Remote Ischemic Preconditioning Applied during Isoflurane Inhalation Provides No Benefit to the Myocardium of Patients Undergoing On-pump Coronary Artery Bypass Graft Surgery

Lack of Synergy or Evidence of Antagonism in Cardioprotection?

Eliana Lucchinetti, Ph.D.,* Lukas Bestmann, Ph.D.,† Jianhua Feng, M.D., Ph.D.,‡ Heike Freidank, M.D.,§ Alexander S. Clanachan, Ph.D.,# Barry A. Finegan, M.B.,‖ Michael Zaugg, M.D., M.B.A.‖

ABSTRACT

Background: Two preconditioning stimuli should induce a more consistent overall cell protection. We hypothesized that remote ischemic preconditioning (RIPC, second preconditioning stimulus) applied during isoflurane inhalation (first preconditioning stimulus) would provide more protection to the myocardium of patients undergoing on-pump coronary artery bypass grafting.

Methods: In this placebo-controlled randomized controlled study, patients in the RIPC group received four 5-min cycles of 300 mmHg cuff inflation/deflation of the leg before aortic cross-clamping. Anesthesia consisted of opioids and propofol for induction and isoflurane for maintenance. The primary outcome was high-sensitivity cardiac troponin T release. Secondary endpoints were plasma levels of N-terminal pro-brain natriuretic peptide, high-sensitivity C-reactive protein, S100 protein, and short- and long-term clinical outcomes. Gene expression profiles were obtained from atrial tissue using microarrays.

Results: RIPC (n = 27) did not reduce high-sensitivity cardiac troponin T release when compared with placebo (n = 28). Likewise, N-terminal pro-brain natriuretic peptide, a marker of myocardial dysfunction; high-sensitivity C-reactive protein, a marker of perioperative inflammatory response; and S100, a marker of cerebral injury, were not different between the groups. The incidence for the perioperative composite endpoint combining new arrhythmias and myocardial infarctions was higher in the RIPC group than the placebo group (14/27 *vs.* 6/28, $P = 0.036$). However, there was no difference in the 6-month cardiovascular outcome. N-terminal pro-brain natriuretic peptide re-

What We Already Know about This Topic

- Remote ischemic preconditioning (RIPC) of the heart is a promising cardioprotective strategy based on ischemic preconditioning, and involves short episodes of ischemia and reperfusion of noncardiac tissue such as the limbs

What This Article Tells Us That Is New

- RIPC applied to the lower extremities during isoflurane inhalation provided no additional protective benefit to the myocardium in patients undergoing on-pump CABG surgery

◆ This article is accompanied by an Editorial View. Please see: Lotz C, Kehl F: Translating volatile anesthetic-induced cardioprotection into systems biology. ANESTHESIOLOGY 2012; 116: 238–9.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are available in both the HTML and PDF versions of this article. Links to the digital files are provided in the HTML text of this article on the Journal's Web site (www.anesthesiology.org)

* Senior Researcher, ‖ Professor, Department of Anesthesiology and Pain Medicine, University of Alberta, Edmonton, Alberta, Canada. # Professor and Chair, Department of Pharmacology, University of Alberta. † Chief Operating Officer, UNILABS, St., Gallen, Switzerland. ‡ Senior Researcher, Department of Radiation Oncology, University of Zurich, Zurich, Switzerland. § Professor and Chief of Laboratory Medicine, University Hospital Basel, Basel, Switzerland.

Received from the Department of Anesthesiology and Pain Medicine, University of Alberta, Edmonton, Alberta, Canada. Submitted for publication June 3, 2011. Accepted for publication October 10, 2011. Supported by grants from the University Hospital Foundation, Edmonton, Alberta, Canada (to Dr. Finegan); the Mazankowski Alberta Heart Institute, Edmonton, Alberta, Canada (to Dr. Zaugg); the Heart and Stroke Foundation of Alberta, Northwest Territories, and Nunavut, Calgary, Alberta, Canada (to Dr. Zaugg); the Swiss National Science Foundation, Berne, Switzerland (grant No. 3200B0–103980/1 to Dr. Zaugg); the 5th Frontiers in Anesthesia Research Award from the International Anesthesia Research Society, Cleveland, Ohio (to Dr. Zaugg); Abbott Laboratories, Ltd. Saint-Laurent, Quebec, Canada (to Dr. Zaugg); and Roche Diagnostics Ltd., Rotkreuz, Switzerland (to Dr. Zaugg). Authors Lucchinetti, Bestmann, Finegan, and Zaugg contributed similarly to this work.

Address correspondence to Dr. Zaugg: Department of Anesthesiology and Pain Medicine, University of Alberta, 8–120 Clinical Sciences Building, Edmonton, Alberta T6G 2G3 Canada. michael.zaugg@ualberta.ca. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. ANESTHESIOLOGY's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

 Anesthesiology 2012; 116:296–310

lease correlated with isoflurane-induced transcriptional changes in fatty-acid metabolism ($P = 0.001$) and DNA-damage signaling ($P < 0.001$), but not with RIPC-induced changes in gene expression.

Conclusions: RIPC applied during isoflurane inhalation provides no benefit to the myocardium of patients undergoing on-pump coronary artery bypass grafting.

REMOTE ischemic preconditioning (RIPC) of the heart is a promising cardioprotective strategy based on ischemic preconditioning and involves short episodes of ischemia and reperfusion of noncardiac tissue such as the limbs.[1] Limb-induced RIPC is of particular interest, as it simply involves the inflation and deflation of a tourniquet applied to the limb before a sustained ischemic period of the heart or other vital organs. RIPC has been shown to effectively reduce cardiac injury associated with ischemia-reperfusion in animal models and patients.[2–4] Whereas local myocardial ischemic preconditioning has not found a routine place in current cardiovascular surgical practice, limb-induced RIPC is noninvasive and has potential clinical applications in prophylactic treatments against myocardial ischemia-reperfusion injury. On the other hand, whole body preconditioning with ether-derived volatile anesthetics was shown to decrease the release of biomarkers associated with myocardial cell death and myocardial dysfunction in patients undergoing coronary artery bypass graft (CABG) surgery.[5–8] One study demonstrated that application of volatile anesthetics for the entire case, mimicking a combination of pre- and postconditioning (anesthetic conditioning), most markedly protected the myocardium of CABG patients.[9] These studies further suggest protective effects of volatile anesthetics on other vital organs and on the perioperative inflammatory response.[6,10]

According to the commonly accepted threshold theory of preconditioning, which implies that a certain degree of stimulation is required to reach the level where a cell or organ is able to activate its endogenous protection program,[11] it would be conceivable that the application of two well-defined preconditioning stimuli should induce a more consistent and effective overall cell protection. Experimental results in the field of ischemic and pharmacologic conditioning provide evidence that cell signaling of both types of conditioning share many critical steps, such as the inhibition of the metabolic enzyme GSK3β and the opening of the mitochondrial K_{ATP} channel.[12–15] Conversely, based on genome-wide transcriptional analyses, striking differences in gene expression patterns elicited by ischemic as compared with pharmacologic preconditioning by isoflurane were detected in the trigger phase (application of the preconditioning stimulus alone), as well as after exposure to ischemia-reperfusion injury.[16] In the present study, we tested whether RIPC executed on the lower limb (second preconditioning stimulus) would protect the myocardium in patients undergoing on-pump CABG surgery with isoflurane anesthesia (first preconditioning stimulus). Specifically, we hypothesized that RIPC in combination with isoflurane inhalation would provide more pronounced protection, *i.e.*, enhance the protection by isoflurane alone, as measured by the perioperative release of the cardiac necrosis marker cardiac troponin T, the primary endpoint of the study.

Materials and Methods

The local ethics committee of the University of Alberta (Edmonton, Canada) approved this study. Written informed consent was obtained from all patients. Fifty-five patients scheduled for elective on-pump CABG surgery were finally enrolled and assigned to RIPC treatment or placebo at the University of Alberta Hospital between September 2008 and July 2010. The trial was registered with ClinicalTrials.gov and issued with the identification number NCT00546390.

Study Criteria

Inclusion criteria were being scheduled for elective on-pump CABG surgery and age of 50–85 yrs. Exclusion criteria were emergency surgery, myocardial infarction within 48 h before surgery as defined by increased plasma concentrations for cardiac enzymes, diabetes mellitus, a body mass index greater than 35, concomitant noncardiac surgery, or severe peripheral vascular disease.

RIPC Protocol and Anesthetic and Surgical Management

Details of the study protocol are given in figure 1. A 1:1 block randomization (block size 10) with no further stratification was generated by an independent person using a computer random number generator, and the results were stored in numbered, sealed, opaque envelopes. Anesthesia was induced with propofol; opioids including fentanyl, sufentanil, or remifentanil; and the muscle relaxant rocuronium. All monitoring lines were inserted and anesthesia was maintained with 0.5–2 minimum alveolar concentration of isoflurane and repetitive doses of opioids and rocuronium. A 15-cm sterile blood pressure cuff was placed around the right thigh and connected to the inflating device, and the patient was draped obscuring the visibility of the cuff. Subsequently, the patient was randomly allocated (by opening of an envelope) to RIPC consisting of four 5-min cycles of lower limb ischemia-reperfusion induced by a tourniquet inflated to 300 mmHg or placebo, *i.e.*, no treatment. This procedure was executed by an operating room technician who also carefully checked the proper functioning of the inflating device before and after usage, but was otherwise not involved in the study. After median sternotomy and pericardiotomy, the right atrium and the ascending aorta were cannulated. Heparin was administered and standard cardiopulmonary bypass (CPB) was started using a disposable hollow fiber oxygenator. Isoflurane was given *via* an Isotec 5 vaporizer (Abbott Canada, Saint-Laurent, Québec, Canada) integrated into the CPB machine. After aortic cross-clamping, cold blood containing cardioplegia was administered antegradely to achieve cardiac arrest. Distal anastomoses were performed during a

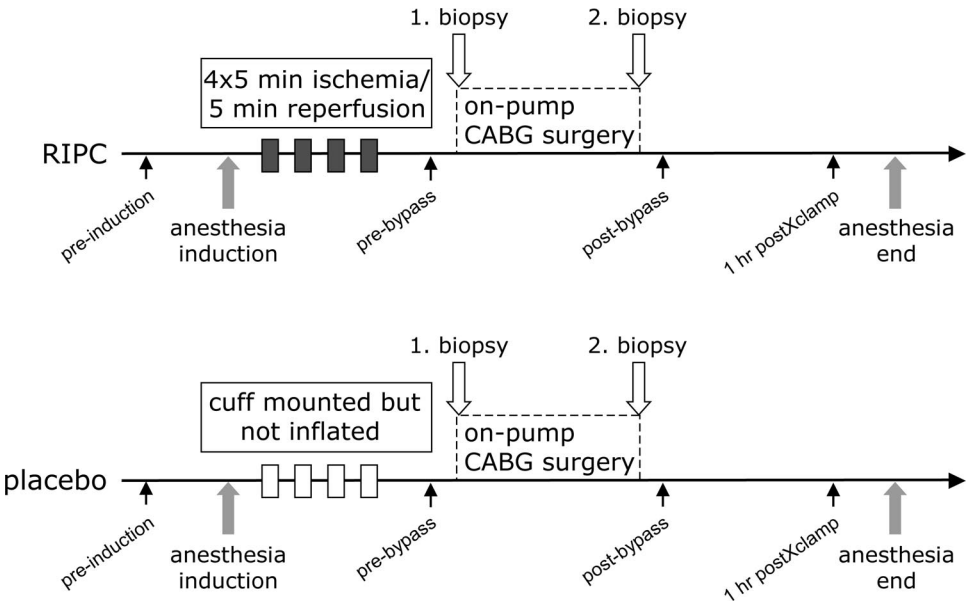

Fig. 1. Study protocol. CABG = coronary artery bypass grafting; RIPC = remote ischemic preconditioning.

single period of aortic cross-clamping, whereas proximal anastomoses were conducted during side clamping. Using α-stat regulation of blood pH, core temperature was allowed to decrease spontaneously. Phenylephrine was administered to maintain on-pump blood pressure greater than 55 mmHg. Atrial tissue samples were collected at the time of cannulation and 15 min after releasing the cross clamp. After CPB, heparin was antagonized. Hemoglobin was maintained at greater than 7 g/dl during CPB and at greater than 9 g/dl after surgery. All patients were transferred to the intensive care unit, where they received the same standardized routine postoperative care. Collection and analyses of all clinical and laboratory data were performed by study personnel blinded for group assignment.

Primary and Secondary Study Outcomes

The primary outcome was high-sensitivity cardiac troponin T (hscTnT) release as measured by peak hscTnT values and area-under-the-curve. Secondary outcomes were plasma levels of N-terminal pro-brain natriuretic peptide (NT-proBNP), high-sensitivity C-reactive protein (hsCRP), S100, and short- and long-term clinical outcomes. The perioperative composite endpoint combining new arrhythmias and new myocardial infarctions was an additional secondary endpoint defined *a priori*.

Determination of Biochemical Markers

Blood samples were drawn preinduction, prebypass, immediately postbypass, 60 min post cross-clamp release, 24, 48, and 72 h after surgery for all patients. They were stored at −80°C until analysis. The following parameters were determined using the Roche Elecsys 2010 (Roche Diagnostics, Mannheim, Germany): cardiac troponin T (cTnT) (electrochemiluminescence sandwich immunoassay), or limit of detection with coefficient-of-variation of 10%: 0.03 ng/ml, reference range/cutoff less than 0.1 ng/ml; hscTnT (electrochemiluminescence sandwich immunoassay), or limit of detection with coefficient-of-variation of 10%: 13 pg/ml, reference range/cutoff less than 14 pg/ml (95% CI, 12.4–24 pg/ml); NT-proBNP (electrochemiluminescence sandwich immunoassay), or limit of detection with coefficient-of-variation of 20%: 50 pg/ml; normal range/cutoff for men: age of 55–64 yrs, less than 210 pg/ml; 65–74 yrs, less than 376 pg/ml; greater than or equal to 75 yrs, less than 486 pg/ml; for women: age of 55–64 yrs, less than 287 pg/ml; 65–74 yrs, less than 301 pg/ml; greater than or equal to 75 yrs, less than 738 pg/ml; hsCRP (particle-enhanced immunoturbidimetric assay), or limit of detection with coefficient-of-variation of 10%: 0.1 ng/ml, reference range/cutoff less than 5 mg/l; S100 (S100A1B and S100BB) (electrochemiluminescence immunoassay), or limit of detection more than 0.005 pg/ml; intra- and interassay coefficients of variance, less than 5%; reference range/cutoff less than 0.105 pg/ml. S100 was only determined in preinduction, 1 h post cross-clamp, 24, 48, and 72 h blood samples.

Clinical Outcome

All medical charts were reviewed, and the caregivers were interviewed daily for the occurrence of adverse events. Any adverse events were diagnosed by the independently managing physicians as opposed to the biomarkers that were determined at the end of the study. Twelve-lead electrocardiograms were obtained postoperatively every day until discharge. The diagnosis of a new postoperative myocardial infarction was made if the criteria of the consensus guidelines for the detection of myocardial infarction as defined by the American Heart Association were

met.[17] The diagnosis of a cerebral stroke required the presence of clinical symptoms and/or a positive computerized tomography scan. The diagnosis of significant renal dysfunction required postoperatively established hemodialysis or hemofiltration. The 6-months follow-up evaluation was performed by structured telephone interviews, and each patient's general physician was also contacted. Hospital charts, if applicable, were further reviewed. The study endpoints were late adverse cardiac events including cardiac death, nonfatal myocardial infarction, unstable angina, intercurrent coronary angioplasty or CABG surgery, arrhythmias requiring rehospitalization, and new episodes of congestive heart failure occurring after the hospitalization for CABG surgery. Death was considered a result of cardiac origin if the patient died of myocardial infarction, arrhythmia, or congestive heart failure. Myocardial infarction and unstable angina were defined as previously reported.[17] The diagnosis of congestive heart failure was based on symptoms and signs of pulmonary congestion and abnormal results on chest radiograph.

Transcriptional Analysis of Atrial Samples

Microarray analyses were performed to confirm the successful translation of the remote ischemic stimulus from the leg to the heart and to identify specific transcriptional changes in the myocardium elicited by RIPC and isoflurane. Microarray analysis was performed following the "minimum information about a microarray experiment" guidelines.[18] The microarray data are available at the Gene Expression Omnibus database under the series number GSE29396. For 11 randomly selected patients from each group, two atrial samples were collected, one at the time of cannulation (T1) and one 15 min after releasing the cross-clamp (T2). Total RNA was isolated using the Qiagen RNeasy MiniKit (QIAGEN Inc., Toronto, Canada) according to the manufacturer's instructions. The quality of the isolated RNA was determined with a NanoDrop ND 1000 (NanoDrop Products, Wilmington, DE) and a Bioanalyzer 2100 (Agilent Technologies, Inc., Santa Clara, CA). The complementary DNA was prepared from total RNA using the WT Ovation Pico System (NuGEN Technologies, Inc., San Carlos, CA). The FL-Ovation complementary DNA Biotin Module V2 (NuGEN) was used to generate biotin-labeled single-stranded complementary DNA samples, which were subsequently fragmented randomly to 35–200 bp at 94°C in Fragmentation Buffer (Affymetrix Inc., Santa Clara, CA) and used to hybridize onto Affymetrix Exon 1.0 ST arrays (Affymetrix Inc.). Exon arrays from Affymetrix contain exon-specific oligonucleotide probes (4 probes per exon). Compared with Affymetrix standard expression arrays, the increased number of probes (for most of the multi-exon genes, 40 probes on average are used to interrogate the same gene) increases the robustness of gene-level expression measurements. Background correction, normalization, and calculation of probe set summaries were based on the custom chip definition files from BrainArray** (Brainarray Version 11.0.1, HuEx10stv2_Hs_ENSG)[19,20] and the Robust Multichip Average method.[21] When computing the Robust Multichip Average, poorly performing probes, *i.e.*, probes with a signal less than 25 (log2 signal threshold 4.644) in all samples were excluded. The gene expression matrix was used as input to Gene Set Enrichment Analysis[22] (GSEA), which is designed to identify genes with coordinate transcriptional regulation within functionally related groups of genes called gene sets. Gene sets are collected in the Molecular Signatures Database†† (MSigDB; Release 3.0, Sept. 2010). GSEA was used (1) to identify pathways that were altered from time T1 (cannulation) to time T2 (after cross-clamp release) independently of the treatment, and (2) to identify differential transcriptional responses to on-pump CABG in RIPC *versus* placebo. To achieve this goal the fold change of each transcript from T1 to T2 was computed and GSEA analysis was performed using a two-phenotype design (FC_RIPC *vs.* FC_placebo). In order to compute the average gene expression changes within a given gene set, the gene expression levels of the enriched genes in a pathway were standardized to a mean of 0 and a variance of 1 across all 44 samples. Validation of chip data using real-time reverse-transcriptase polymerase chain reaction was conducted as previously described.[10]

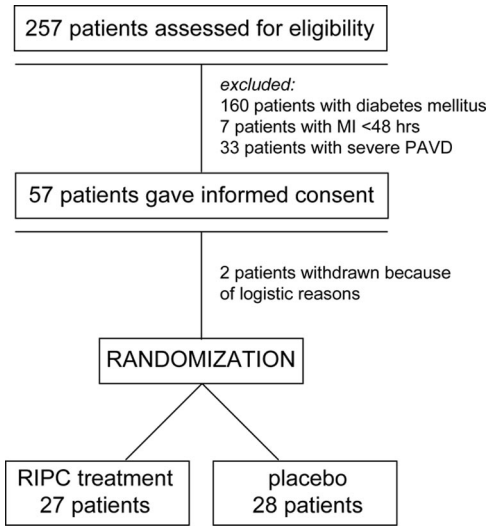

Fig. 2. CONSORT diagram showing the flow of patients through the randomized placebo-controlled remote ischemic preconditioning trial. MI = myocardial infarction; PAVD peripheral arterial vascular disease; RIPC = remote ischemic preconditioning.

** http://brainarray.mbni.med.umich.edu/Brainarray/Database/CustomCDF/genomic_curated_CDF.asp. Accessed April 20, 2011.

†† http://www.broadinstitute.org/gsea/msigdb/index.jsp. Accessed April 20, 2011.

Statistical Analysis

Sample size was calculated based on the results for cTnT reported previously.[2] With an expected difference of 0.22 pg/ml between group means, a SD of 0.25 pg/ml of the means, $\alpha = 0.05$, and $\beta = 0.8$, a sample size of 22 was necessary. Five or six additional patients per group were enrolled to compensate for possible dropouts. The area under the hscTnT concentration time curve was computed using the trapezoidal rule.[2] Continuous data were summarized as mean ± SD or median and quartiles (25% percentile, 75% percentile), where appropriate. Categorical data were summarized using percentage (proportions). The standardized difference (effect size) was computed as the (absolute) difference of the means divided by the SD of all observations (continuous data). In the case of proportions, the standardized difference was computed as: $\text{standardized difference} = \frac{|P_1 - P_2|}{\sqrt{P^*(1-P)}}$, where P_1 and P_2 are the proportions in the placebo and the RIPC group and P is their average. Biochemical parameters were log-transformed and two-way ANOVA was used to evaluate differences over time between groups. All other data including the primary outcome variables (peak hscTnT and area-under-the-curve) were analyzed using the unpaired Student *t* test or the Wilcoxon rank sum test, depending on the underlying data distribution. Categorical variables were compared using the chi-square test, if appropriate. To test the association between NT-proBNP and transcriptional changes, linear regression analysis was performed. In addition, forward stepwise linear regression (F-to-Enter = 4.000, F-to-Remove = 3.996) was applied using NT-proBNP as the dependent variable and transcriptional changes and clinical data as the independent variables. Adjusted squared correlation coefficients (R^2_{adj}) and the *P* values were reported. Differences were considered significant if $P < 0.05$. Analyses were performed using Sigmaplot Version 11.0 (Systat Software, Inc., Chicago, IL).

Table 1. Preoperative Patient Characteristics

	RIPC (n = 27)	Placebo (n = 28)	ES
Demographics			
Age (years)	59 ± 7	62 ± 10	0.33
Gender, male	26 (96)	24 (86)	0.37
Body weight (kg)	90 ± 13	87 ± 13.4	0.23
Height (cm)	173 ± 7	174 ± 9	0.14
Cardiac status			
EF (%)	49.2 ± 10.2	52.8 ± 7.1	0.40
Previous MI	11 (41)	12 (43)	0.04
Angina	21 (78)	19 (68)	0.22
CHF	1 (3.7)	1 (3.6)	0.01
sBP (mmHg)	128.4 ± 16.3	125.7 ± 15.7	0.17
dBP (mmHg)	77.4 ± 10.1	75.4 ± 7.0	0.22
O_2 saturation (%)	95.7 ± 1.2	96.4 ± 2.4	0.34
HR	61.3 ± 11.4	60.7 ± 11.5	0.05
Risk factors and comorbidities			
Hypertension	19 (70)	20 (71)	0.02
Hyperlipidemia	23 (85)	24 (86)	0.02
Current smoker	4 (15)	3 (11)	0.12
History of smoking	21 (78)	20 (71)	0.15
Pack years	17.7 ± 15.5	19.4 ± 14.8	0.11
Cancer	3 (11)	4 (14)	0.10
Medication			
β blockers	25 (93)	25 (89)	0.12
ACE inhibitors	14 (52)	14 (50)	0.04
Angiotensin receptor antagonists	5 (19)	8 (29)	0.24
Calcium blockers	3 (11)	5 (18)	0.19
Statins	26 (96)	27 (96)	0.01
ASA	26 (96)	26 (93)	0.15
Clopidogrel	1 (4)	5 (18)	0.46
Diuretics	6 (22)	5 (18)	0.11

Data are presented as mean ± SD or number (%).

ACE = angiotensin converting enzyme; ASA = aspirin; CHF = congestive heart failure; dBP = diastolic blood pressure; EF = ejection fraction; ES = effect size; HR = heart rate; MI = myocardial infarction; RIPC = remote ischemic preconditioning; sBP = systolic blood pressure.

Table 2. Preoperative Evaluations

	RIPC (n = 27)	Placebo (n = 28)	ES
Angiogram			
Number of diseased vessels	2.9 ± 0.7	3.0 ± 0.7	0.05
LM stenosis	9 (33)	8 (29)	0.10
≥2 vessels with ≥70% occlusion	24 (89)	19 (68)	0.51
Valvular disease			
Tricuspid regurgitation	3 (11)	7 (25)	0.36
Aortic regurgitation	0 (0)	4 (14)	0.55
Aortic stenosis	0 (0)	1 (4)	0.27
Mitral regurgitation	7 (26)	11 (39)	0.28
Mitral stenosis	0 (0)	0 (0)	—
Lab results			
HBG (g/l)	148.7 ± 10.1	147.2 ± 12.5	0.13
PLT (10^9/l)	226.3 ± 37.7	229.1 ± 62.9	0.05
Creatinine (μM)	91.7 ± 15.4	88.0 ± 24.5	0.18

Data are presented as mean ± SD or number (%).

ES = effect size; HGB = hemoglobin; LM = left main coronary artery; PLT = platelet; RIPC = remote ischemic preconditioning.

Results

Demographics, Perioperative Data, and Clinical Outcome

The CONSORT diagram is depicted in figure 2 and patient data are listed in table 1. The RIPC group and the placebo group were similar with respect to all preoperative data including medication and comorbidity. Data from the preoperative assessments are listed in table 2. The number of diseased coronary arteries and the number and the degree of accompanying valvular disease were similar between the groups. Intraoperative data were comparable between groups except for a slightly reduced CPB time in

Table 3. Intraoperative and Postoperative Data

	RIPC (n = 27)	Placebo (n = 28)	*P* Value
Intraoperative data			
Surgery time (min)	227 ± 42	205 ± 35	0.34
Anesthesia time (min)	303 ± 45	281 ± 40	0.33
Elapsed time between biopsies (min)	103 ± 32	93 ± 25	0.32
Bypass time (min)	109 ± 30	94 ± 24	0.05
Cross-clamp time (min)	74 ± 33	63 ± 24	0.16
Ventricular fibrillation after cross clamp	3 (11)	0 (0)	0.11
Atrial fibrillation after cross clamp	1 (4)	0 (0)	0.49
Total bypass grafting	3.6 ± 0.8	3.5 ± 0.6	0.39
Arterial grafting	1.2 ± 0.5	1.2 ± 0.5	0.74
Venous grafting	2.4 ± 1.1	2.3 ± 0.9	0.64
Average [isoflurane] (%) end-tidal	1.02 ± 0.11	1.05 ± 0.08	0.91
Min [isoflurane] (%) end-tidal	0.70 ± 0.29	0.69 ± 0.26	0.91
Max [isoflurane] (%) end-tidal	1.22 ± 0.37	1.29 ± 0.51	0.54
Postoperative data			
At ICU admission			
HGB (g/l)	108 ± 10	109 ± 16	0.88
PLT (10^9/l)	142 ± 42	146 ± 52	0.79
Creatinine (μM)	95 ± 18	90 ± 21	0.39
MAP (mmHg)	75 ± 8	80 ± 13	0.12
CVP (mmHg)	13 ± 12	10 ± 4	0.23
HR	81 ± 13	75 ± 12	0.08
On postop day 1			
HGB (g/l)	98 ± 10	103 ± 12	0.10
PLT (10^9/l)	147 ± 37	154 ± 49	0.54
Creatinine (μM)	90 ± 25	84 ± 21	0.35
MAP (mmHg)	75 ± 8	81 ± 7	0.11
sBP (mmHg)	112 ± 10	119 ± 17	0.17
dBP (mmHg)	67 ± 8	70 ± 11	0.38
CVP (mmHg)	8 ± 3	10 ± 3	0.47
HR	91.56 ± 17.25	88.64 ± 11.37	0.46
On postop day 2			
HGB (g/l)	98 ± 10	103 ± 12	0.10
PLT (10^9/l)	147 ± 37	154 ± 49	0.54
Creatinine (μM)	90 ± 25	84 ± 21	0.35
MAP (mmHg)	75 ± 8	81 ± 7	0.11
sBP (mmHg)	111 ± 10	119 ± 16	0.17
dBP (mmHg)	66 ± 8	70 ± 11	0.38
CVP (mmHg)	8 ± 3	10 ± 3	0.47
HR	92 ± 17	89 ± 11	0.46
On postop day 3			
Creatinine (μM)	92 ± 24	81 ± 21	0.09
Outcome at hospital discharge			
New myocardial infarction	3 (11)	1 (4)	0.35
New atrial fibrillation	10 (37)	5 (18)	0.14
Cerebrovascular insult	0 (0)	0 (0)	—
Hemofiltration/hemodialysis	0 (0)	0 (0)	—
Angiogram prior to discharge	0 (0)	0 (0)	—

Data are presented as mean ± SD or number (%).

CVP = central venous pressure; dBP = diastolic blood pressure; HGB = hemoglobin; HR = heart rate; ICU = intensive care unit; MAP = mean arterial pressure; PLT = platelets; RIPC = remote ischemic preconditioning; sBP = systolic blood pressure.

the placebo group ($P = 0.05$) (table 3). In the RIPC group, three of the patients had a new myocardial infarction and 10 experienced new atrial fibrillation, whereas in the placebo group only one patient had a new myocardial infarction and five patients experienced new atrial fibrillation (table 3). None of the patients required postoperative intraaortic balloon pump therapy, and the vasoconstrictor usage was similar between groups. None of the patients had cerebrovascular injury or renal damage requiring hemdialysis or hemofiltration. The incidence for the perioperative composite endpoint combining new arrhythmias and new myocardial infarctions was higher in the RIPC group than the placebo group (14/27 *vs.* 6/28, $P = 0.036$). However, there was no difference in the long-term cardiovascular outcome between the groups (table 4).

Table 4. All-cause Death and Cardiovascular Long-term Outcome (6 Months)

	RIPC (n = 27)	Placebo (n = 28)	*P* Value
Death	0 (0)	1* (4)	1.00
Rehospitalization	3 (11)	3 (11)	1.00
Heart failure	3	1	
Renal failure	0	1	
New atrial fibrillation	0	1	

Data are presented as mean ± SD or number (%).
* Due to cancer.
RIPC = remote ischemic preconditioning.

Biomarkers for Myocardial Necrosis (hscTnT, cTnT) and Contractile Dysfunction (NT-proBNP) Do Not Demonstrate Cardioprotection with RIPC in Isoflurane-anesthetized Patients

Measurements of hscTnT and cTnT representing the primary endpoint of this study peaked on postoperative day 1 and were similar in the RIPC and the placebo groups (fig. 3A, 3B). The area under the curve was 15,146 (11,708; 25,330) pg · h^{-1} · ml^{-1} in the RIPC group and 9,574 (8,082; 16,597) pg · h^{-1} · ml^{-1} in the placebo group (P = 0.33). Median peak postoperative hscTnT values were 298 (226; 440) pg/ml in the RIPC group compared with 231 (133; 418) pg/ml in the placebo group. Five RIPC patients and two placebo patients had a peak postoperative cTnT concentration greater than 650 pg/ml (5/27 *vs.* 2/28, P = 0.32), indicative of major myocardial damage.[23] NT-proBNP values exhibited a gradual increase from postoperative day 1 to postoperative day 3. There was no difference between the RIPC group and the placebo group (fig. 3C). Median peak postoperative NT-proBNP values were 2,348 (1,638; 3,196) pg/ml in the RIPC group compared with 1,758 (1,234; 3,049) pg/ml in the placebo group.

Biomarker for the Perioperative Inflammatory Response (hsCRP) and Cerebral Injury (S100) Do Not Show Protection with RIPC in Isoflurane-anesthetized Patients

Preoperative hsCRP, a marker for coronary artery plaque inflammation and instability, was similar between RIPC and the placebo group (fig. 4A). Two patients in the placebo group but no patient in the RIPC group had increased baseline preoperative hsCRP levels. Levels of hsCRP peaked on postoperative day 2 (RIPC group: 251 (210; 304) mg/l *vs.* placebo group: 229 (136; 328) mg/l), but there was no difference between groups. S100 measurements only showed a sharp peak 1 h after the release of the cross-clamp (RIPC group: 1.26 (0.79; 1.93) pg/ml *vs.* placebo group: 0.98 (0.45; 1.57) pg/ml) (fig. 4B), which did not correlate with hscTnT measurements (R^2 = 0.12, P = 0.67), implying a different source of release of this biomarker than the heart.

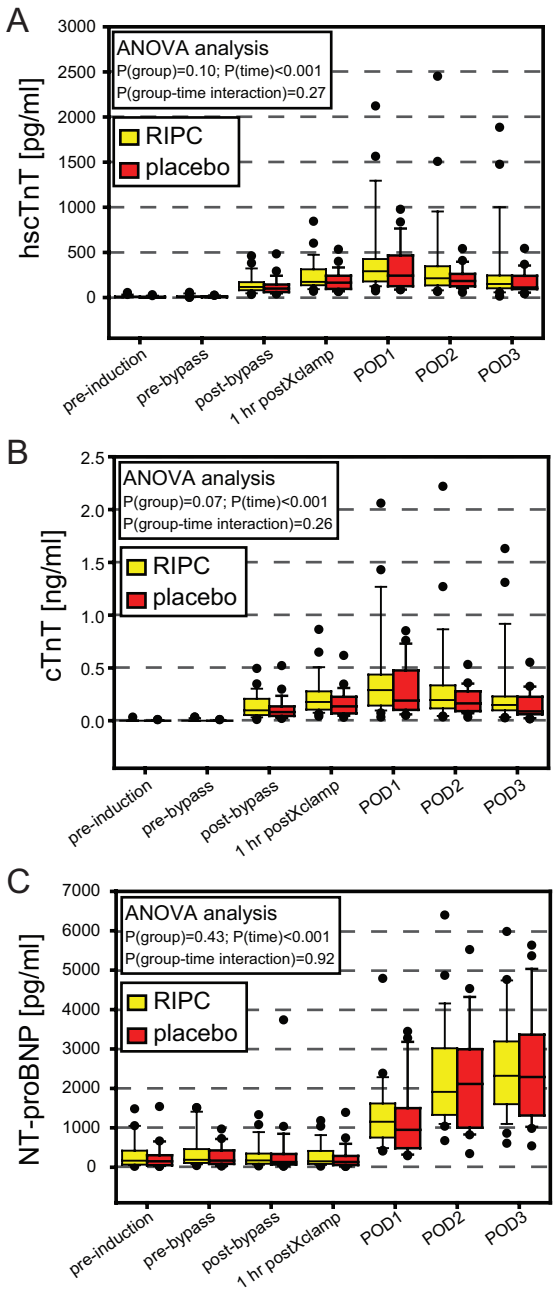

Fig. 3. Cardiovascular biomarkers. (*A*) High-sensitivity cardiac troponin T (hscTnT). (*B*) Cardiac troponin T (cTnT). (*C*) N-terminal pro-brain natriuretic peptide (NT-proBNP). POD = postoperative day; RIPC = remote ischemic preconditioning.

Gene Expression Profiling Unveils that Isoflurane, but Not RIPC-related Transcriptional Footprints, Correlate with the Release of the Biomarker NT-proBNP

For each patient two atrial biopsies were collected, the first sample after induction of anesthesia and chest opening and the second sample after release of the cross-clamp before chest closing. Sample one in both groups mainly reflects the gene expression in the presence of isoflurane, because RIPC was conducted shortly before cannulation and collection of the first tissue sample. Sample two mirrors the expression after ischemia-reperfu-

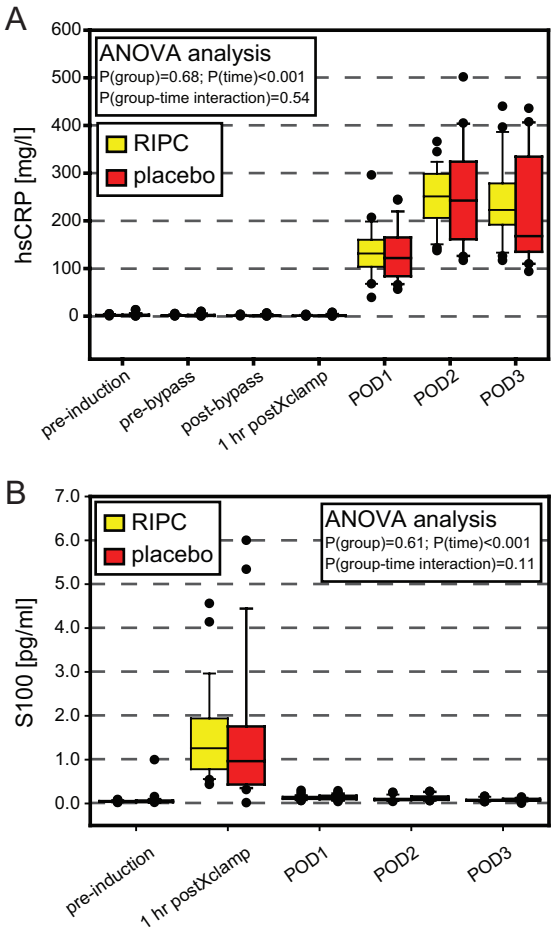

Fig. 4. Inflammatory response and brain injury. (*A*) High-sensitivity C-reactive protein (hsCRP). (*B*) S100 protein. POD = postoperative day; RIPC = remote ischemic preconditioning.

sion with or without the transcriptional effects elicited by RIPC. The expression and direction of transcriptional changes were reliably detected by the microarray as confirmed by real-time quantitative polymerase chain reaction (see Supplemental Digital Content 1, http://links.lww.com/ALN/A796, which is primer information; see Supplemental Digital Content 2, http://links.lww.com/ALN/A797, which is validation status). As a first step, gene sets differentially regulated over time (T1-T2) in both groups were determined. CABG surgery with isoflurane anesthesia induced significant and characteristic changes over time, as detected by GSEA (table 5). Transcripts involved in fatty-acid oxidation and DNA-damage signaling were down-regulated (figs. 5 and 6 and table 5), a transcriptional feature that was previously reported for sevoflurane when compared with propofol in off-pump CABG surgery.[10] There was a close correlation between fatty-acid oxidation and DNA-damage signaling ($R^2_{adj} = 0.89$, $P < 0.001$; see Supplemental Digital Content 3, http://links.lww.com/ALN/A798, which is a graphical representation). Fatty-acid oxidation ($P = 0.001$; fig. 5C) and DNA-damage signaling ($P < 0.001$; fig. 6C) directly correlated with peak NT-proBNP release. There was no correlation with hscTnT. A forward stepwise linear regression was conducted to test whether other factors such as body temperature, bypass time, cross-clamp time, or time elapsed between the collection of the biopsies would predict peak NT-proBNP values. Our analysis shows that only changes in transcripts related to fatty acid metabolism ($P = 0.001$) reliably predict peak NT-proBNP values. Using DNA damage signaling, which strongly correlates with fatty acid metabolism, in a similar analysis, DNA damage signaling ($P < 0.001$) and bypass time ($P = 0.026$) remain as independent predictors of NT-proBNP in the model. Although GSEA detected differences in transcriptional activity between RIPC and placebo (see Supplemental Digital Content 4, http://links.lww.com/ALN/A800, and Supplemental Digital Content 5, http://links.lww.com/ALN/A801, for heat maps of the differentially regulated genes), including gene sets related to tumor necrosis factor signaling,[24] stem cell and progenitor activity,[25] hypertrophy[26] (see table in Supplemental Digital Content 6, http://links.lww.com/ALN/A802, for all upregulated in RIPC), and inner mitochondrial membrane proteins (see table in Supplemental Digital Content 6, http://links.lww.com/ALN/A802, for down-regulated in RIPC), these differences did not correlate with the release of biomarkers (fig. 7 and further graphical representations in Supplemental Digital Content 7, http://links.lww.com/ALN/A803; Supplemental Digital Content 8, http://links.lww.com/ALN/A804; Supplemental Digital Content 9, http://links.lww.com/ALN/A805; and Supplemental Digital Content 10, http://links.lww.com/ALN/A806).

Discussion

The present study tested whether RIPC, when applied following induction of anesthesia, would protect the myocardium against ischemic injury in isoflurane-anesthetized patients undergoing on-pump CABG surgery. Unlike most previous studies,[2,27] the anesthetic was standardized in our study with propofol used for induction and isoflurane for maintenance before, during, and after cardiopulmonary bypass. Here, we report the following salient findings. First, we were unable to detect enhanced cardioprotection by RIPC with any of the used cardiac biomarkers (hscTnT, cTnT, NT-proBNP) in isoflurane-anesthetized patients. Also, there was no decrease in in-hospital and 6-months cardiovascular complications in patients assigned to RIPC. Rather, combining arrhythmias and new myocardial infarction unexpectedly discovered fewer events in the placebo patients. We note that our study is clearly underpowered to detect differences in clinical outcomes, but the sample size used allowed the detection of a 40% difference in the release of troponin between groups with a power of 80%.[2] However, smaller differences may not be detected reliably. Since RIPC potentially impacts not only the heart but also other vital organs,[3] we also evaluated whether RIPC would reduce the release of S100, a marker of cerebral injury, because of CPB-associated emboli[28] and hsCRP, a marker of the perioperative inflammatory response. Again, no differences between groups were

Table 5. Representative Induced and Repressed Pathways in On-pump CABG Surgery with Isoflurane Anesthesia

GSEA Pathway	Brief Description	Normalized Enrichment Score	*P* Value	False Discovery Rate *Q* Value
UZONYI_RESPONSE_TO_LEUKOTRIENE_AND_THROMBIN	Genes upregulated in HUVEC cells (primary endothelium) after stimulation with LTD4 or thrombin for 1 h	1.690	0.002	0.493
SCHLESINGER_METHYLATED_DE_NOVO_IN_CANCER	Genes whose promoters are bound by the polycomb proteins SUZ12 or EED. Epigenetic system that normally has a role in marking embryonic genes for repression	1.769	0.000	0.443
KAAB_FAILED_HEART_ATRIUM_UP	Genes upregulated in atria of failing hearts (DCM and ICM) compared to healthy controls	1.600	0.014	0.475
V$SRF_01	Genes with promoter regions around transcription start site containing the motif ATGCCCATATATGGWNNT, which matches annotation for serum response factor (c-fos serum response element-binding transcription factor)	1.856	0.004	0.636
ADDYA_ERYTHROID_DIFFERENTIATION_BY_HEMIN	Selected genes changed in K562 cells induced by hemin treatment to express erythroid properties	1.813	0.008	0.546
V$STAT3_02	Genes with promoter regions around transcription start site containing the motif NNNTTCCN, which matches annotation for signal transducer and activator of transcription 3 (STAT3)	1.716	0.010	0.493
REACTOME_NUCLEAR_RECEPTOR_TRANSCRIPTION_PATHWAY	Genes involved in the nuclear receptor transcription pathway	1.637	0.019	0.484
REACTOME_FGFR_LIGAND_BINDING_AND_ACTIVATION	Genes involved in fibroblast growth factor receptor ligand binding and activation	1.549	0.019	0.472
GERY_CEBP_TARGETS	Genes changed in NIH 3T3 cells by expression of one or more of CCAAT/enhancer-binding proteins	1.689	0.021	0.467
NEGATIVE_REGULATION_OF_BIOSYNTHETIC_PROCESS	Genes annotated by the GO* term GO:0009890. Any process that stops, prevents, or reduces the rate of the chemical reactions resulting in biosynthesis	1.580	0.022	0.499
GNF2_ATM	Neighborhood of ATM	−1.651	0.004	1.000
DNA_DAMAGE	The process of restoring DNA after damage	−1.706	0.000	1.000
KEGG_FATTY_ACID_METABOLISM	Fatty acid metabolism	−1.555	0.011	1.000
ZHOU_INFLAMMATORY_RESPONSE_FIMA_DN	Genes down-regulated in macrophages by the FimA pathogen	−1.548	0.010	1.000

(*continued*)

Table 5. Continued

GSEA Pathway	Brief Description	Normalized Enrichment Score	*P* Value	False Discovery Rate *Q* Value
KINETOCHORE	Genes annotated by the GO* term GO:0000776. A multi-subunit complex on chromosomes where the spindle fibers attach during cell division	−1.542	0.006	1.000
IVANOVA_HEMATOPOIESIS_INTERMEDIATE_PROGENITOR	Genes upregulated in hematopoietic intermediate progenitor cells from adult bone marrow and fetal liver	−1.536	0.021	1.000
NUCLEAR_ENVELOPE	Genes annotated by the GO* term GO:0005635. The double lipid bilayer enclosing the nucleus and separating its contents from the rest of the cytoplasm	−1.510	0.005	1.000
KEGG_GLUTATHIONE_METABOLISM	Genes involved in glutathione metabolism	−1.505	0.025	1.000
REACTOME_PEROXISOMAL_LIPID_METABOLISM	Genes involved in peroxisomal lipid metabolism	−1.495	0.021	1.000
REACTOME_METABOLISM_OF_RNA	Genes involved in the metabolism of RNA	−1.425	0.039	1.000

False discovery rate is the estimated probability that a set with a given normalized enrichment score represents a false positive finding. A normalized enrichment score is measured as positive = induction/upregulation at time T2, or after aortic cross clamp release; negative = repression/down-regulation at time T2.

* Gene ontology, http://www.geneontology.org. Accessed April 20, 2011.

ATM = ataxia telangiectasia mutated; CABG = coronary artery bypass graft surgery; DCM = dilated cardiomyopathy; EED = embryonic ectoderm development (GeneID = 8,726); GO = gene ontology; GSEA = gene set enrichment analysis; HUVEC = human umbilical vein endothelial cells; ICM = ischemic cardiomyopathy; LTD4 = leukotriene D_4; NIH 3T3 = Mouse embryonic fibroblast cell line established from a National Institutes of Health (Bethesda, Maryland) Swiss mouse embryo; SUZ12 = suppressor of zeste 12 homolog (Drosophila; GeneID = 23512).

detected in these secondary endpoints. Our results of the biomarker analyses were further corroborated with a comprehensive transcriptional analysis in atrial tissue samples collected at the beginning and at the end of the cardiopulmonary bypass. Although previous studies investigated RIPC-induced transcriptional changes in mouse hearts[29] and human blood,[30] our study is the first to assess RIPC-induced transcriptional changes in human hearts. In accordance with the negative results of the biomarker analyses, our genome-wide analysis revealed that peak NT-proBNP release correlated with isoflurane- but not RIPC-induced transcriptional footprints, suggesting that isoflurane rather than RIPC dominated the transcriptome and potentially translated into functional improvement as measured by lower NT-proBNP release. Collectively, we were unable to detect cardioprotective effects elicited by RIPC in isoflurane-anesthetized patients at the biochemical or clinical level.

How can these results be interpreted in the context of the available preconditioning literature? According to the threshold theory of preconditioning, which implies that a certain degree of stimulation is required to reach the level where a cell or organ is able to effectively activate its endogenous protection program, it could be expected that the application of two well defined preconditioning-stimuli, such as RIPC and isoflurane in our study, should indeed induce a more consistent and effective overall cell protection. Conversely, if the maximum preconditioning trigger stimulus has been already reached with approximately 1.0–1.5 minimum alveolar concentration of isoflurane alone, as used in our study, the ischemic stimulus by RIPC may become redundant, and the net result would be a lack of synergy. In support of this concept, Zaugg *et al.*[15] reported a concentration-dependent protection by isoflurane and sevoflurane in isolated rat ventricular myocytes with a ceiling-effect at approximately 1.5 minimum alveolar concentration. Whereas most previous studies of RIPC in nonsurgical patients showed cardioprotection,[31] results from studies in patients undergoing CABG surgery were rather mixed or disappointing.[27] In fact, a recent randomized double-blinded study with 162 patients undergoing on-pump CABG surgery demonstrated no reduction in troponin release or improvement in hemodynamics or any renal or lung protection after exposure to RIPC elicited by three 5-min cycles of 200 mmHg cuff inflation/deflation of the arm.[27] In that study, the patients were exposed to propofol at the time of RIPC and to some (unreported) levels of enflurane and sevoflurane during cardiopulmonary by-

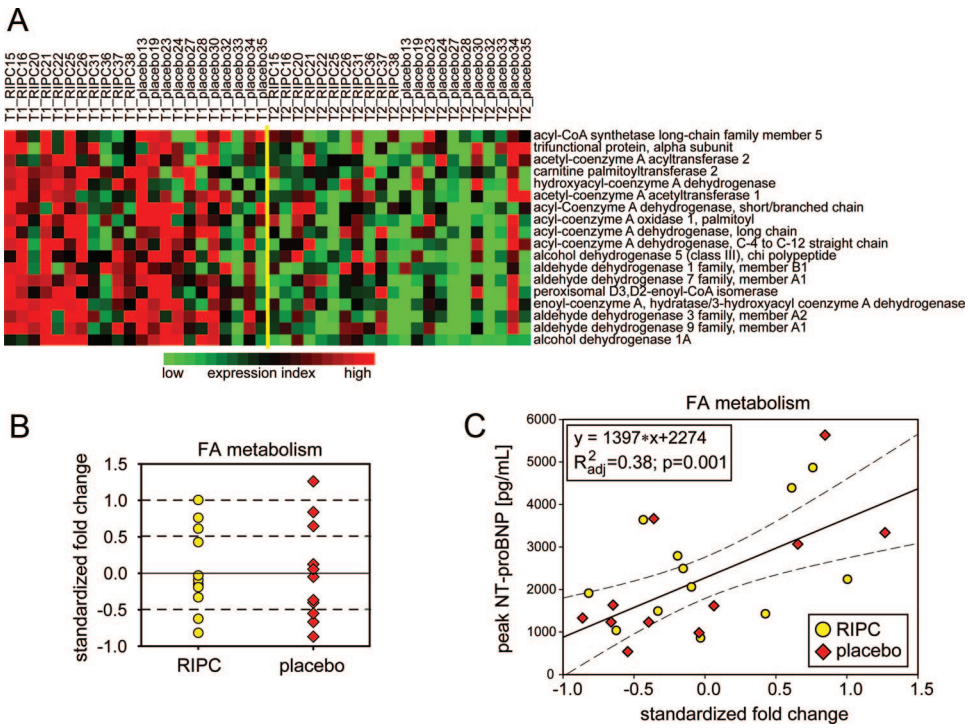

Fig. 5. Fatty-acid metabolism and release of NT-proBNP. (*A*) Fatty-acid metabolism pathway activity at the time of cannulation and after aortic cross clamp release. Each *numbered square* indicates the expression of the indicated transcript in a specific patient. *Red* color indicates high expression, *green* color indicates low expression. There was down-regulation of fatty-acid metabolism-related transcripts (18 of 34) during isoflurane anesthesia and surgery. (*B*) There was no difference in the regulation of fatty-acid metabolism between remote ischemic preconditioning and placebo. Each *dot* represents the standardized fold change of all enriched transcripts in the fatty-acid metabolism signaling pathway. (*C*) Independent of group assignment, fatty-acid metabolism given as standardized fold change closely correlated with peak NT-proBNP release ($P = 0.001$). *Dashed lines* indicate 95% CIs. FA = fatty acids; NT-proBNP = N-terminal pro-brain natriuretic peptide; RIPC = remote ischemic preconditioning; T1 = time of cannulation; T2 = after aortic cross clamp release.

pass. We speculate that application of RIPC under general anesthesia is an ineffective way to achieve cardiac and vital organ protection, because anesthetics are known to mitigate the ischemic response in the human body necessary to elicit the preconditioned state. This may be particularly true for ether-derived volatile anesthetics, which are known to elicit strong preconditioning by activation of the mitochondrial K_{ATP} channel.[13,15] In fact, Lucchinetti *et al.*[32] showed in healthy volunteers that sevoflurane at low sedative concentrations attenuates ischemia-reperfusion-induced activation of leukocytes and protects the endothelium against ischemic injury. Likewise, propofol is known to protect against ischemia/reperfusion damage in a human forearm model of ischemia-reperfusion.[33] What do we know from animal studies? Using a rat model of unilateral nephrectomy and ischemic preconditioning with three 5-min cycles of the contralateral kidney artery, Vianna *et al.*[34] demonstrated that ischemic preconditioning, when applied during isoflurane anesthesia, completely "loses" its renal protection compared to isoflurane anesthesia alone. Similarly, opioids such as remifentanil limit infarct size but attenuate ischemic preconditioning-induced infarct limitation in a rabbit model.[35] Conversely, Toller *et al.*[36] reported in a dog model of coronary artery occlusion synergistic effects of ischemic preconditioning and sevoflurane if administered sequentially and not concomitantly. Taken together, these studies provide evidence of antagonism rather than lack of synergy between different types of preconditioning, *i.e.*, ischemic and pharmacologic preconditioning, and suggest that anesthetics attenuate or even abolish RIPC when administered concomitantly.

Using oligonucleotide microarrays, we previously studied different types of preconditioning for their therapeutic potential in human and rat cardiac tissues.[10,16] Whereas both pharmacologic preconditioning with isoflurane and ischemic preconditioning prevented activation of genes involved in hypertrophy and remodeling, ischemic as opposed to isoflurane preconditioning elicited a postischemic expression profile similar to unprotected cardiac tissue, implying that ischemic preconditioning may be even harmful to the myocardium. Iliodromitis *et al.*[37] reported that RIPC in patients undergoing percuntaneous coronary intervention exacerbates the release of troponin from the heart and that the inflammatory marker C-reactive protein remains high after the intervention especially in patients treated with RIPC. In our study, GSEA,[22] a sophisticated tool for pattern recognition, detected upregulation of gene sets related to hypertrophy and inflammation after RIPC heralding detrimental rather than beneficial effects. The higher incidence of the

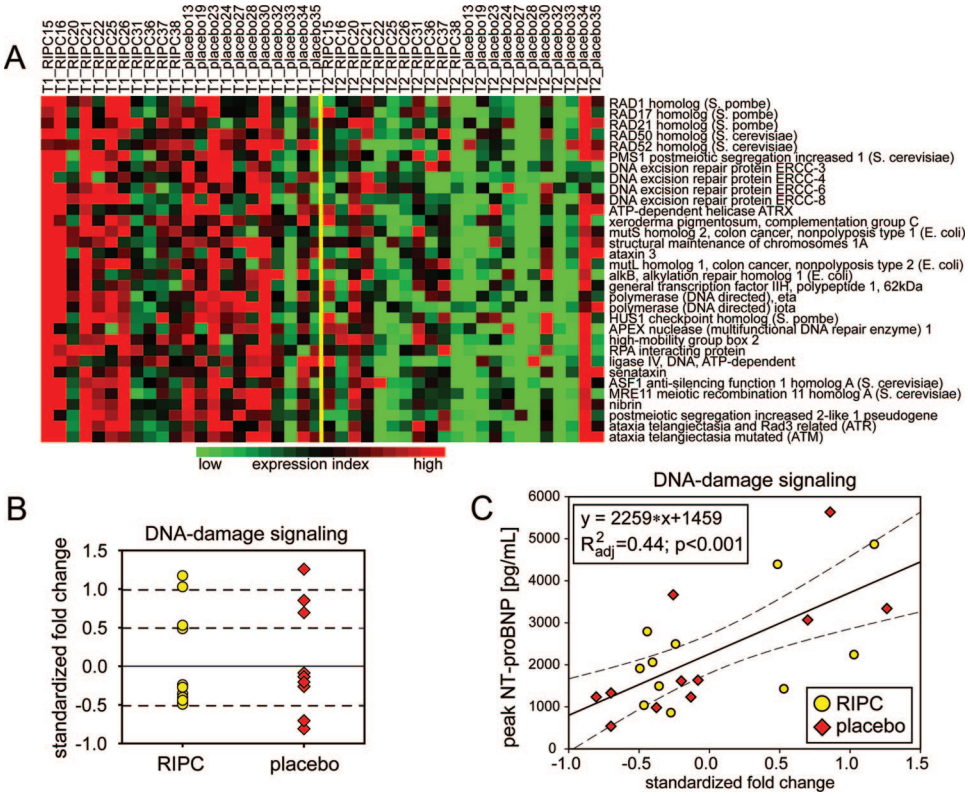

Fig. 6. DNA-damage signaling and release of NT-proBNP. (*A*) DNA-damage signaling pathway activity at the time of cannulation and after aortic cross clamp release. Each *numbered square* indicates the expression of the indicated transcript in a specific patient. *Red* color indicates high expression, *green* color indicates low expression. There was down-regulation of DNA-damage signaling transcripts (32 of 103) during isoflurane anesthesia and surgery. (*B*) There was no difference in the regulation of DNA-damage signaling between remote ischemic preconditioning and placebo. Each *dot* represents the standardized fold change of all enriched transcripts in the DNA-damage signaling pathway. (*C*) Independent of group assignment, DNA-damage signaling given as standardized fold change closely correlated with peak NT-proBNP release ($P < 0.001$). *Dashed lines* indicate 95% CIs. NT-proBNP = N-terminal pro-brain natriuretic peptide; RIPC = remote ischemic preconditioning; RPA = replication protein A; T1 = time of cannulation; T2 = after aortic cross clamp release.

composite endpoint arrhythmias and myocardial infarction in RIPC patients ($P = 0.036$) raises the possibility that RIPC under certain conditions may harm rather than benefit. However, these RIPC-induced transcriptional changes did not correlate with the release of biomarkers. On the other hand, GSEA[22] detected beneficial transcriptional changes previously observed in hearts exposed to volatile anesthetics, including down-regulation of transcripts involved in fatty-acid oxidation.[10] This metabolic shift correlated closely with transcripts involved in DNA-damage signaling (see Supplemental Digital Content 3, http://links.lww.com/ALN/A798) and perioperative cardiac function as determined by NT-proBNP release and confirms previous findings that myocardial substrate metabolism critically affects perioperative cardiac function.[10,38,39] A comparison of the peak NT-proBNP release between this study and the study by Julier *et al.*[6] demonstrates that RIPC and placebo patients in the present study (approximately 2,200 pg/ml) were more similar to the sevoflurane preconditioned group (approximately 1,500 pg/ml) than to the placebo group (without preconditioning) (approximately 3,800 pg/ml) of the Julier study, suggesting that most probably all but not just the RIPC patients, in accordance with isoflurane application to all patients, were preconditioned and hence protected in our study.

Our findings have important clinical implications. RIPC remains a promising strategy to provide protection to the entire body specifically in the nonsurgical setting.[31] But it harbors the risk of plaque ruptures, thrombosis, and embolization. More importantly, the right "dose" of ischemia is unknown specifically during concomitant anesthesia, and experimental studies suggest that diseased hearts may be less amenable to ischemic than pharmacologic preconditioning.[40] Since our study, consistent with previous results,[32] suggests that ischemic and pharmacologic preconditioning antagonize each other rather than act in synergy, organ protection with volatile anesthetics alone may be preferable at least in CABG patients. Another possibility, though less feasible in the clinical setting, could be the sequential application of different types of preconditioning-stimuli, *i.e.*, applying RIPC in the awake patient before anesthesia and surgery. We would like to emphasize that anesthetics may mitigate much less the

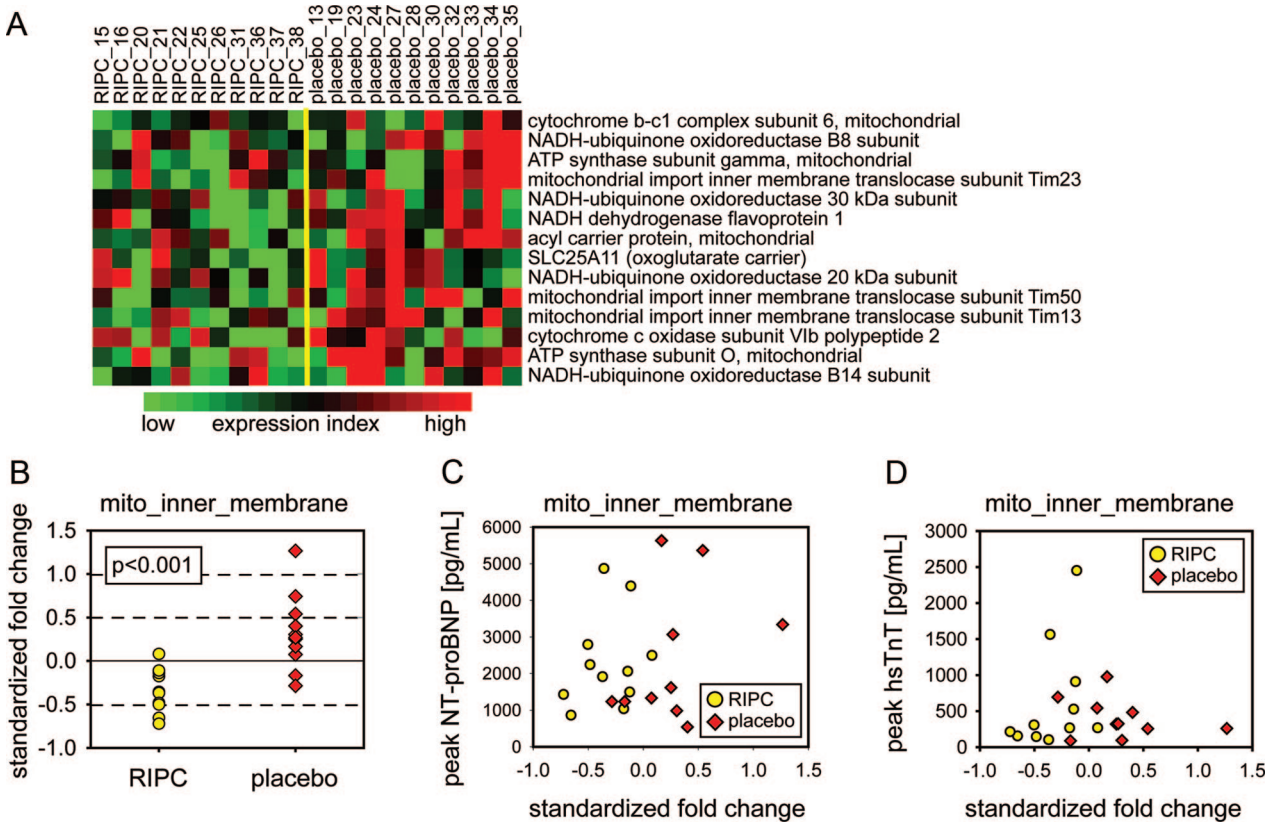

Fig. 7. Absence of correlation between remote ischemic preconditioning-induced transcriptional changes and biomarkers. (*A*) Representative gene set of inner mitochondrial membrane given as fold changes between time of cannulation and aortic cross clamp release. *Red* color indicates upregulation and *green* color indicates down-regulation of transcripts over time. (*B*) There was down-regulation of transcripts related to inner mitochondrial proteins in remote ischemic preconditioning patients as compared with placebo. However, there was no correlation between the release of NT-proBNP (*C*) or hscTnT (*D*). ATP = adenosine-5′-triphosphate; hscTnT = high sensitivity cardiac troponin; NADH = nicotinamide adenine dinucleotide; NT-proBNP = N-terminal pro-brain natriuretic peptide; RIPC = remote ischemic preconditioning.

effects of direct ischemic preconditioning (classic preconditioning), because the ischemic stimulus in this case is much stronger than the anesthetic effects and thus is likely to dominate cell signaling. Whether similar antagonistic effects between volatile anesthetics and RIPC can be observed in patients undergoing abdominal aortic aneurysm repair, *i.e.*, in noncardiac surgery, where RIPC was successfully used for cardioprotection in the past,[41,42] needs to be investigated in future clinical trials. Irrespectively, isoflurane and other halogenated ethers can be safely inhaled during surgery and thus act systemically providing total body protection.

Study Limitations

The negative result of our study could be theoretically because of a failure of our RIPC protocol using leg ischemia as opposed to arm ischemia-induced RIPC. However, this is unlikely, because lower limb-induced RIPC was previously shown to successfully elicit protection in the heart.[41] Also, by using microarray technology in myocardial samples we were able to monitor the effects of leg ischemia-induced RIPC on the cardiac transcriptome, providing evidence that the remote ischemic stimulus was indeed transferred to the heart. Moreover, from a conceptual point of view, leg ischemia should be more effective in activating RIPC than arm ischemia because of the higher release of autacoids. A previous study in rabbits[43] showed that propofol may inhibit desflurane preconditioning if the drugs were administered concomitantly. However, in our clinical study, we used isoflurane and not desflurane, and the results of this animal study cannot be directly translated into the clinical setting. Moreover, in the study by Julier *et al.*[6] propofol was used for induction and maintenance in many patients but did not block sevoflurane protection. Finally, a comparison of the peak NT-proBNP values between our current study and the study by Julier *et al.*[6] suggests that most probably all but not just the RIPC patients, in accordance with isoflurane application to all patients, were preconditioned and hence protected. Therefore, it is unlikely that propofol as the induction agent in our study inhibited any of the preconditioning stimuli (isoflurane or RIPC).

In conclusion, our study suggests that RIPC applied during isoflurane inhalation, and most probably inhalation of other halogenated ethers, provides no additional benefit to the myocardium of patients undergoing on-pump CABG surgery.

The authors thank the cardiac surgeons, anesthesiologists, intensive care physicians and nurses, research assistants, colleagues, and volunteers who facilitated the completion of this trial. The authors would like to thank Catharine Aquino, Ph.D. (Functional Genomics Center Zurich, Zurich, Switzerland), for technical assistance in the hybridization of microarrays.

References

1. Kharbanda RK, Mortensen UM, White PA, Kristiansen SB, Schmidt MR, Hoschtitzky JA, Vogel M, Sorensen K, Redington AN, MacAllister R: Transient limb ischemia induces remote ischemic preconditioning *in vivo*. Circulation 2002; 106:2881–3
2. Hausenloy DJ, Mwamure PK, Venugopal V, Harris J, Barnard M, Grundy E, Ashley E, Vichare S, Di Salvo C, Kolvekar S, Hayward M, Keogh B, MacAllister RJ, Yellon DM: Effect of remote ischaemic preconditioning on myocardial injury in patients undergoing coronary artery bypass graft surgery: A randomised controlled trial. Lancet 2007; 370:575–9
3. Hausenloy DJ, Yellon DM: Remote ischaemic preconditioning: Underlying mechanisms and clinical application. Cardiovasc Res 2008; 79:377–86
4. Weber C: Far from the heart: Receptor cross-talk in remote conditioning. Nat Med 2010; 16:760–2
5. De Hert SG, ten Broecke PW, Mertens E, Van Sommeren EW, De Blier IG, Stockman BA, Rodrigus IE: Sevoflurane but not propofol preserves myocardial function in coronary surgery patients. ANESTHESIOLOGY 2002; 97:42–9
6. Julier K, da Silva R, Garcia C, Bestmann L, Frascarolo P, Zollinger A, Chassot PG, Schmid ER, Turina MI, von Segesser LK, Pasch T, Spahn DR, Zaugg M: Preconditioning by sevoflurane decreases biochemical markers for myocardial and renal dysfunction in coronary artery bypass graft surgery: A double-blinded, placebo-controlled, multicenter study. ANESTHESIOLOGY 2003; 98:1315–27
7. Symons JA, Myles PS: Myocardial protection with volatile anaesthetic agents during coronary artery bypass surgery: A meta-analysis. Br J Anaesth 2006; 97:127–36
8. Yu CH, Beattie WS: The effects of volatile anesthetics on cardiac ischemic complications and mortality in CABG: A meta-analysis. Can J Anaesth 2006; 53:906–18
9. De Hert SG, Van der Linden PJ, Cromheecke S, Meeus R, Nelis A, Van Reeth V, ten Broecke PW, De Blier IG, Stockman BA, Rodrigus IE: Cardioprotective properties of sevoflurane in patients undergoing coronary surgery with cardiopulmonary bypass are related to the modalities of its administration. ANESTHESIOLOGY 2004; 101:299–310
10. Lucchinetti E, Hofer C, Bestmann L, Hersberger M, Feng J, Zhu M, Furrer L, Schaub MC, Tavakoli R, Genoni M, Zollinger A, Zaugg M: Gene regulatory control of myocardial energy metabolism predicts postoperative cardiac function in patients undergoing off-pump coronary artery bypass graft surgery: Inhalational *versus* intravenous anesthetics. ANESTHESIOLOGY 2007; 106:444–57
11. Zaugg M, Lucchinetti E, Uecker M, Pasch T, Schaub MC: Anaesthetics and cardiac preconditioning. Part I: Signalling and cytoprotective mechanisms. Br J Anaesth 2003; 91: 551–65
12. Feng J, Lucchinetti E, Ahuja P, Pasch T, Perriard JC, Zaugg M: Isoflurane postconditioning prevents opening of the mitochondrial permeability transition pore through inhibition of glycogen synthase kinase 3beta. ANESTHESIOLOGY 2005; 103: 987–95
13. Kersten JR, Schmeling TJ, Pagel PS, Gross GJ, Warltier DC: Isoflurane mimics ischemic preconditioning *via* activation of K(ATP) channels: Reduction of myocardial infarct size with an acute memory phase. ANESTHESIOLOGY 1997; 87:361–70
14. Loukogeorgakis SP, Williams R, Panagiotidou AT, Kolvekar SK, Donald A, Cole TJ, Yellon DM, Deanfield JE, MacAllister RJ: Transient limb ischemia induces remote preconditioning and remote postconditioning in humans by a K(ATP)-channel dependent mechanism. Circulation 2007; 116:1386–95
15. Zaugg M, Lucchinetti E, Spahn DR, Pasch T, Schaub MC: Volatile anesthetics mimic cardiac preconditioning by priming the activation of mitochondrial K(ATP) channels *via* multiple signaling pathways. ANESTHESIOLOGY 2002; 97:4–14
16. da Silva R, Lucchinetti E, Pasch T, Schaub MC, Zaugg M: Ischemic but not pharmacological preconditioning elicits a gene expression profile similar to unprotected myocardium. Physiol Genomics 2004; 20:117–30
17. Thygesen K, Alpert JS, White HD, Joint ESC/ACCF/AHA/WHF Task Force for the Redefinition of Myocardial Infarction, Jaffe AS, Apple FS, Galvani M, Katus HA, Newby LK, Ravkilde J, Chaitman B, Clemmensen PM, Dellborg M, Hod H, Porela P, Underwood R, Bax JJ, Beller GA, Bonow R, Van der Wall EE, Bassand JP, Wijns W, Ferguson TB, Steg PG, Uretsky BF, Williams DO, Armstrong PW, Antman EM, Fox KA, Hamm CW, Ohman EM, Simoons ML, Poole-Wilson PA, Gurfinkel EP, Lopez-Sendon JL, Pais P, Mendis S, Zhu JR, Wallentin LC, Fernández-Avilés F, Fox KM, Parkhomenko AN, Priori SG, Tendera M, Voipio-Pulkki LM, Vahanian A, Camm AJ, De Caterina R, Dean V, Dickstein K, Filippatos G, Funck-Brentano C, Hellemans I, Kristensen SD, McGregor K, Sechtem U, Silber S, Widimsky P, Zamorano JL, Morais J, Brener S, Harrington R, Morrow D, Lim M, Martinez-Rios MA, Steinhubl S, Levine GN, Gibler WB, Goff D, Tubaro M, Dudek D, Al-Attar N: Universal definition of myocardial infarction. Circulation 2007; 116:2634–53
18. Brazma A, Hingamp P, Quackenbush J, Sherlock G, Spellman P, Stoeckert C, Aach J, Ansorge W, Ball CA, Causton HC, Gaasterland T, Glenisson P, Holstege FC, Kim IF, Markowitz V, Matese JC, Parkinson H, Robinson A, Sarkans U, Schulze-Kremer S, Stewart J, Taylor R, Vilo J, Vingron M: Minimum information about a microarray experiment (MIAME)-toward standards for microarray data. Nat Genet 2001; 29:365–71
19. Dai M, Wang P, Boyd AD, Kostov G, Athey B, Jones EG, Bunney WE, Myers RM, Speed TP, Akil H, Watson SJ, Meng F: Evolving gene/transcript definitions significantly alter the interpretation of GeneChip data. Nucleic Acids Res 2005; 33:e175
20. Sandberg R, Larsson O: Improved precision and accuracy for microarrays using updated probe set definitions. BMC Bioinformatics 2007; 8:48
21. Irizarry RA, Bolstad BM, Collin F, Cope LM, Hobbs B, Speed TP: Summaries of Affymetrix GeneChip probe level data. Nucleic Acids Res 2003; 31:e15
22. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP: Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 2005; 102:15545–50
23. Carrier M, Pellerin M, Perrault LP, Solymoss BC, Pelletier LC: Troponin levels in patients with myocardial infarction after coronary artery bypass grafting. Ann Thorac Surg 2000; 69:435–40
24. Sana TR, Janatpour MJ, Sathe M, McEvoy LM, McClanahan TK: Microarray analysis of primary endothelial cells challenged with different inflammatory and immune cytokines. Cytokine 2005; 29:256–69
25. Ivanova NB, Dimos JT, Schaniel C, Hackney JA, Moore KA, Lemischka IR: A stem cell molecular signature. Science 2002; 298:601–4
26. Chen Y, Park S, Li Y, Missov E, Hou M, Han X, Hall JL, Miller LW, Bache RJ: Alterations of gene expression in failing myocardium following left ventricular assist device support. Physiol Genomics 2003; 14:251–60
27. Rahman IA, Mascaro JG, Steeds RP, Frenneaux MP, Nightingale P, Gosling P, Townsend P, Townend JN, Green D, Bonser RS: Remote ischemic preconditioning in human coronary artery bypass surgery: From promise to disappointment? Circulation 2010; 122:S53–9
28. Ascione R, Ghosh A, Reeves BC, Arnold J, Potts M, Shah A,

Angelini GD: Retinal and cerebral microembolization during coronary artery bypass surgery: A randomized, controlled trial. Circulation 2005; 112:3833–8

29. Konstantinov IE, Arab S, Li J, Coles JG, Boscarino C, Mori A, Cukerman E, Dawood F, Cheung MM, Shimizu M, Liu PP, Redington AN: The remote ischemic preconditioning stimulus modifies gene expression in mouse myocardium. J Thorac Cardiovasc Surg 2005; 130:1326–32
30. Konstantinov IE, Arab S, Kharbanda RK, Li J, Cheung MM, Cherepanov V, Downey GP, Liu PP, Cukerman E, Coles JG, Redington AN: The remote ischemic preconditioning stimulus modifies inflammatory gene expression in humans. Physiol Genomics 2004; 19:143–50
31. Bøtker HE, Kharbanda R, Schmidt MR, Bøttcher M, Kaltoft AK, Terkelsen CJ, Munk K, Andersen NH, Hansen TM, Trautner S, Lassen JF, Christiansen EH, Krusell LR, Kristensen SD, Thuesen L, Nielsen SS, Rehling M, Sørensen HT, Redington AN, Nielsen TT: Remote ischaemic conditioning before hospital admission, as a complement to angioplasty, and effect on myocardial salvage in patients with acute myocardial infarction: A randomised trial. Lancet 2010; 375:727–34
32. Lucchinetti E, Ambrosio S, Aguirre J, Herrmann P, Härter L, Keel M, Meier T, Zaugg M: Sevoflurane inhalation at sedative concentrations provides endothelial protection against ischemia-reperfusion injury in humans. ANESTHESIOLOGY 2007; 106:262–8
33. Turan R, Yagmurdur H, Kavutcu M, Dikmen B: Propofol and tourniquet induced ischaemia reperfusion injury in lower extremity operations. Eur J Anaesthesiol 2007; 24:185–9
34. Vianna PT, Castiglia YM, Braz JR, Viero RM, Beier S, Vianna Filho PT, Vitória A, Reinoldes Bizarria Guilherme G, de Assis Golim M, Deffune E: Remifentanil, isoflurane, and preconditioning attenuate renal ischemia/reperfusion injury in rats. Transplant Proc 2009; 41:4080–2
35. Kuzume K, Wolff RA, Chien GL, Van Winkle DM: Remifentanil limits infarct size but attenuates preconditioning-induced infarct limitation. Coron Artery Dis 2004; 15:449–55
36. Toller WG, Kersten JR, Pagel PS, Hettrick DA, Warltier DC: Sevoflurane reduces myocardial infarct size and decreases the time threshold for ischemic preconditioning in dogs. ANESTHESIOLOGY 1999; 91:1437–46
37. Iliodromitis EK, Kyrzopoulos S, Paraskevaidis IA, Kolocassides KG, Adamopoulos S, Karavolias G, Kremastinos DT: Increased C reactive protein and cardiac enzyme levels after coronary stent implantation. Is there protection by remote ischaemic preconditioning? Heart 2006; 92:1821–6
38. Lucchinetti E, Wang L, Ko KW, Troxler H, Hersberger M, Zhang L, Omar MA, Lopaschuk GD, Clanachan AS, Zaugg M: Enhanced glucose uptake *via* GLUT4 fuels recovery from calcium overload after ischaemia-reperfusion injury in sevoflurane- but not propofol-treated hearts. Br J Anaesth 2011; 106:792–800
39. Wang L, Ko KW, Lucchinetti E, Zhang L, Troxler H, Hersberger M, Omar MA, Posse de Chaves EI, Lopaschuk GD, Clanachan AS, Zaugg M: Metabolic profiling of hearts exposed to sevoflurane and propofol reveals distinct regulation of fatty acid and glucose oxidation: CD36 and pyruvate dehydrogenase as key regulators in anesthetic-induced fuel shift. ANESTHESIOLOGY 2010; 113:541–51
40. Zaugg M: Is protection by inhalation agents volatile? Controversies in cardioprotection. Br J Anaesth 2007; 99:603–6
41. Ali ZA, Callaghan CJ, Lim E, Ali AA, Nouraei SA, Akthar AM, Boyle JR, Varty K, Kharbanda RK, Dutka DP, Gaunt ME: Remote ischemic preconditioning reduces myocardial and renal injury after elective abdominal aortic aneurysm repair: A randomized controlled trial. Circulation 2007; 116:I98–105
42. Walsh SR, Tang TY, Sadat U, Gaunt ME: Remote ischemic preconditioning in major vascular surgery. J Vasc Surg 2009; 49:240–3
43. Smul TM, Stumpner J, Blomeyer C, Lotz C, Redel A, Lange M, Roewer N, Kehl F: Propofol inhibits desflurane-induced preconditioning in rabbits. J Cardiothorac Vasc Anesth 2011; 25:276–81