

Abstract

In recent years the use of fish oil as a dietary supplement has become exceptionally popular among Americans. Fish oil has been proven to help decrease inflammation in the body, yet the exact mechanism by which it does so is unknown. In this study we will attempt to identify the mechanism by which fish oil works. Recently, it was proposed that two new lipid mediators, resolvins and protectins, are responsible for helping restore homeostasis after an inflammatory reaction by acting in an anti-inflammatory and pro-resolution manner. Both of these compounds are synthesized from the omega-3 fatty acids that are found in fish oil. We will test to see if feeding a diet high in fish oil will decrease the amount of arthritis in mice that are infected with *Borrelia burgdorferi* compared to mice fed a normal diet. The mice will be pre-treated with either a control or fish oil diet for two weeks and then infected with *Borrelia burgdorferi*. At three weeks post-infection, the peak of arthritis severity, we will analyze the development of arthritis, the *Borrelia burgdorferi* loads in different tissues, and the presence of resolvins and protectins. If the inflammation is decreased in the mice which were fed the fish oil diet due to increased resolvins and protectins, that breakthrough could lead to serious implications for the treatment of inflammatory diseases, in addition to finding one of the missing links in the mystery of fish oil's role in limiting inflammation.

Introduction

Inflammation is a key function to the body's immune system, yet it can also be exceedingly detrimental to the body. If inflammation is left unchecked or occurs chronically, it can cause severe tissue damage, as is the case with asthma, inflammatory bowel diseases, and rheumatoid arthritis. Inflammation occurs in response to infections with *Borrelia burgdorferi* and often leads to Lyme arthritis. Inflammation begins when neutrophils are recruited to the site of injury or infection. Once in the tissue, neutrophils phagocytose the bacteria and release their granules in order to clear the infection. Once this inflammatory reaction is no longer needed, this process must be actively terminated in order to prevent damage to the surrounding tissue. Pro-resolution lipoxins are thought to play an important role in terminating inflammation by prohibiting further neutrophil recruitment and commencing apoptosis. Resolvins and protectins are two other compounds that also aid in limiting inflammation by triggering neutrophil apoptosis. Resolvins and protectins are made by the body from the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Fish oil, which is derived from the tissues of predatory fish, is rich in the omega-3 fatty acids DHA and EPA. In addition to several other health benefits, fish oil supplements claim to decrease inflammation by providing these omega-3 fatty acids that the body can use to make pro-resolution compounds.

Materials and Methods

Fish Oil Diet

The fish oil diet contained 18% menhaden fish oil and 2% corn oil. The control diet contained 20% lard. Other diet ingredients included (per kg of diet): 354.6 g corn starch, 230 g casein, 100 g sucrose, 57.4 g fiber, 40.2 g AIN-93 mineral mix, 11.2 g AIN-93 vitamin mix, 3.4 g l-cysteine, 2.9 g choline bitartrate.

Infection

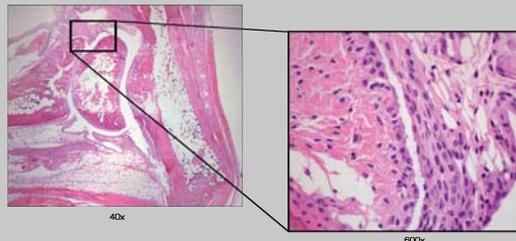
Adult C3H/HeJ mice were inoculated with 1×10^5 of a virulent, low passage, clonal isolate of *Borrelia burgdorferi* strain by injection in both hind footpads.

Quantitative Real Time PCR

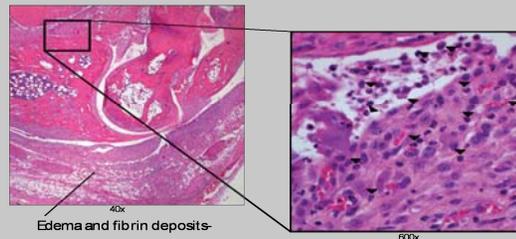
Genomic DNA from ankles and ears on day 21 post infection. The DNA was used to detect the *Borrelia burgdorferi* flagellin gene and was normalized to copies of mouse nidogen gene in the same tube. *Borrelia burgdorferi* was quantified by comparison to a standard curve consisting of known numbers of *Borrelia burgdorferi*.

Histopathology

Uninflamed Tibiotarsal Joint



Inflamed Tibiotarsal Joint



Examples of uninflamed and inflamed tibiotarsal joints are seen in the figures above. The inflamed joint shows large amounts of edema, cellular infiltrates, fibrin deposits and neutrophils (represented by arrow heads).

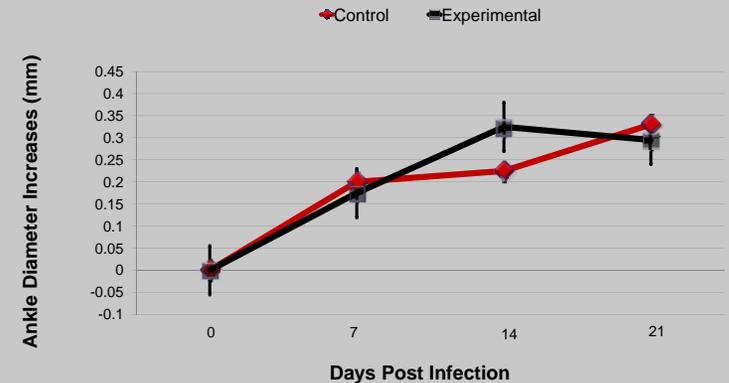


Figure 1: Ankle size due to inflammation in the mice fed fish oil diet was significantly worse than those fed the control diet.

Borrelia burgdorferi Burden in Mouse Tissues

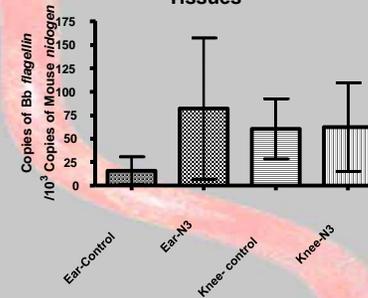


Figure 2: *Borrelia burgdorferi* loads of the ears and knees of control and fish oil mice. The differences are not significant, yet they may indicate that the presence of high levels of fish oil in the diet allow the *Borrelia burgdorferi* to spread to the ears faster than when fed a control diet.

Conclusions

- Ankle swelling in mice fed a diet high in fish oil increases peak inflammation but also induces resolution faster than those fed a control diet. The increased inflammation in fish oil mice may be due to the fact that the omega-3 derivatives in the fish oil block the neutrophils and induce apoptosis before the neutrophils are able to clear the infection.
- Because the omega-3 derivatives block the neutrophils and disable the ability of the immune system to clear the infection, the *Borrelia burgdorferi* are able to migrate to the ear more quickly.
- Mass Spectrometry is currently being done to test for the presence of protectins and resolvins; ELISA will be run to test for the presence of *Borrelia*-specific antibodies; histopathology is also being run to analyze the amount of arthritis that developed in each mouse.