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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*

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Background: **Anesthetic gases modulate gene expression and provide organ protection. This study aimed at identifying myocardial transcriptional phenotypes to predict cardiovascular biomarkers and function in patients undergoing off-pump coronary artery bypass graft surgery.**

Methods: **In a prospective randomized trial, patients undergoing elective off-pump coronary artery bypass graft surgery were allocated to receive either the anesthetic gas sevoflurane (n = 10) or the intravenous anesthetic propofol (n = 10). Blood samples were collected perioperatively to determine cardiac troponin T, N-terminal pro–brain natriuretic peptide, and pregnancy-associated plasma protein A. Cardiac function was measured with transesophageal echocardiography and pulmonary artery thermodilution. Atrial biopsies were collected at the beginning and end of bypass surgery to determine gene expression profiles.**

Results: **N-terminal pro–brain natriuretic peptide and pregnancy-associated plasma protein A blood levels were decreased with sevoflurane treatment. Echocardiography showed preserved postoperative cardiac function in sevoflurane patients, which paralleled higher cardiac index measurements. N-terminal pro–brain natriuretic peptide release was predicted by sevoflurane-induced transcriptional reduction in fatty acid oxidation, whereas changes in cardiac index were predicted by preoperative gene activity of the peroxisome proliferator–activated receptor γ coactivator-1α pathway. Sevoflurane-mediated attenuation of transcripts involved in DNA-damage signaling and activation of the granulocyte colony-stimulating factor survival pathway predicted improved postoperative cardiac index and diastolic heart function, respectively.**

Conclusions: **Anesthetic-induced and constitutive gene regulatory control of myocardial substrate metabolism predicts postoperative cardiac function in patients undergoing off-pump coronary artery bypass graft surgery. The authors' analysis further points to novel cardiac survival pathways as potential therapeutic targets in perioperative cardioprotection.**

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Additional material related to this article can be found on the ANESTHESIOLOGY Web site. Go to http://www.anesthesiology.org, click on Enhancements Index, and then scroll down to find the appropriate article and link. Supplementary material can also be accessed on the Web by clicking on the "ArticlePlus" link either in the Table of Contents or at the top of the Abstract or HTML version of the article.

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OFF-PUMP coronary artery bypass graft (CABG) surgery emerged in recent years as a means to avoid the sequelae of extracorporeal circulation such as whole-body inflammatory response, coagulation disorders, and multiple organ dysfunction. However, this less invasive surgical technique still involves manipulation of the heart and coronaries and apparently does not eliminate irreversible myocardial injury.[1] The release of some cytokines is observed in off-pump CABG surgery to a similar degree as with cardiopulmonary bypass, although with delayed kinetics.[2] Therefore, improving the perioperative management by decreasing myocardial ischemia–reperfusion damage remains of paramount importance and would affect outcome.

Experimental work and recent data from clinical trials provide strong evidence that modern ether-derived halogenated anesthetic gases elicit pronounced protection against myocardial ischemia.[3] In contrast to intravenous anesthetics such as propofol, anesthetic gases exert a multitude of protective effects including pharmacologic preconditioning and postconditioning, two of the most powerful treatments to reduce infarct size and improving postischemic recovery.[4] In a double-blinded placebo-controlled study, brief administration of sevoflurane on

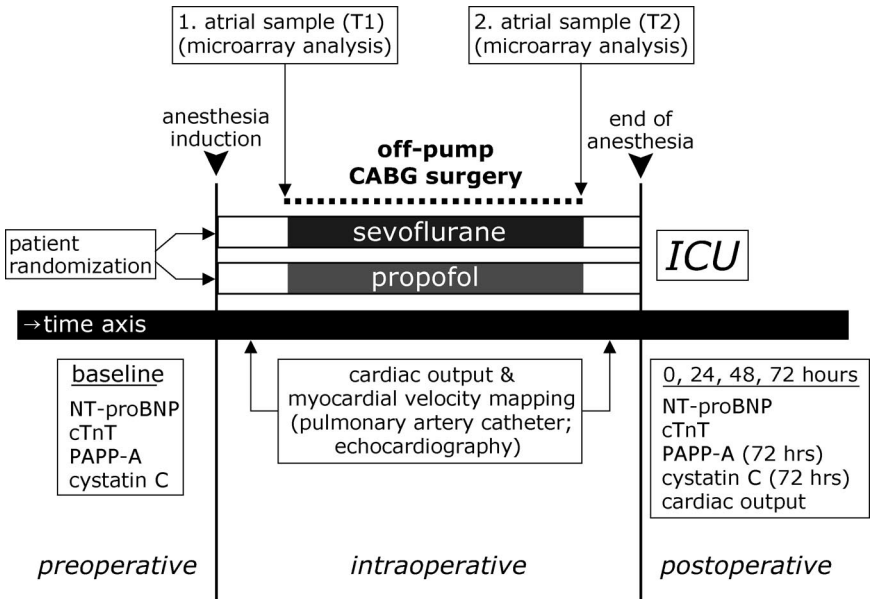

Fig. 1. Study protocol. CABG = coronary artery bypass grafting; cTnT = cardiac troponin T; ICU = intensive care unit; NT-proBNP = N-terminal pro brain natriuretic peptide; PAPP-A = pregnancy-associated plasma protein A.

the cardiopulmonary bypass immediately before induction of cardioplegia improved postoperative cardiac function[5] as well as long-term cardiovascular outcome in patients undergoing CABG surgery.[6] Moreover, administration of anesthetic gases throughout CABG surgery decreased perioperative troponin I release and the duration of intensive care unit and hospital stay.[7]

Using a genome-wide approach, we previously reported that distinct genetic programs are activated by anesthetic gases in isolated rat hearts, which attenuate the adverse consequences of prolonged ischemia.[4,8] This notion is consistent with the concept that anesthetic gases elicit a "second window of protection,"[9,10] which directly depends on altered gene expression.[3] Preliminary results from atrial biopsies suggest that similar genomic reprogramming may occur in patients.[6] To date, no direct comparisons between intravenous anesthetics and anesthetic gases have been performed in human hearts at the gene expression level. In accordance with the reported differences in cardioprotection between various anesthetics, we hypothesized that sevoflurane and propofol would elicit differential genomic responses to cardiac surgery in human hearts. Transcriptional changes were correlated to clinically important cardiovascular biomarkers and to physiologic parameters of cardiac function.

Materials and Methods

The institutional ethics committee of the University Hospital Zurich (Zurich, Switzerland) and the Triemli Hospital (Zurich, Switzerland) approved the study, and written informed consent was obtained from all patients. Twenty patients scheduled to undergo elective off-pump CABG surgery were enrolled between January and June 2005.

Study Criteria

Inclusion criteria were being scheduled for elective off-pump CABG surgery, three-vessel coronary artery disease, male sex, and age of 50–80 yr. Exclusion criteria were diabetes mellitus, concomitant noncardiac surgery, myocardial infarction less than 2 months before CABG surgery, elevated plasma concentrations for cardiac enzymes within 24 h before surgery, unstable angina, left ventricular ejection fraction less than 40%, hemodynamic instability with the need for medical or mechanical inotropic support, serum creatinine greater than 150 μM, and administration of corticosteroids or preconditioning-mimicking/blocking agents such as diazoxide, nicorandil, theophylline, and sulfonylurea drugs, respectively.

Study Protocol

The patients were randomly allocated to the sevoflurane (Sevorane; Abbott, Baar, Switzerland) or the propofol (Diprivan 2%; AstraZeneca, Zug, Switzerland) group (fig. 1). Fentanyl, midazolam, and rocuronium or pancuronium were administered before intubation. Anesthesia was maintained with midazolam, fentanyl, and remifentanil in all patients until the first atrial biopsy was taken (baseline biopsy at T1). Subsequently, anesthesia was maintained with the anesthetic gas sevoflurane or the intravenous anesthetic propofol until the second atrial biopsy was collected shortly before chest closing (biopsy at T2). Sevoflurane and propofol were adjusted to maintain blood pressure and heart rate within 20% of baseline values. During surgical manipulations on the heart, conversion to cardiopulmonary bypass was considered if one of the following criteria was present for at least 15 min: (1) cardiac index less than 1.5 $l \cdot min^{-1} \cdot m^{-2}$, (2) mixed central venous saturation less than 60%, (3) mean arterial pressure less than 50 mmHg, (4) ST-

segment alterations indicative of new myocardial ischemia, or (5) severe arrhythmias. After completion of bypass grafting, remifentanil infusion and repetitive doses of midazolam were used in both groups for sedation until extubation in the intensive care unit. Standard transesophageal echocardiography, tissue Doppler imaging, and hemodynamic measurements using pulmonary artery thermodilution were obtained (fig. 1). Patients from both groups received the same postoperative care.

Cardiac Surgery and Assessment of Myocardial Function

Median sternotomy and pericardiotomy were performed, and the first sample of the right atrium was immediately collected before preparing the grafts. During the surgical manipulation of the heart, hemodynamic stability was achieved by repeated doses of norepinephrine, nitroglycerin, positioning of the operating table, and sequential epicardial stimulation of the heart at a rate of 90 beats/min. After securing the operating field with the aid of a stabilizer (Octopus; Medtronic, Minneapolis, MN), the coronary artery was opened and an intracoronary shunt, which was withdrawn at the end of suturing, was introduced to preserve distal perfusion. The left mammary artery was used to revascularize the left anterior coronary artery. Venous grafts were used for the inferior myocardial wall, and the right internal mammary artery was used for the circumflex branches either through the transverse sinus behind the aorta and connected *in situ* to the circumflex branch, or, if too short, used as a T-graft from the left internal mammary artery. Proximal anastomoses were performed using the heart string device (Guidant, Indianapolis, IN). The quality of all bypasses was controlled by flow measurement (Medistim, Oslo, Norway).

Echocardiographic measurements were obtained before surgery and after completion of coronary bypass grafting. Standard transesophageal echocardiography and tissue Doppler imaging using a Philips SONOS 5500 system with an Omniplane III-TOE probe (Philips Medical Systems, Andover, MA) were performed by a single experienced examiner. The echocardiograms were stored digitally and analyzed *post hoc* without knowing the clinical data or group assignment. Left ventricular end-diastolic area and left ventricular end-systolic area were measured by manual planimetry of the area circumscribed by the leading edge of the endocardial border in the left ventricular transgastric midpapillary short axis view. Left ventricular end-diastolic area was determined as the largest left ventricular cross-sectional area after the R wave, and left ventricular systolic area was determined as the smallest left ventricular cross-sectional area after the T wave. Left ventricular fractional area change (percent) was calculated as $100 \times$ (left ventricular end-diastolic area $-$ left ventricular systolic area)/left ventricular end-diastolic area. Pulsed wave Doppler measurements of transmitral blood flow were obtained with the probe in the midesophageal four-chamber view after placing the sample volume between the tips of the mitral leaflets. Peak early mitral inflow velocity (E wave), deceleration time, peak atrial filling velocity (A wave), and E/A ratio were also measured. Assessment of color M-mode flow propagation was performed with the M-mode cursor aligned parallel to the left ventricular inflow. Main aliased velocity was determined after adjustment to obtain the longest flow column. Tissue Doppler imaging was also obtained from the midesophageal four-chamber view to quantify regional myocardial contractility. Myocardial velocities of the left ventricular septum, the lateral wall, and the mitral annulus were measured. Early systolic, early diastolic, and late diastolic peak velocities were determined using QLAB advanced quantification software version 2.0 (Philips Ultrasound, Bothell, WA). All echocardiographic measurements were recorded as the mean of three consecutive cardiac cycles. Concomitant hemodynamic measurements were obtained before surgery, after completion of coronary bypass grafting, 4 h after arrival in the intensive care unit, and at discharge from the intensive care unit using pulmonary artery thermodilution as the mean of three consecutive ice-water bolus injections.

Diagnosis of Adverse Events

Medical charts were reviewed, and the caregivers were interviewed daily for the occurrence of adverse events. Adverse events (as opposed to myocardial and renal injury markers determined at the end of the study) were diagnosed by the independently managing clinicians. Twelve-lead electrocardiograms were obtained postoperatively every day. The diagnosis of a new postoperative myocardial infarct required a new Q wave, persistent ST-T-segment changes as defined by Minnesota Codes,[11] and/or cardiac troponin T (cTnT) greater than 2.0 μg/l. The diagnosis of a cerebrovascular injury required the presence of clinical symptoms and/or positive computer-assisted tomography scan. The diagnosis of significant postoperative renal dysfunction required newly established postoperative hemodialysis or hemofiltration.

Determination of Cardiovascular Biomarkers

Blood samples were obtained preoperatively, at arrival in the intensive care unit, and 24, 48, and 72 h after surgery and were used to determine cTnT and N-terminal pro brain natriuretic peptide (NT-proBNP) plasma levels. Pregnancy-associated plasma protein A (PAPP-A) and cystatin C plasma levels were only determined preoperatively and after 72 h after surgery. The collected blood samples were stored at -80°C until analysis. The following parameters were determined using the Roche Modular Analytics P or the Roche Modular Analytics

E170 (Roche Diagnostics, Mannheim, Germany): NT-proBNP (electrochemiluminescence sandwich immunoassay)—sensitivity greater than 5 ng/l, intraassay and interassay coefficients of variance less than 3%, normal value (97.5th percentile for men aged > 50 yr) less than 334 ng/l; cTnT (electrochemiluminescence sandwich immunoassay)—sensitivity greater than 0.01 μg/l, intraassay and interassay coefficients of variance greater than 2.5%, normal value less than 0.01 μg/l. PAPP-A assays were purchased from Brahms, Henningsdorf, Germany (immunometric assay based on time resolved amplification cryptate emission)—sensitivity 0.004 U/l, intraassay and interassay coefficients of variance less than 2%, normal range (healthy male) less than 0.01 U/l. Cystatin C assays were purchased from Dako A/S, Goldstrup, Denmark (particle-enhanced turbidimetric assay)—sensitivity greater than 0.2 mg/dl, intraassay and interassay coefficients of variance less than 3.5%, normal range 0.63–1.61 mg/l.

Genomic Analysis of Human Atrial Samples

Microarray analysis was performed following the "minimum information about a microarray experiment" (MIAME) guidelines.[12] Total RNA was prepared from the frozen cardiac tissue using RNeasy Mini Kit (Qiagen AG, Basel, Switzerland). The quality of the isolated RNA was determined with a NanoDrop ND 1000 (NanoDrop Technologies, Wilmington, DE; 260 nm/280 nm ratio between 1.8 and 2.1 and 28S/18S ratio within 1.5–2). Total RNA samples (100 ng) were reverse-transcribed into double-stranded complementary DNA (cDNA) with a Two-Cycle cDNA Synthesis Kit (Affymetrix Inc., Santa Clara, CA). The double-stranded cDNA was purified using a Sample Cleanup Module (Affymetrix Inc.). The purified double-stranded cDNA were *in vitro* transcribed in the presence of biotin-labeled nucleotides using an IVT Labeling Kit (Affymetrix Inc.). The biotinylated complementary RNA (cRNA) was purified using a Sample Cleanup Module (Affymetrix Inc.), and its quality and quantity was determined using NanoDrop ND 1000 and Bioanalyzer 2100. Biotin-labeled cRNA samples (15 μg) were fragmented randomly to 35–200 bp at 94°C in Fragmentation Buffer (Affymetrix Inc.) and were mixed in 300 μl hybridization buffer containing a hybridization Control cRNA and Control Oligo B2 (Affymetrix Inc.), 0.1 mg/ml herring sperm DNA, and 0.5 mg/ml acetylated bovine serum albumin in 2-(4-morpholino)-ethane sulfonic acid (MES) buffer at pH 6.7. Each sample (for a total of 40 samples) was hybridized to an individual Affymetrix GeneChip Human Genome U_133 Plus 2.0 arrays for 16 h at 45°C (Affymetrix Inc.). Arrays were washed using an Affymetrix Fluidics Station 450 EukGE-WS2v5_450 protocol and scanned using an Affymetrix GeneChip Scanner 3000 (Affymetrix Inc.) at a resolution of 3 μm. Raw data processing was performed using the Affymetrix GCOS 1.2 software (Affymetrix Inc.), and all arrays were subjected to quality control according to the parameters established by Affymetrix, such as the assessment of average background and noise value, exogenously added prokaryotic hybridization controls, percent present calls, scaling factors, and internal control genes. Normalization and computation of expression values were performed using the robust multichip average method.[13] The microarray data are available at the Gene Expression Omnibus Database## under the series number GSE4386. For each patient, two atrial samples were collected and used for hybridization. No samples were pooled, *i.e.*, 40 samples corresponding to 40 chips were used in the final analysis. The global gene expression matrix containing the expression values of the 54,675 probe sets for all 40 cardiac samples was used as input for Gene Set Enrichment Analysis (GSEA).[14] GSEA considers predefined gene sets representing pathways of interest and determines whether the members of these sets are overrepresented in a list of genes that has been ordered by their correlations with a specific phenotype or class distinction. The output is a normalized enrichment score that represents a measure of the degree of enrichment of the gene set at the top (highly correlated) or bottom (highly inversely correlated) of the ordered gene list. The normalized enrichment score is used to produce a *P* value that measures the significance of the score itself. The gene sets used for the current analysis (a total of 547) were obtained from the GSEA home page*** or were manually curated. Additional information regarding this is available on the ANESTHESIOLOGY Web site at http://www.anesthesiology.org.

In a first analysis, the "GSEA phenotypes" were defined as follows: SEVO (t = T1), samples collected in the sevoflurane group at time T1; SEVO (t = T2), samples collected in the sevoflurane group at time T2; PROP (t = T1) and PROP (t = T2), samples collected in the propofol group at times T1 and T2, respectively. To define a standardized measure of gene expression (the mean centroid), we normalized the gene expression levels of the enriched genes within a pathway to a mean of 0 and a variance of 1 across all 40 samples. The mean centroid vector of a given pathway is computed as the mean of the normalized expression vectors of each gene in the pathway. A second GSEA analysis was performed using the computed fold changes from T1 to T2 for all Affymetrix probe sets. In this case, two phenotypes (SEVO and PROP) were defined.

GEO database. Available at: http://www.ncbi.nlm.nih.gov/geo/. Accessed March 8, 2006.

*** Available at: http:// www.broad.mit.edu/GSEA. Accessed October 30, 2005.

Validation of Chip Data Using Real-time Reverse-transcriptase Polymerase Chain Reaction

As an independent method of measuring levels of gene expression, real-time reverse-transcriptase polymerase chain reaction was performed for eight selected genes to confirm microarray data (table S1 on the ANESTHESIOLOGY Web site). For this purpose, randomly chosen transcripts with a wide range of fold changes (0.12–8.0), as obtained by gene chip analysis, were selected. First-strand cDNA was synthesized from 1 μg total RNA using Superscript II reverse transcriptase (Invitrogen, Basel, Switzerland) and oligo-dT as primer. Real-time reverse-transcriptase polymerase chain reaction quantification of the selected genes was performed on a Stratagene MX3000 real-time sequence detector instrument (Stratagene Europe, Amsterdam, The Netherlands) using the Brilliant SYBR green QPCR Master Mix (Stratagene Europe). Amplification reactions were conducted with an initial step at 90°C for 3 min followed by 20–35 cycles. Samples were run in triplicates, and ribosomal 18S (a constitutively expressed gene) was used as reference control. Predicted size of amplified products was confirmed by agarose gel electrophoresis.

Statistical Analysis

Sample size was calculated based on the results for NT-proBNP reported previously.[5] For biochemical and physiologic parameters, two-way analysis of variance for repeated measures followed by appropriate multiple comparison procedures (Bonferroni *t* test) was used to evaluate differences over time between groups. An unpaired *t* test was used to compare groups at identical time points, and a paired *t* test was used to compare within groups over time. All other data were analyzed using unpaired *t* tests for parametric data or Mann–Whitney tests for nonparametric data. Categorical variables were summarized by proportions and compared using the Fisher exact test or the chi-square test, if appropriate. *P* values were multiplied by the number of comparisons that were made (Bonferroni correction), and corrected $P < 0.05$ was considered significant. Data are given as mean ± SD or median and quartiles, if appropriate. Forward stepwise linear regression (with F-to-Enter = 4.000 and F-to-Remove = 3.996) was applied using functional/biochemical outcome variables as dependent variables and gene expression measures (mean centroids or fold changes) and clinical data as the independent variables. Adjusted squared correlation coefficients (R^2_{adj}) were reported. Analyses were performed using SigmaStat Version 2 (SPSS Science, Chicago, IL) and StatView version 5 (SAS Institute, Chicago, IL).

Results

Demographics, Perioperative Data, and Clinical Outcome

Patient characteristics and perioperative data are listed in table 1. The sevoflurane and propofol groups were similar with regard to all preoperative data including medical therapy and comorbidity. Intraoperative data were comparable between groups except for the longer anesthesia and surgery time and the smaller amount of administered remifentanil in the sevoflurane group. The cumulative doses of norepinephrine and nitroglycerin used to stabilize hemodynamics during the surgical manipulations were similar in both groups (table 1). None of the patients required conversion to cardiopulmonary bypass or had postoperative myocardial infarction, cerebrovascular injury, or renal damage. No postoperative mechanical or medical inotropic support was needed.

Biomarkers for Contractile Dysfunction (NT-proBNP) and Coronary Plaque Instability (PAPP-A) Indicate Superior Protection by Sevoflurane

Sevoflurane markedly reduced postoperative NT-proBNP release (fig. 2A). Mean peak postoperative NT-proBNP was 3,442 ± 1,458 ng/l in propofol patients compared with 1,392 ± 881 ng/l in sevoflurane patients ($P = 0.0013$). In contrast, no difference was observed in postoperative cTnT release (fig. 2B). However, two propofol patients but none of the sevoflurane patients had a peak postoperative cTnT concentration greater than 0.65 μg/l, indicative of major perioperative myocardial damage. None of the patients experienced postoperative deterioration of renal function (postoperative cystatin C plasma concentrations in sevoflurane patients: 0.63 ± 0.16 mg/l *vs.* propofol patients: 0.64 ± 0.18 mg/l; $P = 0.55$; fig. 2C). Preoperative baseline PAPP-A plasma concentrations were increased in all patients indicating an active inflammatory process in atherosclerotic plaques. Sevoflurane but not propofol abolished the postoperative PAPP-A elevation (mean change in PAPP-A: −1.10 ± 1.10 mU/l in sevoflurane patients *vs.* +6 ± 1.05 mU/l in propofol patients; $P < 0.001$; fig. 2D).

Transesophageal Echocardiography and Hemodynamic Measurements Show Improved Postoperative Myocardial Function after Sevoflurane Anesthesia

Sevoflurane but not propofol preserved inotropy (predominantly lateral wall) and early diastolic lusitropic function (annulus and septum) after CABG surgery (table 2) and thereby successfully maintained global early postoperative cardiac function as determined by cardiac index measurements (table 3). Sevoflurane patients, as opposed to propofol patients, also exhibited increased postoperative ejection fractions compared with preop-

Table 1. Patient Characteristics and Perioperative Data

	Propofol Group (n = 10)	Sevoflurane Group (n = 10)
Age, yr	66.9 ± 7.3	65.2 ± 7.6
Euroscore	2.9 ± 1.5	3.0 ± 2.4
Parsonnet score	4.9 ± 5.0	4.0 ± 4.4
Preoperative ejection fraction, %	66 ± 10 (45–75)	60 ± 13 (42–76)
Previous myocardial infarction, No. of patients	2	2
Preoperative medication, No. of patients		
β Blocker	10	9
Ca^{2+} blocker	3	5
Nitrates	4	5
ACEI	2	3
ATA	4	4
Statins	6	5
Diuretics	6	7
No. of grafts	3.4 ± 0.8 (2–5)	3.9 ± 0.7 (3–5)
Anesthetic drugs		
Propofol, mg*	1,128 ± 364	—
Sevoflurane, vol% end-tidal	—	1.9 ± 0.5 (0.6–2.5)
Midazolam, mg	22.4 ± 6.3	24.5 ± 6.1
Fentanyl, mg	1.4 ± 0.4	1.2 ± 0.4
Remifentanil, mg	4.4 ± 1.5	2.6 ± 0.7‡
Vasoactive drugs		
Norepinephrine, μg	661 ± 936 (0–3,400)	662 ± 881 (0–2,510)
Nitroglycerin, mg	4.84 ± 3.41 (0.6–10)	5.45 ± 2.63 (0.7–8.6)
Volume replacement, ml†	4,272 ± 1,887 (1,830–6,677)	5,090 ± 1,540 (3,430–8,400)
Surgery time, min	222 ± 37	266 ± 44‡
Anesthesia time, min	295 ± 46	352 ± 52‡
Elapsed time between first and second atrial biopsy, min	203 ± 39	174 ± 37

Data are mean ± SD (range), if not otherwise indicated.

* Propofol infusion rate during maintenance of anesthesia: 0.5–2.5 mg · kg^{-1} · min^{-1}. † Total intraoperatively replaced volume (blood products, colloids, and crystalloids). ‡ $P < 0.05$ compared with propofol.

ACEI = angiotensin-converting enzyme inhibitor; ATA = angiotensin II antagonist.

erative values (table 2). Late postoperative hemodynamic measurements obtained in the intensive care unit showed an increase in postoperative cardiac index compared with preoperative measurements in both sevoflurane and propofol patients. However, this increase was more pronounced in sevoflurane patients and further accompanied with a reduced systemic vascular resistance (table 3).

Gene Expression Profiling Shows Pronounced Responses to Off-pump CABG Surgery and Unveils Subtle Differences in Background Energy Metabolism among Patients

For each patient, two atrial samples were collected, the first sample immediately after chest opening (110 ± 45 min after induction of anesthesia; T1) and the second sample shortly before chest closing (180 ± 40 min after obtaining

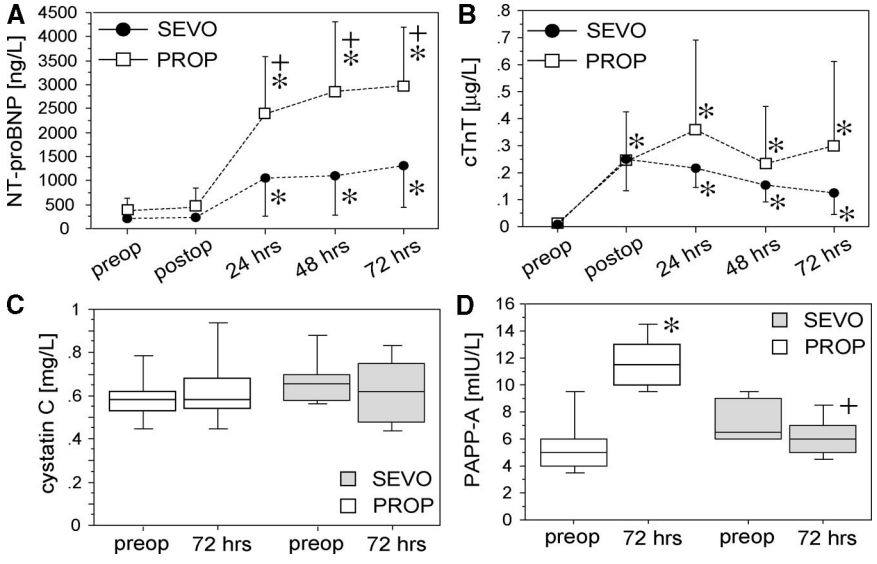

Fig. 2. Cardiovascular biomarkers. Perioperative levels of N-terminal pro brain natriuretic peptide (NT-proBNP) were significantly (time effect $P < 0.001$; treatment effect $P = 0.002$) more elevated in the propofol (PROP) group as compared with the sevoflurane (SEVO) group (*A*). No difference between groups was found in cardiac troponin T (cTnT) (*B*) and in cystatin C (*C*), whereas pregnancy-associated plasma protein A (PAPP-A) was exclusively increased (time effect $P < 0.001$; treatment effect $P = 0.02$) postoperatively in the PROP group (*D*). * Significantly increased compared with baseline values ($P < 0.05$; multiple comparison procedure, Bonferroni-corrected *t* test). + Significantly different between groups ($P < 0.05$; multiple comparison procedure, Bonferroni-corrected *t* test). Data are mean ± SD in *A* and *B*, and medians and 25th and 75th percentile in *C* and *D*.

Table 2. Echocardiographic Parameters

	Before CABG		After CABG	
	Propofol	Sevoflurane	Propofol	Sevoflurane
LV-ESA, cm^2	9.2 ± 3.2	9.6 ± 2.3	7.9 ± 3.4	8.2 ± 3.2
LV-EDA, cm^2	18.3 ± 3.4	18.4 ± 3.2	16.9 ± 4.8	18.8 ± 6.0
LV-FAC, %	50 ± 10	50 ± 10	51 ± 9	56 ± 10*
Diastolic inflow				
E wave, cm/s	62.3 ± 15.2	54.5 ± 16.2	52.1 ± 24.3	50.9 ± 15.2
A wave, cm/s	46.7 ± 20.3	45.3 ± 23.4	40.9 ± 19.1	45.1 ± 15.6
E/A ratio	1.6 ± 0.9	1.5 ± 0.9	1.7 ± 1.4	1.3 ± 0.5
Peak mitral annulus velocity				
Systolic, cm/s	3.8 ± 1.3	3.2 ± 1.0	2.9 ± 1.1	4.2 ± 1.5*
Early diastolic, cm/s	3.7 ± 2.3	3.6 ± 2.2	2.1 ± 1.4*	3.9 ± 1.6†
Late diastolic, cm/s	4.2 ± 2.4	3.5 ± 1.5	3.8 ± 1.4	4.4 ± 1.4*
Myocardial velocity septal				
Systolic, cm/s	2.1 ± 0.8	2.0 ± 1.2	2.1 ± 0.9	3.1 ± 1.8*
Early diastolic, cm/s	2.7 ± 1.0	2.3 ± 0.8	1.7 ± 0.5*	2.9 ± 1.3†
Late diastolic, cm/s	2.4 ± 1.1	2.0 ± 1.1	2.5 ± 1.2	2.3 ± 1.4
Myocardial velocity lateral				
Systolic, cm/s	2.4 ± 1.1	2.3 ± 1.2	1.5 ± 0.5	2.5 ± 0.8†
Early diastolic, cm/s	2.3 ± 1.7	2.4 ± 0.6	1.2 ± 0.6*	2.6 ± 0.9†
Late diastolic, cm/s	2.2 ± 1.2	2.1 ± 1.0	1.5 ± 0.7	1.8 ± 1.0

Data are mean ± SD.

* $P < 0.05$ with values before coronary artery bypass grafting (CABG). † $P < 0.05$ compared with propofol.

A wave = peak late mitral inflow velocity; E wave = peak early mitral inflow velocity; EDA = end-diastolic area; ESA = end-systolic area; FAC = fractional area change; LV = left ventricular.

the first sample; T2). Sample T1 reflects the background gene expression profile before exposure to sevoflurane or propofol (constitutive background activity), whereas sample T2 mirrors the gene responses to surgery in the two anesthetic groups. The existence and direction of gene expression changes were reliably detected by the microarray technology, as confirmed by real-time quantitative polymerase chain reaction (tables S1 and S2 on the ANESTHESIOLOGY Web site). As a first step, the single genes, which were differentially regulated over time (T1–T2) between the two anesthetic groups, were determined (see fig. S1, showing the 50 top-ranked up- and down-regulated genes, on the ANESTHESIOLOGY Web site). Off-pump CABG surgery induced pronounced changes in genes involved in transcriptional regulation and proinflammatory and antiinflammatory actions. Next, the comparison of the differentially and jointly regulated pathways over time (T1–T2) in sevoflurane- and propofol-treated patients showed a similar number of enriched pathways in propofol and sevoflurane patients (fig. 3A and table 4). At T1, GSEA further detected subtle differences (approximately 15%) in activity of genes involved in the peroxisome proliferator–activated receptor γ coactivator 1α (PGC-1α) pathway among patients (normalized enrichment score = 1.37; nominal P value = 0.04, false discovery rate q value = 0.26; fig. 3B). As expected, the PGC-1α pathway correlated with genes involved in oxidative phosphorylation ($R^2_{adj} = 0.71$, $P < 0.00001$) and fatty acid metabolism (uptake and oxidation) ($R^2_{adj} = 0.70$, $P <$

Table 3. Hemodynamic Data

	Before CABG		After CABG		ICU		ICU Discharge	
	Propofol	Sevoflurane	Propofol	Sevoflurane	Propofol	Sevoflurane	Propofol	Sevoflurane
HR, beats/min	57 ± 9	54 ± 6	89 ± 11*	84 ± 7*	91 ± 14*	86 ± 11*	86 ± 11*	82 ± 7*
MAP, mmHg	81 ± 11	82 ± 8	70 ± 24	73 ± 8	76 ± 7	74 ± 8	78 ± 6	77 ± 10
MPAP, mmHg	19 ± 7	24 ± 20	20 ± 7	22 ± 4	22 ± 5	22 ± 6	18 ± 4	17 ± 4
CVP, mmHg	8 ± 6	8 ± 5	8 ± 5	11 ± 4	6 ± 2	9 ± 3	5 ± 2	7 ± 4
PCWP, mmHg	10 ± 5	11 ± 3	10 ± 5	11 ± 4	8 ± 4	9 ± 2	8 ± 4	9 ± 3
CI, $l \cdot min^{-1} \cdot m^{-2}$	3.4 ± 0.6	3.5 ± 0.9	2.9 ± 0.5*	3.8 ± 1.1†	3.9 ± 0.7‡	5.0 ± 1.0*†‡	3.7 ± 0.9‡	4.7 ± 0.7*†‡
SVR, $dyn \cdot s \cdot cm^{-5}$	919 ± 249	928 ± 296	985 ± 237	702 ± 171*†	745 ± 99	558 ± 94*†	846 ± 179	627 ± 174*†
PVR, $dyn \cdot s \cdot cm^{-5}$	116 ± 58	168 ± 222	150 ± 73	120 ± 39	145 ± 67	109 ± 38	134 ± 94	65 ± 39

Data are mean ± SD.

* $P < 0.05$ compared with values before coronary artery bypass grafting (CABG). † $P < 0.05$ compared with propofol. ‡ $P < 0.05$ compared with values after CABG.

CI = cardiac index; CVP = central venous pressure; HR = heart rate (hearts were paced at 90 beats/min during CABG and the early postoperative period); ICU = intensive care unit; MAP = mean arterial pressure; MPAP = mean pulmonary arterial pressure; PCWP = pulmonary capillary wedge pressure; PVR = pulmonary vascular resistance; SVR = systemic vascular resistance.

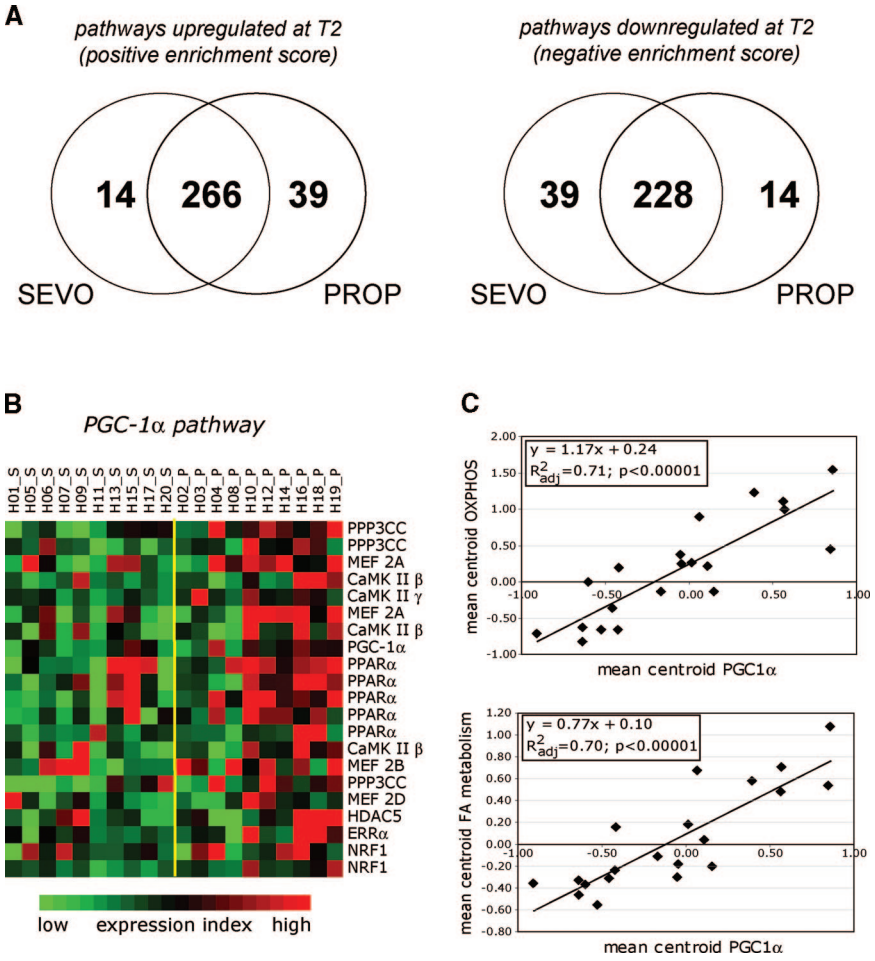

Fig. 3. (*A*) Pathways (gene sets) up-regulated and down-regulated at T2 in response to off-pump coronary artery bypass graft surgery in the two anesthetic treatments. The Venn diagrams show the number of enriched pathways (see also table 4). (*B*) Peroxisome proliferator–activated receptor γ coactivator-1α (PGC-1α) pathway activity at baseline (t = T1). Each numbered square indicates the expression value of the indicated transcript in a specific patient. Subtle differences (approximately 15%) in PGC-1α activity were detected among patients. (*C*) PGC-1α pathway regulates genes involved in oxidative phosphorylation (OXPHOS) and fatty acid (FA) metabolism and was therefore used to express background energy metabolism in the heart (see also fig. S2 on the ANESTHESIOLOGY Web site). CaMK II β = calcium/calmodulin-dependent protein kinase II β; CaMK II γ = calcium/calmodulin-dependent protein kinase II γ; ERRα = estrogen-related receptor α; HDAC5 = histone deacetylase 5; MEF 2A = myocyte enhancer factor 2A; MEF 2B = myocyte enhancer factor 2B; MEF 2D = myocyte enhancer factor 2D; NRF1 = nuclear respiratory factor 1; PPARα = peroxisome proliferator activated receptor α; PPP3CC = calcineurin A γ; PROP = propofol patients; SEVO = sevoflurane patients.

0.00001) (fig. 3C) and hence was subsequently used in the multivariate analysis as an indicator of the myocardial background energy metabolism (fig. S2 on the ANESTHESIOLOGY Web site).

Sevoflurane Favorably Modifies the Transcriptional Responses to Off-pump CABG Surgery

In accordance with the study protocol, short-term changes in gene expression between T1 and T2 should be due to the different anesthetics used. To specifically determine the genomic effects of the anesthetics and to correct for the differences in baseline transcriptional activity, GSEA was applied to fold changes in gene expression between T1 and T2. From this analysis, three major pathways emerged, including fatty acid oxidation (normalized enrichment score = 1.53; nominal *P* value = 0.02, false discovery rate q value = 0.33), DNA-damage signaling (normalized enrichment score = 1.68; nominal *P* value = 0.02, false discovery rate q value = 0.11), and granulocyte-colony stimulating factor (G-CSF) survival pathway (normalized enrichment score = −1.79; nominal *P* value = 0.00, false discovery rate q value = 0.10). Compared with propofol, the anesthetic gas sevoflurane reduced transcriptional activity of genes involved in fatty acid oxidation (*t* test *P* = 0.008) and DNA-damage signaling (*t* test *P* = 0.002) but activated the G-CSF survival pathway (*t* test *P* = 0.017) (fig. 4; for complete list of transcripts, see table S3 on the ANESTHESIOLOGY Web site). Interestingly, the lower activity of transcripts involved in fatty acid oxidation, as observed in sevoflurane patients, was closely correlated with low DNA-damage signaling ($P < 0.001$) and high G-CSF survival pathway activity ($P = 0.006$), implying a possible link between the metabolic phenotype and cardioprotection (fig. 5).

Anesthetic-induced and Constitutive Gene Regulatory Control of Myocardial Energy Metabolism Predicts Postoperative Cardiac Function in Patients Undergoing Off-pump CABG Surgery

Next, we correlated the anesthetic-induced short-term transcriptional changes and the observed differences in constitutive background energy metabolism to postoperative cardiac function. Cardiac function was measured with three independent methods, including NT-proBNP, transesophageal echocardiography, and pulmonary artery thermodilution. Forward stepwise linear regression analysis was applied to determine the best predictors of postoperative cardiac function. Figure 6 shows the regression plots with the best predictors of NT-proBNP, cardiac index, and

Table 4. Induced (Positive NES) and Repressed (Negative NES) Pathways in Off-Pump CABG Surgery

		SEVO			PROP		
GSEA Pathway	Description	NES	Nom. *P* Value	FDR q Value	NES	Nom. *P* Value	FDR q Value
etsPathway (Biocarta)	The Ets transcription factors promote macrophage differentiation	1.69	0.00	0.14	1.94	0.00	0.00
stressPathway (Biocarta)	Tumor necrosis factor–related inflammation and stress-activated kinases	1.67	0.00	0.13	1.69	0.00	0.02
CR_transcription_factors[51]	Cancer related genes that are also transcription factors	1.61	0.00	0.25	1.83	0.00	0.02
MAPK_Cascade (Biocarta)	Mitogen-activated protein kinase pathway	1.56	0.03	0.18	1.67	0.00	0.02
SERUM_INDUCED_MKL_INDEP_SRF[52]	Serum inducible genes identified as a subset of serum response factor (SFR) target genes that are MKL (SRF-specific transcriptional coactivator) independent	1.56	0.00	0.19	1.81	0.00	0.02
LEM_HSC[53]	Stem cell molecular signature	1.54	0.02	0.19	1.78	0.00	0.01
IL6Pathway (Biocarta)	IL-6–related kinases and transcription factors	1.57	0.02	0.19	1.57	0.00	0.06
G-CSF (manually curated)	G-CSF survival pathway	1.46	0.02	0.24	1.52	0.02	0.07
CR_repair[51]	Cancer-related genes involved in DNA repair	−1.58	0.00	0.32	−1.62	0.00	0.20
IL7Pathway (Biocarta)	B- and T-cell development and proliferation	−1.35	0.03	0.68	−1.50	0.00	0.43
atrbrcaPathway (Biocarta)	Double-stranded break repair	−1.52	0.03	0.36	−1.58	0.02	0.29
Fatty_acid_metabolism (GenMAPP)	Fatty acid metabolism	−1.48	0.04	0.14	−1.24	0.1	0.48
OXPHOS (GenMAPP)	Respiratory chain enzymes	−1.41	0.03	0.20	−1.32	0.15	0.43

Fatty acid metabolism and oxidative phosphorylation (OXPHOS) were significantly enriched ($P < 0.05$) in the SEVO group only.

FDR = false discovery rate (FDR is the estimated probability that a set with a given NES represents a false positive finding); G-CSF = granulocyte colony-stimulating factor; GSEA = Gene Set Enrichment Analysis*; IL-6 = interleukin 6; MAPK = mitogen-activated protein kinase; MKL = myocardin-related family of proteins (myocardin, megakaryoblastic leukemia 1, and megakaryoblastic leukemia 2); NES = normalized enrichment score (positive NES indicates up-regulation at T2; negative NES indicates down-regulation at T2); nom. = nominal; PROP = propofol patients; SEVO = sevoflurane patients; SRF = serum response factor.

* For pathway information, refer to Web Enhancement and http://www.broad.mit.edu/GSEA (accessed October 30, 2005).

peak diastolic velocity, a marker of left ventricular relaxation. Interestingly, the G-CSF survival pathway consistently predicted left ventricular diastolic function at different anatomical sites in the hearts. No correlation was found between transcriptional phenotypes and peak systolic velocities. PAPP-A and cTnT did not correlate with cardiac function and transcriptional phenotypes. Together, these data indicate that short-term (by anesthetics) and long-term (constitutive) gene regulatory control of myocardial energy metabolism predicts postoperative cardiac recovery.

Discussion

Here we show for the first time on a genome-wide basis that anesthetics differentially modulate the transcriptional response to cardiac surgery in human hearts. The volatile ether sevoflurane, as compared with the intravenous anesthetic propofol, decreased fatty acid oxidation, which tightly associated with reduced DNA-damage signaling and enhanced activation of the G-CSF survival pathway. These favorable transcriptional changes closely correlated with and predicted improved cardiac functional recovery, as determined by NT-proBNP release, cardiac index, and peak diastolic velocity. However, beside these rather acute transcriptional effects, chronic preoperative background activity of PGC-1α pathway–regulated genes involved in oxidative phosphorylation and fatty acid metabolism, independently predicted postoperative cardiac function. Collectively, our data imply that gene regulatory control of myocardial energy metabolism, either as constitutive background activity or as short-term therapeutic target, plays a pivotal role in perioperative cardioprotection.

We used high-density oligonucleotide microarrays to assess global changes in cardiac gene expression that

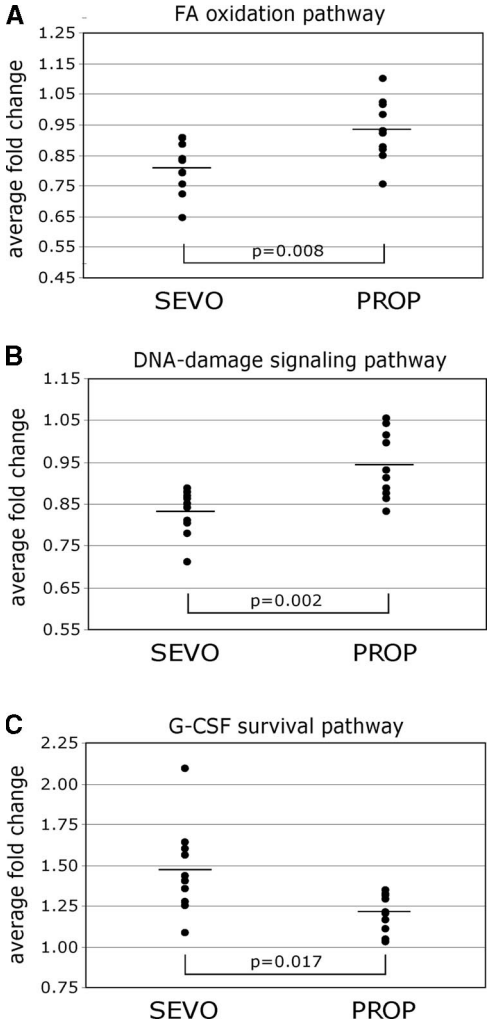

Fig. 4. Anesthetic-induced modulation of gene expression. Using fold changes in gene expression between T1 and T2, Gene Set Enrichment Analysis identified three major pathways, which were differentially regulated by sevoflurane (SEVO) and propofol (PROP). (*A*) Fatty acid (FA) oxidation pathway. (*B*) DNA-damage signaling pathway. (*C*) Granulocyte colony-stimulating factor (G-CSF) survival pathway. The average fold change of all enriched transcripts in the respective pathways was calculated (table S3 on the ANESTHESIOLOGY Web site). The individual *dots* represent changes in pathway activity of each patient. *Horizontal lines* indicate mean values.

result from two widely used anesthetics to gain insight into the underlying mechanisms of perioperative cardioprotection. Based on previously reported differences between the cardioprotective effects of anesthetic gases and intravenous anesthetics,[5,7,15–17] we sought for differential genomic responses in human hearts, which could be correlated to biochemical and functional outcome measures. To broaden the applicability of our results, off-pump CABG surgery was used as a model of human myocardial ischemia, which more closely mimics the situation of general surgical patients experiencing ischemia during noncardiac surgery. To assess the wide spectrum of interacting genes and the complex redundant and antagonistic patterns of transcriptional activation and suppression, we used GSEA, a sophisticated tool for pattern recognition.[14] Single gene approaches are limited to assessing complex pathophysiologic networks and miss important effects on entire pathways. In contrast, GSEA evaluates the microarray data on the level of functionally related genes based on previous biologic knowledge. The unique concept underlying GSEA relates to the fact that even small but coordinate changes in a majority of genes encoding members of a pathway (gene set) may dramatically alter the flux through the pathway. Using this more holistic approach, we were able to track the molecular footprints of anesthetics in the myocardium on a genome-wide basis and to gain novel insights into the mechanisms of perioperative cardioprotection.

In this study, we identified gene regulatory control of myocardial energy metabolism as the major determinant of postoperative cardiac function. A large body of literature supports the concept that myocardial substrate metabolism critically affects cardiac function in the acute and chronic disease state,[18] and that therapeutic strategies switching the metabolic fuel preference away from fatty acid oxidation can indeed improve contractile recovery after ischemia and affect long-term outcome.[19] Under normoxic conditions, the heart predominantly (approximately 60–80%) uses fatty acids as fuel source.[18] However, cardiac substrate utilization is markedly altered during ischemia with glucose as the preferred fuel. Conversely, in the postischemic myocardium, fatty acid oxidation prevails and nearly provides 100% of the cellular adenosine triphosphate (ATP) requirements.[20] Although we did not observe transcriptional changes in glucose transport, glycolysis, or glucose oxidation pathways, the reduction in fatty acid oxidation, *per se* leads to a relative increase in glucose utilization *via* activation of the pyruvate dehydrogenase complex (the glucose–fatty acid cycle by Randle[21]). Favoring the energetically economical glucose (3.17 ATP/oxygen molecule) over fatty acid oxidation (2.83 ATP/oxygen molecule) may be particularly beneficial in the diseased and mechanically stressed heart with only limited oxygen supply. Therefore, high-level fatty acid oxidation either by propofol anesthesia or as constitutive metabolic phenotype may put the heart at higher risk of postoperative contractile dysfunction.

Our study now demonstrates that this protective metabolic phenotype can be induced on a short-term basis in human hearts using anesthetic gases. We previously reported that isoflurane, another halogenated ether, profoundly reduced fatty acid oxidation in an *in vitro* rat heart model (fig. S3 on the ANESTHESIOLOGY Web site),[8] which is consistent with positron emission tomography results from isoflurane-anesthetized mice exhibiting strong and selective myocardial glucose uptake.[22] Likewise, our study patients with constitutive cardiac downregulation of the PGC-1α pathway may have shifted the

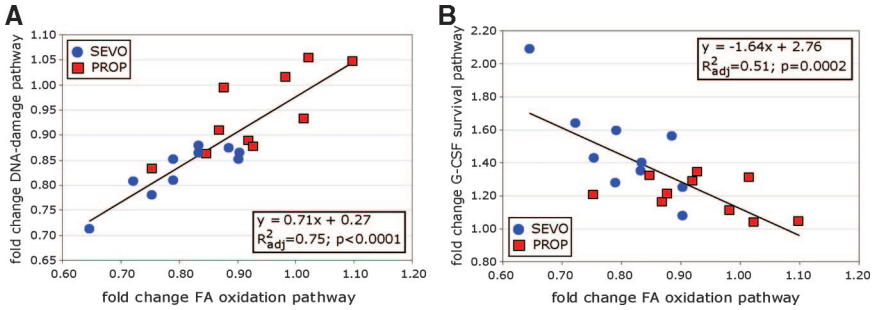

Fig. 5. Relation between anesthetic-induced metabolic changes in fatty acid (FA) oxidation and DNA-damage signaling (*A*), and granulocyte colony-stimulating factor (G-CSF) survival signaling (*B*), respectively. PROP = propofol patients; SEVO = sevoflurane patients.

metabolic fuel preference away from fatty acid oxidation and thus exhibited reduced mitochondrial respiration and oxidative phosphorylation gene activity. The PGC-1α pathway encloses the transcription factors peroxisome proliferator–activated receptor α, estrogen-related receptor α, the nuclear respiratory factors, and the transcriptional coactivator PGC-1α, which critically orchestrate control of cellular energy metabolism.[23] Although the PGC-1α pathway promotes a high energy state with increased contractility,[24] the PGC-1α–induced high ATP turnover may be detrimental under oxygen restriction.[25] Accordingly, hearts lacking peroxisome proliferator–activated receptor α, a key transcription factor of the PGC-1α pathway, exhibit improved ischemic tolerance, whereas hearts with peroxisome proliferator–activated receptor α overexpression only recover poorly after ischemia–reperfusion because of their inability to switch energy substrate.[26] Interestingly, reduced mitochondrial respiration resembles a state of metabolic hibernation, previously identified as a typical feature of the preconditioned state.[27,28] On the other side, anesthetic gases activate ATP-dependent potassium channels,[29,30] key players in the preconditioning process, which are tightly regulated by intermediary metabolites.[31] Together, our data shed new light on the intricate interplay among cellular metabolism, preconditioning, and perioperative cardioprotection and further suggest that modulation of the PGC-1α pathway may be used as a novel anti-ischemic strategy.

DNA-damage signaling and the G-CSF survival pathway closely correlated with fatty acid oxidation. An important question raised by our findings relates to the possible links between these pathways. In the case of DNA-damage signaling, the correlation may merely reflect the protection resulting from metabolic fuel shift. Among others, this pathway encloses the proinflammatory tumor necrosis factor α and the tumor suppressor p53 and the ataxia telangiectasia-mutated (a protein kinase that

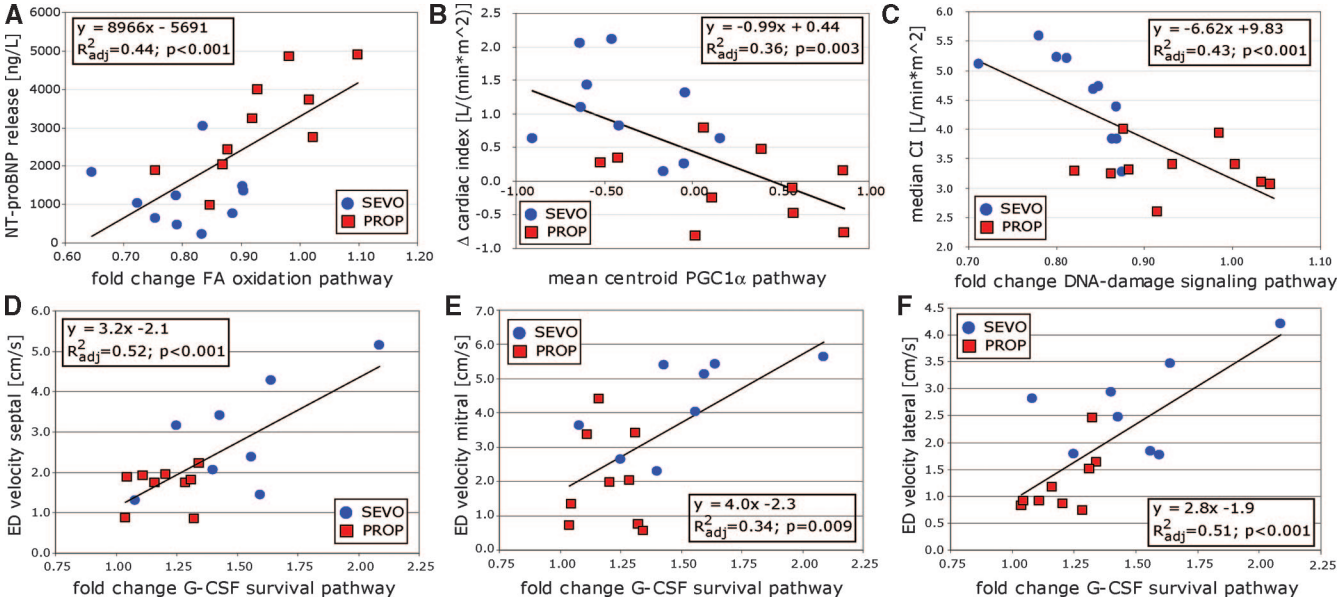

Fig. 6. Regression plots between transcriptional phenotypes and postoperative cardiac function. Multivariate analysis including the explanatory variables peroxisome proliferator activated receptor γ coactivator-1α (PGC-1α) pathway (an index of long-term energy metabolism) and the anesthetic-induced short-term changes in gene expression was used to determine the best predictors of postoperative cardiac function. (*A*) Perioperative changes in N-terminal pro brain natriuretic peptide (NT-proBNP) were best predicted by changes in fatty acid (FA) oxidation pathway activity. (*B*) PGC-1α pathway status best predicted perioperative changes in cardiac index (CI), whereas DNA-damage signaling best predicted postoperative CI (*C*). Granulocyte colony-stimulating factor (G-CSF) survival signaling best predicted postoperative early diastolic (ED) function measured at the septum (*D*), the mitral annulus (*E*), and the lateral wall (*F*). PROP = propofol patients; SEVO = sevoflurane patients.

acts as a tumor suppressor) checkpoint recently shown to be important in ischemia–reperfusion injury in patients undergoing cardiac surgery.[32] In contrast, the G-CSF survival pathway may have a direct causal role in cardiac fuel selection by regulating STAT3-mediated insulin sensitivity in the heart.[33] Representative members of this pathway are JAK2/3, STAT3, vascular endothelial growth factor, and protein kinase B or Akt, which promote cell survival and angiogenesis.[34,35] STAT3 is known to elicit early preconditioning[36] but also has an obligatory role in the context of late preconditioning.[37] Interestingly, changes in left ventricular diastolic function were best predicted by G-CSF survival pathway activity. The diastolic phase of the cardiac cycle imposes the highest energetic demand on the heart, and thus may be particularly sensitive to ischemia but also receptive to protection by this pathway. G-CSF further exerts antiinflammatory actions,[38] which is consistent with our finding that PAPP-A, a marker of coronary plaque inflammation and instability,[39] was not elevated postoperatively in sevoflurane-treated patients. Finally, G-CSF has positive effects on the mobilization and cardiac homing of bone marrow stem cells, which could accelerate postoperative endothelial and myocardial recovery.[40,41] Recent clinical studies emphasize the important role of PAPP-A in the detection of plaque rupture in patients with acute coronary syndrome, even in the absence of biomarkers of necrosis, potentially identifying high-risk patients whose plaque instability might have been otherwise undetected.[42] PAPP-A was also identified as an independent predictor of future cardiovascular events and of the need for future angioplasty or CABG surgery.[43]

Study Limitations

This study is an observational study and does not allow ultimate interpretation of the causal relations. Some of the observed immediate beneficial effects by sevoflurane may be due to direct signaling events related to preconditioning ("first window"), whereas other more medium- and long-term beneficial effects occurring within hours or days after exposure to sevoflurane may be well—at least in part—explained by genomic reprogramming. Our study can not distinguish between these possibilities, but associates the transcriptional changes to functional and biochemical outcomes. The current study was powered to detect differences in postoperative NT-proBNP levels between the SEVO and PROP groups but not to detect differences in clinical outcome measures. Although the DNA microarray technology has limitations with respect to sensitivity, accuracy, specificity, and reproducibility of results, the currently available data suggest that for abundant transcripts the existence and direction of expression changes can be reliably detected by this method.[44] Given the small number of samples compared with the high number of probes on the microarray (tens of thousands), the uncertainty about the statistical power in microarray analysis remains a limitation of large-scale transcriptional profiling. We have used state-of-the-art bioinformatics tools to address these concerns, particularly with respect to correction for multiple comparisons. Nonetheless, our results should be tested in randomized controlled clinical trials. This study is a short-term assessment of myocardial gene expression. However, we have previously shown in patients undergoing on-pump CABG surgery that favorable short-term changes in gene expression elicited by sevoflurane were associated with improved long-term cardiovascular outcome.[6] Although we carefully selected the patients, there always remains a high biologic variability in human studies compared with well-controlled animal experiments. In particular, medication could have affected myocardial substrate metabolism and biased our findings. In fact, sevoflurane patients received less remifentanil, a preconditioning-mimicking opioid, than propofol patients.[45] However, this should have favored cardiac protection in the propofol patients. Furthermore, despite proper randomization and selection of the patients, our analysis showed that the PGC-1α pathway was differentially regulated at baseline between groups. Therefore, we included this pathway in the multivariate analysis. Despite these caveats, we were able to detect robust functional patterns extending beyond this variability. A relatively healthy population of cardiac surgical patients was selected for the study, which might limit the general application of our findings. End-tidal sevoflurane concentrations were measured, whereas propofol concentrations in the blood were not determined, which might complicate the direct comparison between the two anesthetics. However, sevoflurane and propofol were administered in clinically relevant doses to maintain blood pressure and heart rate within 20% of baseline values. The implications of comparing a lipid-based anesthetic (propofol dissolved in Intralipid) with a lipid-free anesthetic on myocardial energy metabolism are unclear. It could be speculated that the lipid component of propofol but not the anesthetic itself might be responsible for some of the observed differences in cardiac energy metabolism. However, most studies showed that the effects of propofol on cardioprotection are independent of the solvent used.[46] Also, experimental data in mice provide evidence that volatile anesthetics *per se* reduce fatty acid oxidation in the heart.[22] Messenger RNA levels do not always directly translate into changes in protein or functional levels, because additional regulatory mechanisms on the posttranslational level exist. However, the gene expression profile underlying the cellular protein contents of enzymes and transporters is an important determinant of the metabolic phenotype. Finally, for ethical reasons, we used human atrial samples rather than ventricular biopsies to examine gene expression changes. Although there are clearly many disparities (but also many similarities) between human atrial and ventricular tissue, it remains elusive whether the gene expression results from atrial samples in this study can be directly extrapolated to left ventricular tissue. However,

one study found that only approximately 1% of genes, predominantly related to contractile function, were differentially expressed between atrium and left ventricle.[47] Using the Affymetrix chips, Barth *et al.*[48] reported a predominance of gene expression related to energy metabolism and oxidative phosphorylation in ventricles, whereas atrial tissue was prominently expressing transcripts related to signal transduction pathways, which might be specifically sensitive to changes in hemodynamic load and neuroendocrine activity. Of note, in the latter study, significant transcriptional differences were also reported between different regions within the same ventricle, and some chamber-specific markers such as the atrial natriuretic factor were also induced in ventricular tissues. Finally, using Affymetrix U95Av2 arrays, Ohki-Kaneda *et al.*[49] were able to predict left ventricular ejection fraction from right atrial gene expression patterns, implying that the atrial transcriptional profiles of our study are likely of clinical significance with respect to left ventricular gene expression and function.

Conclusions

Considerable effort has been focused on improving perioperative outcome in high-risk surgical patients. In a recent landmark article discussing the need for translation of basic science into clinical practice, anesthetic gases were proposed as readily available model drugs to successfully reproduce cardioprotection in patients.[50] Our data clearly support this view and provide a strong rationale for anesthetic gases in the perioperative management of cardiac surgical patients. In summary, using an integrative physiologic approach, we were able to link anesthetic-induced and constitutive gene regulatory control of myocardial energy metabolism to postoperative cardiac function in patients undergoing off-pump CABG surgery. Our analysis further points to the PGC-1α pathway and the G-CSF survival pathway, integral components of the protective program in the heart, as potential therapeutic targets in perioperative cardioprotection.

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