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Mitochondrial Dysfunction as an Arrhythmogenic Substrate

A Translational Proof-of-Concept Study in Patients With Metabolic Syndrome in Whom Post-Operative Atrial Fibrillation Develops

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Objectives This study sought to provide bedside evidence of the potential link between cardiac mitochondrial dysfunction and arrhythmia as reported in bench studies.

Background Atrial fibrillation (AF) is a frequent complication of cardiac surgery. Underlying mechanisms of post-operative atrial fibrillation (POAF) remain largely unknown. Because cardiac mitochondrial dysfunction has been reported in clinical conditions with a high risk of POAF, we investigated whether a causal link exists between POAF onset and pre-operative function of cardiac mitochondria.

Methods Pre-operative mitochondrial respiration and calcium retention capacity, respiratory complex activity, and myocardial oxidative stress were quantified in right atrial tissue from 104 consecutive patients with metabolic syndrome, in sinus rhythm, and undergoing coronary artery bypass graft surgery.

Results In this high-risk population, POAF occurred in 44% of patients. Decreased pre-operative mitochondrial respiration and increased sensitivity to calcium-induced mitochondrial permeability transition pore opening were significantly associated with POAF. Adenosine diphosphate–stimulated mitochondrial respiration supported by palmitoyl-L-carnitine was significantly lower in POAF patients and remained independently associated with AF onset after adjustment for age, body mass index, heart rate, beta-blocker use, and statin medication (multivariate logistic regression coefficient per unit $= -0.314 \pm 0.144$; $p = 0.028$). Gene expression profile analysis identified a general downregulation of the mitochondria/oxidative phosphorylation gene cluster in pre-operative atrial tissue of patients in whom AF developed.

Conclusions Our prospective study identifies an association between pre-operative mitochondrial dysfunction of the atrial myocardium and AF occurrence after cardiac surgery in patients with metabolic disease, providing novel insights into the link between mitochondria and arrhythmias in patients. (J Am Coll Cardiol 2013;62:1466–73) © 2013 by the American College of Cardiology Foundation

Mitochondria are key players in cardiomyocyte energy metabolism, redox state control, and the apoptosis. Recently, a role of the mitochondrial network in arrhythmogenesis has been suggested based on studies in animal models and cardiomyocytes in vitro (1,2). Indeed, increasing evidence indicates that mitochondrial dysfunction can directly alter

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cardiomyocyte excitability and cell-to-cell coupling through the regulation of the adenosine monophosphate protein kinase (3), the adenosine triphosphate–sensitive potassium channel (4), the sarcolemmal sodium channel (5,6).

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia. AF is a frequent complication of cardiac surgery, with the incidence ranging from 10% to 60% (7). Post-operative atrial fibrillation (POAF) is associated with significant increased morbidity and mortality (8,9). Although risk factors for POAF have been identified (i.e., advanced age, structural heart disease, metabolic syndrome, obesity), the pathological mechanisms responsible for the onset and perpetuation of POAF are incompletely understood (7).

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We designed a prospective study exploring the relationship between POAF onset, pre-operative mitochondrial function (i.e., mitochondrial respiration, complex activity and calcium retention capacity, myocardial oxidative stress), and the atrial transcriptome in patients in sinus rhythm (SR) with metabolic syndrome who underwent coronary artery bypass graft (CABG) surgery. Thus, we provide insights into the potential link between mitochondria and arrhythmia by exploring relevant human atrial tissue through pre-operative biopsies just few hours before arrhythmia onset in a population at high risk of atrial arrhythmia (7).

Methods

Patient selection and data collection. From September 2010 to March 2012, patients hospitalized in the heart surgery ward at Lille University Regional Hospital for CABG surgery were included in the study only if they had metabolic syndrome, diagnosed when 3 of the 5 following criteria were present: 1) waist circumference >102 cm in men and >88 cm in women; 2) fasting glycemia level >1 g/l; 3) triglyceride level >1.5 g/l; 4) high-density lipoprotein cholesterol <0.4 g/l in men and <0.5 g/l in women; and 5) hypertension (10). Other inclusion criteria were as follows: patients undergoing on-pump CABG surgery with removal of the right atrial appendage and SR at the time of inclusion. Exclusion criteria were a history of AF or current treatment with class I or III antiarrhythmic drugs, significant valvular disease, left ventricular ejection fraction <50%, patients younger than 18 years of age, or emergency procedure surgery. Clinical, biological, and echocardiographic data of included patients were obtained from the pre-operative consult. POAF was defined as AF lasting >30 s, recorded by continuous wireless rhythmic monitoring, that began immediately after surgery and lasted 8 days. Electrocardiographic data were stored and analyzed after patient discharge. Patients provided informed consent. This study has received authorization from the local Patient Protection Committee.

Atrial appendage biopsy and tissue processing. Human atrial tissue samples were obtained from right atrial appendages before cardiopulmonary bypass (11). Samples were immediately collected in an ice-cold cardioplegic solution for functional studies, with the rest of the tissue biopsy being snap-frozen.

Mitochondrial functional studies and in vitro chain complex activities. PERMEABILIZED MYOCARDIAL FIBER PREPARATION. Thin bundles were separated along atrial trabeculae orientation under stereomicroscopic control in ice-cold BIOPS relaxing solution containing 2.77 mM CaK_2-ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), 7.23 mM K_2EGTA, 6.56 mM $MgCl_2$, 0.5 mM dithiothreitol, 50 mM K-2-(N-Morpholino) ethanesulfonic acid (MES), 20 mM imidazole, 20 mM taurine, 5.3 mM Na_2-adenosine triphosphate (ATP), 15 mM phosphocreatine, pH 7.1. Bundles were then permeabilized for 30 min with saponin solution and washed 3 times for 5 min in respiration medium MiRO5 containing 110 mM sucrose, 20 mM 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid, 10 mM KH_2PO_4, 20 mM taurine, 3 mM $MgCl_26H_2O$, 60 mM MES-K, 0.5 mM EGTA, and 0.1% bovine serum albumin; pH 7.1.

HIGH-RESOLUTION RESPIROMETRY. Respiration rates were measured at 37°C with different substrates and inhibitors in atrial permeabilized fibers, as described previously (11,12). Mitochondrial O_2 consumption measurements were made using the O_2K oxygraph (Oroboros, Innsbruck, Austria). Substrate and inhibitors for respiratory experiments were added to MiRO5 in a step-by-step manner using micro syringes. Chemical agents were sequentially administered. At the end of each experiment, respirometer chambers were calibrated for zero O_2 content with dithionite. Respiration rates were expressed in $pmolO_2 \cdot s^{-1} \cdot mg\ dry\ wt^{-1}$.

Two mitochondrial substrate/inhibitor protocols were applied. In the carbohydrate protocol, substrate combinations were used for electron flow through respiratory chain complexes I and II (Fig. 1A). Complex I–dependent respiration (respiration supported by pyruvate and malate in the absence of adenosine diphosphate) was determined in the presence of 10 mM pyruvate + 2 mM malate without ADP. Complex I–dependent respiration (adenosine diphosphate–stimulated respiration supported by pyruvate and malate) was determined as a phosphorylation-stimulated

Abbreviations and Acronyms

ADP = adenosine diphosphate
AF = atrial fibrillation
ATP = adenosine triphosphate
BMI = body mass index
CABG = coronary artery bypass graft
mCRC = mitochondrial calcium retention capacity
MnSOD = manganese superoxide dismutase
mPTP = mitochondrial permeability transition pore
OXPHOS = oxidative phosphorylation
POAF = post-operative atrial fibrillation
ROS = reactive oxygen species
SR = sinus rhythm
V_{Cox} = complex IV–related uncoupled maximum respiration
$V_{palm+ADP}$ = adenosine diphosphate–stimulated respiration supported by palmitoyl-L-carnitine

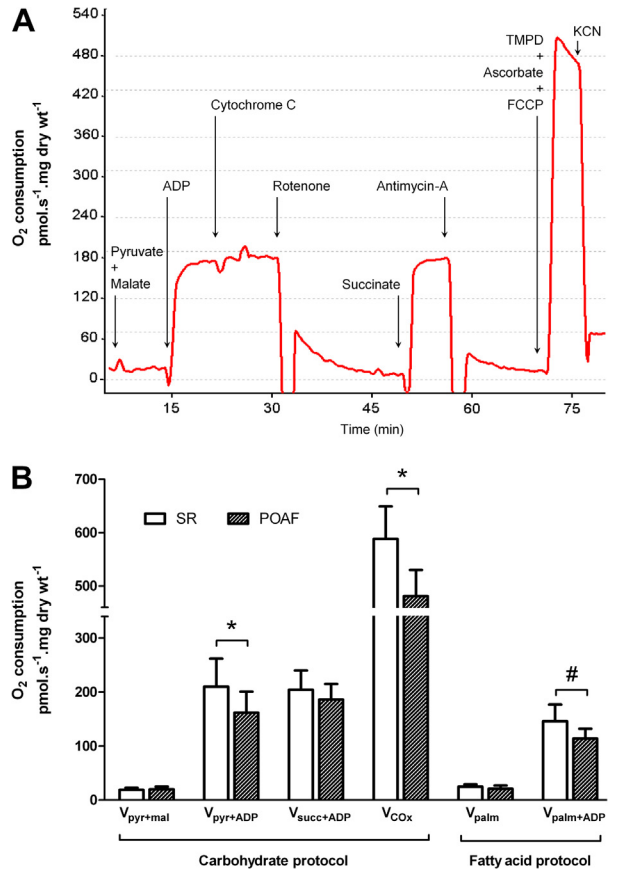

Figure 1 **Pre-Operative Mitochondrial Function in Patients in Whom POAF Is or Is Not Developing, Assessed by O_2 Consumption Rates in Permeabilized Atrial Fibers**

(A) Representative oxygraph trace of a typical O_2 consumption using a substrate/inhibitor combination, as described in the carbohydrate protocol (details in the Methods section). **(B)** Respiration rates in carbohydrate and fatty acid protocols in patients in whom POAF is or is not developing. Five to 7 fibers were explored per protocol for each of the 104 patients. Values are presented as mean ± SD. *$p < 0.05$ versus sinus rhythm. #$p < 0.01$ versus sinus rhythm. ADP = adenosine diphosphate; FCCP = carbonylcyanide-p-trifluoromethoxyphenylhydrazone; KCN = potassium cyanide; POAF = post-operative atrial fibrillation; SR = sinus rhythm; TMPD = N,N,N9,N9-tetramethyl-p-phenylenediamine; V_{Cox} = complex IV–related uncoupled maximum respiration; V_{palm} = respiration supported by palmitoyl-L-carnitine in the absence of ADP; $V_{palm+ADP}$ = ADP-stimulated respiration supported by palmitoyl-L-carnitine; $V_{pyr+ADP}$ = ADP-stimulated respiration supported by pyruvate and malate; $V_{pyr+mal}$ = respiration supported by pyruvate and malate in the absence of ADP; $V_{succ+ADP}$ = ADP-stimulated complex II–dependent respiration.

respiration rate in the presence of 5 mM ADP. In all groups, the lack of a significant increase in respiration after addition of cytochrome c (10 μM) confirmed the integrity of the outer mitochondrial membrane. Subsequently, complex I was inhibited by rotenone (0.5 μM) and complex II–dependent respiration (adenosine diphosphate–stimulated complex II–dependent respiration) was determined in the presence of 10 mM succinate. The complex IV–dependent uncoupled state of respiration (V_{Cox}) was determined in presence of 2 mM ascorbate, 0.5 mM N,N,N9,N9-tetramethyl-p-phenylenediamine (TMPD), the complex III inhibitor antimycin A (2.5 μM), and the oxidative phosphorylation uncoupler carbonylcyanide-p-trifluoromethoxyphenylhydrazone (FCCP) (2 μM) ($V_{TMPD-asc}$). TMPD-ascorbate auto-oxidation (V_{KCN}) was determined as the O_2 consumption in the presence of 1 mM potassium cyanide (KCN) added to the $V_{TMPD-asc}$ medium. V_{Cox} was calculated as $V_{TMPD-asc} - V_{KCN}$. Because antimycin A inhibits complex III, V_{Cox} estimated the complex IV–related uncoupled maximum respiration rate.

In the fatty acid protocol, respiration was measured with palmitoyl-L-carnitine (80 μM) and malate (2 mM) (respiration supported by palmitoyl-L-carnitine in the absence of ADP) followed by ADP (5 mM) (adenosine disphosphate–stimulated respiration supported by palmitoyl-L-carnitine [$V_{palm+ADP}$]) feeding electrons into electron-transferring flavoprotein and complex I.

MITOCHONDRIAL CALCIUM RETENTION CAPACITY. The mitochondrial calcium retention capacity (mCRC) was determined as the capacity of mitochondria to uptake calcium before permeability transition to test the sensitivity of the mitochondrial permeability transition pore (mPTP) opening to calcium. Calcium uptake of permeabilized fibers (ADP-deprived buffer) was quantified by monitoring calcium green (1 μM) fluorescence at 506 to 535 nm in response to 4-nmol pulses of exogenous Ca^{2+}, as previously described (13). mCRC was expressed as total mitochondrial calcium retention in nmolCa^{2+}·mg dry wt^{-1}.

IN VITRO CHAIN COMPLEX ACTIVITIES. Citrate synthase and electron transport chain complex activities (complexes I, II+III, III, and IV) were performed as described in Online Appendix 1.

Myocardial oxidative stress. Reactive oxygen species (ROS) concentrations were measured in frozen tissue by electron paramagnetic resonance spectroscopy, as previously described (14). Manganese superoxide dismutase (MnSOD) and catalase activities were assayed as previously described (15) and expressed as U·mg^{-1} (Online Appendix 1).

Transcriptomic study. Pre-operative right atrial tissues were selected from 8 patients in whom POAF developed and matched to 8 patients without POAF (Online Table 1 in Online Appendix 1). Total RNA was extracted and analyzed with Agilent SurePrint G3 Human Gene Expression 8x60K v2, as described in Online Appendix 1.

Statistical analysis. Continuous variables with a Gaussian distribution are given as mean ± SD. Continuous variables with no Gaussian distribution are given as median (25th to 75th) percentiles. Categorical variables are given as the percentage (number) of patients with the respective attribute. Bivariate comparisons were performed using the *t* test for normally distributed continuous variables or the Mann-Whitney *U* test for variables not normally distributed. Bivariate comparisons of categorical variables were performed with the chi-square test. Potential predictors for POAF onset with a p value <0.15 on bivariate analysis (age,

body mass index [BMI], heart rate, and $V_{palm+ADP}$) were added to the multivariate stepwise logistic regression model using backward variable selection. Beta-blocker and statin medications were also forced into the model because of their clinical relevance. Odds ratios are given with 95% confidence intervals for categorical variables. Coefficients and SDs are given for continuous variables. All analyses were conducted using SAS version 9.2 (SAS Institute, Cary, North Carolina).

Results

Demographic and clinical characteristics. The demographic and clinical characteristics of the study population are presented in Table 1. A total of 104 patients were included in the study. During the 8-day follow-up, AF developed in 46 patients after CABG surgery (44.2%). Patients in whom POAF developed had a significantly higher BMI and pre-operative heart rate than patients who remained in SR. There was a trend for older age in POAF patients. No significant differences in other demographic, clinical, or paraclinical characteristics or pre-operative medications were observed between patients with metabolic syndrome in whom AF developed and those who remained in SR.

Pre-operative mitochondrial function in atrial tissue. In permeabilized cardiac fibers, ADP-stimulated mitochondrial respiration rates supported by either pyruvate malate (adenosine diphosphate–stimulated respiration supported by pyruvate and malate) or palmitoyl-L-carnitine ($V_{palm+ADP}$) and complex IV–related uncoupled maximum respiration (V_{Cox}) were significantly lower in pre-operative atrial myocardium from patients in whom POAF developed compared with those who remained in SR (Fig. 1B). By contrast, ADP-stimulated complex II–dependent respiration was not different between groups (Fig. 1B).

Pre-operative atrial tissues had similar citrate synthase activity in patients in whom POAF developed and those who remained in SR (Table 2). This suggests that altered respiration was likely not related to a modification in mitochondrial content, although mitochondrial DNA or total FCCP-induced respiration were not measured in this study. In vitro, complex II+III and complex III activities (complex activity normalized by citrate synthase) were significantly reduced in POAF patients compared with those who remained in SR, in contrast to complex I and IV activities (Table 2).

The mCRC was significantly decreased in patients with POAF compared with patients who remained in SR (Fig. 2A). Lower mCRC suggests that sensitivity to calcium-induced mPTP opening was increased in patients in whom POAF developed.

Measurements of ROS levels in frozen tissue assessed by electron paramagnetic resonance spectroscopy and mitochondrial MnSOD activity were increased in patients in whom POAF developed (Figs. 2B and 2C). Catalase activity was not significantly different in atrial biopsy specimens from both groups (Fig. 2D).

Table 1 Patient Characteristics

Variables	SR Patients (n = 58)	POAF Patients (n = 46)	p Value
Age, yrs	64.5 ± 10	69 ± 8	0.06
Male	40 (69)	30 (65)	0.83
BMI, kg/m^2	29.8 ± 4.1	32.1 ± 5.4	0.03
Waist circumference, cm	114 ± 10	116 ± 12	0.49
Systolic blood pressure, mm Hg	137 ± 12	134 ± 16	0.36
Diastolic blood pressure, mm Hg	74 ± 9	73 ± 12	0.50
Heart rate, beats/min	67 ± 9	79 ± 14	<0.01
Comorbidities			
Hypertension	54 (93)	40 (86)	0.33
Diabetes	32 (55)	31 (67)	0.23
Dyslipidemia	46 (79)	33 (72)	0.49
Current smoker	12 (21)	13 (28)	0.49
Euroscore	3.2 ± 1.1	3.4 ± 0.9	0.61
Laboratory data			
Creatinine, mg/l	9.8 ± 2.4	10.1 ± 0.9	0.66
Glycosylated hemoglobin, %	7.1 ± 1.3	6.9 ± 0.9	0.17
HDL, g/l	0.32 (0.26–0.53)	0.37 (0.30–0.46)	0.28
LDL, g/l	1.00 (0.85–1.29)	0.90 (0.73–1.16)	0.32
TG, g/l	1.38 (0.97–2.67)	1.42 (1.16–2.48)	0.52
ECG and echocardiographic data			
P-wave duration, ms	111 ± 28	105 ± 21	0.67
Sokolow-Lyon index, mm	19.0 (16–26)	17.8 (15–24)	0.29
LVEF, %	59 ± 8	60 ± 8	0.78
LA diameter, mm	39.5 ± 6	38.9 ± 6	0.67
Time on bypass, min	96 ± 30	94 ± 27	0.79
Pre-operative medication			
Statins	53 (90)	40 (86)	0.53
Diuretics	12 (21)	10 (22)	1.00
ACE inhibitors or ARBs	50 (86)	40 (87)	1.00
Beta-blockers	44 (76)	32 (69)	0.51
Antiplatelet agent	57 (98)	46 (100)	1.00
Oral anticoagulant	1 (2)	2 (4)	0.58
Insulin	2 (3)	2 (4)	1.00
Metformin	19 (32)	20 (43)	0.21
Sulfonylureas	10 (17)	6 (13)	0.59
Fibrates	2 (3)	2 (4)	1.00

Values are mean ± SD, n (%), or median (25th to 75th percentiles).

ACE = angiotensin converting enzyme; ARBs = angiotensin receptor blockers; BMI = body mass index; ECG = electrocardiographic; HDL = high-density lipoprotein; LA = left atrial; LDL = low-density lipoprotein; LVEF = left ventricular ejection fraction; POAF = post-operative atrial fibrillation; SR = sinus rhythm; TG = triglycerides.

Multivariate logistic regression. To understand the impact of pre-operative medication and clinical factors on the association between pre-operative mitochondrial dysfunction (i.e., reduced ADP-stimulated mitochondrial respiration supported by palmitoyl-L-carnitine [$V_{palm+ADP}$]), and POAF, multivariate stepwise logistic regression analysis was performed using backward variable selection. Using this model, $V_{palm+ADP}$ and heart rate were independently associated with POAF onset. Table 3 lists associations between POAF and those parameters. BMI, age, and beta-blocker,

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Table 2 In Vitro Mitochondrial Chain Complex Activities According to Post-Operative Atrial Fibrillation Onset

Substrate	Citrate Synthase	Complex I	Complex II + III	Complex III	Complex IV
SR	201 ± 56	7.3 ± 1.2	13.6 ± 1.4	32 ± 5	60 ± 8
POAF	180 ± 73	5.9 ± 1.5	7.9 ± 1*	24 ± 3*	56 ± 7

Values are mean ± SD. Citrate synthase activity in $nmol \cdot min^{-1} \cdot mg^{-1}$. Complex activities as percentage of citrate synthase activity. *$p < 0.05$ versus sinus rhythm.

Abbreviations as in Table 1.

and statin use were not individually predictive of the occurrence of AF after cardiac surgery.

Gene expression profile associated with POAF onset. Global mRNA expression patterns of atrial biopsies from 8 diabetic patients with no POAF matched with 8 diabetic patients with POAF were measured using high-density oligonucleotide arrays. After filtering data on expression (20% to 100%) to remove minimally expressed genes, a volcano plot graph was generated with a fold change cutoff at 1.2 (Online Fig. 1 in Online Appendix 1). A total of 615 genes (for $p = 0.05$) and 67 genes (for $p = 0.01$) were found to be differentially expressed in patients in whom POAF developed. The gene list, as exported from GeneSpring, was sorted in Excel based on the level (fold change values) of regulation of POAF (down or up) (Online Appendix 2). The top 10 up- and down-regulated genes are presented according to HUGO Gene Nomenclature Committee database in Online Figure 1 in Online Appendix 1.

Up- or down-regulated genes were classified using the functional annotation table in Database for Annotation, Visualization, and Integrated Discovery (DAVID). Although such a classification did not reveal any significant functionally relevant groups in up-regulated genes, down-regulated genes identified a functional group of genes involved in mitochondria/oxphos processes (Fig. 3). In addition, several expressed genes involved in oxidation/reduction reactions were altered in POAF patients, such as glutathione-*S*-transferase, thioredoxin, heme oxidase, peroxiredoxin, cytochrome p-450, and xanthine dehydrogenase (Online Appendix 1).

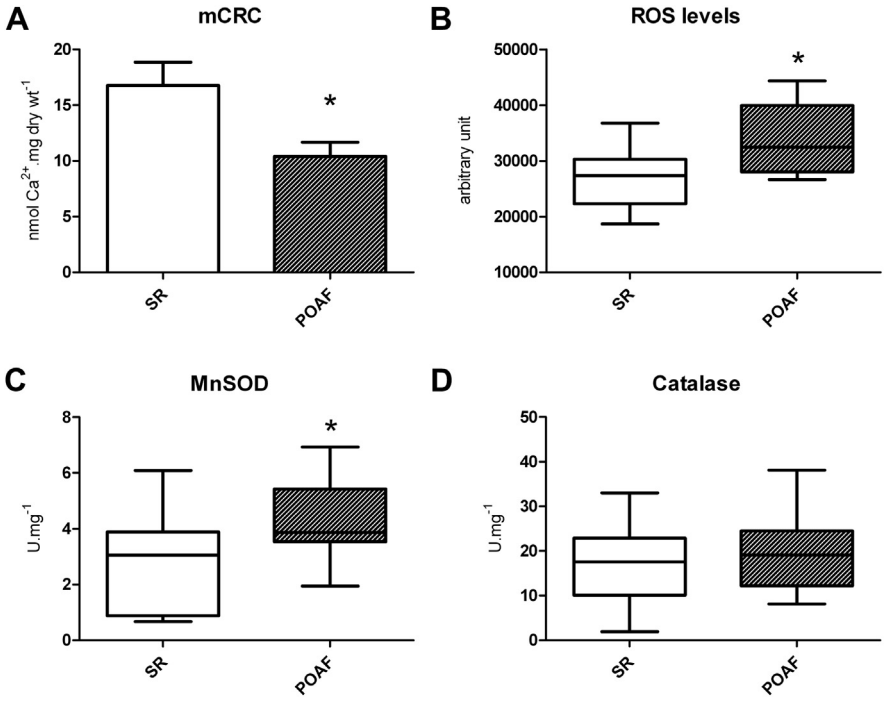

Figure 2 Mitochondrial Permeability Transition Pore Opening Sensitivity and Myocardial Oxidative Stress in Patients in Whom POAF Is or Is Not Developing

(A) Mitochondrial calcium retention capacity (mCRC) determined as the capacity of mitochondria to uptake calcium before permeability transition. Five to seven fibers were explored for each of the 104 patients. **(B)** Reactive oxygen species (ROS) concentration in atrial tissue. Manganese superoxide dismutase (MnSOD) **(C)** and catalase **(D)** activity in atrial tissue. Values are presented as mean ± SD or median, 25th to 75th percentiles, and range. n = 83 to 104 patients. *$p < 0.05$ versus SR. Abbreviations as in Figure 1.

Table 3 Pre-Operative Variables Associated With Post-Operative Atrial Fibrillation Onset

	Coefficient	SD	p Value
$V_{palm+ADP}$, per unit	−0.314	0.144	0.028
Heart rate, beats/min	0.065	0.031	0.048

Coefficients and SDs are given for continuous variables.
$V_{palm+ADP}$ = adenosine diphosphate–stimulated respiration supported by palmitoyl-L-carnitine (in $pmolO_2 \cdot s^{-1} \cdot mg\ dry\ wt^{-1}$).

Discussion

The present translational study provides the first direct evidence of a role of perturbations of cardiac mitochondrial function in clinical arrhythmia pathophysiology. More specifically, our results suggest that pre-operative mitochondrial dysfunction of the atrial myocardium may be an important determinant of AF risk after CABG surgery in patients with metabolic syndrome. We provide data arguing for a transcriptional downregulation of the oxidative phosphorylation (OXPHOS)/mitochondrial genes as a mechanism of this mitochondrial dysfunction in diabetic patients with metabolic syndrome in whom POAF develops.

Mitochondria and arrhythmia. The heart is a tissue with one of the highest rates of energy conversion in the body, being critically dependent on mitochondrial oxidative phosphorylation as a major source of ATP (16). Thus, cardiac mitochondria represent a key actor of the biological systems addressed to prevent any mismatch between ATP production and use and thus any major disruption in the energy available for function.

Potential roles of mitochondria in cardiac electrical function have recently emerged from in vitro studies showing that modulations of the mitochondrial respiration, membrane potential, and ion channels alter sarcolemmal ion channels involved in action potential genesis. Inhibition of the mitochondrial respiratory chain by antimycin A, a complex III inhibitor, or by an increased ratio between the reduced and the oxidized form of the intracellular nicotinamide adenine dinucleotide, has been shown to inhibit the sarcolemmal sodium current in cardiomyocytes (5,6). Brown et al. (17) showed that cardiac arrhythmias in isolated perfused heart induced by glutathione oxidation can be inhibited by ligands of the mitochondrial benzodiazepine receptor, which stabilize mitochondrial membrane potential and prevent mitochondrial depolarization. In accordance, our study identified elements of mitochondrial dysfunction (i.e., impairment in maximal capacity to oxidize both carbohydrate- and lipid-based substrates and increased sensitivity to mPTP opening) associated with arrhythmia onset in patients. The sarcolemmal K_{ATP} channel and adenosine monophosphate protein kinase are sensors of cellular energy levels that have been reported as candidates for the downstream mediators of metabolic stress, namely, the link between cardiomyocyte excitability and mitochondrial metabolism (3,4). Their specific role in POAF was not tested here and warrants further investigation.

POAF and mitochondrial dysfunction in the metabolic syndrome. In case-control studies, Anderson et al. (13,18) and Niemann et al. (19) showed that mitochondrial function is abnormal in the atrial myocardium of obese patients and/or those with diabetes. Our prospective study provides data showing that this mitochondrial dysfunction is meaningful and associated with clinically relevant events in patients with metabolic syndrome.

The exact pathological mechanisms responsible for the onset and perpetuation of POAF are incompletely understood. POAF-facilitating factors are typically separated into acute factors directly related to surgery (i.e., inflammation, ischemia injury, beta-adrenergic activation) and factors that reflect chronic and progressive cardiac remodeling (7). Pre-existing factors can initiate AF and/or aggravate the underlying conditions able to perpetuate AF. Among these latter factors are alterations in cellular Ca^{2+} and K^{+} ion channels, age-dependent atrial remodeling, and structural heart diseases (7). Indirect evidence suggests a role for altered energy metabolism in POAF pathology. In case-control studies, lower oxidative phosphorylation and altered expression of genes involved in cardiac mitochondrial energy production have been observed in human atrial biopsy specimens from patients with chronic AF (20,21). Using a permeabilized fiber approach on atrial tissue obtained before CABG surgery and thus before arrhythmia onset, we demonstrate here that mitochondrial respiration rates supported by both carbohydrate- and lipid-based substrates are reduced in patients in whom AF developed compared with those who remained in SR. Consistently, the individual activity of complex II+III was reduced in frozen atrial biopsy specimens from POAF patients. Taking into account the established biological and clinical mediators of AF, we demonstrate that reduced mitochondrial respiration is an independent predictor of POAF. Mitochondria-targeted tools such as mitochondria-targeting antioxidants and myocardial preconditioning should be tested as promising strategies in POAF prevention in patients with metabolic syndrome.

Mechanisms of cardiac mitochondrial dysfunction in patients in whom POAF develops. Recent evidence shows that increased ROS production and, more specifically, increased superoxide anion production derived from the atrial nicotinamide adenine dinucleotide phosphate oxidase are independently associated with a higher risk of POAF (22,23). In line with these studies, we found that high levels of ROS production in pre-operative biopsy specimens discriminated between patients in whom AF developed from those who maintained SR in the post-operative period. We also identified several differentially expressed genes in POAF patients. A significantly enriched cluster of down-regulated genes is involved in oxidation/reduction reactions and contributes to cellular oxidative stress. In addition, atrial tissue from patients in whom POAF developed exhibited an upregulation of mitochondrial MnSOD activity and an increased sensitivity of mPTP opening. Both can be regarded as indirect evidence of increased mitochondria-targeted ROS. If mitochondrial oxidases are not a major source of ROS in POAF, as observed

A

	Search Term	Genes Down-Regulated in POAF Patients count [%]	P-value
GO term BP FAT	cellular respiration	19 [7,5%]	$4.4E^{-17}$
GO term BP FAT	electron transport chain	18 [7%]	$1.6E^{-14}$
GO term BP FAT	ATP synthesis coupled electron transport	13 [5%]	$1.5E^{-12}$
SP PIR Keywords	mitochondrion	66 [25%]	$2.1E^{-35}$
SP PIR Keywords	electron transport	18 [7%]	$2.7E^{-15}$
SP PIR Keywords	respiratory chain	16 [6%]	$4.1E^{-15}$

B

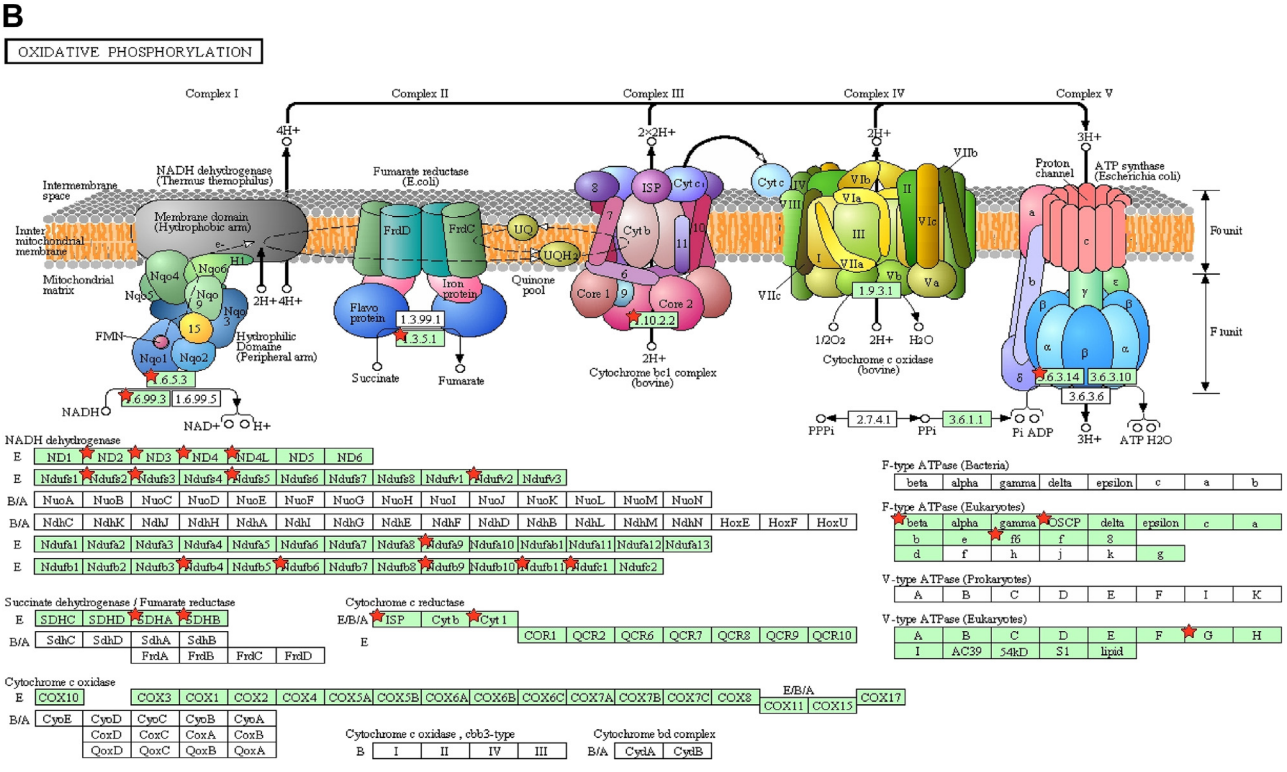

Figure 3 **Altered Expressed Genes in Atrial Samples From 8 Diabetic Patients With No POAF Matched With 8 Diabetic Patients With POAF**

(A) Gene Ontology (GO)-term enrichment analysis of down-regulated genes in post-operative atrial fibrillation (POAF) patients. Official gene symbols corresponding to down-regulated genes (fold change >1.2, $p < 0.05$) were uploaded into the Database for Annotation, Visualization, and Integrated Discovery (DAVID) portal and analyzed using medium stringency. Top-ranking annotations according to the Biological Process Functional Annotation Tool (BP FAT) and the Protein Information Resource (PIR) keywords are shown. Count: number of genes included (% involved genes/total genes). P Value: modified Fisher exact test p value. ATP = adenosine triphosphate. **(B)** Representative scheme of genes altered in the "oxidative phosphorylation" functional pathway (Kyoto Encyclopedia of Genes and Genomes [KEGG] pathway). *indicates significant down-regulated gene expression in POAF patients versus patients in SR. Gene count is 21 (8.3%) with a p value of $1.1E^{-15}$. All abbreviations correspond to the official gene symbol. A detailed description can be found at http://www.genecards.org/.

by Reilly et al. (24), nicotinamide adenine dinucleotide phosphate oxidase–derived ROS can target and damage mitochondrial DNA and protein complexes, the observed mitochondrial dysfunction being the link between previously reported ROS imbalance and arrhythmia (1,2).

Transcriptomic analysis of mRNA from atrial biopsy specimens from diabetic patients in whom AF developed in the post-operative period revealed that nuclear and mitochondrial genes involved in mitochondria/OXPHOS processes were down-regulated. Molecular mechanisms for this concomitant down-regulation of OXPHOS have not been identified. Several hypotheses, warranting further investigation, can be put forward to explain this phenomenon. An upstream transcriptional regulator controlling this OXPHOS network may be altered in diabetic cardiomyocytes. Alternatively, but not exclusively, the microRNA expression pattern may be altered in the clinical situations associated with POAF.

Study limitations. The present study does not prove a causal association between mitochondrial dysfunction and POAF; therefore, further mechanistic studies are required. Concerning our statistical analysis, the sample size may have limited our ability to fully account for clinical factors, and the multivariate model is slightly overfitted. Finally, our results of a monocentric study are restricted to patients with metabolic syndrome who were selected because of their high susceptibility to AF after CABG surgery and marked alterations of cardiac mitochondria in their atrial myocardium. Although the population studied is representative of nearly 50% of patients referred to the hospital for CABG surgery, our results should be tested in the general population.

Conclusions

We demonstrate in a prospective study that pre-operative mitochondrial dysfunction of the atrial myocardium is associated with AF occurrence after cardiac surgery, identifying the mitochondrium as a potential key player in clinically relevant arrhythmia and thus as a new promising target in POAF prevention strategies.

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Key Words: atrial fibrillation ■ cardiac surgery ■ diabetes ■ gene expression ■ mitochondria.

APPENDIX

For supplemental material, a figure, and a table, please see the online version of this article.