion, and continued for 15 min after balloon deflation. In study 2, hypothermia with reperfusion (4°C, 8 ml/min) was initiated from 60 min after occlusion and compared with reperfusion without hypothermia. Blood samples were obtained at baseline and at the end of the protocol. Coronary flow measurements were performed at baseline, 60 min and 180 min after reperfusion. B. Angiographic frame showing the location of catheters, thermister (black arrow), and over-the-wire type percutaneous transluminal coronary angioplasty balloon (white arrow). C. Schematic representation of regional myocardial hypothermia in the study.

**Figure 2.** Changes to baseline in study 1 (A) and in study 2 (B). Data are expressed as mean  $\pm$  SEM.

**Figure 3.** Time course of hemodynamic parameters in study 1. The P value by two-way ANOVA: group difference  $<0.01$  for  $LVdP/dt_{max}$ . \* indicates  $P<0.05$  vs. Normothermia group at the same stage by Bonferroni's multiple-comparison *t* test. Data are expressed as mean  $\pm$  SEM. MAP: Mean arterial pressure, HR: Heart rate, CO: Cardiac output,  $LVdP/dt_{max}$ : Peak positive first derivative of left ventricular pressure, tau: Time constant of LV relaxation.

**Figure 4.** Serial changes in coronary flow reserve (CFR) in study 1 (A) and in study 2 (B). P values by two-way ANOVA. Group  $<0.001$ ; time course  $<0.001$ , group-time course interaction  $<0.001$  for

CFR from both studies. Data are expressed as mean  $\pm$  SEM. \* indicates  $P < 0.05$  vs Normothermia or Reperfusion group at the same stage, and † indicates  $P < 0.05$  vs baseline within the same group by Bonferroni's multiple-comparison *t* test.

**Figure 5.** Comparisons of  $\Delta$ CKMB,  $\Delta$ cTnT, and  $\Delta$ 8-iso-PGF<sub>2 $\alpha$</sub>  for the two groups in study 1 (A) and in study 2 (B). CKMB: Creatinin kinase MB isozyme; cTnT: Cardiac troponin T; 8-iso-PGF<sub>2 $\alpha$</sub> : 8-iso-prostaglandin F<sub>2 $\alpha$</sub> .  $\Delta$  indicates the change in values between baseline and 3hr after reperfusion.

Data are expressed as mean  $\pm$  SEM.

**Figure 6.** Ischemic risk area and necrotic area in study 1 (A) and study 2 (B). LV: Left ventricle,

Data are expressed as mean  $\pm$  SEM.

**Figure 7.** Scatter plot of % necrotic area and % ischemic risk area in study 1.

Regional hypothermia dramatically reduced the necrotic area regardless of the size of the ischemic risk area.

**Figure 8.** Ischemic and necrotic myocardium of representative cases. Upper panels show the ischemic risk myocardium (not stained blue), and lower panels the necrotic myocardium (white region). The heart treated with Normothermia showed a larger area of necrosis than did the one treated with Hypothermia.

**Figure 9.** Tissue water content of myocardium from subendocardial and epicardial side in Reperfusion and Hypothermia-reperfusion groups. Data are expressed as mean  $\pm$  SEM.

# Figure 1

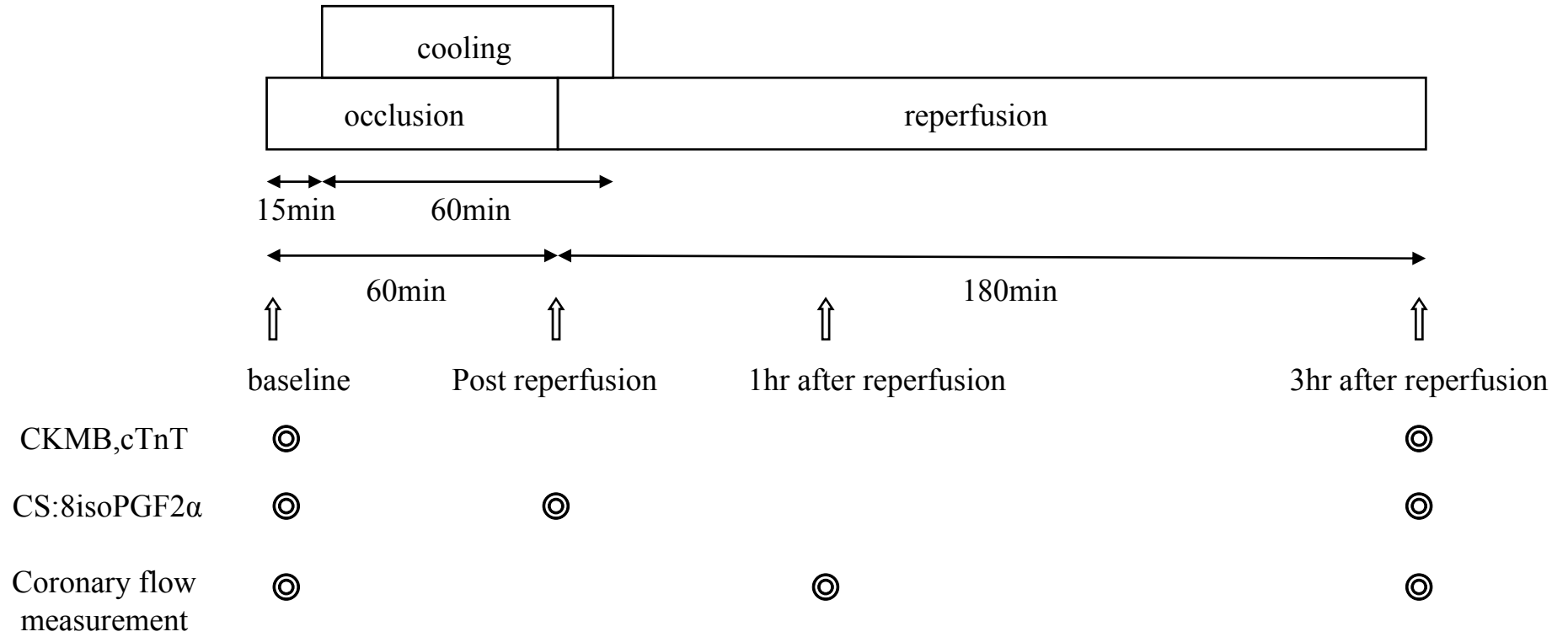


Figure 2

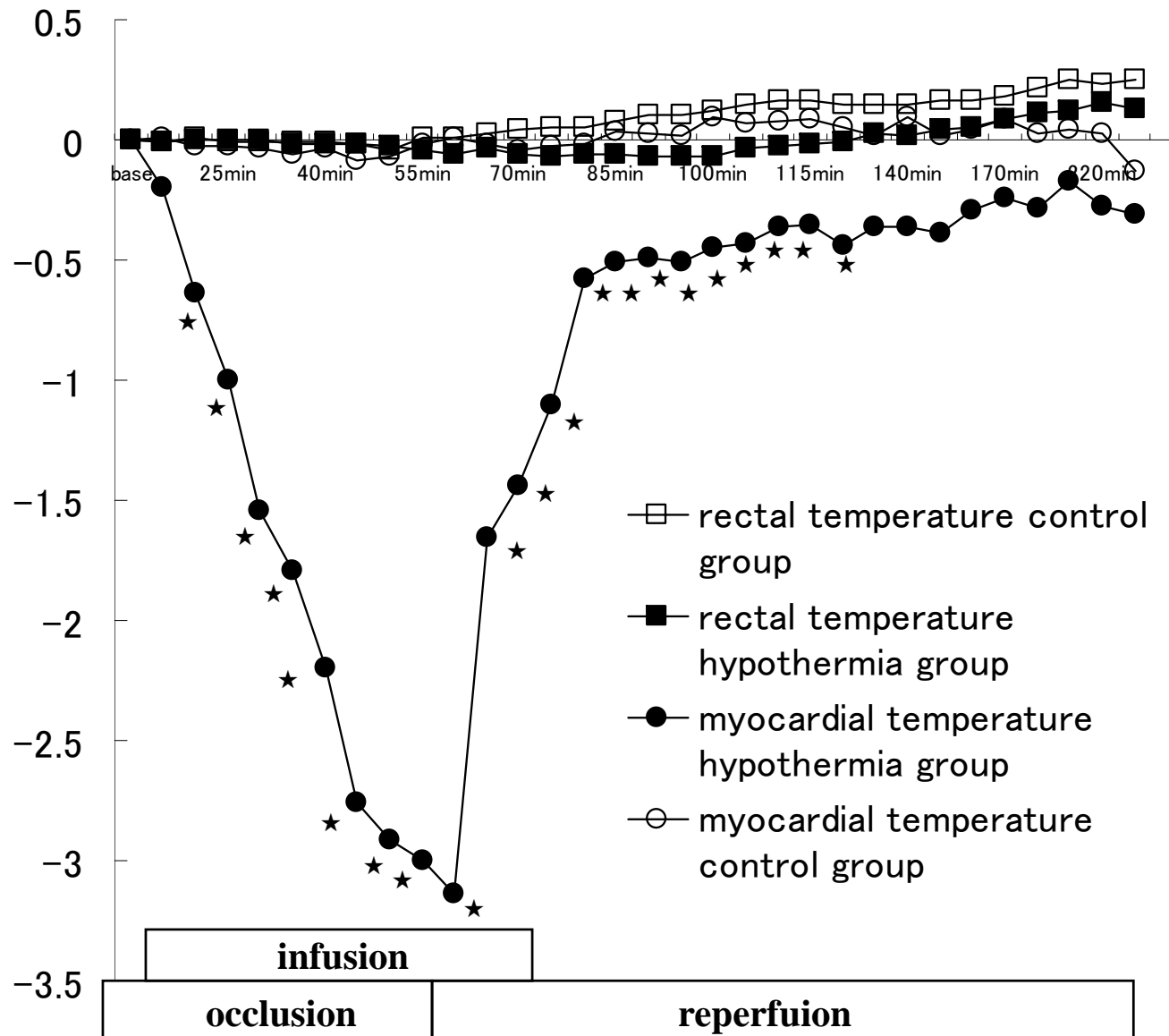
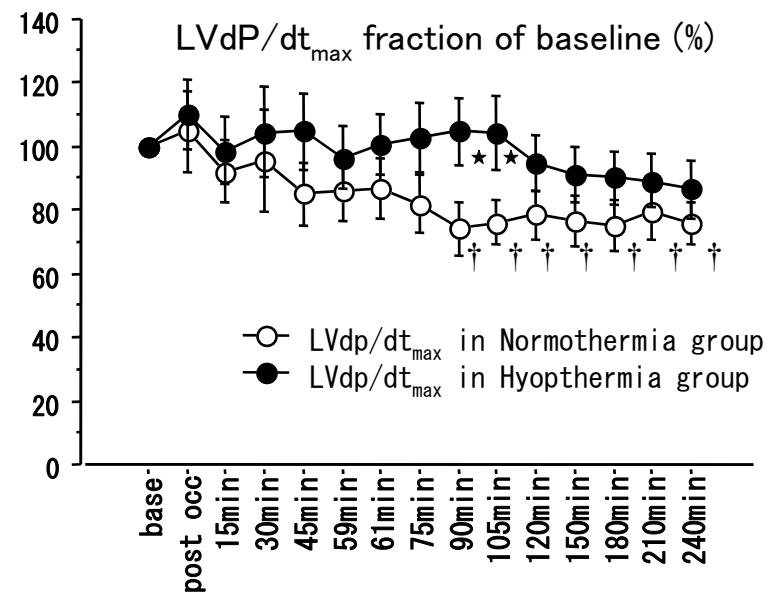
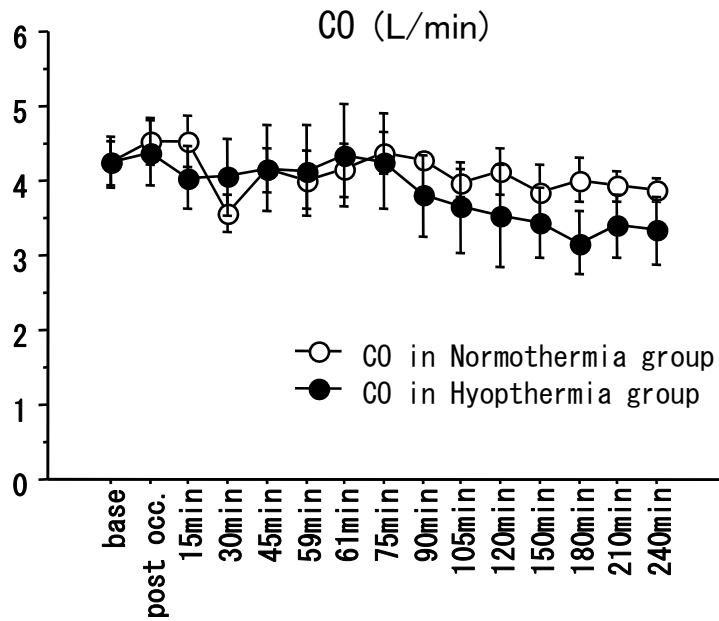
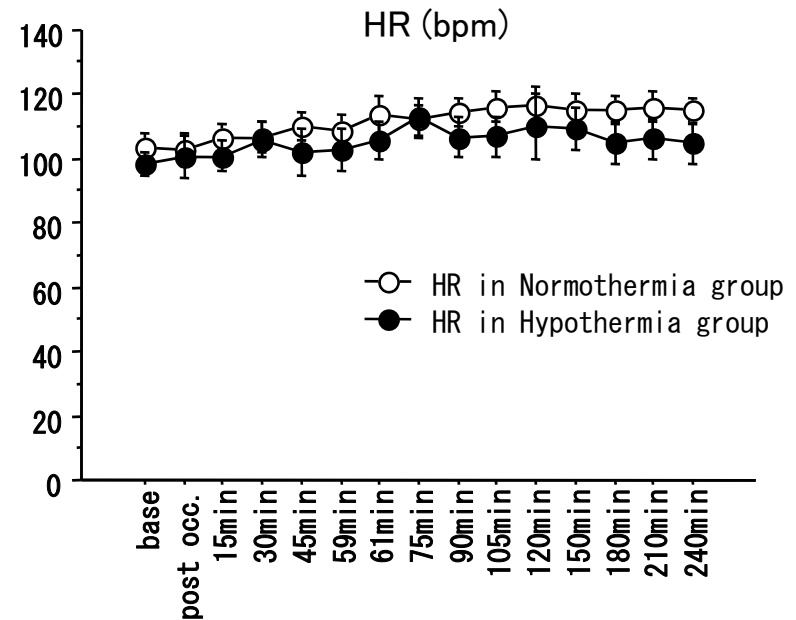
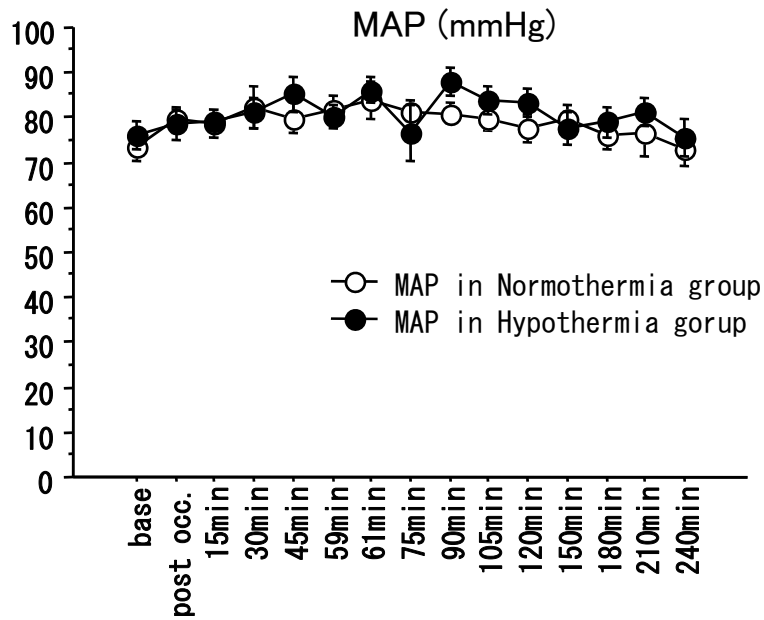
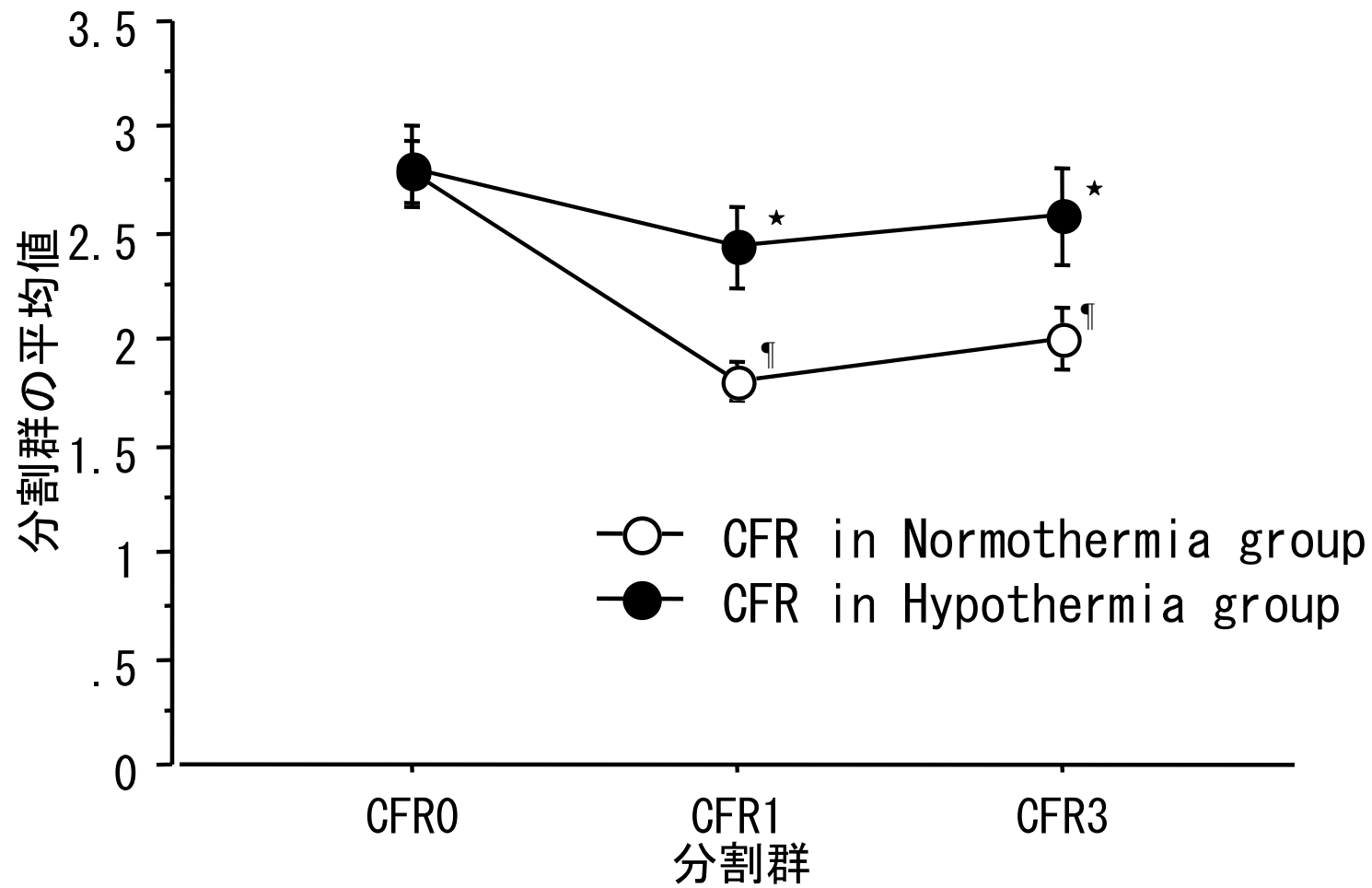




Figure 3: Serial changes of Hemodynamic parameters

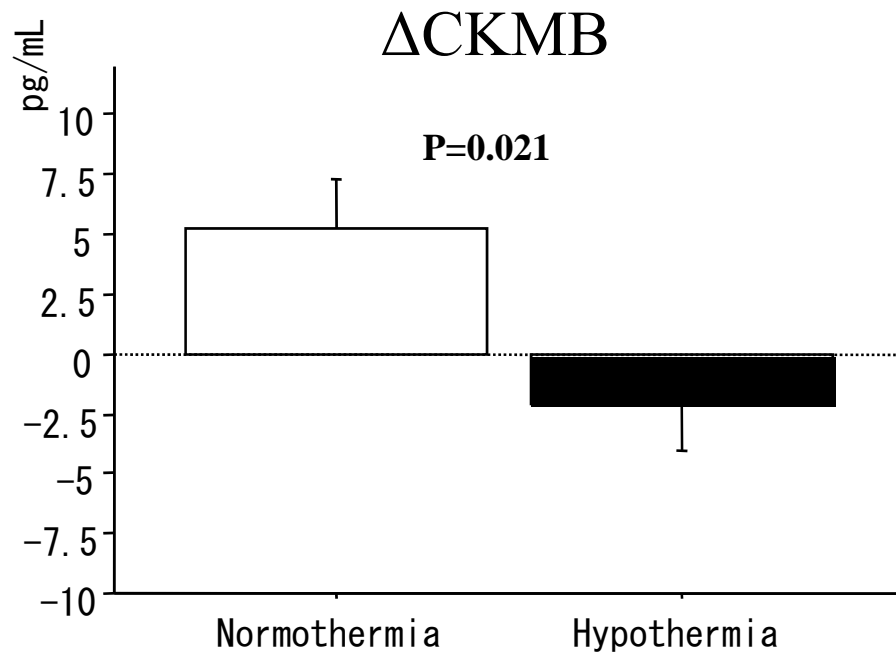
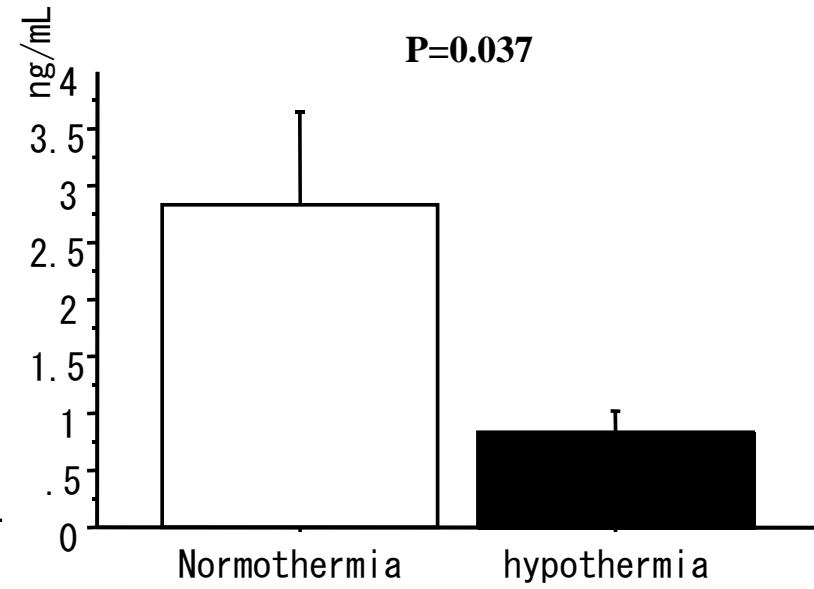
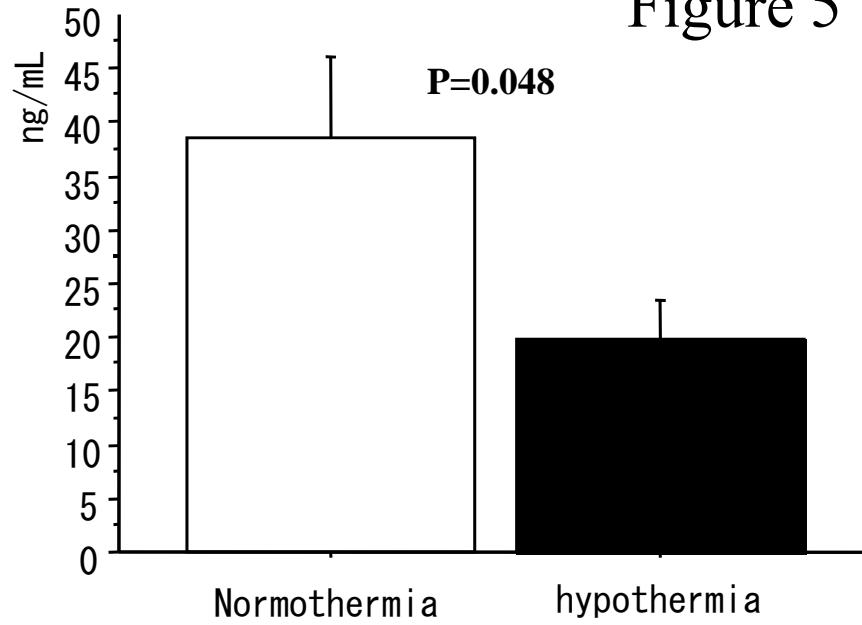




★ P<0.05 vs control under same condition.

‡ P<0.05 vs baseline within same group.

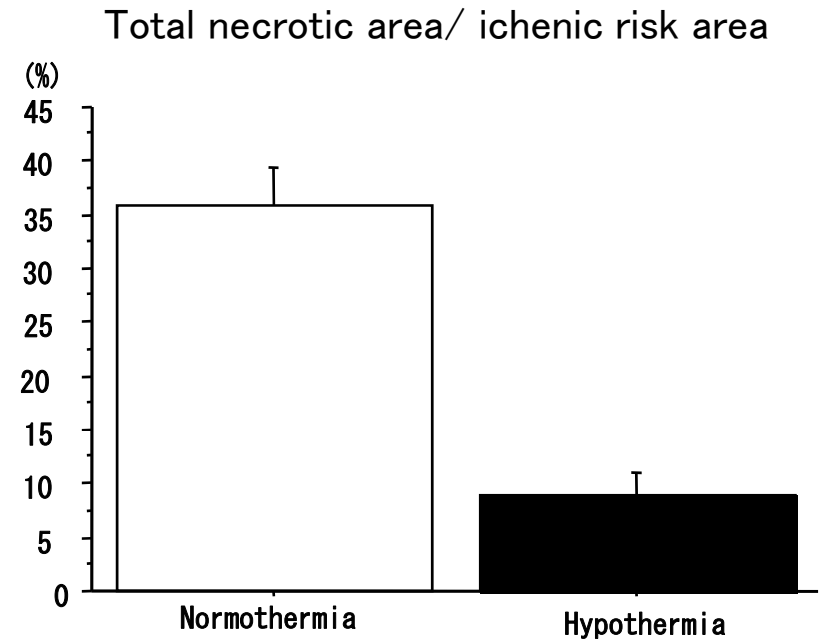
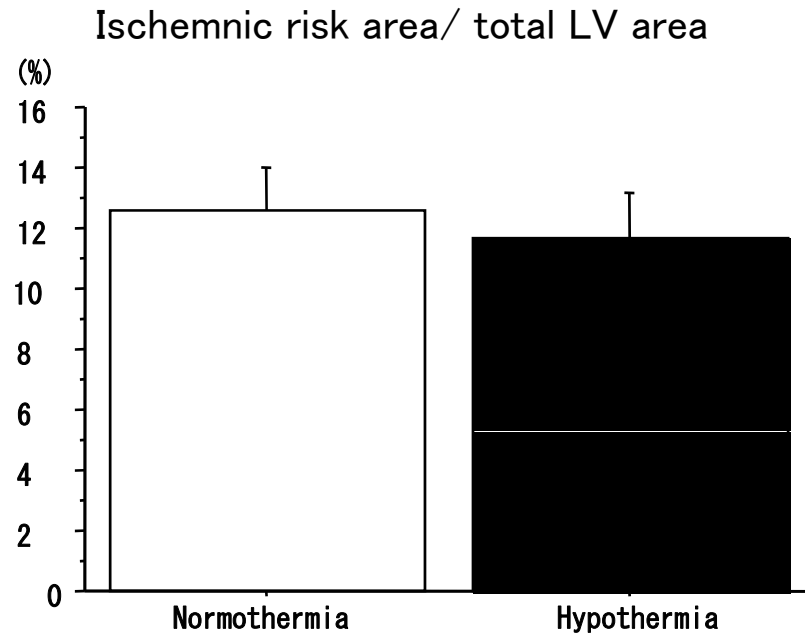
Figure 5



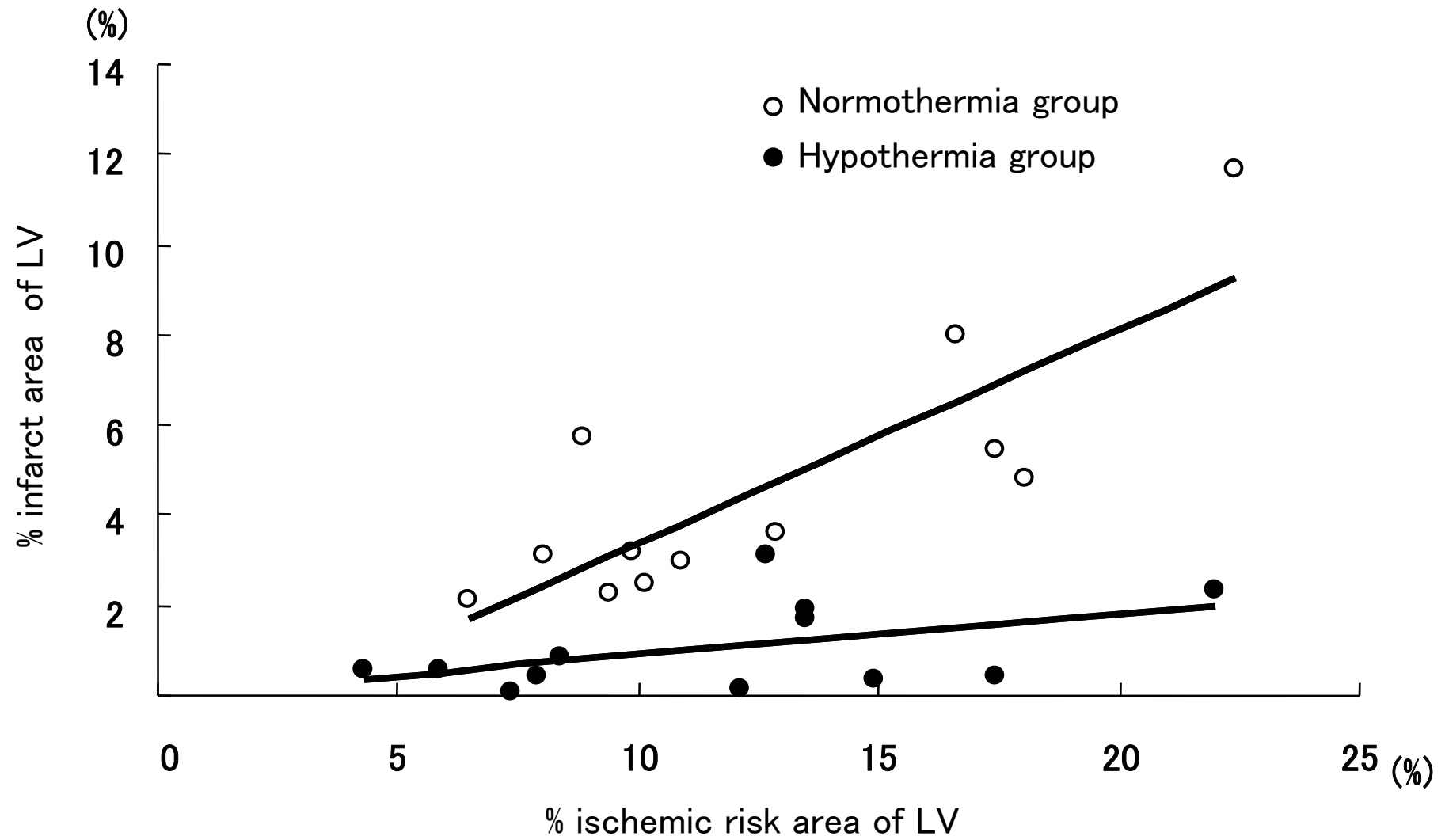
$\Delta$ trop T

$\Delta$ 8iso-PGF2 $\alpha$

Figure 6 ischemic risk area and necrotic area in H and N group



**Figure 7: Risk area and infarct area (% of LV)**



**Figure 8: representative case of ischemic myocardium and infarct myocardium.**

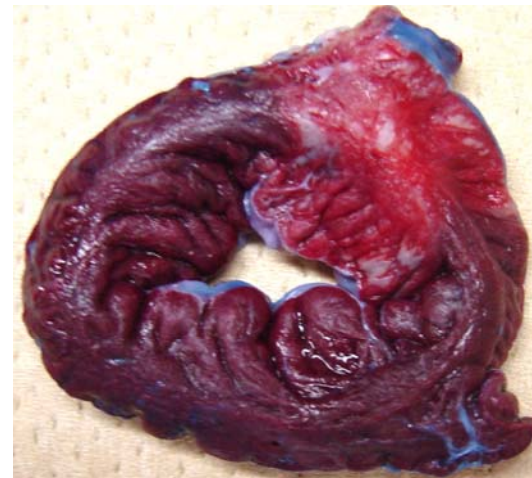
Normothermia group

Hypothermia group

Ischemic myocardium



Necrotic myocardium



# Figure 9

