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Omega-3 Polyunsaturated Fatty Acids Prevent Atrial Fibrillation Associated With Heart Failure but Not Atrial Tachycardia Remodeling

Masao Sakabe, MD, PhD; Akiko Shiroshita-Takeshita, MD, PhD; Ange Maguy, PhD; Chloe Dumesnil, BSc; Anil Nigam, MD; Tack-Ki Leung, MD; Stanley Nattel, MD

Background—There is epidemiological evidence that omega-3 polyunsaturated fatty acids (PUFAs) reduce the risk of atrial fibrillation (AF), but clinical data are conflicting. The present study assessed the effects of PUFA on AF in experimental models.

Methods and Results—We studied the effects of oral PUFA supplements in 2 experimental AF paradigms: electrical remodeling induced by atrial tachypacing (400 bpm for 1 week) and congestive heart failure–associated structural remodeling induced by ventricular tachypacing (240 bpm for 2 weeks). PUFA pretreatment did not directly change atrial effective refractory period (128 ± 6 [mean \pm SEM] versus 127 ± 2 ms; all effective refractory periods at 300-ms cycle lengths) or burst pacing–induced AF duration (5 ± 4 versus 34 ± 18 seconds). Atrial tachypacing dogs had shorter refractory periods (73 ± 6 ms) and greater AF duration (1185 ± 300 seconds) than shams (119 ± 5 ms and 20 ± 11 seconds; $P < 0.01$ for each). PUFAs did not significantly alter atrial tachypacing effects on refractory periods (77 ± 8 ms) or AF duration (1128 ± 412 seconds). PUFAs suppressed ventricular tachypacing–induced increases in AF duration (952 ± 221 versus 318 ± 249 seconds; $P < 0.05$) and attenuated congestive heart failure–related atrial fibrosis (from $19.2 \pm 1.1\%$ to $5.8 \pm 1.0\%$; $P < 0.001$) and conduction abnormalities. PUFAs also attenuated ventricular tachypacing–induced hemodynamic dysfunction (eg, left ventricular end-diastolic and left atrial pressure from 12.2 ± 0.5 and 11.4 ± 0.6 mm Hg, respectively, to 6.4 ± 0.5 and 7.0 ± 0.8 mm Hg; $P < 0.01$) and phosphorylation of mitogen-activated protein kinases (extracellular-signal related and P38 kinase).

Conclusions—PUFAs suppress congestive heart failure–induced atrial structural remodeling and AF promotion but do not affect atrial tachycardia–induced electrical remodeling. The beneficial effects of PUFAs on structural remodeling, possibly related to prevention of mitogen-activated protein kinase activation, may contribute to their clinical anti-AF potential. (*Circulation*. 2007;116:2101-2109.)

Key Words: arrhythmia ■ atrium ■ fatty acids ■ heart failure

Atrial fibrillation (AF), the most frequent sustained arrhythmia in clinical practice, has the potential for serious medical consequences.^{1,2} However, the utility of conventional antiarrhythmic agents is limited by inefficacy and side effects, especially proarrhythmia at the ventricular level. Recent advances in our understanding of atrial remodeling provide the possibility of novel therapeutic approaches targeting the atrial substrates that permit AF maintenance.¹

Clinical Perspective p 2109

Increased fish consumption is associated with reduced risks of cardiovascular death^{3–5} and possibly ventricular tachyarrhythmia.^{6,7} Epidemiological studies initially suggested benefit from fish oils in AF prevention,⁸ but subsequent analyses have not been confirmatory.^{9,10} The active element in fish oils is

largely believed to be omega-3 polyunsaturated fatty acids (PUFAs). Acutely administered PUFAs have a number of effects on cardiac ion channels and transporters, which may differ in important ways from chronically administered PUFAs that are gradually incorporated into the cell membrane.¹¹ PUFAs may have complex effects that are proarrhythmic, antiarrhythmic, or neutral, depending on the mode of administration and arrhythmia mechanism.^{11,12} Acutely administered PUFAs have been reported to reduce stretch-induced susceptibility to AF in isolated rabbit hearts¹³ and to suppress short-term (several-hour) atrial tachycardia remodeling in dogs.¹⁴ However, no published information is available on sustained oral PUFA administration, as occurs with dietary fish oil, on animal models of clinically relevant AF substrates like those induced by longer-term atrial tachypacing (ATP) and ventricular tachypacing (VTP)–induced

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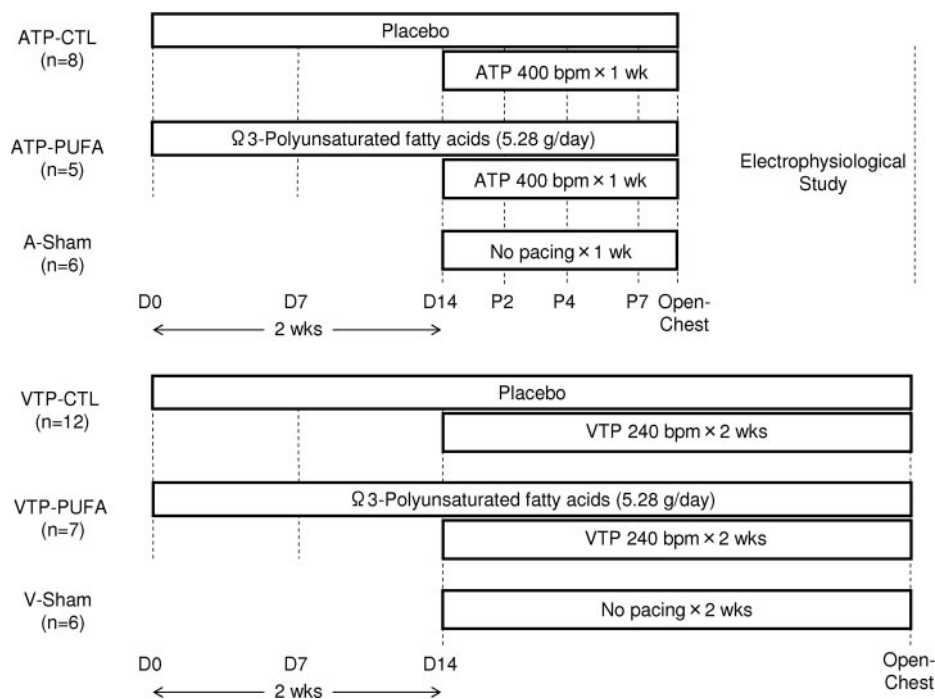


Figure 1. Schematic of groups and interventions. D0, D7, and D14 indicate days 0, 7, and 14 of drug administration before tachypacing; P2, P4, and P7, days 2, 4, and 7 of ATP; and vertical dashed lines, electrophysiological study days.

congestive heart failure (CHF).¹⁵ We therefore undertook the present study to evaluate the effects of sustained oral PUFA administration in the ATP and VTP canine models.

Methods

Animal Models and Experimental Groups

Animal handling procedures were approved by the local Animal Research Ethics Committee following National Institutes of Health guidelines. Forty-four mongrel dogs (weight, 20 to 37.5 kg) were divided into 6 groups (Figure 1): (1) ATP-PUFA dogs subjected to 7 days of ATP (400 bpm with atrioventricular block and ventricular demand pacing at 80 bpm to control ventricular rate) during oral treatment with 5.28 g/d omega-3 fatty acids/docosahexaenoic acid and eicosapentaenoic acid in a 1:1 ratio beginning 2 weeks before ATP onset and continued through the tachypacing period (n=5); (2) ATP-control (CTL) dogs subjected to 7 days of ATP during oral treatment with placebo (n=8); (3) A-Sham dogs, sham-operated dogs undergoing the same instrumentation as ATP-PUFA and ATP-CTL dogs but without tachypacing (n=6); (4) VTP-PUFA dogs that underwent 2 weeks of VTP (240 bpm) and were treated with PUFAs (5.28 g/d) beginning 2 weeks before VTP onset and continuing throughout VTP (n=7); (5) VTP-CTL dogs subjected to 2 weeks of VTP during oral treatment with placebo (n=12); and (6) V-Sham dogs, dogs with the same instrumentation as VTP-PUFA and VTP-CTL dogs but without tachypacing (n=6). Investigators were blinded to treatment assignment until analyses had been performed.

Dogs were subjected to ATP and VTP as previously described.¹⁶ In ATP dogs, unipolar pacing leads were inserted into the right ventricular apex and right atrial (RA) appendage under fluoroscopic guidance. The leads were connected to a ventricular-demand pacemaker (Medtronic, Minneapolis, Minn), and a custom-modified atrial tachypacemaker was implanted in the neck. A bipolar electrode was inserted into the RA appendage for stimulation and recording during serial electrophysiological study. AV block was created by radiofrequency ablation. The right ventricular pacemaker was programmed to 80 bpm. In VTP dogs, a unipolar lead was inserted into the right ventricular apex and connected to a custom-modified

ventricular tachypacemaker in the neck. A bipolar electrode was inserted into the RA appendage for electrophysiological study.

Study Protocols

After a 24-hour recovery period, a baseline closed-chest electrophysiological study was performed under ketamine (5.3 mg/kg IV)/diazepam (0.25 mg/kg IV)/isoflurane (1.5%) anesthesia. Dietary interventions were then started and continued throughout the protocol in all groups except sham-operated dogs.

In ATP study dogs, closed-chest electrophysiological studies were repeated at 7 and 14 days of the pretachypacing period and then after 2, 4, and 7 days of ATP. A final open-chest electrophysiological study was performed on day 8 under morphine (2 mg/kg SC)/ α -chloralose (120 mg/kg IV), followed by 29.25 mg \cdot kg⁻¹ \cdot h⁻¹ anesthesia. In VTP dogs, closed-chest electrophysiological studies were performed during the pre-VTP period and after 14 days of VTP. Open-chest electrophysiological studies were performed on day 15.

Atrial effective refractory period (ERP) was measured with 10 basic (S1) stimuli, and the mean of 3 ERP determinations at each basic cycle length was used. AF was induced by burst pacing (10 Hz, 4 times threshold, 2-ms stimuli, 1 to 10 seconds). To estimate mean AF duration in each dog, AF was induced 10 times for AF \leq 20 minutes and 5 times for 20 to 30 minutes of AF. AF \geq 30 minutes was considered sustained and was cardioverted with synchronized DC shock. A 30-minute rest period was then allowed before experimentation was resumed. After 2 cardioversions in a given dog, no further AF inductions were performed.

On open-chest study days, dogs were anesthetized and ventilated mechanically. Body temperature was maintained at 37°C. A femoral artery and both femoral veins were cannulated for pressure monitoring, fluid infusion, and drug administration. A median sternotomy was performed, and bipolar electrodes were hooked into the RA and left atrial (LA) appendages for recording and stimulation. Five silicon sheets containing 240 bipolar electrodes were sutured onto the atrial surfaces as previously described.¹⁶ Electrophysiological mapping was conducted with the Cardiomap system (Research Center, Sacré-Coeur Hospital and Biomedical Engineering Institute, École Polytechnique and Université de Montréal, Montreal, Quebec, Canada). After completion of open-chest studies, atrial tissue samples were removed from several RA and

Table. Group Characteristics and Hemodynamic Data

	A-Sham (n=6)	ATP-Control (n=8)	ATP-PUFA (n=5)	V-Sham (n=6)	VTP-Control (n=12)	VTP-PUFA (n=7)
Body weight, kg						
Before pacing	29.2±0.7	29.6±0.1	27.7±1.1	31.3±1.9	27.2±1.1	28.3±1.9
After pacing	...	29.2±0.2	28.1±1.1	...	28.5±1.4	30.2±2.0
Pressures, mm Hg						
Systolic	116±3	116±6	117±3	111±13	84±3*	98±8
Diastolic	65±2	55±3	61±3	60±10	48±3	58±6
LVEDP	5.8±0.4	5.7±0.4	3.8±0.3	5.5±0.6	12.2±0.5†	6.4±0.5‡
LAP	4.8±0.5	5.3±0.4	4.0±0.3	5.3±0.5	11.4±0.6†	7.0±0.8‡
RAP	4.7±0.5	5.0±0.4	3.0±0.3	5.5±0.5	9.6±0.6†	5.3±0.6‡

LVEDP indicates left ventricular end-diastolic pressure; LAP, mean LA pressure; and RAP, mean RA pressure.

* $P < 0.05$, † $P < 0.01$ vs V-sham; ‡ $P < 0.01$ vs VTP-CTL.

LA zones and divided. Samples from each area were immersed in 10% neutral-buffered formalin for 24 hours for subsequent embedding and staining, and other samples were snap-frozen in liquid N₂ at -80°C for biochemical analysis.

Histology

From each tissue zone, blocks were sectioned along longitudinal and transverse planes. Sections (5- μ m thickness) were cut at room temperature and stained with Masson's trichrome. Microscopic images were digitized (Scion Image Software, Frederick, Md) and analyzed with Sigmascan 4.0 (Jandel, Leighton Buzzard, UK). Connective tissue content was quantified as percent surface area, excluding blood vessels.

Western Blot Analyses of Mitogen-Activated Protein Kinases

Quick-frozen LAs of VTP-sham, VTP-CTL, and VTP-PUFA dogs were pulverized in liquid N₂ and resuspended in chilled protein extraction buffer [25 mmol/L Tris-HCl, 150 mmol/L NaCl, 10% glycerol, 5 mmol/L MgCl₂, 1 mmol/L EGTA, 1 mmol/L Na₃VO₄, 25 mmol/L NaF, 10 μ g/mL leupeptin, 10 mmol/L benzamide, 1 μ mol/L microcystin LR, 0.1 mmol/L 4-(2-aminoethyl)-benzenesulfonyl-fluoride hydrochloride, 10 mmol/L dithiothreitol, and 1 mL/20 mg powder Triton-X 100 1%]. The suspension was homogenized and incubated on ice (30 minutes). Samples were then centrifuged (10 minutes at 3000 rpm and 4°C). The supernatant was collected. The pellet was resuspended, homogenized, and centrifuged (10 minutes at 3000 rpm and 4°C). This supernatant was collected and pooled with the first. This step was repeated 3 times to optimize protein extraction. Pooled supernatants were centrifuged (10 minutes at 14 000 rpm and 4°C). Protein concentrations were quantified by Bradford assay (Bio-Rad Laboratories, Hercules, Calif). Proteins were separated with SDS-PAGE by loading 50- μ g protein samples on 10% polyacrylamide gels and transferring them onto nitrocellulose membranes. Primary antibodies were mouse anti-phospho-P38-mitogen-activated protein (MAP) kinase, anti-phospho P44/42-MAP kinase and anti-phospho-c-Jun-N-terminal kinase, mouse anti-P38 MAP kinase, anti-P44/42 MAP kinase, and anti-JNK (Cell Signaling Technology, Danvers, Mass; 1/2000 dilution, overnight incubation). Mouse anti-GAPDH antibody was used at 1/10 000 dilution (1-hour incubation, room temperature). Horseradish peroxidase-conjugated anti-mouse IgG (Santa Cruz Biotechnology, Inc, Santa Cruz, Calif), the secondary antibody, was revealed with chemiluminescence. Quantification was achieved with Quantity-One software (Bio-Rad). All expression data are provided relative to GAPDH bands for the same samples on the same gels.

Data Analysis

Phase-delay analysis was performed in VTP-CTL, VTP-PUFA, and V-sham dogs to evaluate local conduction abnormalities, as previously reported.¹⁶ The phase-delay range (P₅₋₉₅) represents the differ-

ence between fastest- and slowest-conducting zones. The variation coefficient (P₅₋₉₅/P₅₀) is a conduction heterogeneity index independent of conduction velocity. Atrial ERPs were measured at multiple basic cycle lengths in RA and LA appendages and at a basic cycle length of 300 ms at 6 additional sites: RA and LA posterior walls, RA and LA inferior walls, and RA and LA Bachmann's bundles. AF vulnerability was the percentage of sites at which AF (>1 second) was inducible with single extrastimuli.

All results are expressed as mean±SEM. Multiple-group comparisons were obtained with 1-way ANOVA or repeated-measures ANOVA as appropriate. All data satisfied statistical criteria for normal distribution except for AF duration, which satisfied normal distribution criteria after logarithmic transformation and were so analyzed. When 1-way ANOVAs revealed significant effects, Bonferroni-adjusted pairwise comparisons were performed by multiplying pairwise probability values by 3. For repeated-measures ANOVAs, Bonferroni-corrected probability values were computed as follows. First, if group effects were statistically significant, pairwise comparisons were conducted between groups, and each probability value was multiplied by 3. Second, if the within-factor effect was statistically significant, pairwise comparisons between the levels of the within-factor effect were conducted, and each probability value was multiplied by the number of comparisons. Third, if the interaction between group and the within-factor effect was statistically significant, pairwise comparisons between groups were conducted at each level of the within-factor effect, and each probability value was multiplied by 3 times the number of levels of the within-factor effect. A 2-tailed value of $P < 0.05$ was considered statistically significant.

The authors had full access to and take responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Overall group characteristics and hemodynamic data at final open-chest study are presented in the Table. There were no significant hemodynamic differences among ATP groups. VTP-CTL dogs had greater left ventricular end-diastolic and atrial pressures and smaller systolic pressures than V-shams. PUFA-treated VTP dogs showed significant hemodynamic improvement compared with VTP-CTL dogs, with values no longer significantly different from V-sham dogs.

Direct Electrophysiological Effects of PUFAs

During the 14-day medication administration period before tachypacing, PUFAs did not change atrial ERPs (Figure 2A) or the duration of burst pacing-induced AF (Figure 2B). P-wave duration, an index of atrial conduction, was unaffected by PUFA therapy, averaging 39 ms at all time points.

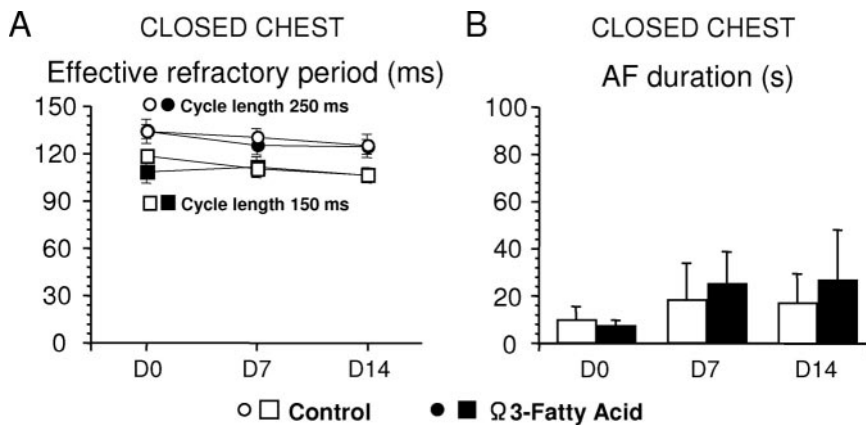


Figure 2. ERP (A) and mean duration of induced AF (B) at 0 (D0), 7 (D7), and 14 (D14) days of oral PUFA therapy before VTP. Open symbols indicate control dogs; solid symbols, PUFA dogs.

Effects of PUFAs on Atrial Tachycardia-Induced Remodeling

AF progression data for dogs subjected to ATP in the presence or absence of PUFAs are shown in Figure 3A. Mean AF duration increased progressively over time. There were no significant ATP response differences between PUFA and placebo groups. Changes in ERP during serial closed-chest studies in ATP study groups are shown in Figure 3B. ERPs shortened similarly with ATP and reached nearly steady-state values within 4 days in both groups.

Figure 4 shows differences in mean AF duration (Figure 4A), AF vulnerability (Figure 4B), regional atrial ERP values (Figure 4C), and conduction velocity (Figure 4D) at the final open-chest study. ATP-CTL dogs had significantly increased AF duration compared with A-sham dogs (Figure 4A). PUFAs did not alter the substrate for AF maintenance caused by ATP-induced remodeling; AF duration of ATP-PUFA dogs was not significantly different from ATP-CTL dogs. Persistent AF requiring cardioversion for termination was induced in 62.5% (5 of 8) of the ATP-CTL, 60% (3 of 5) of the ATP-PUFA, and 0% (0 of 7) of the A-sham dogs. Single atrial extrastimuli induced AF at about two thirds of the atrial sites in both the ATP-CTL and ATP-PUFA groups, substantially greater than in A-sham dogs ($13 \pm 3\%$; $P < 0.01$; Figure 4B). ERP shortening caused by ATP was regionally variable (Figure 4C), with similar patterns for ATP-CTL and ATP-PUFA. ATP did not alter conduction

velocity (Figure 4D) and did not alter atrial fibrous tissue content (Figure 5A through 5C).

Effects of PUFAs on CHF-Associated Structural Remodeling

In V-sham dogs (Figure 5D), the atria were grossly normal and showed small amounts of interstitial fibrous tissue. In VTP-CTL dogs (Figure 5E), there was extensive interstitial fibrosis. In VTP-PUFA dogs (Figure 5F), VTP-induced fibrosis was strongly attenuated to a level not significantly different from V-sham. Figure 5G shows the overall mean percentage of fibrous tissue in the atria for each group. Fibrous tissue was increased ≈ 6 -fold in VTP-CTL dogs, a response attenuated by PUFA therapy.

Figures 6 and 7 provide mean data regarding electrophysiological properties of VTP dogs at open-chest study. Mean AF duration was 33 ± 10 seconds in V-sham dogs (Figure 6A) versus 952 ± 221 seconds in VTP-CTL dogs ($P < 0.01$), with sustained AF in 0 of 5 V-sham dogs (0%) and 5 of 12 of VTP-CTL dogs (42%). PUFA therapy attenuated AF promotion induced by VTP (AF duration, 318 ± 249 seconds; sustained AF in 1 of 7 dogs [14%]). As in our previous work,¹⁶ AF vulnerability was not significantly affected by VTP (with or without PUFA). There were no significant ERP differences between VTP-CTL and VTP-PUFA dogs (Figure 6B).

The results of phase-delay analyses are shown in Figure 7. The phase-delay range reflecting slow conduction zones (P_{5-95} ; Figure 7A) and the conduction heterogeneity index (P_{5-95}/P_{50} ; Figure 7B) were significantly increased in VTP-CTL dogs compared with V-sham dogs. PUFA treatment substantially attenuated the P_{5-95} and P_{5-95}/P_{50} changes induced by VTP, consistent with the histopathology results showing decreased fibrosis in PUFA-treated animals. Overall conduction velocity was not significantly altered by VTP (Figure 7C).

Effects on MAP Kinase Expression and Phosphorylation

Figure 8 illustrates the results of Western blot analysis of VTP effects on MAP kinase expression in the presence and absence of PUFA therapy. Figure 8A shows representative Western blot gels for ERK (left) and P38 (right). Figure 8B provides mean data for total (top) and phosphorylated (bottom) isoform band intensities. Both molecular mass isoforms (42 and 44 kDa, designated P42- and P44-kinase) of ERK

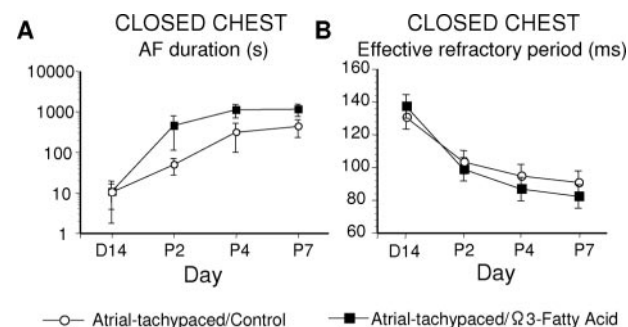


Figure 3. Time-dependent changes in mean AF duration (A) and ERP at a cycle length of 300 ms (B). Time, but not group, was a highly significant determinant of ERP and AF duration, with statistically significant alterations ($P < 0.01$) beginning by day 2 for both AF duration and ERP. D14 indicates day 14 of drug therapy before tachypacing; P2, P4, and P7, days 2, 4, and 7 after ATP onset.

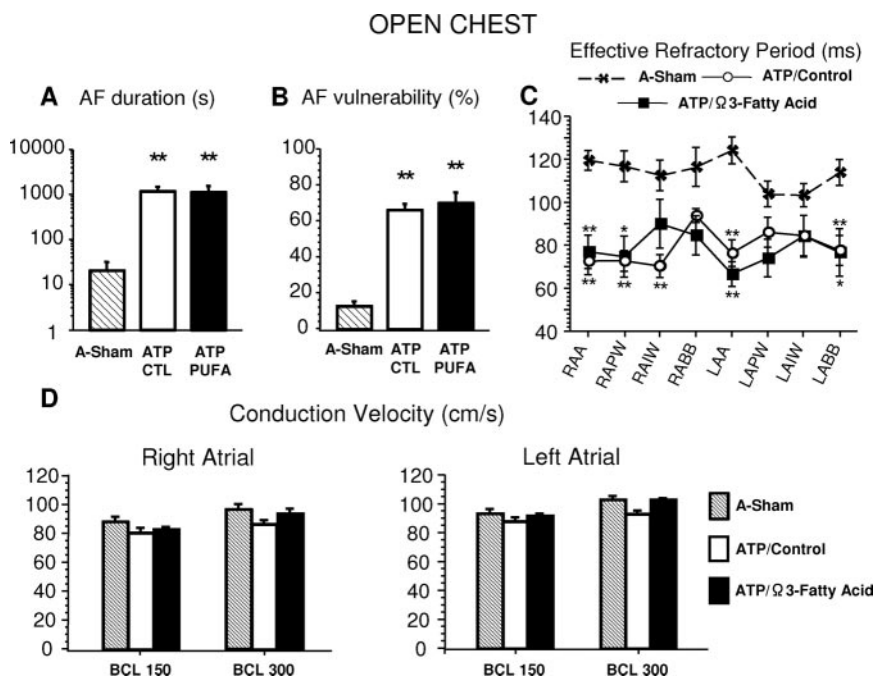


Figure 4. A, AF duration. B, Atrial vulnerability. C, ERPs at 300-ms cycle length. D, Conduction velocity. RAA indicates RA appendage; RAPW, RA posterior wall; RAIW, RA inferior wall; RABB, RA Bachmann’s bundle; LAA, left atrial appendage; LAPW, left atrial posterior wall; LAIW, left atrial inferior wall; LABB, left atrial Bachmann’s bundle; and BCL, basic cycle length. * $P < 0.05$, ** $P < 0.01$ vs A-sham.

were well detected by antibodies specific to phosphorylated ERK (Figure 8A, top left) and were detected by an antibody that reacts with both phosphorylated and nonphosphorylated ERK (total P44 and P42, middle left). VTP significantly increased the expression of both phosphorylated and total ERK in non-PUFA-treated dogs (black bars). In PUFA-treated VTP dogs, phosphorylated ERK expression was no longer significantly different from sham dogs for both isoforms (cross-hatched bars) and was significantly reduced compared with VTP-CTL for (p)-P44. VTP enhancement of total ERK was not significantly affected by PUFAs. P38 bands were clearly enhanced in VTP-CTL dogs (Figure 8A, right), associated with statistically significant increases (Figure 8B, right). PUFA ther-

apy significantly attenuated VTP enhancement of phosphorylated P38 without significantly changing total P38 expression relative to VTP-CTL. No statistically significant differences in JNK were seen between the VTP-CTL and VTP-PUFA groups (Figure I of the online-only Data Supplement).

Discussion

We have found that orally administered long-chain omega-3 PUFAs prevent CHF-induced atrial structural remodeling and AF promotion without altering the development of the AF substrate caused by atrial tachycardia remodeling and that PUFAs attenuate CHF-related phosphorylation of the MAP kinases ERK and P38.

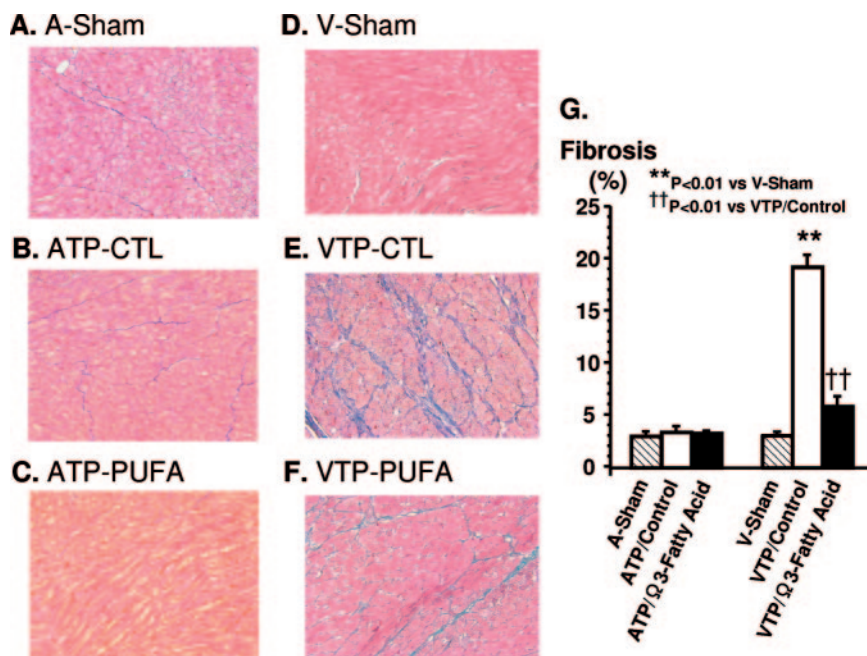


Figure 5. Masson’s trichrome-stained LA light micrographs ($\times 400$) from representative A-Sham (A), ATP-CTL (B), ATP-PUFA (C), V-Sham (D), VTP-CTL (E), and VTP-PUFA dogs (F). G, Mean \pm SEM percent connective tissue.

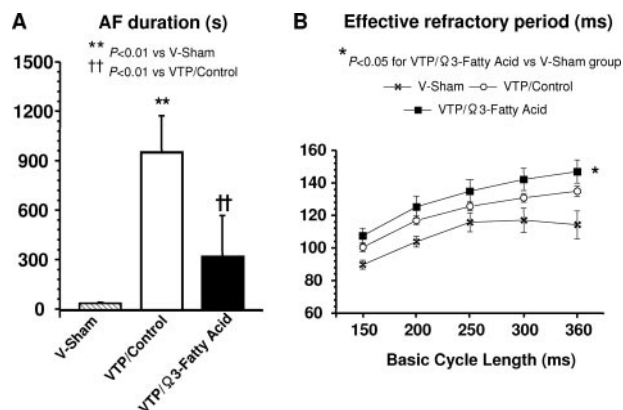


Figure 6. Electrophysiological properties at open-chest electrophysiology study in VTP study groups.

Comparison With Previous Literature Regarding PUFA Effects on Cardiac Electrophysiology and Arrhythmias

The ability of dietary lipids to influence ventricular arrhythmia occurrence in animal models has been recognized for >25 years.¹⁷ Epidemiological evidence for a beneficial effect of omega-3 PUFAs on sudden death rates in humans was first presented ≈20 years ago.⁷ A rich and complex literature has since developed regarding the effects of PUFA on cardiac electrophysiological function and on arrhythmias in experimental models (for an excellent recent review, see Reference 11). Many experimental studies have assessed the effects of acute administration of PUFAs in vivo or in vitro; however, it is clear that the results of sustained oral dietary PUFA ingestion (which causes a gradual but important increase in membrane PUFA content) may be quite different from the effects of acute PUFA exposure, which produces a much higher ratio of extracellular to membrane PUFA. For example, acute PUFA exposure decreases Na⁺, rapid delayed rectifier K⁺, and transient outward K⁺ currents while substantially altering cellular Ca²⁺ handling, whereas all of these are unaltered by dietary PUFA ingestion.¹¹ In conjunction with varying mechanisms of arrhythmias in different experimental models, these considerations may explain why the observed effects of PUFAs may be neutral or even proarrhythmic as well as antiarrhythmic.^{11,12}

Mozaffarian et al⁸ first reported that increased dietary consumption of fatty fish was associated with a lower incidence of new-onset AF in elderly individuals at higher risk of this condition. Subsequent epidemiological studies have failed to confirm this observation, possibly because of differences in population composition, high baseline fish consumption, or other confounding factors.^{9,10} A recent randomized, nonblinded study found a reduced incidence of post-coronary artery bypass graft AF in patients receiving PUFA capsules beginning at least 5 days before coronary artery bypass graft versus a routine-therapy control group.¹⁸

Dietary supplementation with fish oil had beneficial effects against AF promotion by atrial stretch in a Langendorff-perfused rabbit heart model, with PUFA-enriched hearts requiring a greater increase in atrial pressure to demonstrate the same degree of AF promotion compared with control hearts.¹³ PUFA admin-

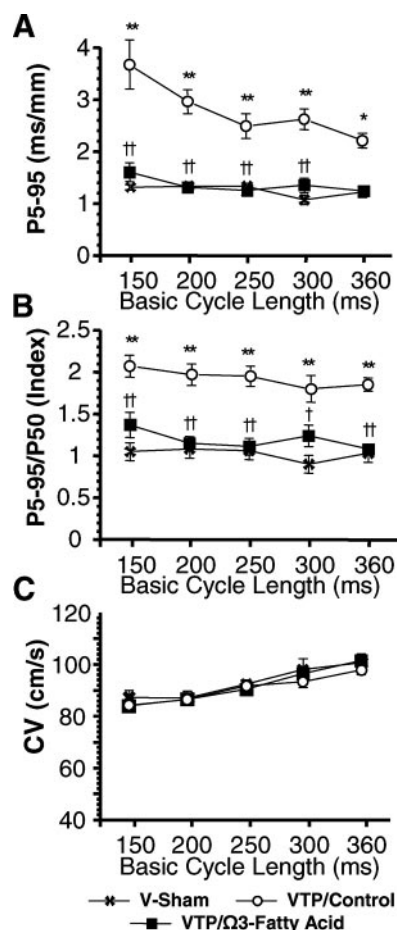


Figure 7. Mean ± SEM absolute heterogeneity (P₅₋₉₅; A), heterogeneity index (P₅₋₉₅/P₅₀; B), and conduction velocity (CV; C) in VTP series dogs. **P*<0.05, ***P*<0.01 vs V-Sham; †*P*<0.05, ††*P*<0.01 vs VTP-CTL.

istration did not alter ERP, consistent with our observation of a lack of significant direct electrophysiological effects on atrial electrophysiology. We cannot exclude the possibility that a reduced AF-promoting response to atrial stretch contributed to the AF-preventing effect of PUFAs in our CHF dogs. However, normalization of atrial volumes after recovery from VTP-induced CHF fails to prevent sustained AF,¹⁹ suggesting that the atrial stretch response is not an essential contributor to AF in this model and that the PUFA-induced prevention of atrial fibrosis and conduction abnormalities that we observed is a stronger candidate to explain AF suppression.

A recent study by da Cunha et al¹⁴ showed that acute intravenous administration of PUFAs prevented atrial electrical remodeling caused by several hours of ATP in dogs. Intravenous PUFAs did not directly affect ECG intervals, atrial ERP, or atrial conduction as indicated by P-wave duration. In contradistinction to the study by da Cunha et al, we were unable to show any beneficial effects of PUFAs on atrial tachycardia remodeling. This discrepancy is likely due to study design. We studied chronic oral administration of PUFAs, mimicking dietary PUFA intake that causes gradual PUFA incorporation into cardiac cell membranes,¹² whereas da Cunha et al studied PUFAs on acute intravenous administration. In addition, we studied the evolution of atrial

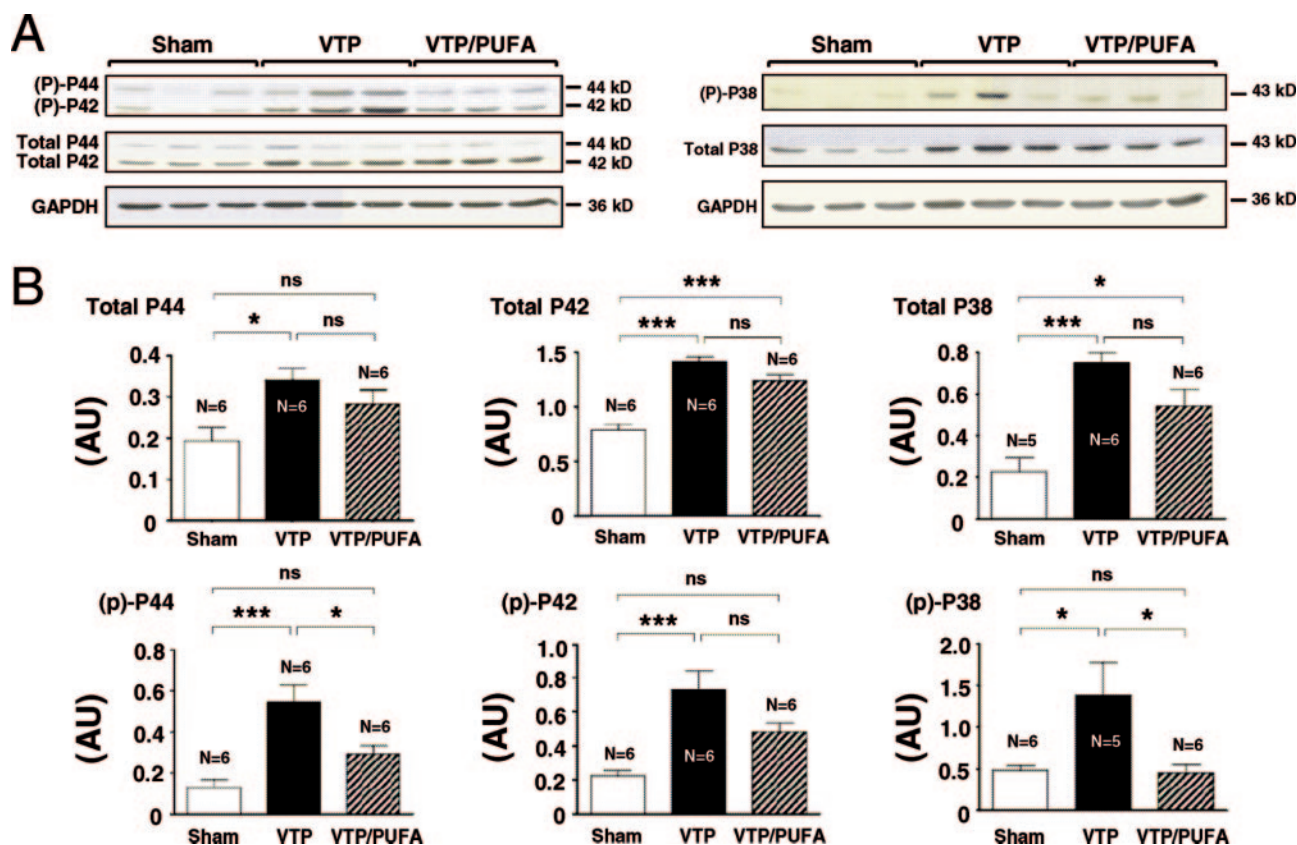


Figure 8. Western blot determination of MAP kinase expression and phosphorylation in V-Sham (Sham), VTP-CTL (VTP), and VTP-PUFA dogs. A, Left, Representative gel showing extracellular-signal related (ERK) bands from 3 dogs in each group. Both 42- and 44-kDa isoforms (P42 and P44) were detected by both the phosphospecific, designated (p)-P42 and (p)-P44, antibody and the antibody that detects both phosphorylated and nonphosphorylated ERK (total P42 and P44). Right, Representative gel showing P38 kinase bands with both phosphospecific [(p)-P38] and nonphosphospecific (total) antibodies from 3 dogs in each group. B, Mean \pm SEM band intensity of ERK and P-38 signals relative to GAPDH for each group indicated. AU indicates arbitrary units; N, number of dogs/analysis. * $P < 0.05$, ** $P < 0.001$ for comparison shown.

tachycardia remodeling over a prolonged period of tachycardia (between 2 and 7 days) in the closed-chest, unanesthetized state, whereas da Cunha et al limited their observations to several hours in anesthetized dogs. There are several other examples in which drugs were found to attenuate atrial tachycardia-induced short-term atrial ERP remodeling (several hours)^{20,21} but to be without effect on longer-term remodeling occurring over periods >24 hours.^{22,23}

Potential Mechanisms of Structural Remodeling Prevention by PUFAs

A number of studies have shown that PUFAs are capable of preventing disease-induced MAP kinase phosphorylation. The suppression of P38 phosphorylation may contribute to the effects of PUFA in ischemia-reperfusion models.^{24,25} MAP kinases play important roles in cardiac remodeling, with P38 kinase appearing to be particularly important in producing tissue fibrosis.²⁶ There is evidence for an important role of MAP kinases in AF patients.²⁷ MAP kinase phosphorylation evolves over time during CHF-related atrial remodeling.²⁸ Increased phosphorylation of ERK, JNK, and P38 kinase appears within 6 hours. JNK phosphorylation reverts toward normal levels after 24 hours, but enhanced ERK and P38 kinase phosphorylation persists for up to 5 weeks of VTP. Angiotensin-converting enzyme inhibition with enala-

pril attenuates VTP-induced structural remodeling and suppresses ERK phosphorylation.^{28,29} Although it is difficult to compare the results of different studies, PUFA-induced suppression of atrial fibrosis in the present trial seemed somewhat greater than that previously observed with enalapril,²⁹ perhaps because PUFAs suppressed the phosphorylation of both ERK and P38, in contrast to enalapril, which prevented only ERK phosphorylation.

In addition to the suppression of MAP kinase phosphorylation that we noted, PUFAs have a number of other reported actions that may favorably influence the progression of CHF and associated atrial structural remodeling. PUFAs reduce blood pressure in hypertensive rat models³⁰ and in clinical trials³¹; however, the lack of a blood pressure-lowering effect of PUFAs in both ATP and VTP dogs argues against a significant contribution of vasodilation in the present work, as does the lack of effect of a clearly effective vasodilatory drug combination (hydralazine/long-acting nitrate) on CHF-related atrial remodeling in a previous study.²⁹ Dietary PUFAs reduce the peripheral vascular resistance response to angiotensin³² and thus have angiotensin-antagonizing actions. Orally ingested PUFAs also have been reported to increase left ventricular ejection fraction by enhancing ventricular filling³³ and to reduce oxygen consumption.³⁴

Potential Significance

CHF is one of the most important clinical predisposing factors for AF, and AF is implicated in complications and impaired prognosis.^{35,36} In addition, structural remodeling is a common motif in clinical AF resulting from a variety of causes.³⁷ Because of the important limitations of currently available drug therapy approaches for AF, there is intense interest in new therapeutic targets, including agents that prevent the development of the AF substrate.³⁸ PUFAs are an attractive possibility because they are naturally occurring substances with minimal or no significant toxicity.

The observations in the present study provide some insights into the potential mechanisms by which PUFAs could influence the likelihood of AF. PUFAs did not directly alter atrial electrophysiology and failed to affect electrical remodeling caused by a week of ATP. These results suggest that PUFAs may not beneficially influence the “domestication of AF” by which repeated AF paroxysms lead to persistent AF,³⁹ the progressive resistance to antiarrhythmic drugs caused by atrial tachycardia remodeling,⁴⁰ or the enhanced vulnerability to AF recurrence that occurs after AF cardioversion.⁴¹ On the other hand, PUFAs attenuated CHF-associated atrial structural remodeling and AF promotion. Other agents with similar actions in experimental models include angiotensin-converting enzyme inhibitors,^{28,29} angiotensin receptor blockers,²³ and statins.⁴² Although the efficacy of these agents in preventing clinical AF has yet to be proved in ongoing large-scale, randomized, double-blind prospective trials, retrospective database analyses are encouraging, particularly in CHF populations.^{43,44} The potential value of PUFAs in AF occurring in structurally remodeled atria such as those of patients with significant left ventricular dysfunction also warrants further assessment.

Our observation of the differing effects of PUFAs on atrial tachycardia-related versus CHF-related remodeling is consistent with an emerging body of data indicating that the pharmacological response of different forms of AF can vary importantly.^{45,46} Differing effects of PUFAs in AF of different mechanisms may contribute to the variable results reported in previous epidemiological studies of the effects of PUFA.^{8–10} This notion underscores the need for careful planning and evaluation of clinical trials in AF with respect to the homogeneity of the target population.

Potential Study Limitations

We studied the effects on PUFAs on 1-week ATP. With longer periods of atrial tachycardia, additional factors come into play,⁴⁷ and the effects of PUFAs could be different. We induced CHF by VTP. Extrapolation to other causes of CHF and to pathophysiology in other species, including humans, should be done cautiously. PUFAs significantly improved left ventricular hemodynamic indexes in VTP dogs; thus, PUFA atrial protective effects may have been mediated indirectly by hemodynamic improvement. The left ventricular protective effects of PUFAs merit further consideration in future studies.

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Disclosures

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CLINICAL PERSPECTIVE

Atrial fibrillation (AF), an extremely common clinical arrhythmia that is challenging to treat, contributes to a variety of important sources of cardiovascular morbidity and mortality. There is currently great interest in novel “upstream” therapies that target the development of the vulnerable AF substrate. Clinical evidence regarding the effects of omega-3 polyunsaturated fatty acids (PUFAs) on AF is conflicting. The present study assessed the effects of PUFA on 2 clinically relevant AF substrate experimental models: electrical remodeling induced by 1 week of rapid atrial tachypacing to simulate AF and congestive heart failure (CHF)-associated structural remodeling induced by 2 weeks of ventricular tachypacing (240 bpm for 2 weeks). PUFA pretreatment had no direct atrial electrophysiological effects and did not alter atrial refractoriness abbreviation or AF promotion caused by atrial tachycardia. On the other hand, PUFAs suppressed the atrial profibrillatory remodeling associated with CHF, including atrial fibrosis, associated conduction abnormalities, and spontaneous AF maintenance. PUFAs also attenuated the hemodynamic dysfunction caused by ventricular tachycardia and reduced the associated phosphorylation of mitogen-activated protein kinases (extracellular-signal related and P38 kinase). Thus, PUFAs suppress atrial profibrillatory structural remodeling caused by CHF, possibly by preventing mitogen-activated protein kinase activation, and should be studied further for clinical anti-AF action, especially in CHF and other contexts associated with atrial structural remodeling. In addition, the potential cardioprotective actions of PUFAs in CHF bear further study. Conversely, their lack of effect on atrial tachycardia-induced atrial electrical remodeling indicates that PUFAs cannot be expected to be helpful in all clinical AF paradigms.