Is fish oil really good for your heart?

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Abstract

Fish oil, a rich source of omega-3 polyunsaturated fatty acids (PUFA), is one of the most widely used supplements worldwide due to the conception that dietary fish oil reduces the risk of cardiovascular diseases. However, this belief has been challenged by multiple recent clinical trials which failed to demonstrate the benefits of dietary fish oil[1]. Instead, studies consistently revealed that fish oil supplements, especially at large dosages, are associated with an increased risk of atrial fibrillation (AF), a common cardiac arrythmia[2]. However, the link between fish oil and AF remains poorly understood. In this study, we assessed whether PUFA associated ferroptosis, a type of cell death, contributes to AF pathology.

Keywords

polyunsaturated fatty acids; atrial fibrillation; ferroptosis; omega-3 fatty acids; arachidonic acid; lipid peroxides

Introduction

Cardiovascular disease is the leading cause of death worldwide, accounting for 1 in every 5 deaths in the United States[3]. Fish oil, a rich source of omega-3 fatty acids, has been recommended by the National Institutes of Health (NIH) for the past 20 years as a dietary measure to prevent cardiovascular disease[4]. The belief that dietary fish oil can reduce the risk of cardiovascular disease has fueled a lucrative global market for these supplements. As of 2023, the market for omega-3 fatty acids has reached $2.62 billion dollars[5].

Despite being historically recommended by public health authorities, the health benefits of omega-3 fatty acid supplements are questionable. Specifically, omega-3 supplements may increase the risk of atrial fibrillation (AF). In the STRENGTH randomized clinical trial, 13,078 patients at high risk for cardiovascular disease were randomized to receive either 4 g/d of an omega-3 fatty acid formulation or corn oil, which served as an inert comparator. After a median follow-up of 42 months, no significant difference was observed in a composite outcome of major adverse cardiovascular events. However, new-onset AF was more common in patients receiving the omega-3 formulation[6]. In the REDUCE-IT randomized clinical trial, patients with hypertriglyceridemia were randomized to receive either 4 g/d of eicosapentaenoic acid, an omega-3 fatty acid found abundantly in fish oil, or placebo. After a median follow-up of 4.9 years, the incidence of major adverse cardiovascular events was found to be lower in patients that received the supplement compared to those who received only placebo. Still, as in the STRENGTH trial, the researchers also observed a significant increase in AF events (3.1% vs 2.1%) in the group which received the omega-3 supplement[7].

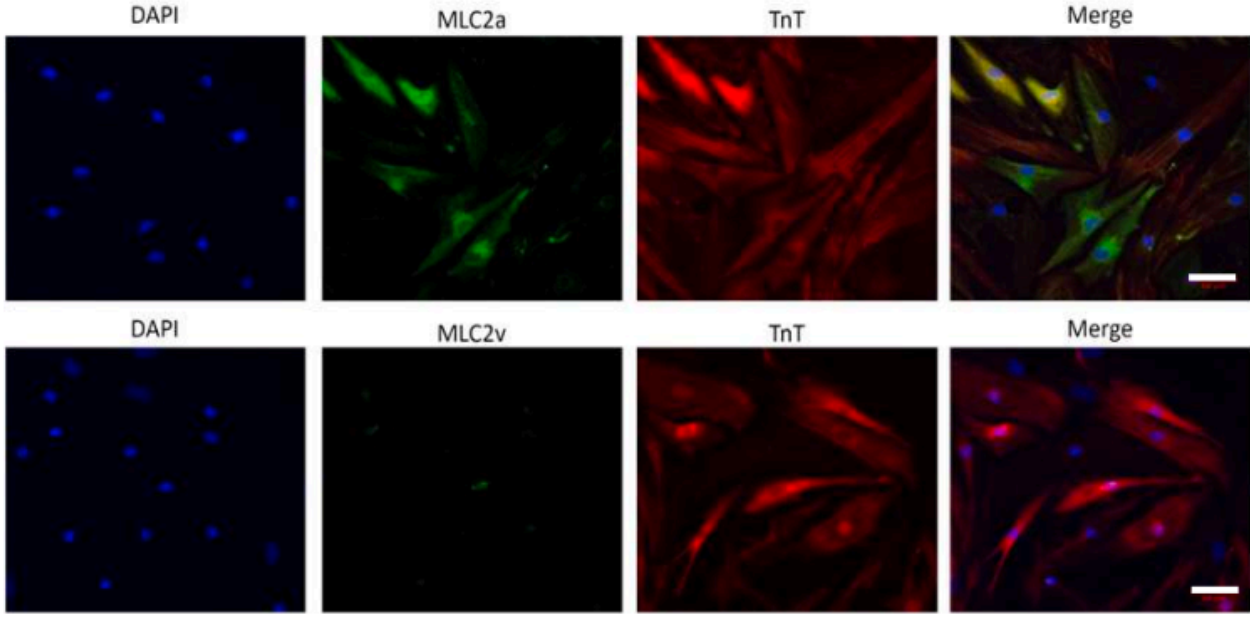
Very little is understood about the link between omega-3 fatty acids and AF pathogenesis. In this report, we analyzed the effect of dietary omega-3 intake on ferroptosis, a distinct type of programmed cell death associated with PUFA peroxides. Through *in vitro* treatment of omega-3 fatty acids to a line of immortalized atrial cardiomyocyte (iAM) cells, we discovered a novel relationship between free PUFA levels and ferroptosis. Additionally, by performing western blot analysis of tissue samples from different cohorts of mice, we found that metformin attenuates ferroptotic stress *in vivo*.

Methods

Functional Analysis of Atrial Cells

In order to perform detailed functional analyses and downstream mechanistic studies, it is crucial to establish an atrial cell line that resembles the properties of primary cells. In collaboration with Dr. Antoine de Vries from Leiden University in the Netherlands, we have established an immortalized rat atrial cell line (iAM), originating from primary rat atrial cardiomyocytes transduced with a lentiviral vector conferring doxycycline (Dox)-controlled expression of SV40 large T antigen[8]. These cells proliferate in the presence of Dox and start differentiating once Dox is removed. We have extensively validated the phenotypes of these cells and confirmed that differentiated iAM cells express sarcomere proteins, and atrial cardiomyocytes-specific markers including myosin light chain 2a (MLC2a) and atrial natriuretic factor (ANF), but not the ventricular cardiomyocyte marker MLC2v (Fig 1A). Since iAM cells have a significantly narrower action potential duration (APD) and propagate electrical signals at a much faster rate, they are a better model for atrial cardiomyocytes than HL1 cells. More importantly, high frequency pacing monolayer iAM cells can result in re-entrant conduction and generate “AF in a dish”. Therefore, we believe that iAM cells are a unique and powerful tool that can be used for detailed functional analyses and downstream mechanistic studies.

(A)



(B)

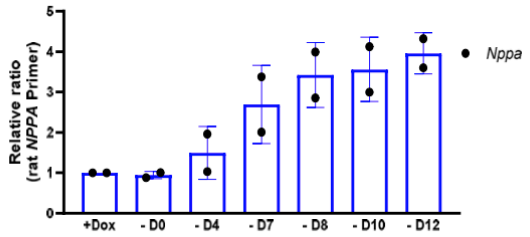


Figure 1: (A) Representative immunofluorescence images of iAM cells after seven days of differentiation. Images show the expression of the atrial and ventricular specific markers MLC2a and MLC2v (green), troponin (red), and Hoechst (blue). Scale bar = 50um. **(B)** qRT-PCR analysis of atrial natriuretic peptide (NPPA) expression.

To prevent variability from genomic instability, an aliquot of the iAM cell line was used for only 4 weeks before a fresh batch of cells was thawed and propagated for experimental use.

Characteristics of the Mouse Model

To analyze ferroptotic stress on atrial and ventricular tissue *in vivo*, we used three cohorts of mice. All mice were fed on a chow diet until they reached the age of 8 weeks. Then, one group of mice (n = 5) was fed ad libitum with a chow diet while the other two groups (n=3 and n=3) were fed ad libitum with a high fat diet (24% (w/w) fat, 41% (w/w) carbohydrate, and 24% (w/w) casein protein). After 4 weeks, the third group began to receive metformin. 4 weeks later, the mice were euthanized at the end of the dark cycle.

All experiments described in this work were approved and conducted under the oversight of the University of Texas (UT) Southwestern Institutional Animal Care and Use Committee. All mice were housed in colony cages in a room with a 12-h light/12-h dark cycle.

PUFA Treatment Procedure

To analyze the effect of omega-3 treatment on ferroptosis, differentiated iAM cells were treated with varying dosages of arachidonic acid (AA), a polyunsaturated omega fatty acid which is abundant in fish oil. We selected DMSO as a control for our experiment because it was the solvent that the AA was dissolved into. All cells were treated for a period of 24 hours.

Based on baseline measurements of free AA levels in the human bloodstream, the dosages of AA which we selected were physiologically relevant.

Ferroptosis was evaluated quantitatively by measuring the expression level of oxidized peroxiredoxin 3 (PRDX3), a specific ferroptosis marker, using Western blot.

Results

AA Treatment Increases Ferroptosis Marker in iAM Cells

Our preliminary data revealed that AA treatment increased oxidized PRDX3 levels in atrial cells in a dose-dependent manner.

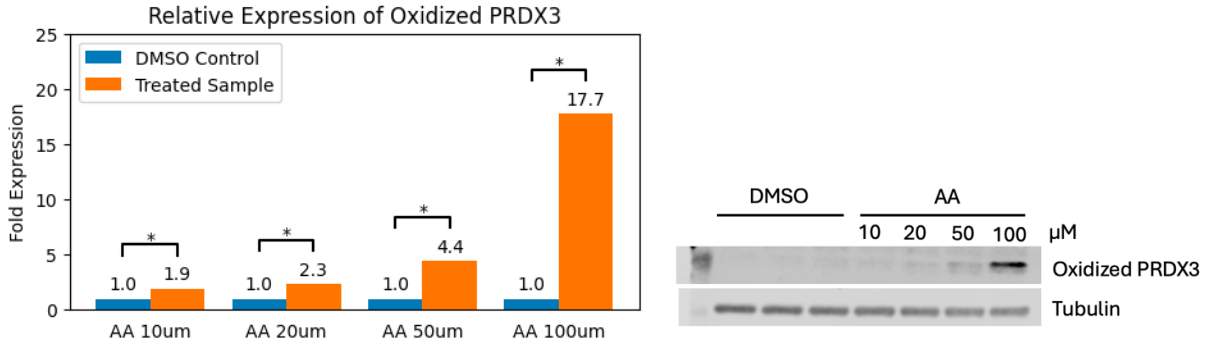


Figure. Relative expression of oxidized PRDX3 when compared to the baseline DMSO control.

Metformin Treatment Attenuates Ferroptosis Marker *in vivo*

Our preliminary data revealed that metformin treatment decreased oxidized PRDX3 levels in atrial cells of mice on a high fat diet.

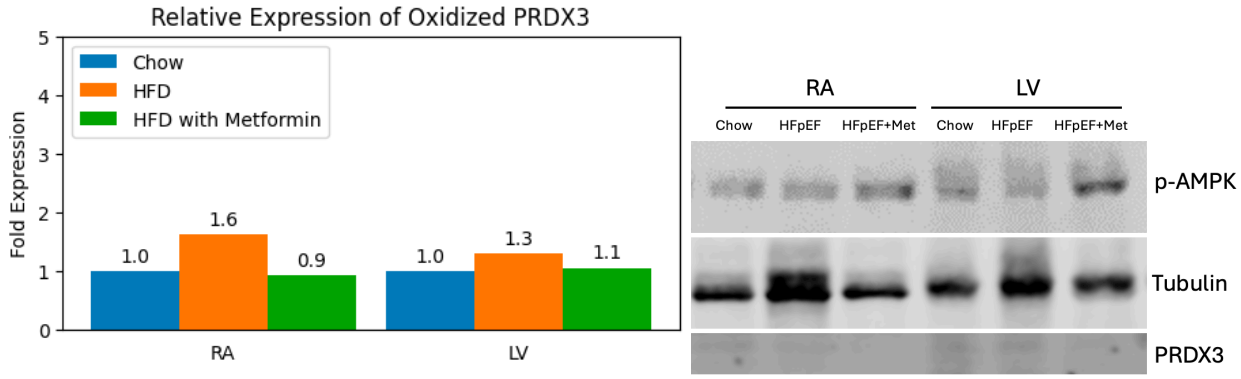


Figure. Relative expression of oxidized PRDX3 in chow, HFD, and HFD with Metformin mice. Data is representative of tissues sampled from two mice in the same cohort. Quantified PRDX3 was scaled based on level of tubulin expression.

Discussion

Fish Oil Induces Ferroptosis in Atrial Cardiomyocytes

Our data revealed that oxidized peroxiredoxin 3 (PRDX3), a specific marker of ferroptosis, is significantly upregulated after treatment with omega fatty acids. This suggests that fish oil supplements increase the risk of AF by promoting atrial cardiomyocyte ferroptosis. This could explain why the prevalence of AF is increased in patients who are taking fish oil supplements.

The Effect of Metformin

Based on our preliminary data, metformin may protect against ferroptosis in the heart. Since metformin is the second most extensively used therapeutic agent for type 2 diabetes[9], it is important to consider whether a patient is taking this drug when deciding whether fish oil is a good idea. Overall, one must balance the benefits for against the associated AF risk when deciding whether to take fish oil supplements.

Limitations

Our study has certain limitations. First, we only tested the effect of AA without considering other types of omega-3 and omega-6 fatty acids. It will be advantageous to test whether other fatty acids yield similar results. Secondly, the mechanism behind how PUFAs induce ferroptosis in cardiac cells remains unclear. We suspect that increased PUFA content in the cell membrane can drive lipid peroxidation and accumulation of excess iron, leading to the iron-dependent production of reactive oxygen species (ROS)[10]. ROS has been shown to lead to destruction of barrier function, causing cell death[11]. Our next experiment would be to validate this theory by testing whether co-treatment with an iron chelator[12] could attenuate ferroptosis.

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Conflict of Interest

The authors declare no conflict of interest.

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