Synergistic effects of remote perconditioning with terminal blood cardioplegia in an in vivo piglet model.

Takayuki Abe[1], Kiyozo Morita[1,*], Gen Shinohara[1], Kazuhiro Hashimoto[1] and Masako Nishikawa[2]

[1]Department of Cardiac Surgery, Jikei University School of Medicine, Tokyo, Japan.

[2]Biotatistician, Clinical Research Support Center, Jikei University School of Medicine, Tokyo, Japan.

Corresponding author:

Kiyozo Morita

Department of Cardiac Surgery

Jikei University School of Medicine

Tokyo, Japan

Tel: +81-3-34331111

Fax: +81-3-34331175

E-mail: kiyozo@jikei.ac.jp

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23 **ABSTRACT**

24 **Objectives:** This study tested the hypothesis that remote perconditioning offers effective and
25 synergistic cardioprotection to terminal warm blood cardioplegia for prompt ventricular
26 recovery after prolonged cardioplegic arrest in an *in vivo* piglet model.

27 **Methods:** Twenty-four piglets were subjected to 120 min of single-dose cardioplegic arrest, and
28 were divided into 4 groups according to the mode of reperfusion: control (simple aortic
29 unclamp), remote perconditioning, terminal warm blood cardioplegia, or remote
30 perconditioning + terminal warm blood cardioplegia; remote perconditioning (4 cycles of 5-min
31 ischemia-reperfusion of the lower limb) was applied prior to aortic unclamping. Left-ventricular
32 systolic and diastolic functions were assessed by pressure-volume loop analysis at baseline and
33 after 60 min of reperfusion. Biochemical injury was evaluated by plasma troponin-T level.

34 **Results:** The control group showed decreased end-systolic elastance, preload recruitable stroke
35 work and inverse of end-diastolic P-V relationship of 51.3±14.0%, 46.1±22.5% and 34.8±14.9%,
36 respectively. Percentage recovery of end-systolic elastance and preload recruitable stroke work
37 were significantly better with terminal warm blood cardioplegia (with or without remote
38 perconditioning) (end-systolic elastance: 95% confidence interval, 38.6-84.1; preload
39 recruitable stroke work: 95% confidence interval, 0.4-54.3). Percentage recovery of inverse of
40 end-diastolic P-V relationship was significantly better in the remote perconditioning groups

(with or without terminal warm blood cardioplegia) (95% confidence interval, 1.6-41.6). No synergistic effects of remote perconditioning and terminal warm blood cardioplegia on troponin-T release were noted.

Conclusions: Remote perconditioning offers promising synergistic cardioprotection to terminal warm blood cardioplegia, implicating potential clinical benefit by contributing to prompt left ventricular functional recovery during pediatric open-heart surgery.

Keywords: remote perconditioning; cardioplegic arrest; terminal warm blood cardioplegia; reperfusion injury; ischemic conditioning; cardioprotection

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INTRODUCTION

Remote perconditioning (rPerC) was first proposed in 2007 by Schmidt et al. [1] as a novel endogenous cardioprotective strategy in which brief, repeated ischemic stimuli are applied to a distant organ (i.e., a limb) during ischemia of a target organ (myocardium) prior to the onset of reperfusion. In an *in vivo* porcine model of 40 min of left anterior descending coronary artery occlusion with 120 min of reperfusion, Schmidt et al. [1] demonstrated that rPerC at the time of established myocardial ischemia reduced the extent of infarction and associated with improved functional indexes by a K_{ATP} channel-dependent mechanism; they proposed the term “remote perconditioning”. The protocols for remote ischemic per- and postconditioning have been extensively investigated and numerous studies of *in vivo* coronary ligation models in various species have revealed a universal protective effect against myocardial infarction [2]. More recently, this strategy has been adopted in humans and clinical studies of percutaneous coronary intervention [3] and cardiac surgery [4]. The benefits of rPerC on troponin release have been demonstrated.

However, its effects on myocardial function other than the anti-necrotic effect have not been evaluated in previous investigations [2,3,4]. Therefore, we argue that the real clinical role of rPerC during open-heart surgery can be confirmed practically if its benefit on global myocardial function is demonstrated in conjunction with the clinically available myocardial protection

strategies. Terminal warm blood cardioplegia (TWBCP), first advocated by Buckberg et al. [5], is a well-established representative procedure for reperfusion injury to resuscitate damaged myocardium during ischemia and constitutes an essential part of "integrated myocardial protection" in clinical practice [6]. The current study tested the hypothesis that rPerC in the lower limb offers effective and synergistic cardioprotection with TWBCP for prompt ventricular recovery after prolonged cardioplegic arrest in an *in vivo* piglet model, especially relevant to pediatric patients.

MATERIALS AND METHODS

All experimental animals received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals" (National Institutes of Health Publication No. 85-23, revised 1996). The present study was approved by the Animal Care and Use Committee of the Jikei University of Medicine.

Experimental Preparation

Twenty-four White-Mandrake-Durex female piglets (weight, 16-19 kg; age, 7-9 weeks) were initially anesthetized with intramuscular medetomidine hydrochloride and butorphanol tartrate.

After tracheostomy, each piglet was ventilated with a volume-cycled respirator using oxygen, air, and 1-1.5% isoflurane. After midline sternotomy, a conductance catheter (Millar Instruments, Houston, TX, USA) was inserted into the left ventricle (LV) from the apex to measure cardiac volume and pressure. A snare was passed around the inferior vena cava for preparing reduction of preload to change pressure-volume (P-V) loops. The Mills Pressure-Volume Loop System (Millar Instruments, Houston, TX, USA) and Power Lab (AD Instruments, Sydney, Australia) were used to collect data on a series of LV pressure-volume correlation data during vena cava occlusion. After heparinization (0.3 ml/kg), a 12-Fr arterial cannula was positioned in the aortic arch via the right carotid artery and a 24-Fr cannula was placed in the right atrium. The cardiopulmonary bypass (CPB) circuit was primed with heparin sodium, hydroxyethylated starch, D-mannitol, sodium bicarbonate, 20% albumin, and methylprednisolone (30 mg/kg) without homologous blood transfusion. The CPB system consisted of an extracorporeal membrane oxygenator (HPO-06RHF-CP; Senko Medical Instrument Mfg., Tokyo, Japan) and extracorporeal pump (HAS-P100; Senko Medical Instrument Mfg.). An 8-Fr cannula was advanced into the left atrium for venting the left side of the heart during cardiac arrest. An aortic root cannula with side branches for administration of cardioplegic solution, pressure monitoring, and venting was inserted into the aortic root.

106 **Experimental Protocol**

107 Following animal preparation and instrumentation, baseline control measurements of all
108 parameters were obtained. CPB was initiated at a flow rate of approximately 2.2 L/min/m^2,
109 representing 70 ml/kg/min. Arterial blood pressure was maintained at 60 mmHg, with a pH of
110 7.35 to 7.45, oxygen tension greater than 150 mmHg, and carbon dioxide tension of 35-45
111 mmHg, under systemic normothermia. An aortic cross-clamp was applied approximately 10 min
112 after starting CPB, and cardioplegic arrest was initiated with 400 mL of St Thomas Hospital II
113 solution (Miotector; Mochida, Tokyo, Japan) infused within 3 min at an aortic root pressure of
114 50-80 mmHg. Temperature of cardioplegia was 8 °C. Topical cooling with ice shavings was
115 performed. The heart was subjected to 120 min of global ischemia without additional
116 cardioplegia. No inotropic or vasoactive drugs were used. The heart was kept in an empty
117 beating state for 30 min after reperfusion, then the piglet was weaned from CPB and observed
118 for 30 min up to 60 min of total reperfusion period. After post ischemic cardiac function
119 measurements and blood sampling, the piglets were sacrificed under deep anesthesia.

120

121 **Experimental Groups**

122 Piglets were divided into the following groups on the basis of the method of reperfusion (Fig.
123 1).

Group I (Control): This group underwent uncontrolled reperfusion. In 6 piglets, the cross clamp was simply removed, and the heart was reperfused at a perfusion pressure of 50-70 mmHg. No cardioprotective technique was applied with reperfusion.

Group II (rPerC): In 6 piglets, a rPerC technique in the lower limb was performed 40 min before reperfusion. Remote perconditioning consisted of 4 cycles of 5 min of lower limb ischemia using a digital tourniquet. Cuff pressure was set at 250 mmHg during ischemia. This protocol for rPerC was determined based on previous studies that compared different perconditioning algorithms [7] or tested different allocation timings [1,4,7,8].

Group III (TWBCP): In 6 piglets, after 120 min of ischemia, TWBCP (37°C) was performed 5 min prior to the onset of aortic unclamping, at a dose of 10 ml/kg/min for 5 min and an aortic pressure of 30-40 mmHg. For TWBCP, the St. Thomas Hospital Solution II was used, with added aspartic acid, bicarbonate, and a citrate-phosphate-dextrose solution to make a hypocalcemic (0.54 ± 0.03 mmol/l), alkalotic (pH: 7.361 ± 0.092), and substrate-enriched blood cardioplegia solution (4:1; blood/crystalloid, Hb level, 7.8 ± 2.2 g/dl). Aspartic acid is added to replenish the substrate depleted during ischemia.

Group IV (rPerC+TWBCP): Both rPerC and TWBCP techniques were performed as described above.

Evaluations

Myocardial performance

A series of LV P-V loops was obtained by the Mills Pressure-Volume Loop System and Power Lab through transient occlusion of the inferior vena cava by tightening and releasing a tape snare during an 8-s period of apnea at baseline before the onset of CPB and after 60 min of reperfusion. LV performance was assessed by P-V loop analysis with LabChart7 software (AD Instruments, Sydney, Australia) and functional recovery of values after reperfusion were assessed as percentages of the respective baseline values.

Left-ventricular contractility: LV contractility was assessed from the slope of the end-systolic pressure-volume relationship by linear regression analysis, end-systolic elastance (Ees) and preload recruitable stroke work (PRSW) as the slope of the linear regression between EDV vs. LV stroke work.

Diastolic compliance: The slope of the end-diastolic P-V relationship (EDPVR) was calculated from a series of P-V loops through transient occlusion of the inferior vena cava, and LV compliance was assessed as the inverse of EDPVR.

Biochemical parameters: plasma troponin-T

Arterial blood samples were acquired just after initiation of CPB, and after 1, 10, 30 and 60 min of reperfusion or unclamping. Biochemical myocardial injury was determined by measuring plasma troponin-T concentrations using the electrochemiluminescent immunoassay method.

160

161 **Statistical Analysis**

162 The sample size was calculated based on the inverse of EDPVR. A total sample size of 24
163 animals was required to detect a mean increase of recovery of 25% with the rPerC treatment,
164 assuming a common standard deviation (SD) of 20% and a power of 80% with a two-sided
165 significance level of 0.05. We also assumed that the effect of the combination treatment when
166 compared with TWBCP alone is the same as that of the single treatment with rPerC compared
167 with control. As functional assessments were not feasible due to hemodynamic instability after
168 the termination of CPB in one experiment in group II, those data were imputed as the half value
169 of the worst data among all the piglets to reflect a low value. Data are summarized as mean
170 value and SDs, or 95% confidence intervals (CIs) for continuous variables.

171 The primary analysis was conducted by using the two-sample *t*-test to compare the rPerC
172 treatments versus non-rPerC treatments. For the sensitivity-analyses, we performed analysis of
173 variance (ANOVA) with rPerC (with/without) and TWBCP (with/without) as factors. If rPerC
174 was indicated as a significant factor, we perform ANOVA with the same two factors and their
175 interaction. As some data were imputed, the normality of the residual distribution was examined
176 using the normal quantile-quantile plot to confirm the assumption of the parametric analyses.
177 All statistical analyses were two-sided, and a p value <0.05 was considered statistically

178 significant. Analyses were performed with SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

179

180 **RESULTS**

181 Five piglets out of the total 29 piglets used in this study did not tolerate surgical preparation or
182 experienced technical difficulty in measuring correct cardiac function. The remaining 24 piglets
183 were allocated to 4 groups for analyzing data. Baseline data were analyzed by performing
184 one-way ANOVA and no significant difference existed in baseline absolute values among
185 groups.

186

187 **Left Ventricular Systolic Function: End-systolic Elastance**

188 TWBCP with or without rPerC showed a significant effect on systolic functional recovery,
189 percentage recovery in Ees (mean difference, 61% recovery; 95% CI, 38.6 - 84.1, $p<0.0001$,
190 ANOVA) (Fig. 2). No statistically significant difference in Ees was found between the group
191 treated with and that treated without rPerC (mean difference, 10.1% recovery; 95% CI, -25.0 -
192 45.1, $p=0.56$, t-test). RPerC in conjunction with TWBCP showed a modest effect in comparison
193 with the effect of TWBCP alone (122.8%recovery vs. 99.9%recovery).

194 **Left Ventricular Systolic Function: Preload Recruitable Stroke Work**

Significant effects on systolic functional recovery as a percentage recovery of PRSW were found (mean difference, 20.9% recovery; 95% CI, 0.4 - 54.3, p=0.047, ANOVA) (Fig. 3). No statistically significant difference in PRSW was found between the group treated with rPerC and that treated without rPerC (mean difference, 20.9% recovery; 95% CI, -8.0 - 49.8, p=0.15, *t*-test). RPerC in conjunction with TWBCP showed a modest effect in comparison with the effect of TWBCP alone (94.3%recovery vs. 70.3%recovery).

Left Ventricular Diastolic Function: Inverse of End-Diastolic Pressure-Volume Relationship

RPerC showed a significant effect on diastolic compliance recovery; percentage recovery of the inverse of EDPVR (p=0.036, *t*-test and; p=0.035, ANOVA, respectively) (Fig. 4). The mean difference in percentage recovery between the group treated with rPerC and that treated without rPerC was 21.6% (95% CI, 1.6 - 41.6 (*t*-test) and 1.7 - 41.2 (ANOVA), respectively). RPerC showed a marked percentage recovery in the inverse of EDPVR both with and without TWBCP (67.5%recovery vs. 65.2%recovery).

For examining the normality of the residual distribution, the *t*-test and the ANOVA were robust. Next, we performed ANOVA with the same two factors and their interaction; however, the

interaction was not significant. The Wilcoxon rank sum test was performed as a sensitivity analysis and similar results were obtained for both systolic and diastolic function.

Biochemical Parameters

The profile of plasma troponin-T levels over time is presented in Fig. 5. We performed a repeated measures analysis of variance, where with/without rPerC and with/without TWBCP were between-subject effects, and time, all interactions of time, and between-subject effects were within-subject effects. Troponin-T showed a clear significant increase over time. There was no significant difference in the upward trend with or without rPerC or TWBCP.

DISCUSSION

The present study demonstrates that rPerC, induced by intermittent limb ischemia administered in the late phase of established myocardial ischemia prior to the onset of reperfusion, exerts synergistic cardioprotective effects with TWBCP on LV systolic and diastolic functional recovery after global ischemia/reperfusion in an *in vivo* piglet model of prolonged single-dose cardioplegic arrest simulating pediatric open heart surgery. This study represents a novel experimental investigation testing the benefit of rPerC after global myocardial ischemia in an *in vivo* study using CPB, in contrast to previous studies [1,2]. We confirmed clinical benefits of

this novel reperfusion strategy, as a rescue or trouble-shooting strategy in situations where ischemic intervals are unexpectedly prolonged or myocardial protection is technically inadequate.

Remote vs local ischemic conditioning

We recently demonstrated that ischemic postconditioning (IPO) promotes LV systolic and diastolic function recovery after prolonged cardioplegic arrest [9]. Local IPO to the heart is a simple and reproducible procedure, but may have the disadvantage of inducing additional myocardial ischemia. In practical terms, the invasive technique of repetitive manipulation of the aortic cross clamp may increase the risk of aortic dissection and crucial embolism in clinical cardiac surgery. Given these concerns about the application of IPO, a novel, alternative approach to reduce ischemia reperfusion injury of a target organ (i.e., myocardium) is "remote ischemic conditioning" (rIC) induced by brief repetitive ischemia and reperfusion in distant organs, as first reported in 2000 by Oxman et al. [10]. Remote conditioning stimuli in various organs have been experimentally evaluated, including occlusion of the renal artery [11], carotid artery [12], femoral artery [13], and the opposite left coronary artery (intra-organ remote conditioning) [14], in addition to the upper or lower limbs as the most noninvasive method [2, 10].

Mode of remote ischemia conditioning: the rationale of rPerC and its applications

Application of rIC can be made before target organ (i.e., myocardial) ischemia (remote preconditioning) or at the onset of reperfusion (remote postconditioning), and can also even be applied during target organ ischemia (rPerC). Among the different settings (timings), rPerC, first proposed in 2007 by Schmidt et al. [1] offers even more beneficial features from a practical perspective, since an adequate set-up interval must be provided to provoke a protective pathway or mechanism within the target organ from the remote ischemic stimulus at the onset of reperfusion injury. Basalay et al. [13] compared the efficacy of remote conditioning at different timings (i.e., remote pre-, per-, and post-conditioning) in a rat coronary occlusion model, and demonstrated comparable reductions in infarct size between protocols, but not with delayed application of post-conditioning. Since the first report in 2007, experimental studies on the protective effects of rPerC against infarct size have been reported in various species [2]. To date, the majority of clinical applications of rPerC have been randomized clinical trials in myocardial infarction patients who underwent primary percutaneous coronary intervention or thrombolysis [2,3,15]. Transient upper or lower limb ischemia using simple blood pressure cuffs is a simple noninvasive stimulus with important potential clinical applications and an attractive cost-benefit ratio. Application of rPerC has recently been translated to cardiac surgery. Li et al. [4] applied either remote preconditioning (rIPC) or rPerC by cuff occlusion of the lower limb in adult patients undergoing valve replacement. Although peak plasma troponin-I levels and the

incidence of ventricular fibrillation in the rPerC group were lower compared with either control or rIPC groups, beneficial effects on myocardial function recovery and hemodynamic status have not been established. To date, no additional data is available regarding the influence of rPerC on myocardial function recovery particularly concerning the clinical protocol of myocardial protection. The benefits demonstrated in the present experimental study cannot be reproducible in clinical trials with a standard protection protocol. Nevertheless we think that this combination strategy with TWBCP and rPerC deserves consideration as a viable option for rescue or trouble-shooting strategy, if rPerC is proved as a safe intervention in a standard clinical setting of cardioplegia.

Optimal algorithms and timing of rPerC

The most important factors influencing the outcomes of perconditioning are assumed to be the timing of treatment and algorithms (length of cycles). In contrast to local postconditioning, in which optimal algorithms are largely species-dependent [9], an almost universal protocol has been successfully applied for rPerC to provide infarct reduction among different species [2]. Xin et al. [7] compared different perconditioning algorithms in an experimental study and demonstrated that only 3-4 cycles of 5-min ischemic-reperfusion (I-R) resulted in reduced infarct size, whereas no cardioprotection was provided by 1-2 cycles of 10-min I-R or four cycles of 1- to 3-min I-R. Several other studies, including the present analysis (4 cycles of 5 min

each I-R), have confirmed these findings [2]. Among different allocation timings (i.e., early, late or continuous) [1,4,7,8], late-phase timing is the most widely used method, because the influence of mediators induced by remote conditioning can reach the myocardium only after unclamping of the aorta. The highest concentration of transportable mediators can be achieved by this procedure.

Based on information available in the literature, we applied rPerC (4 cycles of 5 min) using lower limb compression during the last phase of 120 min of ischemia from 40 min prior to the onset of reperfusion (aortic unclamping) to obtain maximum benefit. In order to trigger the mechanism linking distant and target organs, an adequate stimulus from the remote organ is essential. In our pilot study, remote stimuli induced by occlusion of the femoral artery or abdominal aorta did not provide any benefits, unlike tourniquet compression of the lower limb; such results may imply the importance of the intensity of remote-organ ischemia over the extent of the ischemic area in provoking a conditioning pathway.

Functional and biochemical outcome

The key finding in the present study was that rPerC in addition to TWBCP reduced both systolic and diastolic LV dysfunction after prolonged cardioplegic arrest. In a coronary ligation model, Schmidt et al. [1] demonstrated that rPerC preserves global systolic and diastolic function, and showed that the protective effects of rPerC were abolished by administration of glibenclamide a

302 K_{ATP} channel inhibitor. TWBCP, which was advocated by Buckberg et al. [5,6] is a standard
303 and well-known representative procedure for reperfusion injury and constitutes an essential part
304 of 'integrated myocardial protection' in clinical practice [6]. The rationale of using TWBCP is
305 the active resuscitation of the ischemically damaged, substrate-depleted heart by maximizing the
306 kinetics of repair and minimizing oxygen demands by maintaining arrest [5,16]. Although
307 TWBCP has been shown to accelerate myocardial metabolic recovery characterized by a more
308 rapid shift to aerobic metabolism and better preservation of tissue ATP concentration [16], it
309 may be insufficient to protect against inevitable reperfusion injury after prolonged ischemia
310 with this modality alone. The present study did not confirm rPerC effects on myocardial
311 biochemical injury.
312 We speculate that the short time span for measuring troponin release might be one of the reasons
313 for the absence of biochemical benefits of any of the tested interventions, in addition to the
314 small number of experiments and the large variability of data. This also explains the inconsistent
315 results between the clinical report on open-heart surgery and the present result regarding
316 enzyme release.
317
318 **Clinical Implications**
319 It might be argued that prolonged single-dose crystalloid cardioplegic arrest may not seem

clinically relevant, as multi-dose blood or crystalloid cardioplegia is widely used as a routine practice in most cases. The present experimental model (120 min of single-dose crystalloid cardioplegic arrest) is intended to simulate critical clinical conditions of incomplete or inappropriate myocardial protection in which ischemic intervals are unexpectedly prolonged or myocardial protection is inadequate owing to structural or practical difficulties in optimal cardioplegic delivery. Furthermore, recent trends for the prevalence of a certain type of single-dose cardioplegia (i.e., del Nido cardioplegia) performed for practical reasons in adults (i.e., minimally invasive cardiac surgery) and pediatric cardiac surgery may increase the risk of incomplete myocardial protection from prolonged ischemia especially during normothermic CPB without topical cooling.

The modality examined in the present study may be useful as a rescue or trouble-shooting strategy under these conditions where TWBCP alone may not be very helpful. This easy-to-use, low-risk and promising protective strategy can be applied in every elective and unscheduled high-risk situation, whenever a greater danger of developing reperfusion injury exists. In addition to cardiac surgery, organ transplantations during donor surgery might be a good candidate indication for use of this promising method. In the present study, the clinical role of rPerC during extended cardiac surgery (i.e., 4-6 h) with standard myocardial protection using multi-dose blood cardioplegia has not been established, because of the technical and practical

338 difficulties in the experimental setting of prolonged use of CPB per se (>4 h). These limitations
339 regarding the nature of the acute animal experiment would necessitate further investigation in a
340 well-designed randomized clinical trial in cardiac surgery.
341
342 **Study Limitations**
343 The issue of limited study size is a shortcoming of the present investigation that may cause
344 potential statistical type-II error. The very short time span of blood sampling for troponin
345 release is another shortcoming of the present protocol. Longer observation is essential to assess
346 the real net troponin release because it is usually reported to peak 8 hours after acute myocardial
347 infarction. Nevertheless, in the present study, the reason for restricting the reperfusion period to
348 only 60 minutes is an inevitable situation of post bypass pathophysiology induced by
349 ischemia-reperfusion or CPB per se in *in vivo* animal experiments with CPB. Prolonged
350 ischemia frequently precluded sustained observation sufficiently long after the termination of
351 CPB. Furthermore, the main objective of the present study was to elucidate the functional
352 benefit of the new intervention in the surgical arena in terms of contributing to prompt left
353 ventricular function recovery, especially in a pediatric surgery rather than effects on enzyme
354 release. Histopathological examination of cardiac specimens was not included in this study and
355 should be considered in future investigations. To elucidate the specific mechanism of rPerC,

pharmacological manipulation, as reported by Schmidt and colleagues [1], who tested whether glibenclamide would block the K_{ATP} channel mechanism, might be of interest and useful for future investigation.

CONCLUSION

RPerC offers promising synergistic cardioprotection in combination with TWBCP, implicating potential clinical benefit by contributing to prompt LV systolic and diastolic function recovery, especially myocardial dysfunction induced by prolonged cardioplegic arrest during pediatric open-heart surgery. Since no distinct adverse effect on the myocardium has been demonstrated in this intervention, unlike local ischemic conditioning, remote perconditioning can be safely applied as a supplemental reperfusion strategy to the standard clinical BCP strategy in addition to enhance post-bypass myocardial function recovery contributing to reductions in postoperative morbidity.

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375 **CONFLICT OF INTEREST STATEMENT**

376 None declared.

FIGURE LEGENDS

Figure 1. Experimental protocols

Twenty-four piglets were divided into the following groups on the basis of the method of reperfusion: Groups I (Control), II (rPerC), III (TWBCP), IV (rPerC+TWBCP).

CPB: cardiopulmonary bypass; rPerC: remote perconditioning; TWBCP: terminal warm blood cardioplegia

Figure 2. Percentage recovery of Ees at 60 min after reperfusion. Data are expressed as mean and 95% confidence interval for Groups I (Control), II (rPerC), III (TWBCP), IV (rPerC+TWBCP). TWBCP showed significant difference in percentage recovery of Ees (mean difference, 61% recovery; 95% confidence interval, 38.6 - 84.1, $p<0.0001$, ANOVA).

Ees, end-systolic elastance; rPerC: remote perconditioning; TWBCP: terminal warm blood cardioplegia.

Figure 3. Percentage recovery of PRSW at 60 min after reperfusion. Data are expressed as mean and 95% confidence interval for Groups I (Control), II (rPerC), III (TWBCP), IV (rPerC+TWBCP). TWBCP showed significant difference in percentage recovery of PRSW (mean difference, 20.9% recovery; 95% confidence interval, 0.4 - 54.3, P=0.047, ANOVA).

395 PRSW, preload recruitable stroke work; rPerC: remote perconditioning; TWBCP: terminal
396 warm blood cardioplegia.
397
398 Figure 4. Percentage recovery of inverse of left-ventricular EDPVR; as left-ventricular
399 compliance at 60 min after reperfusion. Data are expressed as mean and 95% confidence
400 interval for Groups I (Control), II (rPerC), III (TWBCP), IV (rPerC+TWBCP). RPerC showed
401 significant difference in percentage recovery of inverse of EDPVR (mean difference, 21.6%;
402 95% confidence interval, 1.6 - 41.6; p=0.036, *t*-test).
403 EDPVR: end-diastolic pressure-volume relationship; rPerC: remote perconditioning; TWBCP:
404 terminal warm blood cardioplegia.
405
406 Figure 5. Plasma troponin-T level over time after reperfusion in Groups I (Control), II (rPerC),
407 III (TWBCP), and IV (rPerC+TWBCP). Data are expressed as mean and standard deviation.
408 Significant differences were observed in troponin-T over time.
409 rPerC: remote perconditioning; TWBCP: terminal warm blood cardioplegia.
410

411 **Table 1: Baseline data**

	Control	rPerC	TWBCP	rPerC+TWBCP
Ees (mmHg/ml)	**8.4 ± 5.2**	**9.3 ± 1.1**	**6.7 ± 4.1**	**6.9 ± 2.0**
PRSW (g*m/ml)	**78 ± 49**	**47 ± 20**	**53 ± 22**	**48 ± 15**
Inverse of EDPVR	**3.1 ± 1.0**	**1.7 ± 0.2**	**2.5 ± 0.5**	**2.4 ± 0.9**

412 All values are expressed as mean ± standard deviation.

413 rPerC: remote perconditioning; TWBCP: terminal warm blood cardioplegia; Ees: end-systolic

414 elastance; PRSW: preload recruitable stroke work; EDPVR: end-diastolic pressure-volume

415 relationship

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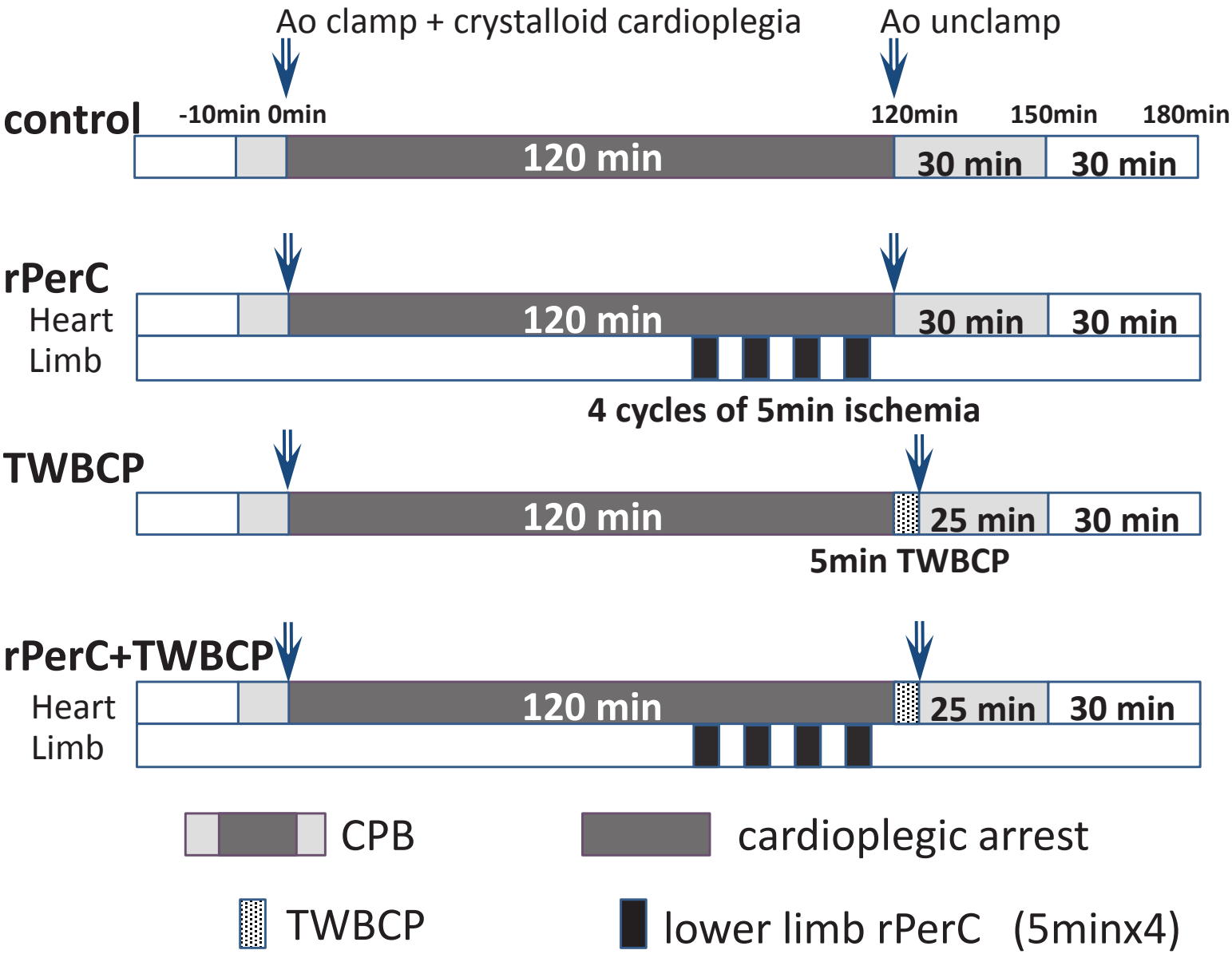
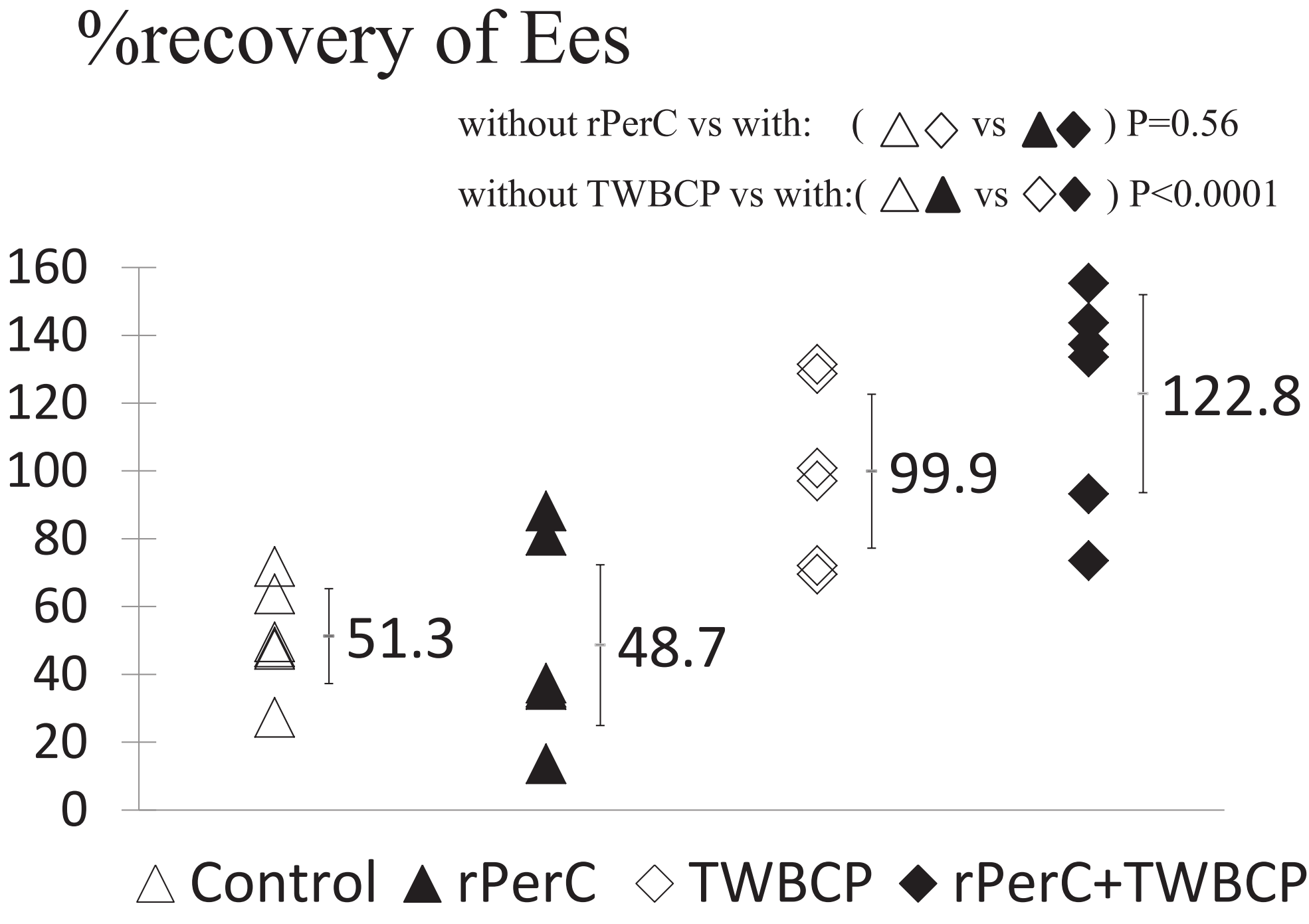
Protocols:


Ao clamp + crystalloid cardioplegia
Ao unclamp
control
-10min 0min
120min
150min
180min
120 min
30 min
30 min
rPerC
Heart
Limb
120 min
30 min
30 min
4 cycles of 5min ischemia
TWBCP
120 min
25 min
30 min
5min TWBCP
rPerC+TWBCP
Heart
Limb
120 min
25 min
30 min
CPB
cardioplegic arrest
TWBCP
lower limb rPerC (5minx4)


%recovery of Ees
without rPerC vs with: (△◇ vs ▲◆) P=0.56
without TWBCP vs with:(△▲ vs ◇◆) P<0.0001
160
140
120
100
80
60
40
20
0
51.3
48.7
99.9
122.8
△ Control ▲ rPerC ◇ TWBCP ◆ rPerC+TWBCP

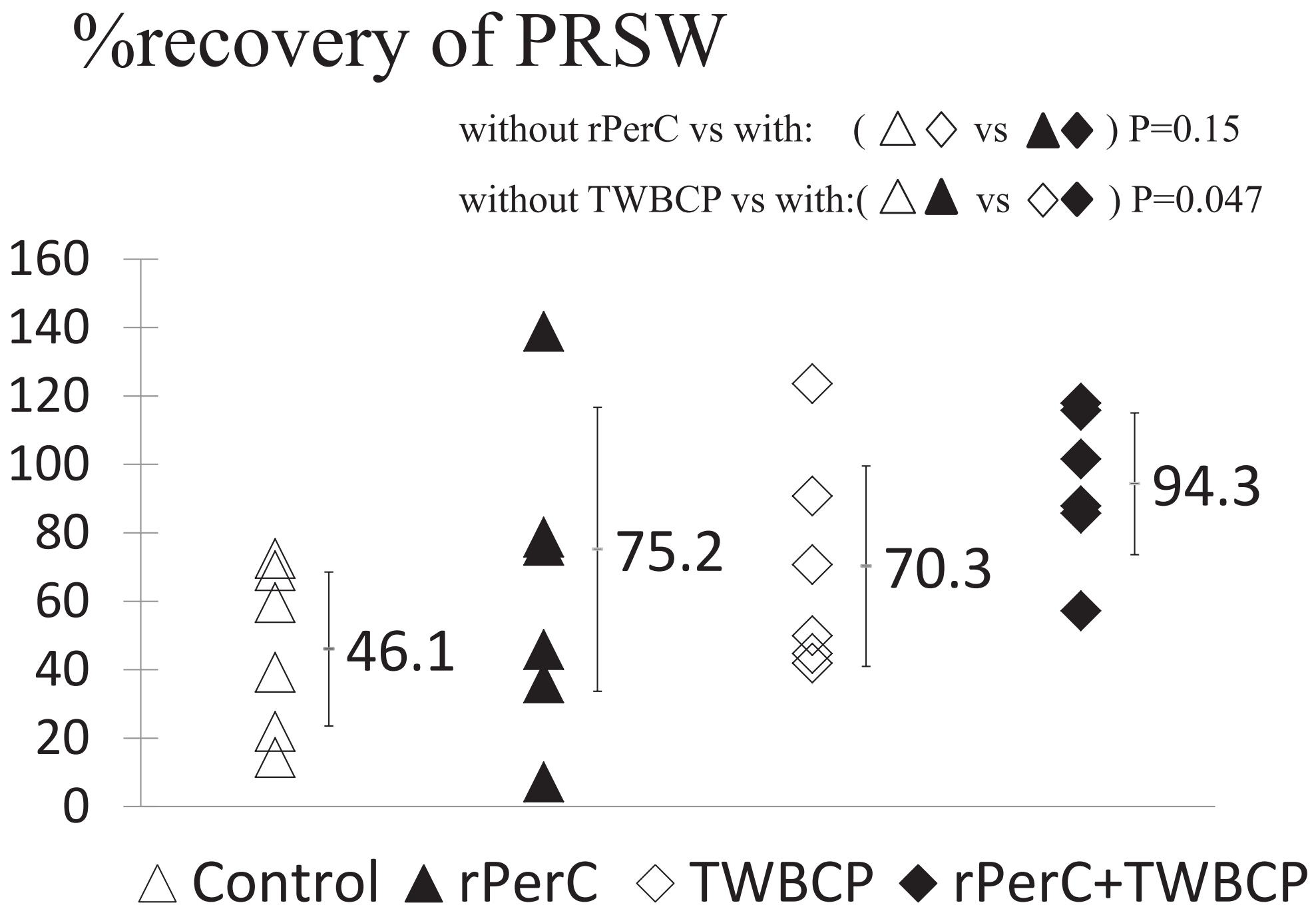

%recovery of PRSW
without rPerC vs with: (△◇ vs ▲◆) P=0.15
without TWBCP vs with:(△▲ vs ◇◆) P=0.047
160
140
120
100
80
60
40
20
0
46.1
75.2
70.3
94.3
△ Control ▲ rPerC ◇ TWBCP ◆ rPerC+TWBCP

Figure 4

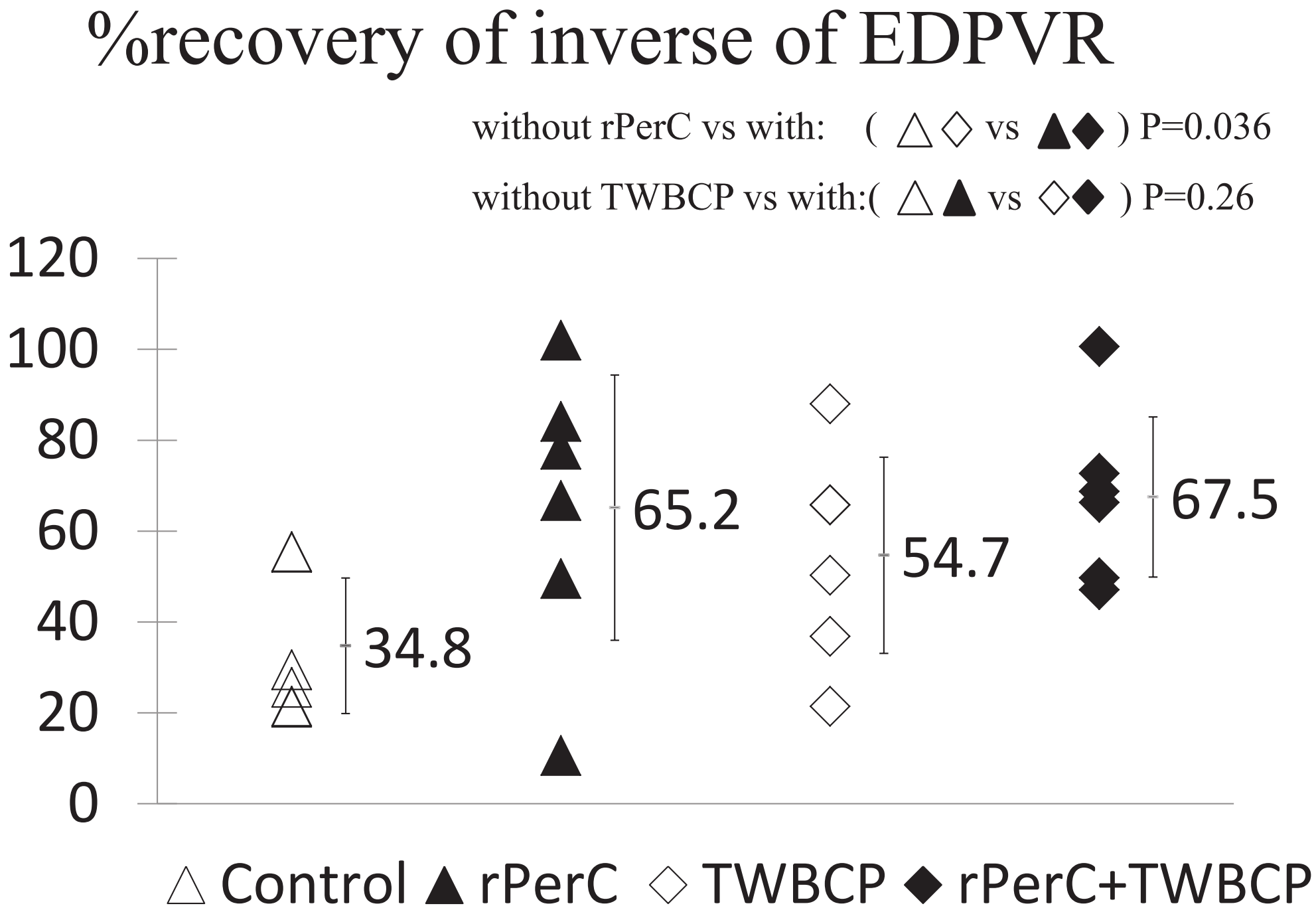
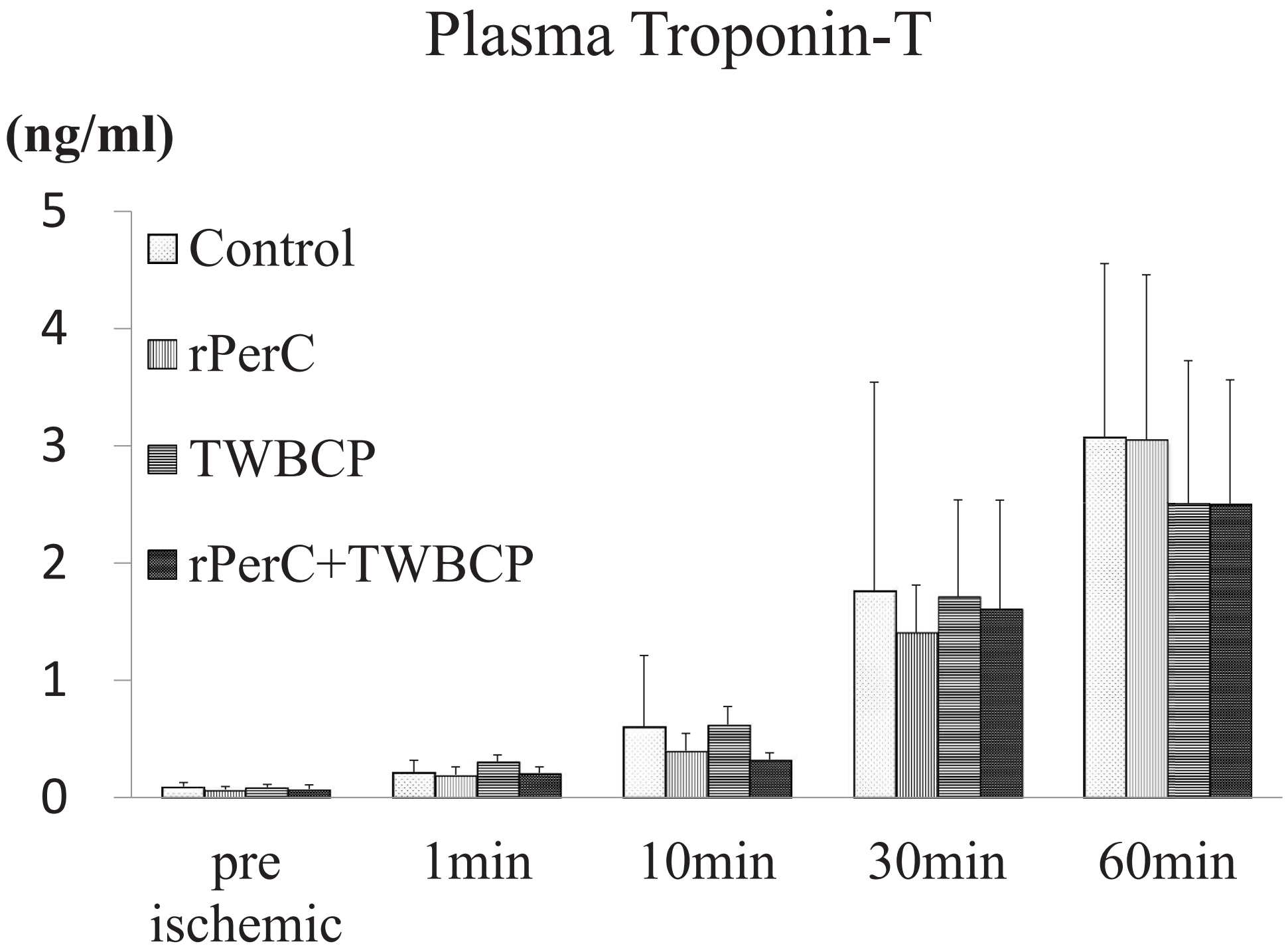

%recovery of inverse of EDPVR
without rPerC vs with: (△◇ vs ▲◆) P=0.036
without TWBCP vs with:(△▲ vs ◇◆) P=0.26
120
100
80
60
40
20
0
34.8
65.2
54.7
67.5
△ Control ▲ rPerC ◇ TWBCP ◆ rPerC+TWBCP

Figure 5


Plasma Troponin-T
(ng/ml)
5
4
3
2
1
0
Control
rPerC
TWBCP
rPerC+TWBCP
pre ischemic
1min
10min
30min
60min