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Heart Rhythm Disorders

A Randomized Controlled Trial to Prevent Post-Operative Atrial Fibrillation by Antioxidant Reinforcement

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Objectives This study was designed to assess whether the reinforcement of the antioxidant system, through n-3 fatty acids plus antioxidant vitamin supplementation, could reduce the incidence of post-operative atrial fibrillation.

Background Therapy to prevent post-operative atrial fibrillation remains suboptimal. Although oxidative stress plays a key role in the pathogenesis of this arrhythmia, antioxidant reinforcement has produced controversial results.

Methods A total of 203 patients scheduled for on-pump cardiac surgery were randomized to placebo or supplementation with n-3 polyunsaturated fatty acids (2 g/day) (eicosapentaenoic acid:docosahexaenoic acid ratio 1:2), vitamin C (1 g/day), and vitamin E (400 IU/day). The primary outcome was the occurrence of post-operative atrial fibrillation. Secondary outcomes were the biomarkers related to oxidative stress and inflammation.

Results Post-operative atrial fibrillation occurred in 10 of 103 patients (9.7%) in the supplemented group versus 32 of 100 patients (32%) in the placebo group ($p < 0.001$). Early after surgery, placebo patients presented with increased levels of biomarkers of inflammation and oxidative stress, which were markedly attenuated by antioxidant supplementation. The activity of catalase, superoxide dismutase, and glutathione peroxidase in atrial tissue of the supplemented patients was 24.0%, 17.1%, and 19.7% higher than the respective placebo values ($p < 0.05$). The atrial tissue of patients who developed atrial fibrillation showed NADPH oxidase p47-phox subunit protein and mRNA expression 38.4% and 35.7% higher, respectively, than patients in sinus rhythm ($p < 0.05$).

Conclusions This safe, well-tolerated, and low-cost regimen, consisting of n-3 polyunsaturated fatty acids plus vitamins C and E supplementation, favorably affected post-operative atrial fibrillation, increased antioxidant potential, and attenuated oxidative stress and inflammation. (Prevention of Post-Operative Atrial Fibrillation: Pathophysiological Characterization of a Pharmacological Intervention Based on a Novel Model of Nonhypoxic Pre-Conditioning; ISRCTN45347268) (J Am Coll Cardiol 2013;62:1457–65) © 2013 by the American College of Cardiology Foundation

Atrial fibrillation following cardiac surgery is considered a major risk factor of morbidity and mortality in the surgical setting (1). Currently, the available conventional therapies for preventing post-operative atrial fibrillation (POAF) are suboptimal (2). Accumulated evidence suggests a role for oxidative stress in ischemia-reperfusion injury (3–6). Moreover, oxidative stress has been implicated in the pathophysiology of several cardiovascular disorders (7–10). Therefore, it is of interest to study specific biomarkers derived from reactive oxygen species (ROS) for assessing POAF risk (11). Specifically, atrial NADPH oxidase shows increased activity during cardiac ischemia-reperfusion (6,12) and is independently associated with increased risk for POAF (13). Also, serum peroxide levels have been associated with POAF risk (14).

Interestingly, low to moderate ROS levels enhance the endogenous antioxidant response (15) through activation of nuclear factor erythroid 2–related factor 2 transcription

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Abbreviations and Acronyms

DHA = docosahexaenoic acid

EPA = eicosapentaenoic acid

MDA = malondialdehyde

POAF = post-operative atrial fibrillation

PUFA = polyunsaturated fatty acid

ROS = reactive oxygen species

factor (16). This mediator up-regulates gene expression of cardiomyocyte antioxidant enzymes such as catalase (CAT) and glutathione peroxidase (GSH-Px) (17,18). Hence, n-3 polyunsaturated fatty acids (n-3 PUFAs) can act as indirect antioxidants by inducing low to moderate increases in ROS levels and decreasing vulnerability of myocardial tissue to a subsequent oxidative challenge.

Previously we proposed a hypothesis of nonhypoxic cardiac pre-conditioning to patients scheduled for cardiac surgery (4). The paradigm consists of a synergistic indirect antioxidant effect of n-3 PUFAs to elicit antioxidant enzyme induction followed by the direct antioxidant effect of vitamins C and E. Some clinical trials have used vitamins C and E to prevent POAF (5,19,20) and to reduce early recurrence rates after electrical cardioversion (21). n-3 PUFAs also have demonstrated preliminary favorable effects with this aim (22–24). However, other similar studies (25–27) and the large multicenter OPERA (Omega-3 Fatty Acids for Prevention of Post-Operative Atrial Fibrillation) trial (28) clearly found no evidence of a beneficial effect of this treatment, thus giving rise to a controversy (29–31). Therefore, the molecular bases of this effect are needed to clarify these issues. An extended background is presented in the Online Appendix.

The present study was aimed at testing the hypothesis that reinforcement of the antioxidant system leads to a cardioprotective effect against the occurrence of POAF in patients subjected to on-pump cardiac surgery.

Methods

Design overview. This randomized, double-blind, placebo-controlled trial was conducted in patients undergoing on-pump cardiac surgery to test whether reinforcement of their antioxidant status reduced the occurrence of POAF. A population of 307 patients was admitted for cardiac surgery at the University of Chile Clinical Hospital and San Juan de Dios Hospital between February 2010 and December 2011. The research protocol was approved by the institutional ethics committees, according to the Helsinki Declaration of the World Medical Association (2000). All patients gave written informed consent.

Setting and participants. The patients were 18 years of age or older, scheduled for coronary artery bypass graft, valve surgery, or mixed, all in sinus rhythm (Fig. 1). Exclusion criteria comprised a previous history of any arrhythmia, previous myocardial infarction, current use of amiodarone or sotalol, severe congestive heart failure (New York Heart Association class III or IV), presence of prosthetic valves, congenital valvular disease, or left atrial diameter >50 mm. We also excluded patients with conditions associated with oxidative stress or inflammation such as chronic rheumatic or neoplastic diseases, liver insufficiency, severe chronic kidney disease (serum creatinine >2.0 mg/dl), and recent infections. In addition, patients receiving nonsteroidal anti-inflammatory drugs, corticosteroids, antioxidant vitamins, or fish oil supplements 3 months prior to surgery were excluded. Demographic and clinical characteristics were carefully recorded (Table 1).

Randomization and interventions. Randomization of participants to placebo or supplemented groups was performed 7 days before surgery; the randomization was central, nonstratified, block based (4-patient block size), and computer generated. Surgical access was through a median sternotomy incision, and the same induction and anesthesia protocol was used for all patients. All anastomoses were sutured by hand. Protection of myocardial tissue was accomplished with crystalloid cold potassium cardioplegic solution.

Treatment with n-3 PUFAs at a dose of 2 g daily was initiated immediately after randomization. The formulation contained eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids in a 1:2 EPA:DHA ratio, as previously reported (22). Two days before surgery, vitamin C (1 g/day) plus vitamin E (400 IU/day) were added. The whole supplementation regimen continued until hospital discharge. The placebo group received an equal number of identical capsules containing caprylic/capric triglyceride (825 mg/capsule), inert microgranules (500 mg), and vegetable oil (400 mg) replacing the n-3 PUFAs, vitamin C, and vitamin E, respectively.

Outcomes and follow-up. The primary outcome of the study was the occurrence of electrocardiographically (ECG) confirmed POAF from surgery until hospital discharge. Continuous ECG monitoring was performed 24 to 48 h after the operation to detect new-onset atrial fibrillation. Subsequently, a Holter monitoring device was placed on the patients until the fourth post-operative day. Between the removal of the Holter monitoring device and discharge, a 12-lead ECG was performed every 12 h or whenever arrhythmia symptoms occurred. The presence of ECG-documented atrial fibrillation for at least 1 min was recorded and analyzed as "post-operative atrial fibrillation." All symptomatic episodes detected by the patients or clinical residents were confirmed by a 12-lead ECG. The secondary outcomes included oxidative stress–related biomarkers in atrial tissue/plasma, as well as blood inflammation indexes. Blood samples were drawn during the morning hours at a fasting state on the day of enrollment (day −7), 5 days after n-3 PUFAs administration (day −2), 15 min before starting extracorporeal circulation (time 0), 6 to 8 h after the surgical operation (day +1), and post-operative day 5 (day +5). Right atrial appendage samples were obtained immediately before starting extracorporeal circulation. Sample management and analysis methods are reported in the Online Appendix.

Statistical analyses. Sample size calculation was performed for the primary outcome, with an expected POAF occurrence of 35% in the placebo group and 15% in the

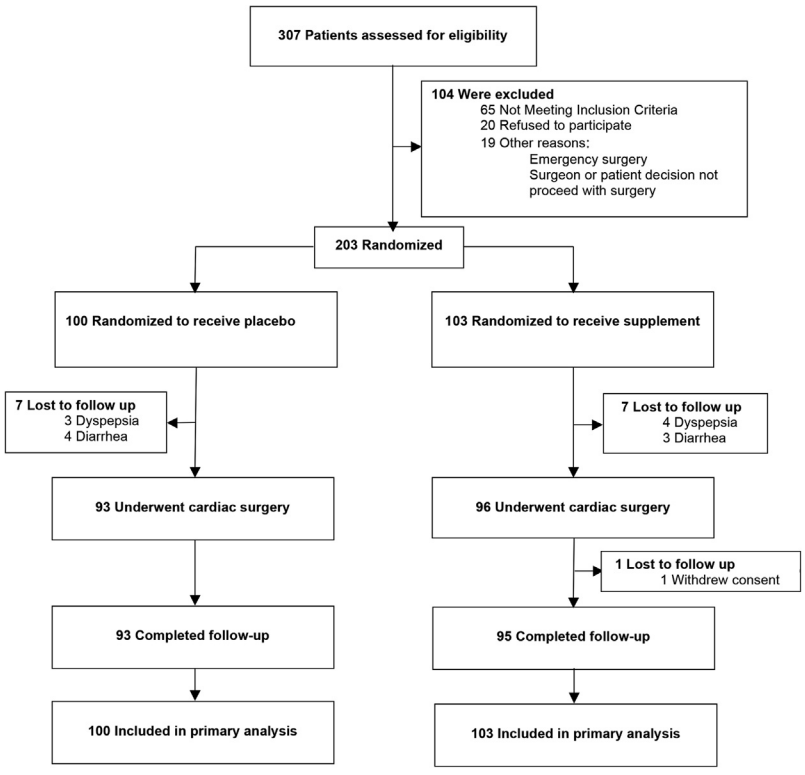

Figure 1 **Disposition of Patients**

Randomization, treatment, and follow-up of participants.

supplemented group, a significance level of 0.05 and 80% test power. The resulting sample size was 91 patients per group (Stata version 10.1 for Windows; Stata Corporation, College Station, Texas). The Shapiro-Wilk test and distribution plots were used to test the normality of distribution. The results are expressed as the mean ± SD for parametric variables and median plus interquartile range for nonparametric variables. The significant differences for normally distributed variables were compared by Student *t* test or repeated-measures analysis of variance, and the post-hoc Bonferroni test was applied. The significant differences for nonnormally distributed variables were compared using the Mann-Whitney *U* test. Categorical variables are expressed as numbers and frequencies (%). The Fisher exact test with Katz approximation was used to compare adverse event frequencies. The primary analysis, based on confirmed atrial fibrillation events, was by intention to treat, including all patients according to treatment assigned at randomization, independently of the duration of treatment and the follow-up period. Time-to-first-event analysis was calculated by the Kaplan-Meier method and compared using the log-rank test. Cox proportional hazards models with the Breslow method for ties were used to calculate the hazard ratio in the survival curves between the groups. The Spearman rank-order coefficient was estimated in correlation analysis. All reported p values are 2-sided. The difference between the groups was considered significant with a p value <0.05. Statistical analysis was performed using Stata version 10.1 (Stata Corporation).

Results

Primary outcomes. The study population consisted of 203 patients with comparable baseline characteristics (Table 1). POAF occurred in 10 of 103 patients (9.7%) of the supplemented group versus 32 of 100 patients (32%) of the placebo group (relative risk [RR]: 0.28; 95% CI: 0.14 to 0.56; $p < 0.001$). The Kaplan-Meier survival curves illustrate the occurrence of POAF (Fig. 2). The 30 patients (71.4%) who developed atrial fibrillation between the second and third post-operative days belonged mostly to the placebo group (24 of 30 patients [80%]; log-rank test $p < 0.001$). The mean time of POAF occurrence was 3.3 ± 0.7 days in patients assigned to the supplementation group and 2.9 ± 0.4 days in the placebo group. The placebo patients had 3.62 times more risk for POAF at any day compared with the supplemented patients (hazard ratio: 3.62; 95% CI: 1.78 to 7.36; $p < 0.001$). The number needed to treat to prevent one atrial fibrillation event was 4.7 patients (95% CI: 3.3 to 11.4). POAF was diagnosed in

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Table 1 Baseline Characteristics of Enrolled Patients

Characteristic	Placebo (n = 100)	Supplemented (n = 103)	p Value
Age, yrs	58.5 (56.6–61.0)	61 (58.0–62.0)	0.438
Male	88 (88.0)	85 (82.5)	0.272
Body mass index, kg/m^2	24.8 (23.9–25.6)	25.4 (24.6–26.2)	0.439
Pre-operative heart rate on resting, beats/min	73 (70–74)	75 (73–77)	0.428
Post-operative heart rate (day +1), beats/min	63 (59–65)	61 (58–63)	0.595
Pre-operative systolic blood pressure, mm Hg	127 (124–131)	131 (127–134)	0.567
Pre-operative diastolic blood pressure, mm Hg	78 (75–81)	74 (71–79)	0.775
Left ventricular ejection fraction, %	55 (55–59)	55 (55–60)	0.474
Left atrial AP dimension, mm	39.6 (37.1–41.0)	40 (39.2–43.1)	0.454
Comorbidities			
Systemic hypertension	29 (29.0)	32 (31.0)	0.748
Diabetes mellitus	57 (57.0)	62 (60.2)	0.644
Hypercholesterolemia	45 (45.0)	47 (45.6)	0.928
Smoking history	36 (36.0)	39 (37.9)	0.783
Chronic obstructive pulmonary disease	11 (11.0)	13 (12.6)	0.830
NYHA functional class I	45 (45.0)	48 (46.6)	0.901
NYHA functional class II	17 (17.0)	12 (11.6)	0.428
Coronary artery disease			
1 compromised vessel	5 (5.0)	6 (5.8)	0.978
2 compromised vessels	26 (26.0)	24 (23.3)	0.754
3 compromised vessels	33 (33.0)	40 (38.8)	0.587
4 compromised vessels	3 (3.0)	4 (3.9)	0.521
Valvular dysfunction			
Mitral stenosis	25 (25.0)	24 (23.3)	0.874
Mitral regurgitation	14 (14.0)	12 (11.7)	0.682
Aortic stenosis	8 (8.0)	7 (6.8)	0.795
Other valvulopathy	5 (5.0)	5 (4.9)	0.618
Perioperative features			
Revascularization	48 (48.0)	55 (53.4)	0.484
Valve replacement	33 (33.0)	29 (28.2)	0.542
Mixed etiology	19 (19.0)	19 (18.4)	1.000
Cross-clamp time, min	79 (75.0–95.0)	78 (71.1–85.0)	0.571
CPB time, min	92 (87.0–97.0)	89 (87.0–94.9)	0.515
Pharmacological treatment			
Aspirin	69 (69.0)	68 (66.0)	0.650
Atorvastatin	37 (37.0)	37 (35.9)	0.873
Other statins	11 (11.0)	6 (5.8)	0.169
ACE inhibitors	54 (54.0)	67 (65.0)	0.109
ARBs	21 (21.0)	19 (18.4)	0.647
Diuretics	25 (25.0)	28 (27.2)	0.723
Beta-blockers	64 (64.0)	61 (59.2)	0.484
Inhibitors of platelet aggregation	12 (12.0)	7 (6.8)	0.122
Calcium channel blockers	7 (7.0)	9 (8.7)	0.646
Nitrates	2 (2.0)	3 (2.9)	0.675
Insulin	3 (3.0)	2 (1.9)	0.627
Sulfonylurea	7 (7.0)	10 (9.7)	0.486
Biguanides	25 (25.0)	29 (28.2)	0.611

Values are median (interquartile range) or n (%).

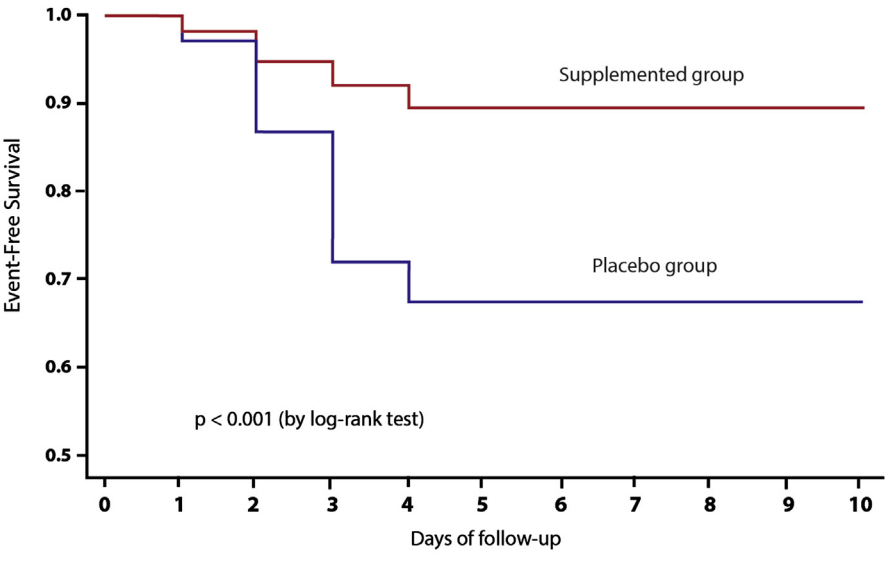
ACE = angiotensin-converting enzyme; AP = anteroposterior; ARB = angiotensin receptor blocker; CPB = cardiopulmonary bypass; NYHA = New York Heart Association.

the coronary or intensive care unit in 6 patients (60%) in the supplemented group and in 14 patients (43.7%) in the placebo group ($p = 0.758$). The remaining cases were detected by means of Holter monitoring (after discharge from the intensive care unit, a Holter monitoring device was placed until day 4) or by ECG recordings. The mean duration of POAF was 10.8 ± 0.7 h in patients assigned to supplementation and 11.2 ± 1.5 h in the placebo patients ($p = 0.06$). The length of stay in the intensive care unit and in the hospital was 2.87 ± 0.44 versus 3.08 ± 0.54 days ($p = 0.76$) and 8.77 ± 0.37 versus 9.57 ± 0.66 days ($p < 0.05$) for the supplemented versus placebo groups,

respectively. Spontaneous conversion to sinus rhythm without any intervention occurred in 3 supplemented patients and 4 placebo patients developing atrial fibrillation (Fisher exact test $p = 0.20$). Cardioversion with amiodarone was achieved in 5 supplemented and 25 placebo patients (Fisher exact test $p = 0.096$). Electrical cardioversion was successfully performed in 2 supplemented and 3 placebo patients (Fisher exact test $p = 0.340$). There were no significant differences in therapy drugs in both groups between pre-operative and post-operative data; however, beta-blocker dose showed an expectable trend of being increased in both groups, rising from 64% to 71% in the placebo group (Fisher exact test $p = 0.361$) and from 59.2% to 71.8% in the supplemented group (Fisher exact test $p = 0.438$). The frequency of use of beta-blockers in the post-operative period did not show a statistically significant difference between the groups (Fisher exact test $p = 0.521$).

Secondary outcomes. OXIDATIVE STRESS AND INFLAMMATION-RELATED BIOMARKERS. Oxidative stress biomarkers, including lipid peroxidation assessed as malondialdehyde (MDA) levels in blood throughout the protocol and atrial tissue on the day of surgery (time 0), are illustrated in Figures 3A and 3B, respectively. After 5 days of n-3 PUFA exposure, the MDA levels were 59.6% higher than baseline values ($p < 0.01$) and 45.6% higher than placebo values ($p < 0.01$). However, there was no significant difference in MDA levels at time 0 following the addition of antioxidant vitamins. Early after surgery (day +1), the placebo group exhibited 47.5% higher MDA levels than the supplemented group, with the levels being 3.7-fold and 3.5-fold greater than the baseline (day −7) and pre-operative (time 0) values, respectively ($p < 0.01$). The supplemented group presented 2.8-fold and 2.2-fold increased plasma levels of MDA compared with the baseline (day −7) and pre-operative (time 0) values, respectively ($p < 0.01$) (Fig. 3A). The supplemented group presented 26.1% lower MDA levels ($p < 0.01$) in atrial tissue on the day of surgery compared with the placebo group (Fig. 3B). The correlation between plasma and atrial tissue lipid peroxidation at the time of surgery is depicted in Figure 3C. Interestingly, these 2 variables demonstrated a strong direct correlation in both the placebo ($r = 0.96$; $p < 0.01$; $n = 50$) and supplemented ($r = 0.88$; $p < 0.01$; $n = 50$) groups, with no significant differences between the groups. In addition, patients who suffered POAF presented with significantly higher levels of atrial MDA (4.47 vs. 3.85 μmol/mg protein; $p < 0.01$) at the time of surgery, compared with patients who did not develop POAF.

Inflammation-related variables throughout the protocol are shown in Figures 3D and 3E.



Follow-up	day	0	1	2	3	4	5	6	7	8	9	10	TOTAL
At risk (n)	POAF	0	3	13	17	9	0	0	0	0	0	0	42
	No POAF	203	200	187	170	161	161	161	161	161	161	161	161

Figure 2 Freedom From Atrial Fibrillation

The Kaplan-Meier survival curve depicts the occurrence of post-operative atrial fibrillation following cardiac surgery in the placebo and supplemented groups. POAF = post-operative atrial fibrillation.

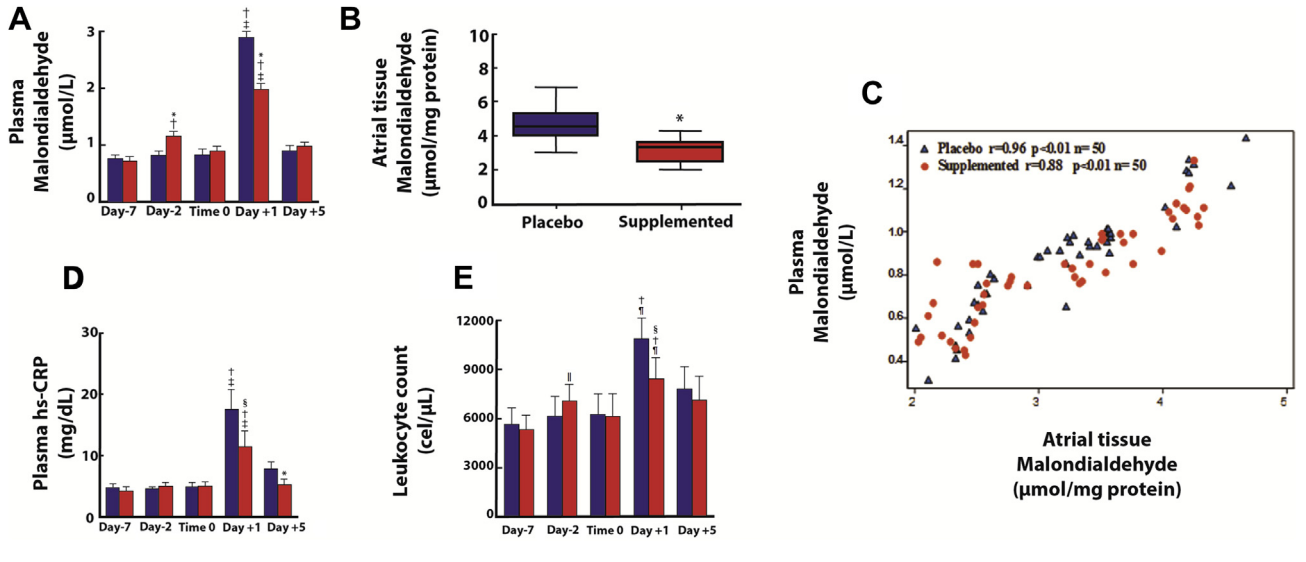


Figure 3 Oxidative Stress and Inflammation Biomarkers in Blood Throughout the Protocol and Lipid Peroxidation in Atrial Tissue on the Day of Surgery

(A) Oxidative stress in blood assessed by malondialdehyde (MDA) concentration (n = 50 for each group). **(B)** Oxidative stress in atrial tissue (time 0), assessed by MDA concentration (n = 50 for each group). **(C)** Scatter diagram shows the Spearman correlation of MDA concentration in plasma versus atrial tissue. **(D)** Plasma high-sensitivity C-reactive protein (hs-CRP) levels for supplemented and placebo patients (n = 50 for each group). **(E)** Blood leukocyte counts throughout the protocol for supplemented and placebo patients (n = 50 for each group). *p < 0.01 versus placebo. †p < 0.01 versus baseline. ‡p < 0.01 versus pre-operative. §p < 0.05 versus placebo. ‖p < 0.05 versus baseline. ¶p < 0.05 versus pre-operative. Day −7 = moment of starting n-3 polyunsaturated fatty acid supplementation or placebo. Day −2 = moment of starting the anti-oxidant vitamin supplementation or placebo. Time 0 = day of surgery. Day +1 = 6 to 8 h after surgery. Day +5 = post-operative fifth day.

Early after surgery (day +1), the placebo and supplemented groups presented higher serum high-sensitivity C-reactive protein levels, 3.6-fold and 2.2-fold greater than the pre-operative values ($p < 0.01$). However, the supplemented group exhibited values 35.4% lower than those of the placebo group ($p < 0.05$) (Fig. 3D). Following 5 days of n-3 PUFA treatment (day −2), the leukocyte count in the supplemented group was 32.5% higher than the baseline values ($p < 0.05$). Early after surgery (day +1), the placebo and supplemented groups presented a 73.8% and 36.2% increase in leukocyte count, respectively, compared with pre-operative values ($p < 0.05$). In addition, the leukocyte count in the supplemented group at this time was 22.2% lower than that in the placebo group ($p < 0.05$) (Fig. 3E).

CHARACTERIZATION OF ATRIAL TISSUE REDOX STATUS ON DAY OF SURGERY. The activity and mRNA expression of the antioxidant enzymes CAT, superoxide dismutase (SOD), and GSH-Px in the atrial tissue of supplemented and placebo patients on the day of surgery are presented in Figures 4A, 4B, and 4C. The activity of antioxidant enzymes CAT, SOD, and GSH-Px in the atrial tissue of the supplemented patients was 24.0%, 17.1%, and 19.7% higher than the respective placebo values ($p < 0.05$). Accordingly, the assessment of enzyme gene expression levels in the supplemented group indicated 52.9%, 42.5%, and 34.5% higher levels, respectively, compared with the placebo group ($p < 0.01$).

The p47-phox NADPH oxidase subunit protein and mRNA expression in atrial tissue are shown in Figures 4D and 4E. Patients with POAF presented 115.5% higher protein expression and 65.8% higher mRNA levels (Fig. 4D) of this subunit compared with patients in sinus rhythm ($p < 0.05$). In addition, the supplemented group presented protein expression and mRNA levels 41.3% and 36.4% lower than the respective placebo group values ($p < 0.05$) (Fig. 4E).

Further results concerning plasma oxidative stress–related biomarkers, erythrocyte thiol index, and atrial tissue xanthine oxidase (XO) activity plus nuclear factor kappaB (NF-kappaB) Western blot and electrophoretic mobility shift assay analyses are shown in Online Figures 1 and 2, respectively. A detailed explanation of these data is also presented in the Online Appendix.

Adverse events. Adverse events occurred in 7 supplemented and 7 placebo patients, with no significant differences as determined by Fisher exact test (RR: 0.97; 95% CI: 0.35 to 2.67; $p = 0.586$). The most frequent events were dyspepsia (4 vs. 3 events; RR: 1.58; 95% CI: 0.34 to 6.76; $p = 0.71$) and diarrhea (3 vs. 4 events; RR: 0.44; 95% CI: 0.21 to 2.73; $p = 0.47$). One patient of the supplemented group withdrew consent. No hemorrhagic or major adverse cardiovascular events were observed during the hospital follow-up. No patients died during the hospital follow-up; however, during the following 6 months after discharge, 8 patients died (5 supplemented vs. 3 placebo;

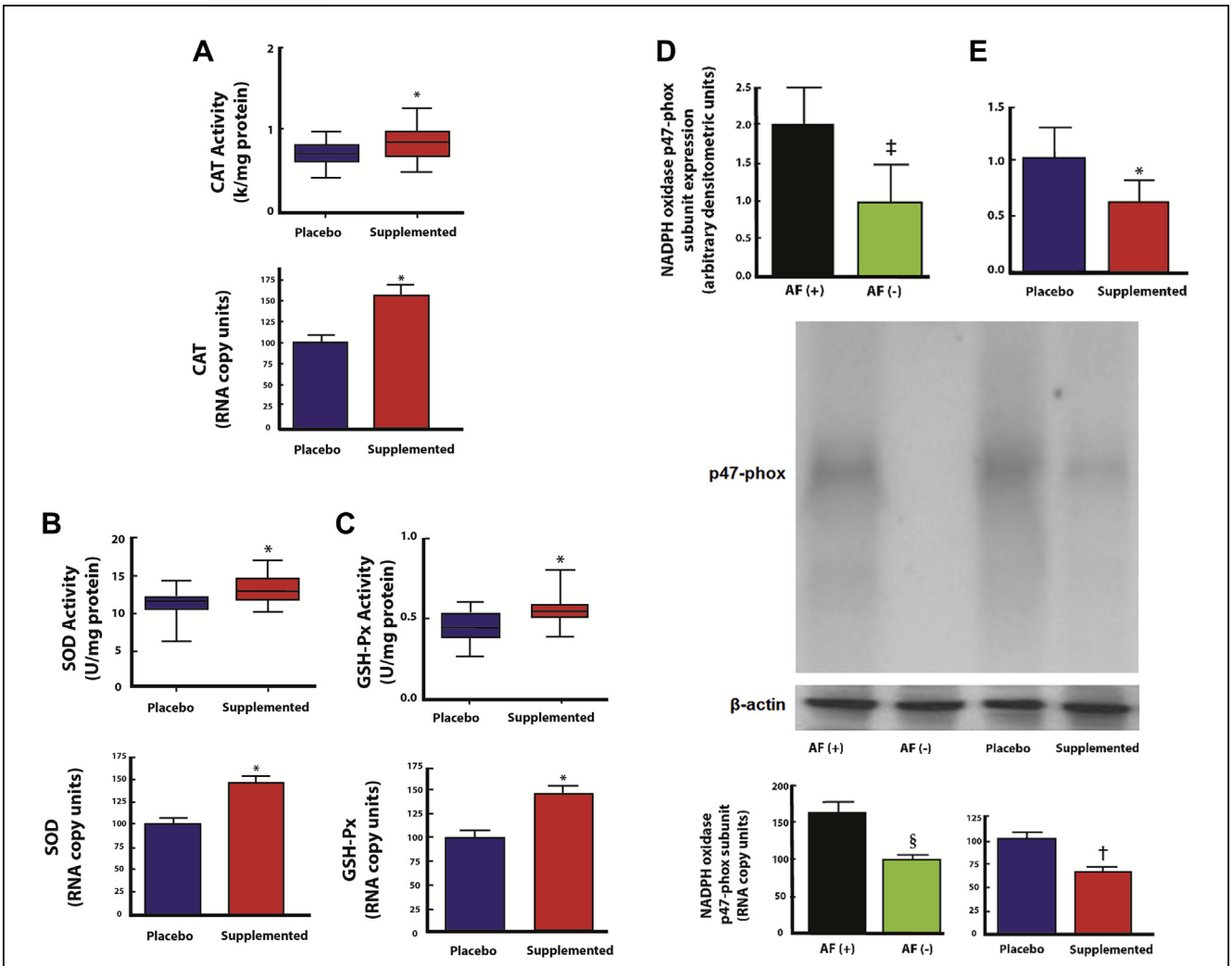


Figure 4 **Characterization of Atrial Tissue Antioxidant Enzymes (CAT, SOD, and GSH-Px) in Supplemented and Placebo Groups and Pro-Oxidant Enzymes (NADPH Oxidase) Profile on the Day of Surgery (Time 0)**

Comparison between supplemented versus placebo plus atrial fibrillation versus sinus rhythm patients. **(A)** Catalase (CAT) enzyme activity and mRNA expression (n = 50 for each group). **(B)** Superoxide dismutase (SOD) enzyme activity and mRNA expression (n = 50 for each group). **(C)** Glutathione peroxidase (GSH-Px) enzyme activity and mRNA expression (n = 50 for each group). **(D)** Association between the occurrence of atrial fibrillation and NADPH oxidase p47-phox subunit protein and mRNA expression (n = 6 for each group). **(E)** NADPH p47-phox subunit protein and mRNA expression in supplemented versus placebo groups (n = 12 for each group). *$p < 0.05$ versus placebo. †$p < 0.01$ versus placebo. ‡$p < 0.05$ versus sinus rhythm. §$p < 0.01$ versus atrial fibrillation. AF(+) = atrial fibrillation; AF(−) = sinus rhythm.

RR: 1.63; 95% CI: 0.40 to 6.65; p = 0.37), representing a mortality rate of 3.94% of the randomized patients.

Discussion

This study demonstrated that a novel strategy of antioxidant reinforcement was effective against POAF, as previously hypothesized (4,6). The combination of indirect antioxidant effects of n-3 PUFAs plus direct antioxidant effects of vitamins C and E decreased the vulnerability of atrial tissue to develop arrhythmia, showing a noteworthy 66% reduction. Through assessment of oxidative stress and inflammation biomarkers, we determined the contribution of these processes throughout the study protocol. The increased ROS status induced by n-3 PUFAs was confirmed on day −2 in the supplemented group by the elevation of plasma MDA levels (Fig. 3A), as well as the reduction of either ferric-reducing ability of plasma or erythrocyte thiol index (Online Figs. 1A and 1B, respectively). Early after surgery, lipid peroxidation (Fig. 3A) and inflammation biomarker levels (Figs. 3D and 3E) increased significantly in both groups but less markedly in the supplemented group.

Interestingly, this response could be due to increased ROS depuration and scavenging, as well as decreased ROS production. The molecular bases supporting this view could be related to the metabolic status of atrial tissue. The supplemented group showed up-regulation of CAT, SOD, and GSH-Px activity and mRNA expression, accounting for an

improvement in the ability of atrial tissue to depurate ROS, an effect attributable to n-3 PUFA exposure. In addition, the finding of decreased protein and mRNA expression of the NADPH oxidase p47-phox subunit in the supplemented group (Fig. 4E) might be due to down-regulation of this enzyme activity following vitamin C and E exposure, consistent with studies of the aorta in a rat model (32). Remarkably, the higher expression of this enzyme subunit in patients developing atrial fibrillation (Fig. 4D) strongly supports a pathogenetic role of oxidative stress, as reported in experimental models (33). Previous trials demonstrated that patients experiencing POAF exhibit higher pre-operative NADPH oxidase activity (13). Although a recent study failed to demonstrate a beneficial effect of vitamin C supplementation on POAF (34), it should be stressed that vitamin C could enhance its antioxidant potential administered together with vitamin E because both act synergistically (5,20,35). We found detectable atrial XO activity, another enzyme contributing to superoxide production (12), that was not significantly different between supplemented and placebo groups but was higher in patients with POAF compared with those in sinus rhythm (Online Figs. 1C and 1D). Enhanced XO activity should be expected following ischemia-reperfusion, but we determined this activity only at the onset of the surgical procedure.

Concerning n-3 PUFA supplementation, all randomized placebo-controlled studies that failed to demonstrate a beneficial effect used a formulation containing 1.24 EPA:DHA ratio (25–28). In contrast, data reported here were obtained with an EPA:DHA ratio equal to 0.5. Clinical trials performed with this same EPA:DHA ratio also reported a beneficial effect in POAF prevention (22–24), although of a lesser magnitude. This difference could be due to the additional effect of vitamins C and E in our study. In support of the present data, a meta-regression analysis showed a trend toward a benefit from n-3 PUFA supplementation when the EPA:DHA ratio was 0.5 (36). More recently, a study including all published controlled trials concluded that n-3 PUFA therapy decreased the risk of developing POAF in post-surgical cardiac patients (37).

The molecular mechanism accounting for the effectiveness of this EPA:DHA ratio remains to be determined, but it could be related to the kinetics of incorporation of these fatty acids into cell membranes. A previous incorporation study in human atrial tissue indicated a more rapid incorporation for DHA than EPA, reaching the DHA half-maximal incorporation during the first week of fish oil supplementation (38). Notably, our study reported a greater preventive effect against POAF compared with other previous attempts for this purpose. Possibly these results arose from the pleiotropic effects of n-3 PUFAs administered in an optimal 1:2 EPA:DHA ratio plus the direct antioxidant effects of vitamins C and E. Furthermore, our data support the role of oxidation and inflammation in the pathophysiology of POAF.

Study limitations. The study population showed a male predominance, with younger patients compared with other related studies. We did not assess n-3 PUFA pleiotropic effects other than those related to oxidative stress and inflammation. Therefore, we cannot rule out that additional antiarrhythmic mechanisms are operative in this setting. Given that continuous monitoring was performed only until the fourth post-operative day, some asymptomatic arrhythmia episodes after that day may have been undetected.

Conclusions

This study presented a strategy that successfully reduced the occurrence of POAF based on an indirect antioxidant effect of n-3 PUFAs (1:2 EPA:DHA ratio) plus a direct antioxidant effect of vitamins C and E. Oxidative stress amelioration was accompanied by a reduction in the vulnerability of myocardial tissue to the oxidative challenge known to occur during ischemia-reperfusion of this clinical model. This short-term, safe, low-cost treatment containing readily available substances represents a cost-effective strategy that could improve the outcome of patients who undergo on-pump cardiac surgery, as well as other surgeries, involving atrial fibrillation risk, thereby reducing major cardiovascular adverse events, the length of hospital stay, and the overall cost.

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Key Words: antioxidants ■ inflammation ■ n-3 fatty acids ■ oxidative stress ■ post-operative atrial fibrillation ■ vitamin C ■ vitamin E.

APPENDIX

For expanded Methods and Discussion sections and supplemental figures, please see the online version of this article.