During cardiac surgery the myocardium is exposed to ischemia-reperfusion (IR) injury, which is known to be associated with an adverse outcome.\textsuperscript{1,2} Although the off-pump technique enables avoidance of myocardial injury caused by cardioplegic arrest and aorta cross-clamping,\textsuperscript{3–5} complications such as perioperative myocardial infarction still accompany cardiac surgery.\textsuperscript{6,7} Thus, efforts to devise an effective myocardial protective strategy continue for patients undergoing off-pump coronary artery bypass graft surgery (OPCAB).

Several recent clinical trials have shown that remote ischemic preconditioning (RIPC) has a powerful protective effect on myocardial injury.\textsuperscript{8,9} The concept of RIPC is that transient ischemia in 1 tissue protects distant tissues from sustained ischemia. In previous studies, RIPC was induced by inflating a blood pressure cuff on an upper or lower limb. This method was found to provide a straightforward, cheap, non-invasive, and powerful means of myocardial protection during cardiac surgery.

In our previous study, however, RIPC (4 cycles of 5-min ischemia and 5-min reperfusion on an upper limb) failed to significantly reduce myocardial injury in patients undergoing OPCAB.\textsuperscript{10} This result was not expected, because the RIPC technique used was similar to that adopted in previous studies in which RIPC was seen to have a powerful myocardial protective effect.\textsuperscript{8,9} We expected that this result may have been caused by smaller\textsuperscript{3} and more variable\textsuperscript{11} myocardial enzyme elevations during OPCAB, and that a more intense RIPC technique might be required to induce a distinct myocardial protective effect in patients undergoing OPCAB.

In the present study, we conducted a randomized clinical trial on patients undergoing OPCAB. To augment the protective effect of RIPC, RIPC was given as lower limb ischemia, rather than upper limb ischemia, and remote ischemic postcon-
Remote Ischemic Preconditioning and OPCAB

Methods

Patients
This trial was approved by the institutional review board and all patients provided informed written consent before enrollment. Adult patients scheduled for elective OPCAB were recruited. The following exclusion criteria were applied: age >80 years; a major combined operation, such as carotid surgery; unstable angina; recent myocardial infarction (within 7 days); severe hepatic, renal, or pulmonary disease; recent systemic infection (within 7 days); use of an inotropic agent or a mechanical assist device; left ventricular ejection fraction <30%; or peripheral vascular disease affecting lower limbs. Oral hypoglycemic agents such as sulphonylurea were stopped 3 days before surgery, because they have been shown to affect ischemic preconditioning. Patients were randomly assigned to an RIPC+RIPostC group or to a control group using a computer-generated random list and numbered envelopes. The envelopes were opened immediately before surgery by an anesthesiologist who was not involved in patient treatment, who performed the RIPC+RIPostC procedures. Surgeons and anesthesiologists were not told of patient assignments.

Treatment and Procedures
RIPC+RIPostC was induced by 4 cycles of 5-min ischemia and 5-min reperfusion in a lower limb using a blood pressure cuff inflated to 200 mmHg. After sterilization of both legs, an aseptic blood pressure cuff was applied to 1 side of the thigh that was less expected to be used for saphenous vein harvest. RIPC was applied twice, just after anesthesia induction (RIPC) and just after completion of anastomoses (RIPostC). Patients in the control group wore the same cuff on a lower limb, and the same maneuver to inflate the cuff was carried out as in the RIPC+RIPostC group. A 3-way stopcock between the pneumatic cuff and the cuff inflator was opened, however, and no pressure was applied.

Anesthetic and surgical techniques were standardized during the trial. All the included patients were treated by a single anesthesiologist and a single surgeon. Anesthesia was induced with i.v. midazolam (0.15 mg/kg), vecuronium (0.12 mg/kg), and sufentanil (1 μg/kg). The trachea was intubated and lungs were mechanically ventilated with oxygen and medical air to maintain an end-tidal carbon dioxide tension of 35–40 mmHg. Anesthesia was maintained by target-controlled propofol (1.5–3.5 μg/ml) and remifentanil (8–20 ng/ml) infusion. Propofol infusion was adjusted to achieve a bispectral index of 40–60, and remifentanil infusion was titrated according to the clinical situation. Vecuronium (1 μg·kg⁻¹·min⁻¹) was used for muscle relaxation. No inhalation agent, such as sevoflurane, was used. Patients received continuous monitoring consisting of 5-lead electrocardiography, pulse oximetry, invasive radial artery pressure measurement, pulmonary artery pressure measurement, nasopharyngeal temperature measurement, and transesophageal echocardiography.

All patients underwent OPCAB involving an internal mammary artery graft or a saphenous vein graft after systemic heparinization (to maintain an activated clotting time of >300 s). During distal anastomoses, intracoronary shunts (Axius™ Coronary Shunt; Guidant, Cupertino, CA, USA) were used regardless of grade of coronary stenosis or coronary artery territories. Intraluminal occluders (Flo-Rester®, Synovis Surgical Innovations, St Paul, MN, USA), however, were used in coronary arteries with total occlusion, especially in small vessels (diameters 1–1.25 mm) at left circumflex or right coronary artery territories, when there was some difficulty in performing anastomosis while the shunt was in place. After completing anastomoses, heparin was reversed with protamine sulfate. Cardiac index was maintained at >2.0 L·min⁻¹·m⁻² using i.v. fluids or catecholamine infusion under trans-esophageal echocardiographic guidance. After surgery, patient-controlled i.v. morphine was provided for 48 h. Aspirin (100 mg/day) was continued until the day of surgery and resumed with ticlodipine (200 mg/day) on the first postoperative day. Patients underwent early follow-up coronary angiograms regardless of angina symptoms.

Outcomes
The primary endpoint of this study was myocardial injury reduction as assessed on serum troponin I release. The secondary endpoints were postoperative renal and pulmonary injury as assessed on serum creatinine level and PaO₂/FiO₂ ratio, respectively. Serum troponin I concentrations were determined preoperatively, and at 1, 6, 12, 24, 48, and 72 h postoperatively. Renal dysfunction was defined as a postoperative serum creatinine level >2.0 mg/dl accompanied by an increase of >0.7 mg/dl from the preoperative baseline. Postoperative myocardial infarction was diagnosed based on the definition provided by the European Society of Cardiology/American College of Cardiology/American Heart Association/World Health Federation (ESC/ACC/AHA/WHF), as cardiac biomarker values >5-fold the upper reference limit associated with new Q waves, new left bundle branch block, or an angiographically documented new graft or native coronary artery occlusion within 72 h.12 Stroke was defined as a neurologic deficit lasting >24 h, as confirmed by a neurologist based on appropriate imaging.

Statistical Analysis
Sample size was calculated based on area under the curve (AUC) for postoperative troponin I, which was 84.5±61.3 h·ng⁻¹·ml⁻¹ in our pilot study. RIPC was found to reduce AUC by 26% in our previous study,10 and we expected a greater reduction for RIPC combined with RIPostC. To detect a 50% reduction in AUC (42.25 h·ng⁻¹·ml⁻¹), randomization of 70 patients was needed for a power of 80% and a significance of 5%. Data are expressed as mean±SD or as median (lower–upper quartiles). The significance of differences between the 2 groups was assessed using the Student t-test or Mann-Whitney U-test. Categorical data were compared using the chi-squared test or Fisher’s exact test. Considering the correlation among repeated measures within a subject, the generalized estimating equation (GEE) method using first-order autoregression was applied to compare troponin I, creatinine levels and PaO₂/FiO₂ ratios. The interaction between group and time was tested using a 0.05 level of significance. When a significant interaction was found between group and time, multiple comparisons were performed at each time point and adjusted using Bonferroni correction. All tests were 2-tailed, and P<0.05 was considered statistically significant. AUC was determined using the standard trapezoidal method. Statistical analysis was performed using SPSS version 17.0 (SPSS, Chicago, IL, USA).

Results
Patients scheduled for elective OPCAB were screened for eligibility. Of the 87 patients who underwent screening, 10 did...
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87 Patients were screened

- 10 Did not meet eligibility criteria
- 77 Were eligible

- 5 Underwent combined major surgery
- 2 Unable to informed consent

- 70 Were randomised

- 35 Received RIPC + RIPostC
- 35 Received control

- 35 Analysed

**Figure 1.** Trial flowchart. RIPC, remote ischemic preconditioning; RIPostC, remote ischemic postconditioning.

**Table 1. Patient Characteristics**

<table>
<thead>
<tr>
<th></th>
<th>RIPC+RIPostC</th>
<th>Control</th>
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<tbody>
<tr>
<td><strong>Baseline characteristics</strong></td>
<td></td>
<td></td>
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<tr>
<td>Patients (n)</td>
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<td>35</td>
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<tr>
<td>Age (years)</td>
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<td>Weight (kg)</td>
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<tr>
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<tr>
<td>NYHA class</td>
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<td>Left ventricular function*</td>
<td>2 (1–2)</td>
<td>1 (1–2)</td>
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<tr>
<td>Sulphonylurea**</td>
<td>9 (26)</td>
<td>12 (34)</td>
</tr>
</tbody>
</table>

Data given as mean±SD, n (%), or median (lower-upper quartiles).
*1, good; 2, moderate; 3, poor. **Sulphonylurea was stopped 3 days before surgery.

RIPC, remote ischemic preconditioning; RIPostC, remote ischemic postconditioning; NYHA, New York Heart Association; ACE, angiotensin-converting enzyme.

not meet the eligibility criteria, and 7 were excluded (5 underwent combined major surgery and 2 were unable to provide informed consent). Seventy patients were randomized to either the RIPC+RIPostC group (n=35) or to the control group (n=35). The study was completed in all 70 patients (Figure 1) and the RIPC+RIPostC protocol was applied without any complications.

In the RIPC+RIPostC group, mean time between completion of RIPC and onset of graft placement was 18.1±8.2 min, and mean time between completion of anastomoses and initiation of RIPostC was 6.2±2.8 min. Intracoronary shunts or intraluminal occluders were used during distal anastomosis. Intraluminal occluders were used in 13 of 35 patients in the control group and in 15 of 35 patients in the RIPC+RIPostC group (P=0.626). Proximal snaring was not performed in any patients.

The baseline characteristics of the 2 groups were comparable (Table 1), and the groups were similar in terms of operative characteristics and intensive care unit (ICU) and hospital stay (Table 2). In addition, sites of graft placement were comparable, and no patient in either group received a graft on the proximal left anterior descending artery, which may be associated with a large volume of at-risk myocardium.

Patient-controlled morphine consumption was comparable in the 2 groups (RIPC+RIPostC, 67.5±20.2 mg vs. control, 69.1±19.7 mg; P=0.534). Postoperative stroke occurred in 1 patient in each group (P=1.000). No patient developed postoperative myocardial infarction, and no postoperative 30-day or in-hospital mortality occurred.

Postoperative coronary angiograms were performed in 98.6% of patients (69/70) on postoperative day 1.4±1.1. Graft occlusion occurred in 1 patient in the control group, and the patient underwent reoperation for the graft interposition. There was no patient who developed graft occlusion in the RIPC+RIPostC group, but there was no difference in early graft patency rate (97.1% vs. 100%; P=1.000).
Baseline serum troponin I levels before surgery were 0.02 ng/ml (0.01–0.04 ng/ml) in the RIPC+RIPostC group and 0.02 ng/ml (0.02–0.03 ng/ml) in the control group (P=0.231). In both groups, troponin I levels increased postoperatively (Figure 2) and peaked at 6h postoperatively, indicating that most myocardial injury had occurred during surgery.

No significant interaction was found between group and time in troponin I levels (P=0.111) and so the GEE method was used. Troponin I levels were significantly lower in the RIPC+RIPostC group (P=0.001). The AUC of the postoperative troponin I levels was 21.3 h · ng⁻¹ · ml⁻¹ (16.5–53.1 h · ng⁻¹ · ml⁻¹) in the RIPC+RIPostC group and 41.5 h · ng⁻¹ · ml⁻¹ (24.6–90.2 h · ng⁻¹ · ml⁻¹) in the control group (P=0.020), indicating that RIPC+RIPostC reduced troponin I release by 48.7%. Data presented as median and quartiles. Error bars indicate the 90th and 10th percentiles.

Myocardial Injury
Baseline serum troponin I levels before surgery were 0.02 ng/ml (0.01–0.04 ng/ml) in the RIPC+RIPostC group and 0.02 ng/ml (0.02–0.03 ng/ml) in the control group (P=0.231). In both groups, troponin I levels increased postoperatively (Figure 2) and peaked at 6h postoperatively, indicating that most myocardial injury had occurred during surgery.

No significant interaction was found between group and time in troponin I levels (P=0.111) and so the GEE method was used. Troponin I levels were significantly lower in the RIPC+RIPostC group (P=0.001). The AUC of the postoperative troponin I levels was 21.3 h · ng⁻¹ · ml⁻¹ (16.5–53.1 h · ng⁻¹ · ml⁻¹) in the RIPC+RIPostC group and 41.5 h · ng⁻¹ · ml⁻¹ (24.6–90.2 h · ng⁻¹ · ml⁻¹) in the control group (P=0.020), indicating that...
RIPC+RIPostC reduced troponin I release by 48.7%.

Renal and Pulmonary Injury
Figure 3A shows serum creatinine levels before and after surgery. The interaction between group and time was significant (P=0.001), and therefore multiple comparisons were tested. In multiple comparisons with Bonferroni correction, there was no difference in creatinine levels at each time point. Renal dysfunction occurred in 4 patients in the control group and in 1 patient in the RIPC+RIPostC group after surgery, and all required hemodialysis. This difference, however, did not reach statistical significance (P=0.356). Among these 5 patients, 2 patients in the control group and 1 patient in the RIPC+RIPostC group had preoperative chronic renal dysfunction. Another 2 patients in the control group had no preoperative renal disease and developed acute renal injury postoperatively.

In terms of pulmonary function assessment (Figure 3B), no significant interaction was found between group and time (P=0.202) and so the GEE method was used. No overall difference in PaO₂/FiO₂ ratio was observed between the groups (P=0.168), and duration of mechanical ventilation in the ICU was comparable in the 2 groups (Table 2).

Discussion
In the present study, myocardial injury, as assessed by tropo-
ning I level, was reduced by almost half by RIPC+RIPostC using lower limb ischemia in patients undergoing OPCAB.

**RIPC and Myocardial Protection**

Przyklenk et al found that brief episodes of ischemia in the circumflex artery reduced the myocardial infarct generated by occlusion of the left anterior descending artery in dogs. Since then, the concept of regional ischemic preconditioning has been extended to RIPC, which can be applied to organs distant from the heart, such as kidneys and skeletal muscles. In recent clinical trials, RIPC has been found to exhibit myocardial protective effects. Cheung et al induced RIPC by lower limb ischemia in children undergoing congenital heart defect repair, and found that it attenuated myocardial injury. Hausenloy et al found that myocardial injury in patients undergoing on-pump CABG was reduced by RIPC. Not all RIPC studies, however, have been as successful. In a study by Rahman et al, RIPC (by upper limb ischemia) did not reduce troponin T levels in 162 patients undergoing on-pump CABG. In our previous study, we used upper limb RIPC and also failed to significantly reduce myocardial injury in patients undergoing OPCAB.

**Modified RIPC Techniques**

The present study was conducted using the same surgical, anesthetic technique, and medical personnel as in our previous study. The only difference, excepting the RIPC technique changes, was that total i.v. anesthesia was used in the present study and inhalation anesthesia in the previous study. In the previous study, we achieved only a 26% postoperative troponin I reduction, which was not significant (P=0.281), whereas in the present study, a significant 48.7% reduction was observed (P=0.020). We believe that this difference was caused by the changes made to the RIPC technique.

First, in the present study, RIPC was applied by lower limb ischemia, and the greater mass of the lower limb is likely to have resulted in the release of greater amounts of humoral factors. Loukogeorgakis et al found that IR injury was reduced more by lower limb ischemia than by upper limb ischemia. In addition, they also demonstrated that RIPostC by lower limb ischemia can reduce IR injury in man. These results suggest that the protective effects of RIPC and RIPostC by lower limb ischemia are greater than those induced by upper limb ischemia.

Second, in the present study, we used RIPC and RIPostC in combination, and it is known that myocardial protection can also be provided by ischemic postconditioning (IPostC). Experimental studies have shown that RIPostC reduces myocardial injury in animals and endothelial IR injury in man. Interestingly, in a recent study on percutaneous coronary intervention, remote ischemia (3 cycles of 4-min ischemia and 4-min reperfusion) on the upper limb was started 10min before balloon inflation. Therefore, remote ischemia was applied before, during and after ischemia. This “perconditioning” technique was found to have a myocardial protection effect as evaluated on ST segment resolution and troponin I levels.

It is unclear whether preconditioning and postconditioning have an additive protective effect. In experimental animal studies, Yang et al found that ischemic preconditioning (IPC) and postconditioning provided additional cardioprotection, but Manijeveld et al did not. In clinical practice, De Hert et al found that the protective effect of combined preconditioning and postconditioning is more powerful than that achieved by preconditioning only, based on observations that the cardio-protective effects of sevoflurane were most apparent when it was administered throughout surgery.

**Systemic Effects of RIPC and RIPostC**

Both RIPC and RIPostC are believed to have a systemic protective effect on various organs at risk of IR injury. In patients undergoing surgery for abdominal aortic aneurysm repair, RIPC was found to reduce the incidence of renal impairment. Furthermore, Cheung et al found that RIPC lowered airway resistance in children undergoing cardiac surgery. These results support the notion that RIPC has beneficial effects on different organs and tissues. In the present study, however, RIPC+RIPostC did not improve renal or pulmonary outcomes. We believe that the number of patients recruited was probably too small to enable detection of differences in these parameters, because the primary endpoint of the present study was troponin I reduction. For example, although renal dysfunction occurred in 4 patients in the control group and in only 1 patient in the RIPC+RIPostC group, this difference did not reach statistical significance. Based on the present results, 617 patients per group (with a power of 80% and an alpha level of 0.05) are required to compare incidences of renal failure. Furthermore, renal and pulmonary function may be preserved better by OPCAB than by conventional CABG, which means that a larger scale trial is required to evaluate possible protective effects in renal and pulmonary injury in patients undergoing OPCAB.

**Study Limitations**

In the present study we modified the RIPC technique in 2 ways, that is, we used lower limb ischemia and RIPostC to enhance protective effects. It is difficult, however, to compare the present results with those of our previous study to determine the effects of these modifications, because they are 2 different studies. Furthermore, because the 2 modifications were implemented simultaneously, we could not separate the possible beneficial effects of lower limb ischemia and of RIPostC.

In the present study, we included patients with several factors, such as, old age, diabetes mellitus, hypertension, dyslipidemia, and medications, which might have modified the preconditioning effect. For example, antecedent angina may have had a preconditioning effect on some patients. Furthermore, we cannot rule out the possibility that the control group was also preconditioned by such factors. Unfortunately, subgroup analysis could not be conducted to identify these factors due to the relatively small number of patients. No difference was observed, however, between the 2 study groups in terms of average age or the proportions affected by diabetes mellitus, hypertension, or dyslipidemia (Table 1). In addition, we excluded patients older than 80 years and those with unstable angina, and we stopped oral hypoglycemic agents.

Cardiac enzymes, such as, troponin I and troponin T, are highly sensitive, specific markers of myocardial damage, and their release is associated with adverse outcome. Increases in the level of serum troponin I per se, however, do not imply an adverse postoperative outcome, and the present study was not sufficiently powered to assess clinical outcome. Thus, although we found that RIPC+RIPostC markedly reduced troponin I level, we suggest that a larger scale randomized multicenter study is required to evaluate the clinical effects of RIPC+RIPostC.

**Conclusions**

RIPC+RIPostC by lower limb ischemia was found to reduce
myocardial enzyme elevation by almost half. RPC+RIPostC by lower limb ischemia has a powerful protective effect in patients undergoing OPCAB. A larger study is required to assess the ability of RPC+RIPostC to improve clinical outcome.

Acknowledgments
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Disclosures
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References