Dietary ω-3 fatty acids reduced atrial fibrillation vulnerability via attenuating myocardial endoplasmic reticulum stress and inflammation in a canine model of atrial fibrillation

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Research

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Abstract

Background: It is known that dietary consumption of ω-3 fatty acids is correlated with a reduced incidence of cardiovascular events. Here, we investigated the effect of dietary ω-3 fatty acids on atrial fibrillation (AF) vulnerability in a canine model of AF and explored the related mechanisms.

Methods: 24 male beagle dogs (weight, 8-10 kg) were randomly divided into four groups: (a) sham-operated group (normal chow), (b) AF group (AF with normal chow), (c) AF+FO (AF and chow supplemented with fish oil (FO, 0.6 g ω-3 PUFA/kg/day)), (d) FO (chow supplemented with FO). Daily oral administration FO was initiated 1 week before surgery and continued throughout the study period.

Results: Dietary FO significantly reversed atrial electric remodeling post rapid atria pacing (RAP). FO treatment also attenuated the elevated levels of biomakers for ER stress (all $P<0.05$ vs. AF). RAP-induced upregulation of inflammation factors IL-1β, IL-6, TNF-α in LA were significantly attenuated by FO treatment (all $p<0.05$ vs. AF). In addition, Masson staining revealed increased interstitial fibrosis in LA was significantly reduced in AF+FO group as compared to AF group ($P<0.01$). FO treatment also alleviated RAP-induced myocardial apoptosis ($p<0.05$).

Conclusions: Dietary ω-3 fatty acids reduced RAP-induced AF vulnerability, possibly via attenuating myocardial ER stress and inflammation in this canine model of AF.

Background

Atrial fibrillation (AF) is presently the most common persistent clinical dysarrhythmia. The endoplasmic reticulum (ER) stress, also known as the unfolded protein response (UPR), is considered to play a key role in the stress response, such as inflammation, hypoxia or nutrient deprivation [1]. ER stress related to the initiation and progression of numerous diseases [1, 2]. Recent study demonstrated that ER stress may act as a potential target to weaken cardiac remodeling in AF [3].

n-3 polyunsaturated fatty acids (PUFAs), especially eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA,C22:6n-3) were served as the active components of fish oil (FO) [4]. It is known that dietary of consumption ω-3 fatty acids is related to reduced risk of major cardiovascular events [5, 6]. In addition, FO was reported to exhibite anti-inflammatory, anti-oxidant effect, and attenuated ER stress in numerous deceases [7–9]. Previous studies demonstrated that PUFAs could decrease AF vulnerability [10–12]. However, the mechanism of the anti-arrhythmic effect of ω-3 fatty acids is not fully clear. Here, we investigated the effect of dietary ω-3 fatty acids on a canine AF model and tested if ω-3 fatty acids reduced AF vulnerability was related to ER stress changes.

Materials And Methods

Animal Preparation

This study was conducted in strict accordance with the recommendations in Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The Committee governing the Ethics of Animal Experiments of the Central South University approved the protocol. Dogs were anesthetized with 3% sodium pentobarbital and ventilated with a positive-pressure respirator (MAO01746; Harvard Apparatus, Holliston, MA); initial dose was 1 ml/kg and an additional 2 ml/h was administered. All efforts were made to minimize suffering.

Group setting

24 male beagle dogs (weight, 8-10 kg) were randomly divided into four groups (n=6 each): (a) sham-operated group (normal chow), (b) AF group (AF with normal chow), (c) AF+FO (AF and chow supplemented with fish oil, (d) FO (normal chow supplemented with FO). Alignment with previous reports [13], daily oral administration of FO (FO, containing 0.6 g/kg/day EPA/DHA) was initiated 1 week before surgery and continued throughout the study period.

Canine Model of atrial fibrillation

AF was induced in canine using long-term RAP [14]. In brief, a programmable pacemaker (AOO, Harbin University of Science and Technology, China) was implanted and used for continuous atria pacing at 400 bpm for 4 weeks to induce AF. The success of this procedure was confirmed by electrocardiography. Sham-operated dogs were implanted with a same instrument but were maintained without pacemaker activation.

Atrial electrophysiological study

Standard ECG limb leads were recorded at baseline and after 4-week RAP. We dissected the right femoral vein and inserted a 4 Fr multielectrode catheter into the high right atrium (HRA). The electrophysiological parameters, such as effective refractory period (ERP), window of vulnerability (WOV) and AF duration were detected at the HRA site. Programmed stimulation of atrial was performed by the computer-based Lab System (Lead 7000; Jingjiang, Chengdu City, China). ERP was determined by programmed pacing with 8 consecutive stimuli (S1-S1=300 ms) followed by a premature stimulus (S1-S2) at 2×threshold, the S1-S2 interval was decreased from 160 ms initially in decrements of 10 to 2 ms when approaching ERP. The difference between the longest and shortest S1-S2 interval, where AF was induced at each bipolar pair, was defined as WOV, which serves as a quantitative measure of AF inducibility [15]. AF was defined as an irregular atrial rate faster than 500 beats/min correlated with irregular atrioventricular conduction lasting 5s [16]. To determine AF vulnerability, 10 consecutive bursts of rapid atrial pacing (cycle length 60 ms) at HRA for 2 s were implemented with 30 s intervals. AF duration induced by burst pacing from all episodes in each dog was analyzed. Operators were performing the electrophysiological study blinded to the treatment group for the dogs.

After 4 weeks RAP, anaesthetized animals were euthanized and hearts were removed. Left atrial posterior wall tissue samples were collected and immersed in 4% paraformaldehyde or snap frozen at −80˚C for

further analysis. Fasting blood samples were collected at baseline before pacemaker implantation and before measuring electrophysiological parameters and stored at −80°C for further analysis.

Histological study

LA samples were fixed in 4% paraformaldehyde, embedded in paraffin and sliced into 5-μm-thick sections. Masson-stained sections was used to estimate the interstitial fibrosis of LA which was showed as percentage of total area occupied by blue-stained interstitial tissue. For each section, 5 optical fields (400×magnification) were examined using Image-Pro 6.2 software, and the data averaged.

TUNEL (TdT-mediated dUTP Nick-End Labeling) assay

To detect apoptosis, paraffin-embedded LA was cut transversely into 5-μm-thick sections. TUNEL assay was performed with DeadEnd Fluorimetric TUNEL System (Promega), according to the manufacturer's protocol. Cell nuclei were counterstained with DAPI (4',6-diamidino-2-phenylindole). The number of TUNEL-positive nuclei was manually counted. Image-Pro 6.2 software was used to determine the total number of nuclei by automatically counting the DAPI.

Western blot analyses

Protein-extracts of snap-frozen LA were prepared using standard procedures. Bicinchoninic acid (BCA) assay (ASPEN, USA) was used to measure the protein concentrations of supernatants. After separating on SDS-polyacrylamide gels, proteins were transferred to PVDF membranes. The primary antibodies, rabbit monoclonal anti-GAPDH antibody (diluted 1:10000), rabbit polyclonal anti-GRP78 antibody (diluted 1:500; Abcam), rabbit monoclonal anti-PERK antibody (diluted 1:1000; Abcam), rabbit monoclonal anti-p-PERK antibody (diluted 1:1000; Bioss), rabbit polyclonal anti-CHOP antibody (diluted 1:1000;Bioss), rabbit polyclonal anti-Caspase12 antibody (diluted 1:500; Abcam), followed by secondary goat anti-rabbit antibody (diluted 1:10000, Abcam) were used. The ECL detection reagent (ASPEN, USA) was used to detect the antibody binding. Bands were quantified with ImageJ software.

Real-time quantitative PCR

According to the manufacturer's protocol, the RNA extraction of LA tissues proceeded after homogenization in TRIZOL (Invitrogen, Carlsbad, CA). Reverse transcription was performed with RevertAid First strand cDNA Synthesis Kit (Thermo Scientific), and gene-specific primers and SYBR Green PCR Master Mix (Applied Biosystems) were used for quantitative PCR. Real-time qPCR analysis was carried out with the StepOne Real-Time PCR (Life technologies, Alameda, CA). The primer of gene β-actin, IL-1β, IL-6, TNF-α, RyR2, SERCA2a, NCX, CACNA1C was synthesized from GeneCreate (Wuhan, China). We listed the primer sequences used in Table 1 and used the amount of β-actin to determine and normailize the amount of each gene. The differences among groups were evaluated through the relative expression quantity 2-ΔΔCt value.

Statistical Analysis

We used R-3.4.3 (https://www.r-project.org/) for statistical analysis. All values were expressed as mean±SD. Statistically significant differences between means were assessed by ANOVA and Tukey HSD for comparisons between four groups. P<0.05 was considered statistically significant.

Results

Fish oil attenuated atrial electrical remolding and AF vulnerability

Electrophysiological parameters were detected at high right atrium (HRA). After 4-week rapid atrial pacing, ERP in AF group (76.00 ± 13.84 ms) was significantly decreased compared to sham-operated group (125.83 ± 13.83 ms) and FO group (126.83 ± 6.62 ms) ($p < 0.05$, respectively). ERP in AF + FO groups was (102.16 ± 12.34 ms), higher than that in AF group but still lower than that in sham-operated group and FO group ($P < 0.05$, respectively) (Fig. 1A).

There was a significant increase of WOV in AF group (53.33 ± 8.17 ms) compared to sham-operated group (3.33 ± 8.17 ms) and FO group (6.67 ± 10.32 ms) ($P < 0.01$, respectively), which was significantly low in AF + FO group (31.67 ± 7.53 ms) than in AF group ($P < 0.05$, Fig. 1B).

In addition, AF duration following burst pacing in AF group (7.22 ± 0.33 s) was significantly higher than in sham-operated group (2.3 ± 0.52 s) and FO group (2.2 ± 0.47 s) ($P < 0.05$, respectively), which was significantly lower in AF + FO group (5.03 ± 0.80) ($P < 0.01$ vs. AF group)AF (Fig. 1C). AF inducibility in the AF group (88.33 ± 9.83%) was significantly higher than that in the sham-operated group (10 ± 10.95%) and FO group (18.33 ± 13.29%) ($P < 0.05$, respectively), which was significantly lower in AF + FO group (60 ± 16.73%) than in AF group ($P < 0.05$) (Fig. 1D).

To detect the molecular mechanism of fish oil in this AF model, the mRNA expression level related to Ca^{2+} handling were analyzed in LA tissue sample. Compared to sham-operated and FO groups, mRNA expression level of RyR2 and NCX was significantly upregulated, and the expression of SERCA2a and CACNA1c was significantly downregulated in AF group (all $p < 0.05$), above changes, except RyR2, were reversed in AF + FO group. (Fig. 3D, C, E and F). These data imply that fish oil could attenuate RAP-induced atrial electric remodeling and AF vulnerability.

Fish oil suppressed ER stress activation in LA during AF

To evaluate whether ER stress activation in atrial myocardium and the effect of fish oil on atrial ER stress during AF, the protein levels related to ER stress were measured. As shown in Fig. 2A, the protein expressions levels of GRP78, CHOP, cleaved-Caspase12 and phosphorylation of PERK were significantly upregulated in the AF group than those in the sham-operated and FO groups, which were significantly downregulated in the AF + FO group (all $p < 0.05$ vs AF group) (Fig. 2B, C, D and E). These data indicated that fish oil suppressed RAP-induced ER stress activation in this model.

Fish oil reduced AF induced atrial inflammation, fibrosis and apoptosis

To evaluate the effect of fish oil on atrial inflammation in this AF model, the mRNA expression of proinflammatory factors were detected. The mRNA expression of IL1β, TNFα, IL-6 were significantly upregulated in the AF group than those in the other three groups ($p < 0.05$, respectively) (Fig. 3A, B and C). The mRNA level of these factors were remarkably lower in AF + FO group than in AF group ($p < 0.05$). In addition, FO group had lower level of those factors than Sham-operated group ($p < 0.05$, respectively).

As shown in Fig. 4A and C, the Masson staining evidenced increased interstitial fibrosis in AF group (10.12 ± 0.62%) compared to that in the sham-operated group (2.53 ± 0.41%) and FO group (2.42 ± 0.36%) ($P < 0.05$, respectively), fish oil treatment significantly reduced atrial fibrosis (4.87 ± 0.27% vs. 10.12 ± 0.62%, $P < 0.05$).

As shown in Fig. 4B and D, the proportions of TUNEL–positive cells was significantly higher in AF group (13.31 ± 0.36%) than in other three groups (all $p < 0.05$), which was significantly lower in AF + FO group than in AF group ($p > 0.05$). These dates indicated that fish oil reduced RAP-induced atrial proinflammatory response, fibrosis, and the level of atrial myocardium apoptosis in this model.

Discussion

The present study results show that the administration of fish oil (FO) could reduce atrial electrical remolding and AF vulnerability in RAP-induced canine model, possibly by attenuating myocardial ER stress in atrium. To our best knowledge, this is the first report linking the beneficial effect of fish oil therapy on AF vulnerability in RAP-induced canine model with the potential role of reduced myocardial ER stress in atrium. FO treatment also decreased proinflammatory adipokine IL1β and TNF-α, and apoptotic factors C/EBP homologous protein (CHOP) and Caspase12 in the atrium. Thus, our results hint that FO treatment could inhibit the ER stress, reduce inflammatory responses and apoptosis in this RAP-induced AF model.

Recently, experimental findings showed that the omega-3 PUFA, main active components of FO, could attenuate AF vulnerability in pacing-induced AF in dogs and reduced the incidence of post-operative AF (POAF) in patients undergoing cardiac surgery [10, 12, 17–19]. Previous studies indicated that the above mentioned anti-arrhythmic effects of omega-3 PUFAs might be associated with modulation of ion channel and membrane properties as well as anti-inflammatory and anti-fibrotic effects [20]. It is known that the initiation and maintenance of AF is connected with the changes in cardiac sarcolemmal ion channels known as electrical atrial remodeling. In some pathological status, the inhibition of sarcolemmal ion channels may stabilize electrical activity and prolong the relative refractory period of the cardiomyocytes. In line with above hypothesis, we observed reduced AF vulnerability and decreased AF duration post fish oil treatment, thus fish oil could attenuate electrical remodeling in this RAP-induced AF model.

FO treatment induced reduced endoplasmic reticulum stress (ERS) in atrium tissue of this canine AF model. Endoplasmic reticulum (ER) is known to play important roles in the pathogenesis of AF. Recent studies demonstrated that ERS pathways were activated in the atrial tissue of human or animal model during AF [21–24]. ERS marker protein such as glucose-regulated protein78 (GRP78) was significantly increased in the tachypacing induced AF model and atrial myocardium obtained from AF patients [23, 24]. UPR effectors like protein kinase R-like ER kinase (PERK) could destabilize voltage-gated cardiac Na + channel α-subunit (SCN5A) and Kv4.3 channel expression, which were important mediators of electric remodeling[22]. Interestingly, researchers have reported that docosahexaenoic acid (DHA) could reduce ER stress in vitro and in vivo [25, 26]. DHA could protect against monocrotaline-injured pulmonary hypertension by reducing ER stress, suppressing cell proliferation and inflammation [25]. In line with this finding, present study found that the protein expressions levels of GRP78 and phosphorylation of PERK were significantly higher in the canine atrial of AF group. FO treatment could significantly decrease the elevated level of GRP78 and phosphorylation of PERK in this AF model.

Besides the reduced ER related pathways, FO treatment also reduced inflammation, apoptosis and Ca2 + handling related proteins. All contributed actively in the pathogenesis of AF. FO treatment could reverse the elevated level of ERS related apoptotic factors CHOP and Caspase12 in the AF group. Daily oral administration of FO also significantly decreased the proportions of TUNEL–positive cells in the fibrillating atrial myocardium. Besides, the mRNA expression of proinflammatory factors like IL1β, TNFα, IL-6 and the interstitial fibrosis were remarkably lower in AF + FO group than AF group. Taken together, our result indicate that FO treatment could attenuate the ER stress, inflammation, apoptosis, these effects might contribute to the observed beneficial role of reducing the AF vulnerability and electrical remodeling in this canine AF model.

Conclusion

RAP induced AF could increase the ERS, inflammation and interstitial fibrosis which could be successfully reversed by oral administration of FO. Meanwhile, FO could attenuate atrial electrical remolding and AF vulnerability. Thus, our results suggest that dietary ω-3 fatty acids could reduce RAP-induced AF vulnerability, possibly through attenuating myocardial ER stress, inflammation and apoptosis.

Declarations

Ethics approval and consent to participate

All animal studies followed the National Institutes of Health guidelines with the approval by the Central South University Animals Research Ethics Committee.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Tao Tu, Biao Li and Xuping Li contribute for design, data analysis, drafting article and data collection; Baojian Zhang, Jiayi Li, Fen Qin, Na Liu and Chao Sun contribute for data analysis, data collection and Statistics; Qiming Liu and Shenghua Zhou for design, data analysis, critical revision and approval of article. All authors read and approved the final manuscript.

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Abbreviations

AF: Atrial fibrillation; CHOP: C/EBP homologous protein; DHA: docosahexaenoic acid; EPA: Eicosapentaenoic acid; ER: Endoplasmic reticulum; ERP: effective refractory period; ERS: endoplasmic reticulum stress; FO: Fish oil; GRP78: glucose-regulated protein78; HRA: High right atrium; IL1β: interleukin 1 beta; IL-6: interleukin 6; RAP: Rapid atria pacing; PERK: protein kinase R-like ER kinase; PUFAs: n-3 polyunsaturated fatty acids; SCN5A: voltage-gated cardiac Na+ channel α-subunit; TNFα: tumor necrosis factor-alpha; WOV: window of vulnerability

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Table

Table 1. Sequences of primers used for real-time PCR

Primer	Forward (5’ →3’)	Reverse (5’ →3’)
β-actin	ATGACGATATCGCTGCGCTT	TCGTCCCAGTTGGTGACGAT
IL-1β	CATCCAGTTGCAAGTCTCCCA	ACTTGCAGTCCACCGATTGC
IL-6	TGATGGCTACTGCTTTCCCTAC	TTGAACCCAGATTGGAAGCAT
TNF-α	GATAGTGCCGTCAGATGGGTT	GGACTCGGCAAAGTCCAGAT
RyR2	AACCCTGCCCAGAAGACTCC	CCACAATGGCTTCTATCCGG
SERCA2a	TTGCTCGAGTCGAACCTTCC	CCACGATGGTGGAGAAGTTGT
NCX	GCCCATTCTGGGAGAACACA	TCCCAACCACAAGGGCTAAG
CACNA1C	GGTGTCTGGAGTCCCAAGTCTC	CATCTTCTGCTGGAACATCTGC

Figures

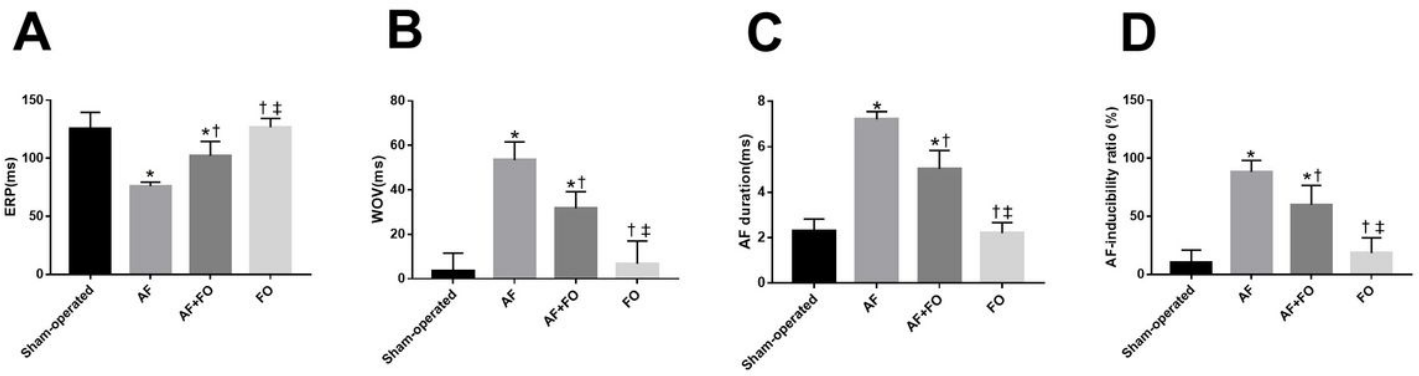

Figure 1

Effect of fish oil on atrial electrical remolding and AF vulnerability The electrophysiological parameters were detected at high right atrium in the four groups: sham-operated group, AF group, AF+FO group, FO group, each group has 6 dogs(n=6). (A, B) The change of ERP and WOV. (C, D) The mean AF duration and AF inducibility (atrial tachyarrhythmia ≥5s) after ten times burst pacing. *P <0.05 compared to the Control group; †P <0.05 compared to the AF group, ‡P <0.05 compared to the AF+FO group. ERP=effective refractory period; WOV= window of vulnerability.

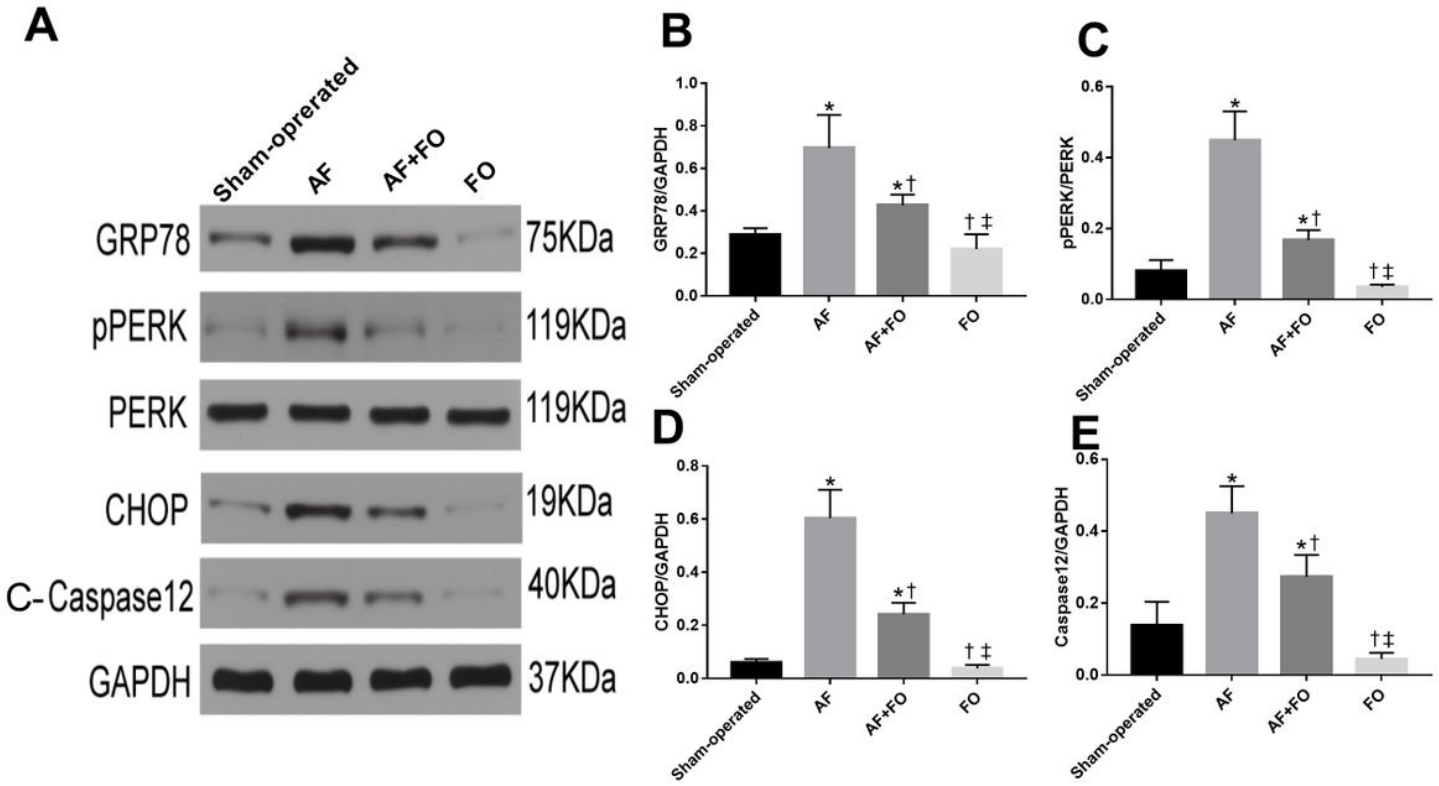

Figure 2

Effect of fish oil on atrial ER stress during AF (A) Representative immunoblots for the expression of the ER stress markers in four groups, each group has 6 dogs (n=6). (B, C, D and E) Quantitative analysis protein level of GRP78, Chop, cleaved Caspase12 and PERK/pPERK ratio. ER stress activation during AF, fish oil treatment suppressed ER stress. *P <0.05 compared to the sham-operated group, †P <0.05 compared to the AF group, ‡P<0.05 compared to the AF+FO group.

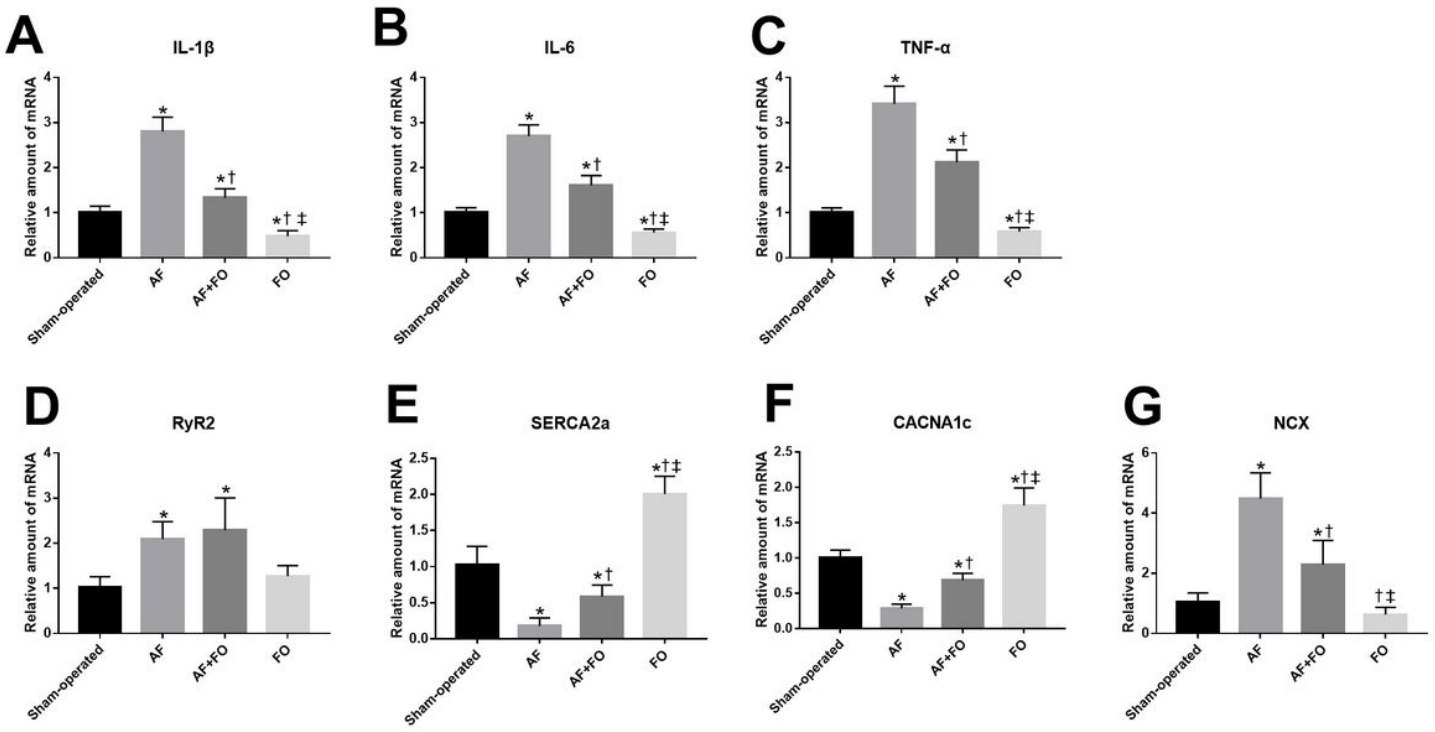

Figure 3

Effect of fish oil on atrial inflammation and Ca2+ handling mRNA expression of inflammation factors (IL-1β, IL-6 and TNF-α) and Ca2+ handling (RyR2, SERCA2a, CACNA1c and NCX) in the left atrium of four groups, each group has 6 dogs (n=6). β-actin was used for normalization. (A, B, and C) IL-1β, IL-6, TNF-α expression increased in AF group, fish oil treatment attenuated atrial inflammatory response in AF+FO group. (D, E, F and G) mRNA expression level of RyR2 and NCX increased, while the expression of SERCA2a and CACNA1c decreased in AF group, fish oil treatment reversed these changes except RyR2. *P <0.05 compared to the sham-operated group, †P <0.05 compared to the AF group, ‡P <0.05 compared to the AF+FO group.

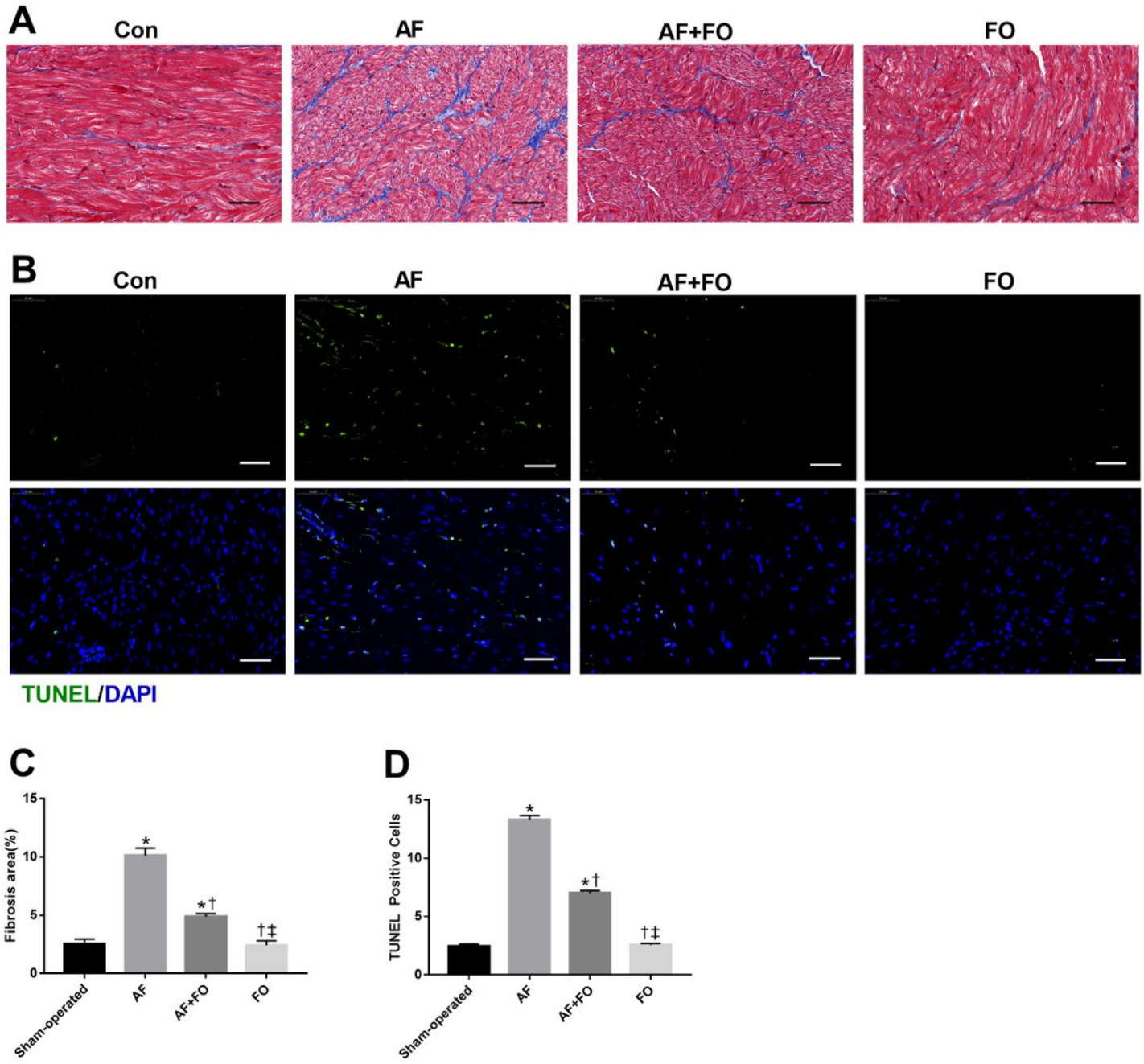

Figure 4

Effect of fish oil on atrial inflammation and Ca2+ handling mRNA expression of inflammation factors (IL-1β, IL-6 and TNF-α) and Ca2+ handling (RyR2, SERCA2a, CACNA1c and NCX) in the left atrium of four groups, each group has 6 dogs (n=6). β-actin was used for normalization. (A, B, and C) IL-1β, IL-6, TNF-α expression increased in AF group, fish oil treatment attenuated atrial inflammatory response in AF+FO group. (D, E, F and G) mRNA expression level of RyR2 and NCX increased, while the expression of SERCA2a and CACNA1c decreased in AF group, fish oil treatment reversed these changes except RyR2. *P <0.05 compared to the sham-operated group, †P <0.05 compared to the AF group, ‡P <0.05 compared to the AF+FO group.