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Impact of Omega-3 Fatty Acid Supplementation on Baseline Levels of Inflammatory Markers in the General Population

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IMPACT OF OMEGA-3 FATTY ACID SUPPLEMENTATION ON BASELINE
LEVELS OF INFLAMMATORY MARKERS IN THE GENERAL
POPULATION

by

ALEX T. NHAN

A thesis submitted in partial fulfillment of the requirements
for the Honors in the Major Program in Biomedical Sciences
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at the University of Central Florida
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ABSTRACT

Inflammation is a complex physiological response normally initiated by the innate immune system, often as a response to exposure to otherwise harmful stimuli. While generally useful in humans as a protective response to foreign matter, chronically elevated quantities of associated inflammatory factors C-reactive protein, TNF- α , IL-6, and IL-1 β have been linked in literature with decreased overall lifespan and well-being in humans via inflammatory processes. It is possible that by lowering these associated factors, increased well-being and lifespan may be experienced by the general population. One common health supplement with such promise is fish oil, which, through compounds eicosapentaenoic acid and docosahexaenoic acid, has been observed to decrease levels of secreted inflammatory markers in cell culture. In addition, molecular pathways have since been discovered which demonstrate possible means for which this physiological response may occur. However, despite the promise of such health benefits, studies attempting to discern the impact EPA/DHA supplementation has on inflammatory markers within humans have since emerged with mixed results. The aim of this study is to provide a meta-analysis across a number of studies to determine whether or not an impact exists through EPA/DHA supplementation in healthy populations, and if one exists, to what degree the respective inflammatory factors may be lowered.

TABLE OF CONTENTS

LIST OF FIGURES	iv
LIST OF TABLES	v
LIST OF ABBREVIATIONS/NOMENCLATURE/ACRONYMS:	vi
INTRODUCTION	1
LITERATURE REVIEW	9
OBJECTIVES	18
METHODS	19
RESULTS	21
DISCUSSION	48
REFERENCES	53

LIST OF FIGURES

Figure 1. Elongation and Desaturation of Endogenous Fatty Acids in Humans Cannot Produce ALA, EPA, or DHA.....	10
Figure 2. Fatty Acid Synthesis in Humans of EPA and DHA from ALA.	11
Figure 3. EPA Metabolic Pathway. Rv Represents Resolvin.	13
Figure 4. DHA Metabolic Pathway. Rv Represents Resolvin.	14
Figure 5. EPA Supplementation Fit Plot for CRP (Assumed Independence).....	24
Figure 6. DHA Supplementation Fit Plot for CRP (Assumed Independence).	25
Figure 7. Residual by Regressors for CRP	26
Figure 8. Contour Fit Plot for CRP.....	27
Figure 9. EPA Supplementation Fit Plot for TNF- α (Assumed Independence)	30
Figure 10. DHA Supplementation Fit Plot for TNF- α (Assumed Independence)	31
Figure 11. Residual by Regressors Plot for TNF- α	32
Figure 12. Contour Fit Plot for TNF- α	33
Figure 13. EPA Supplementation Fit Plot for IL-6 (Assumed Independence).....	36
Figure 14. DHA Supplementation Fit Plot for IL-6 (Assumed Independence).....	37
Figure 15. Residual by Regressors Plot for IL-6	38
Figure 16. Contour Fit Plot for IL-6	39
Figure 17. EPA Supplementation Fit Plot for IL-1 β (Assumed Independence).....	42
Figure 18. DHA Supplementation Fit Plot for IL-1 β (Assumed Independence).....	43
Figure 19. Residual by Regressors Plot for IL-1 β	44
Figure 20. Contour Fit Plot of IL-1 β	45

LIST OF TABLES

Table 1: Potential Sources of Low-Grade Chronic Inflammation	2
Table 2: Sample of Associated Benefits of Omega-3 Consumption in Literature	3
Table 3: Sample of Various Prostaglandin Roles in Humans	9
Table 4: Sample List by which EPA/DHA May Downregulate Inflammation	16
Table 5: Normal Ranges for Inflammatory Markers	17

LIST OF ABBREVIATIONS/NOMENCLATURE/ACRONYMS:

ALA – α -Linolenic Acid

CRP – C-Reactive Protein

DHA – Docosahexaenoic Acid

EPA – Eicosapentaenoic Acid

IL-1 β – Interleukin 1 beta

IL-6 – Interleukin 6

NF- κ B – Nuclear factor kappa-light-chain-enhancer of activated B cells

TNF- α – Tumor necrosis factor alpha

INTRODUCTION

In general, inflammation is a reaction of the body's immune response which protects an individual from foreign debris [1]. This is performed through a number of cellular responses, through which the body can more effectively remove harmful stimuli and initiate healing among damaged tissues. In general, inflammation may be classified into two subcategories - acute and chronic inflammation [2, 3]. While both types of inflammation result as a response to stimuli within the human body, acute inflammation generally tends to be characterized by a rapid onset, high degree of severity, and quick resolution, whereas chronic inflammation tends to be more low-grade and prolonged [2]. Chronic inflammation is generally less severe and is characterized by being unresolved, which may lead to the development of health problems in the long term [2, 4].

Acute inflammation often initiates shortly after tissue injury. This process is often characterized by pain, redness/swelling, heat, and loss of function, depending upon the affected tissue [1]. The function of acute inflammation is to help eliminate necrotic cells and pathogens, and to help start the healing process for the injured tissue [3]. Three common characteristics associated with acute inflammation is arteriole dilation, increased capillary permeability, and neutrophil movement into interstitial spaces [2]. As suggested by name, the duration of acute inflammation is generally short-lived, although if inflammation persists for more than a few weeks, it may develop into chronic inflammation [3].

Chronic inflammation results from ongoing inflammatory processes within the body, occurring when the body is unable to rid itself of the stimuli instigating the inflammatory response [4]. One common characteristic of chronic inflammation is an increased level of

circulating inflammatory factors throughout the bloodstream [5]. As chronic inflammation is often linked to a number of chronic diseases, it may interest the general population to decrease the overall circulating levels of inflammatory markers associated with potential long-term diseases in hopes of warding off development of chronic disease [3, 4, 6, 7]. Even otherwise perceived “healthy” populations may experience systemic low-grade chronic inflammation, which may be caused by a variety of factors, listed below.

Table 1: Potential Sources of Low-Grade Chronic Inflammation

Source of low-grade systemic inflammation	Supporting Literature
Sedentary Lifestyle	[8-10]
Increased Bodyweight	[11, 12]
Diets with high glycemic load, low fiber, or vitamin/mineral deficiencies	[13-15]

Two compounds with potential anti-inflammatory effects include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), two fatty acids commonly sourced from fatty fish that may have anti-inflammatory properties. The acids themselves are categorized as “omega-3 fatty acids,” although additional forms of omega-3s (hereafter referred to as n-3, as is literature standard) outside of EPA/DHA do exist in nature. The consumption of these aforementioned compounds is often reputed by literature to contain numerous health benefits, as seen on the following page in Table 2.

Table 2: Sample of Associated Benefits of Omega-3 Consumption in Literature

Supplementation Benefits	Supporting Literature
Decreased Triglycerides	[16-18]
Decreased Blood Pressure	[16, 19, 20]
Increase in HDL-C	[16-18]
Decrease in Cortisol	[21-23]
Decreased Cognitive Decline	[24, 25]
Improved Well-Being	[26-28]

In regards to diet, EPA/DHA serum concentration in humans generally correlates with fatty fish consumption (e.g., mackerel, salmon) [29]. Fatty fish are defined as fish which store a majority of their lipids in their flesh, rather than their liver [30]. For individuals with vegetarian/vegan diets, other means exist to obtain these fatty acids. DHA concentrations in algae oil have been found comparable to DHA concentrations found in fish, with algae oil being additionally suitable for human consumption [31]. Another method to obtain dietary DHA concerns the consumption of foods rich in alpha-linolenic acid (ALA), which can be converted in humans to EPA/DHA [32]. The conversion rate is measured to range from 2-10% after such consumption, although this is subject to individual variability [32, 33].

One of the many purported benefits of EPA/DHA supplementation is the downregulation of several inflammatory markers, for which several possible mechanisms have since been discovered [34, 35]. This suggests that increased EPA/DHA consumption may decrease levels of inflammatory markers in both baseline and elevated states, potentially holding a number of applications in the fields of sports science, general health, and human longevity [36]. In regards

to sports science, inflammation is one of the major factors in determining recovery periods for athletes [37]. Previous studies have found higher n-3 PUFA levels to be linked with lower all-cause mortality, for which chronic inflammation could be a contributing factor [38-40]. Levels of systemic low-grade chronic inflammation have also been strongly correlated with mortality rates among older adults [37, 41, 42]. While acute inflammation does yield a number of benefits, such as fighting off infection, healing injury, and scavenging debris, states of chronic inflammation may lead to a number of diseases and disorders which negatively affect the overall health of the individual, such as arthritis, atherosclerosis, and diabetes [43-48]. It is with this in mind that EPA/DHA supplementation may positively contribute to both lifespan and quality of life in individuals.

However, despite these potential benefits, many studies regarding inflammation reduction after EPA/DHA supplementation have since emerged with mixed results. While some studies do suggest that a decrease of inflammation occurs after a period of EPA/DHA supplementation, other studies propose that EPA/DHA supplementation does not affect inflammation at all [18, 20, 49-58]. It is with this perspective that this study aims to more clearly define how EPA/DHA supplementation may affect baseline levels of inflammation in the human body, by assessing changes in levels of circulating inflammatory markers within humans as subjects.

The mechanisms by which EPA/DHA downregulate the inflammatory process often involve receptor complexes with EPA/DHA n-3 derivatives, resulting in decreased production of tumor necrosis factor- α (TNF- α) and nuclear factor light-chain enhancer of activated B-cells (NF- κ B), two markers of inflammation [59, 60]. In studies assessing the impact of EPA/DHA on inflammation, circulating levels of C-reactive protein (CRP), interleukin-1 β (IL-1 β), TNF- α , and

interleukin-6 (IL-6) were most commonly measured, as these compounds were likely to have a significant impact on the body if affected by supplementation. Notably, supplementation of 1.8 g/d of EPA/DHA has been observed to impact the expression of at least 1040 genes, including genes involved in inflammatory, atherogenic, and NF-kB pathways [61].

If one wishes to apply the results of the aforementioned studies to the general public, an issue arises regarding the diverse conditions of the populations who underwent supplementation. In many of the studies, the subjects themselves had pre-existing medical conditions (e.g., cancer, heart disease), which is generally not representative of most populations [18, 20, 49-58]. EPA/DHA dosages were also varied across studies, potentially affecting patient inflammatory responses. As such, this study seeks to look at the effect of EPA/DHA supplementation in otherwise healthy populations, to examine its impact on circulating inflammatory factors, and to form a conclusion regarding EPA/DHA supplementation on inflammation that may be extended to the general population looking to ward off chronic disease. To the extent of this study's knowledge, such an analysis has never been previously performed.

In regards to the inflammatory factors themselves, CRP can bind lysophosphatidylcholine, activating the classical pathway of complement and contributing to general inflammation [62]. IL-1 β is a pyrogenic cytokine which helps modulate cell proliferation, differentiation, apoptosis, and activation of the PTGS2/COX2 pathway [63-65]. TNF- α is an acute-phase protein with pyrogenic properties, capable of initiating cell death among a variety of other actions [66]. Its main function is to regulate immune cells through the binding of the receptors TNFR1 and TNFR2 [67]. IL-6 enhances the immune response and is

generally associated with inflammation, although it has since been found to exhibit anti-inflammatory properties as a myokine [67].

For the purpose of this project, paired t-tests and F-tests were used to examine the data. Contour plots were generated afterwards to model the data pooled for each inflammatory marker. In general, paired t-tests can function to examine differences in means among a population, while the F-tests function to examine differences in variance among multiple populations, which can be used to give data about population means [68]. Although a paired t-test could potentially have been used multiple times in place of an F-test, repeated usage of t-tests holds the risk of an increased chance Type I errors, whereas F-tests allow the testing of multiple populations with a single test [68].

Paired t-tests are performed by first calculating the difference (d_i) between all observations, accounting for all positive and negative differences.

$$d_i = y_i - x_i$$

Next, the mean difference (\bar{d}) across all observations is calculated, followed by the standard deviation of the differences (s_d). From here, the standard error of the mean difference is calculated, given by the following equation:

$$SE(\bar{d}) = \frac{s_d}{\sqrt{n}}$$

The t-statistic is then calculated, which is determined by:

$$T = \frac{\bar{d}}{SE(\bar{d})}$$

This value T may then be compared to a t-distribution table under t_{b-1} , which will yield a p-value for the paired t-test [68]. As this value will help determine whether a significant difference in

change of means occurred before and after supplementation with EPA/DHA, this would be a viable test to see whether supplementation has any effect on the average level of circulating inflammatory markers in the human body. As a result, paired t-tests were used in this study to examine the general effect of supplementation on specific inflammatory markers.

F-tests may be performed by first finding the sample mean (\bar{x}) of an entire data set, which is determined by adding up all observations in a data set and dividing by the number of observations. Then, the sample means are calculated for each treatment ($\bar{x}_1, \bar{x}_2, \bar{x}_3, \text{etc.}$). Sample variance is then calculated for each treatment as follows, where n = sample size, x_i = term in data set, and \bar{x} is the sample mean:

$$s^2 = \frac{\sum (x_i - \bar{x})^2}{n - 1}$$

From here, one may then compute the mean square due to treatment (MST) and mean square due to error (MSE), as follows, where k is the number of populations tested:

$$MST = \frac{n_1(\bar{x}_1 - \bar{x})^2 + n_2(\bar{x}_2 - \bar{x})^2 + \dots + n_k(\bar{x}_k - \bar{x})^2}{k - 1}$$

$$MSE = \frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2 + \dots + (n_k - 1)s_k^2}{n - k}$$

After calculation of the MST and MSE, the F-test statistic may be calculated as follows:

$$F = \frac{MST}{MSE}$$

This determined F-value may then be compared to a F-distribution table to find out the statistical significance of the F-test, and consequently calculated into a p-value [68]. The advantage of using an F-test over paired t-tests for means is that it is able to assess multiple population means with the use of one test rather than using a paired t-test repeatedly [68]. Repeated use of a paired

t-test will lead to an increased chance of a Type I error or a false positive, which in the context of this study would mean that EPA/DHA supplementation would have a measurable effect on circulating inflammatory markers when there is none.

The aforementioned statistical analyses was performed using Statistical Analysis System (SAS) software, version 9.4, courtesy of the UCF Data Mining Lab. A total of 24 studies were collected in this analysis, totaling 2087 subjects for comparison. Analysis of specific CRP, TNF- α , IL-6, and IL-1 β values utilized 1850, 1231, 1290, and 150 subjects for each marker, respectively.

LITERATURE REVIEW

The requirement for n-3 acids in the human diet is due to the absence of a $\Delta 15$ desaturase enzyme, requiring humans to either directly consume EPA, DHA, or ALA to obtain the EPA/DHA products in the bloodstream [69]. Metabolically, humans can only desaturate fatty acids up to the $\Delta 9$ position, making them unable to synthesize EPA/DHA without utilizing an ALA carbon backbone from diet (Figure 1, Figure 2) [69]. DHA conversion from ALA is rate-limited in humans by the enzyme $\Delta 6$ desaturase, although increased DHA production may be induced through consumption of fucoxanthin, a xanthophyll found in brown algae [70-72]. If low serum levels of DHA are present, downregulation of phospholipases A₂ (PLA₂s) and cyclooxygenase-1 (COX1) can occur, leading to reduced prostaglandin synthesis [73, 74]. As prostaglandins play a diverse number of roles in the body, relatively low amounts of prostaglandins within cells may have a detrimental effect on an organism. Various prostaglandin roles in humans are provided below:

Table 3: Sample of Various Prostaglandin Roles in Humans

Prostaglandin Function	Supporting Literature
Vasodilation	[75]
Lipolysis Inhibition	[76]
Uterine Contraction	[77]
Bronchodilation	[78]
↑ Autonomic Transmitters	[79]

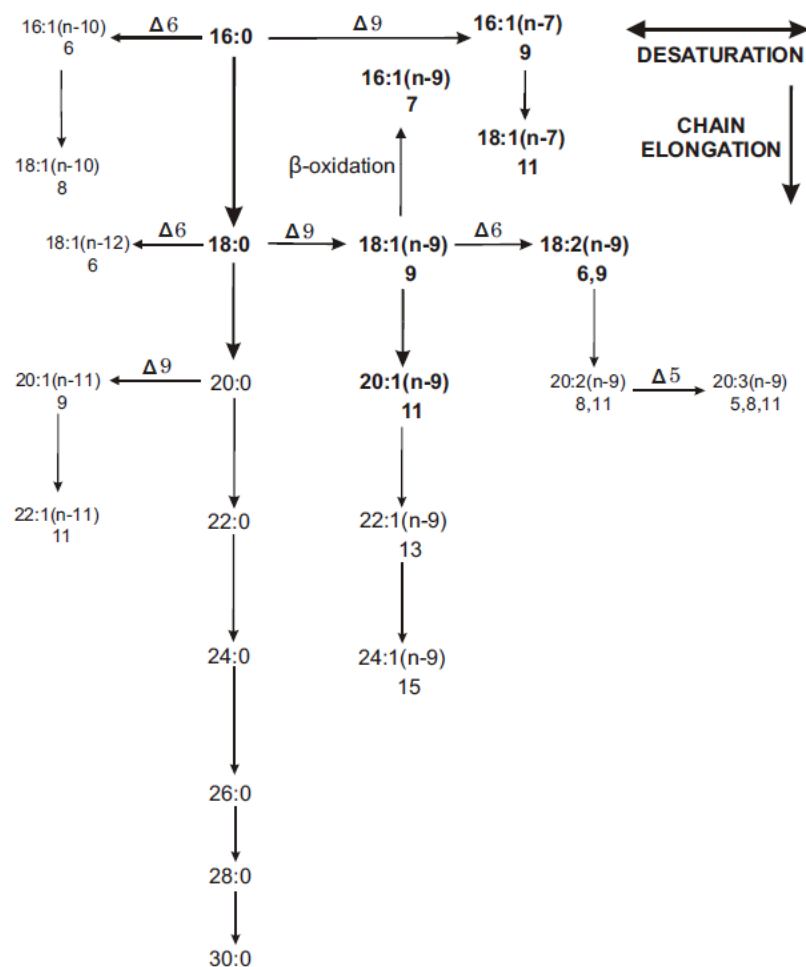


Figure 1. Elongation and Desaturation of Endogenous Fatty Acids in Humans Cannot Produce ALA, EPA, or DHA.

In the above figure, “Δn” catalysts represent the desaturase used to convert one product to the next. Numbers below the compounds represent the carbon position of the double-bonds within the fatty acid molecule. For reference, nomenclature used for ALA/EPA/DHA is 18:3(n-3), 20:5(n-3), and 22:6(n-3), respectively. As can be observed, neither ALA, EPA, nor DHA is present in the above figure, as none of these compounds can be synthesized endogenously under normal conditions.

Adapted from “FATTY ACIDS AS BIOCOMPOUNDS: THEIR ROLE IN HUMAN METABOLISM, HEALTH AND DISEASE – A REVIEW. PART 1: CLASSIFICATION, DIETARY SOURCES AND BIOLOGICAL FUNCTIONS,” by E. Tvrzicka et al., 2011, *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*, 155, p. 117-130. Copyright 2011 by E Tvrzicka et al. Adapted with permission.[69].

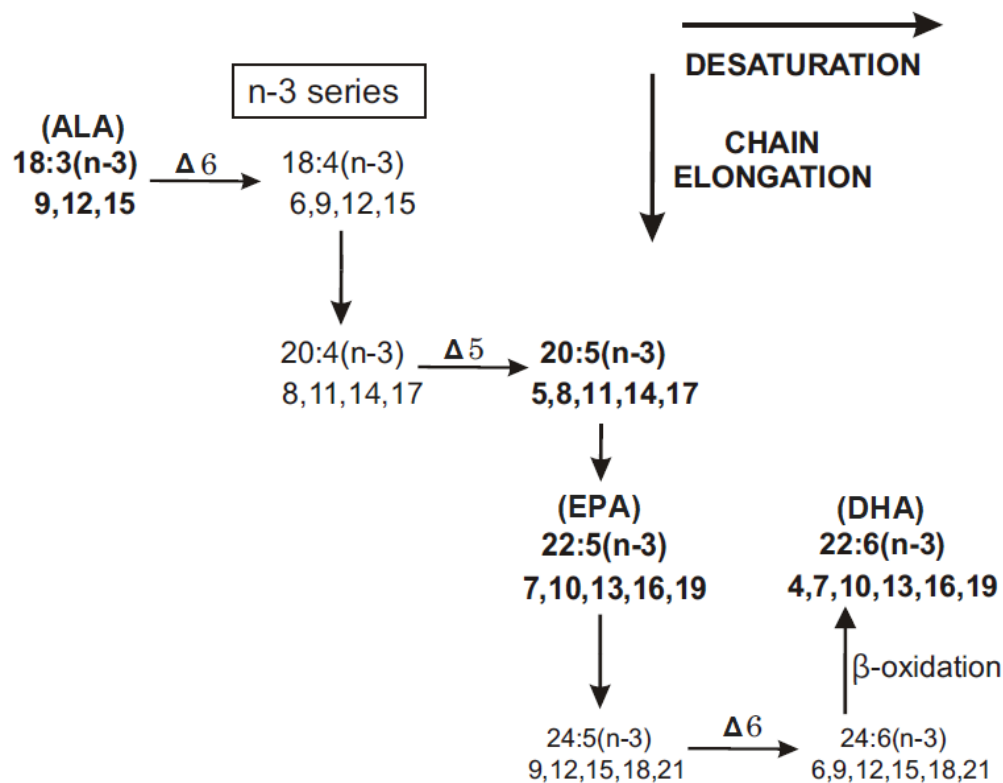


Figure 2. Fatty Acid Synthesis in Humans of EPA and DHA from ALA.

In the above figure, number catalysts (Δn) indicate the desaturase used in humans to convert one product to the next. Numbers listed below the compounds represent the carbon position of the double-bonds within the respective fatty acid molecule. As can be observed in conjunction with the previous figure, only direct consumption of ALA, EPA, or DHA results in EPA/DHA being produced or utilized in the body. This is due to the necessity of the ALA carbon backbone.

Adapted from "FATTY ACIDS AS BIOCOMPOUNDS: THEIR ROLE IN HUMAN METABOLISM, HEALTH AND DISEASE – A REVIEW. PART 1: CLASSIFICATION, DIETARY SOURCES AND BIOLOGICAL FUNCTIONS," by E. Tvrzicka et al., 2011, *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*, 155, p. 117-130. Copyright 2011 by E Tvrzicka et al. Adapted with permission. [69]

PLA2s function to release the central fatty acid of a glycerol molecule, while COX1 converts free arachidonic acid to prostaglandin H₂ [80, 81]. As DHA is a substrate for both of these enzymes, this may result in lifespan extension of an average DHA molecule in the body [82]. Notably, increases of dietary DHA have also been found to decrease DHA synthesis *in vivo*, suggesting that the body attempts to maintain steady circulating levels of DHA [83]. In regards to n-3 deficiencies, issues have only been observed in studies where multi-generational depletion of EPA/DHA has occurred. The impact of such deficiencies appears to primarily affect the eyes and brain [84, 85].

Individual cellular processes contributing to inflammation can be mediated through derivatives of EPA and DHA known as resolvins [6]. The production of resolvins occurs through the initial EPA/DHA conversion to 15-HEPE and 15-H(p)DHA, respectively [86, 87]. These compounds can then be converted into their respective EPA and DHA derivatives, each with individual receptor interactions. The resulting EPA derivatives (E series, RvE1, RvE2) are the result of two pathways, including a lipoxygenase pathway (R isomers) and a COX2/P450 pathway (S isomers), diagrammed on the following page (Figure 2) [87, 88]. RvE1 and RvE2 have been observed to help promote microbial clearance and reduce general inflammatory response [89-91]. Resulting DHA derivatives (D series, RvD1, RvD2) may undergo a LOX pathway (R isomers) or an aspirin-inducible pathway (S isomers), provided in the next following pages (Figure 3) [87, 92-94]. RvD1 and RvD2 have been shown to be anti-inflammatory through downregulation of TNF- α related cytokine expression in microglia of the immune and nervous system [93]. It is also believed that both RvE1 and RvD1 may act as ligands of the ChemR23

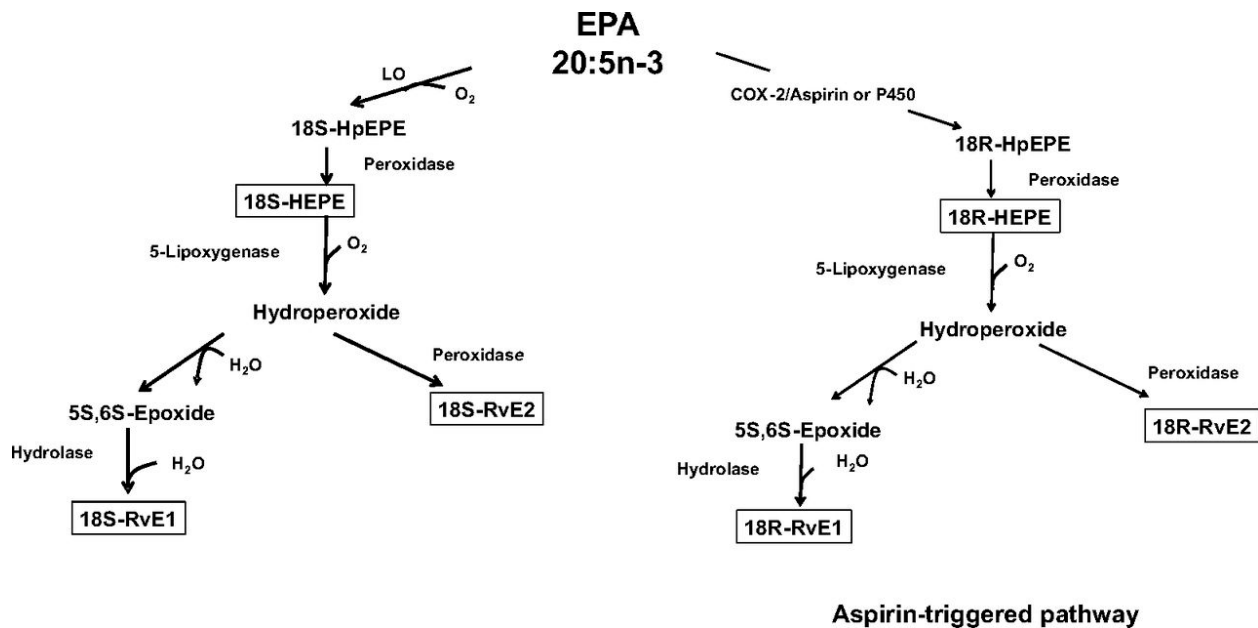


Figure 3. EPA Metabolic Pathway. Rv Represents Resolvin.

Adapted from “Resolvins D1, D2, and Other Mediators of Self-Limited Resolution of Inflammation in Human Blood following n-3 Fatty Acid Supplementation,” by E. Mas et al., 2012, *CLINICAL CHEMISTRY*, 58, p. 1476-1484. Copyright 2012 by The American Association for Clinical Chemistry. Adapted with permission. [87]

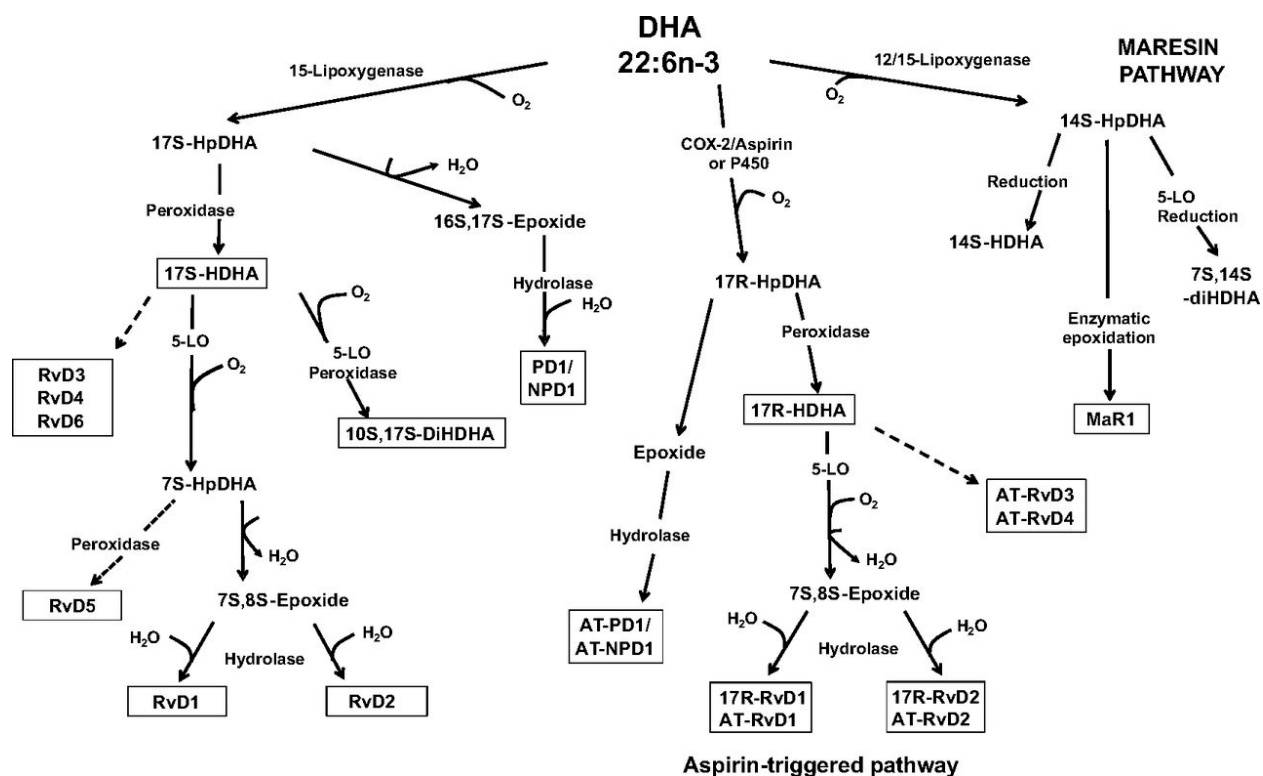


Figure 4. DHA Metabolic Pathway. Rv Represents Resolvin.

Adapted from "Resolvins D1, D2, and Other Mediators of Self-Limited Resolution of Inflammation in Human Blood following n-3 Fatty Acid Supplementation," by E. Mas et al., 2012, *CLINICAL CHEMISTRY*, 58, p. 1476-1484. Copyright 2012 by The American Association for Clinical Chemistry. Adapted with permission. [87]

GPRC receptor, resulting in analgesic effects [95-97].

Hydrolysis of cellular triglycerides via phospholipases A₂ is stimulated in times of metabolic or cellular stress [98, 99]. Hydrolysis can potentially release DHA from a glycerol backbone, permitting synthesis of RvD1 and RvD2 [80]. DHA cleavage from a triglyceride produces 17SH(p)DHA, which is converted to a 16(17)-epoxide which rearranges into neuroprotectin D1 (NPD1) [100]. NPD1 impairs IL- β based COX2 induction, resulting in a reduced inflammatory response and decreased β -amyloid aggregation via a PPAR γ dependent mechanism [101]. An alternate DHA derivative which has also been found to help downregulate inflammation is Maresin 1, produced by 12-lipoxygenase [102].

EPA and DHA have also been found to decrease NF- κ B signaling in macrophages, which provides another route by which inflammation can be suppressed by n-3 supplementation [60, 103]. The n-3 fatty acids are believed to bind to free fatty acid receptors, leading to anti-inflammatory effects [104]. Adipocytes have also been found to secrete fewer inflammatory cytokines under n-3 supplementation [50]. EPA/DHA have been observed to reduce IL-2 secretion, potentially decreasing inflammation further as IL-2 stimulates TNF- α and IL-1 α/β secretion [105]. Another mechanism by which EPA/DHA may work to reduce inflammation does not involve inflammatory cytokines at all. EPA/DHA supplementation has been found to decrease levels of leukotriene B₄, a strong lipid chemoattractant which recruits leukocytes to areas of inflammation [106]. Decreased expression of this lipid mediator may lead to decreased cell motility, which can consequently reduce inflammation levels in living tissue, although this may not be desired in times of illness [107]. Notably, in murine models, it has been noted that n-3 supplementation has led to delayed recovery time from influenza, although direct trials on

humans have yet to be conducted [108-110]. A sample table of proposed mechanisms have been provided below summarizing the literature of how EPA/DHA supplementation may reduce overall inflammation:

Table 4: Sample List by which EPA/DHA May Downregulate Inflammation

Method	Supporting Literature
EPA/DHA suppress NF- κ B signaling in macrophages	[60, 103]
EPA derivatives RvE1/RvE2 promote microbial clearance and reduced inflammatory response	[89-91]
DHA derivatives RvD1/RvD2 downregulate TNF- α related cytokine expression in microglia of immune system	[93]
DHA derivative NPD1 impairs COX2 induction, initiated by IL-1 β , resulting in a reduced inflammatory response	[101]
DHA derivative Maresin 1 holds strong anti-inflammatory properties	[102]
EPA/DHA decrease IL-2 secretion, a cytokine which stimulates TNF- α and IL-1 α/β production	[105]
EPA/DHA supplementation decreases levels of leukotriene B ₄ , a strong leukocyte chemoattractant	[106]
High EPA doses of EPA (500mg/kg) reduce PPAR α /PPAR γ expression in muscle cells and reduce production of TNF- α /IL-6	[111]

Primary markers for measuring inflammation include CRP, TNF- α , IL-6, and IL-1 β . CRP is an acute-phase protein associated with inflammation that can be induced by IL-6 secretion [62-67]. CRP serves to bind lysophosphatidylcholine, activating the classical pathway of complement [62]. TNF- α is another acute-phase protein with pyrogenic properties, capable of initiating cell death among a variety of other actions [66]. Its main function is to regulate immune cells through the binding of the receptors TNFR1 and TNFR2 [67]. IL-6 enhances the immune response and is generally associated with inflammation, although it has since been found to exhibit anti-inflammatory properties as a myokine [67]. IL-1 β is a pyrogenic cytokine which helps modulate cell proliferation, differentiation, apoptosis, and activation of the

PTGS2/COX2 pathway [63-65]. A suggested normal range for inflammatory markers CRP, TNF- α , IL-6, and IL-1b in the human bloodstream is provided in the table below.

Table 5: Normal Ranges for Inflammatory Markers

Inflammatory Marker	Normal Range
CRP	0-3 mg/dL [112, 113]
TNF- α	0-60 pg/mL [113-115]
IL-6	0-300 pg/mL [113, 114]
IL-1 β	0-.03 pg/mL [113]

OBJECTIVES

Current literature is mixed regarding the influence of EPA/DHA supplementation on the inflammatory markers CRP, TNF- α , IL-6, and IL-1 β . This project aims to conduct a meta-analysis to determine the general effect of EPA/DHA supplementation on circulating inflammatory markers in healthy populations. The goals of this project are:

- 1). To locate a number of studies via Pubmed written in English for which inflammatory markers in otherwise healthy people were recorded before and after a period of EPA/DHA supplementation, in which the dosage and frequency of EPA/DHA supplementation have been provided, and no conflicts of interest were declared. This was to be performed by searching for terms “fish oil inflammation,” “EPA inflammation,” and “DHA inflammation.”
- 2.) To conduct a systematic analysis of the aforementioned studies to determine if there is an association between increased EPA/DHA supplementation and circulating inflammatory markers. This is to be performed using SAS v.9.4, courtesy of UCF’s data mining lab.
- 3.) To determine the degree in which inflammatory markers were affected, relative to the level of EPA/DHA consumed by the subjects across all studies.
- 4.) To suggest a therapeutic threshold or scale of EPA/DHA consumption to reduce inflammation based upon the observed studies, given an association exists.

METHODS

For this project, a database search of Pubmed was performed using the keywords “fish oil inflammation,” “EPA inflammation,” and “DHA inflammation.” The resulting articles were screened and reviewed for studies written in English for which EPA/DHA dosage had been provided, inflammatory markers before and after supplementation had been recorded for at least a month, and for which no conflicts of interest had been declared. For studies that used repeated subjects for varied dosages, a minimum 4-week wash-out period was required for use in this study. The subjects used in the studies were deemed otherwise “healthy” by the reviewers, and populations with pre-existing chronic conditions were excluded for the purpose of this analysis. Starting with a total of 2,666 hits, the database produced 24 studies after screening. The studies themselves were recorded for a percentage increase or decrease of inflammatory factor levels after EPA/DHA supplementation was administered. For the purposes of this study, results were considered significant at $P < .05$.

The resulting data pooled from the studies were analyzed via paired t-tests and F-tests using the SAS system to determine how circulating inflammatory markers were affected by EPA/DHA supplementation. Paired t-tests were used to assess whether a statistically significant difference in the respective marker levels was present pre- and post-EPA/DHA supplementation, while F-tests were used to assess for the potential independence and dependence of the effects of EPA and DHA supplementation on the measured inflammatory markers. As paired t-tests function to analyze whether a statistically difference in means is present within a population, it was considered an appropriate test for analysis. With F-tests examining changes in variance and elucidating data about subpopulation means, it was similarly considered an appropriate test to

run statistical analysis. In addition, the F-test had the advantage of being able to analyze more than two populations at once, diminishing the probability of a Type I error present if t-tests had been used instead [68]. A resulting contour plot from the pooled studies for each inflammatory marker was generated afterwards to provide a predictive model regarding EPA/DHA supplementation. By developing multiple models assuming dependence or independence of EPA or DHA and comparing the results from each to actual data, a general idea of the interaction between EPA and DHA could be obtained.

Since independence or dependence of the two compounds on inflammatory factors was unknown prior to analysis, F-tests for independence were run first, and linear models based on the resulting data were generated. Afterwards, a residual by regressors plot was included to check whether or not the resulting plots for EPA and DHA, assuming independence, were a reasonable model for the data. F-test data also found a likely interaction assuming dependence. Based on this finding, a resulting contour plot modeling the study data was then generated for each inflammatory marker, which revealed that the prior linear models generated for EPA/DHA supplementation were not adequate to account for the observed study outcomes regarding EPA and DHA supplementation. As the contour plot was based upon prior study outcomes, it provided projections regarding anticipated circulating inflammatory marker levels under different EPA/DHA dosages. The analyses and models assuming EPA and DHA independence were included to provide a more complete view of the project, as well as to provide models for comparison, from which the study conclusions were drawn.

RESULTS

A total of 24 studies were collected for this study, resulting in a total of 2087 individuals and 2234 observations. The total study population was approximately 59% male. The average subject age was 51.1 with a BMI <28.6. It was hypothesized that supplementation with EPA/DHA, if effective, would decrease the levels of inflammatory markers within the participants after treatment. For the purpose of this analysis, paired t-tests were used, which analyze whether a difference in means is present within a population. By checking the means before and after EPA and DHA supplementation, it was found that only circulating levels of TNF- α were significantly affected by EPA/DHA supplementation (CRP, $p=.6894$; TNF- α , $p=.0068$; IL-6, $p=.5030$; IL-1 β , $p=.1756$). Afterwards, F-tests were run to assess for whether or not EPA and DHA effects were independent. F-tests allow for the testing of differences in variance among more than two groups, which can reveal data about changes in population means [68]. Notably, after running F-tests with the assumption that EPA and DHA effects were independent, EPA and DHA supplementation were generally found to affect all measured inflammatory markers with statistical significance (CRP: EPA, $p=0.0140$, DHA, $p<.0001$; TNF- α : EPA, $p=.5550$, DHA, $p<.0001$; IL-6: EPA, $p<.0001$, DHA, $p<.0001$; IL-1 β : EPA, $p=.0116$, DHA, $p<.0001$). Linear models were found to be adequate in modeling both the effects of EPA and DHA assuming independence, as observed in the randomly scattered data within the generated residual by regressors plot. The F-test data for EPA/DHA dependence also found that EPA/DHA likely influence each other in regards to determining circulating inflammatory marker levels (CRP, $p<.0001$; TNF- α , $p<.0001$; IL-6, $p<.0001$; IL-1 β , $p=.0015$). To model this EPA/DHA interaction, a contour plot was generated based upon the study data. The resulting

contour plot suggested that the previously generated linear models were inadequate to account for the patterns emerging from EPA/DHA study outcomes. Data for each inflammatory marker before and after supplementation is provided in the pages that follow.

C-reactive protein

A total of 17 studies [18, 20, 54, 57, 58, 116-127] were used for analysis of CRP levels, yielding 1850 individuals and 1997 observations. The average subject age was 48 years with a BMI of 26.8. Paired t-testing, which was used to assess for a statistically significant change in mean inflammatory marker levels before and after EPA and DHA supplementation, revealed no significant impact of EPA/DHA supplementation on circulating CRP levels ($p=.6894$). An F-test was run afterwards to assess for potential individual or combined effects of EPA/DHA supplementation on marker levels. The F-test assuming EPA independence resulted in a statistically significant p-value ($p=.0140$), indicating an effect on CRP levels assuming EPA held independent effects from DHA. A linear model was found adequate in modeling an independent EPA interaction. F-test data assuming DHA independence similarly resulted in a statistically significant p-value ($p<.0001$). As with EPA, a linear model was found adequate in modeling an independent DHA interaction. Running a final F-test assuming EPA/DHA dependence resulted in a statistically significant p-value ($p<.0001$), indicating EPA/DHA may influence each other's overall effect. A resulting contour plot derived from the study data was then generated, which indicated that the prior linear models were no longer adequate predictors of the effect of EPA/DHA on inflammatory marker levels when both compounds were analyzed for dependent effects. Individual effect sizes and graphs depicting the data spread have been provided on the following pages.

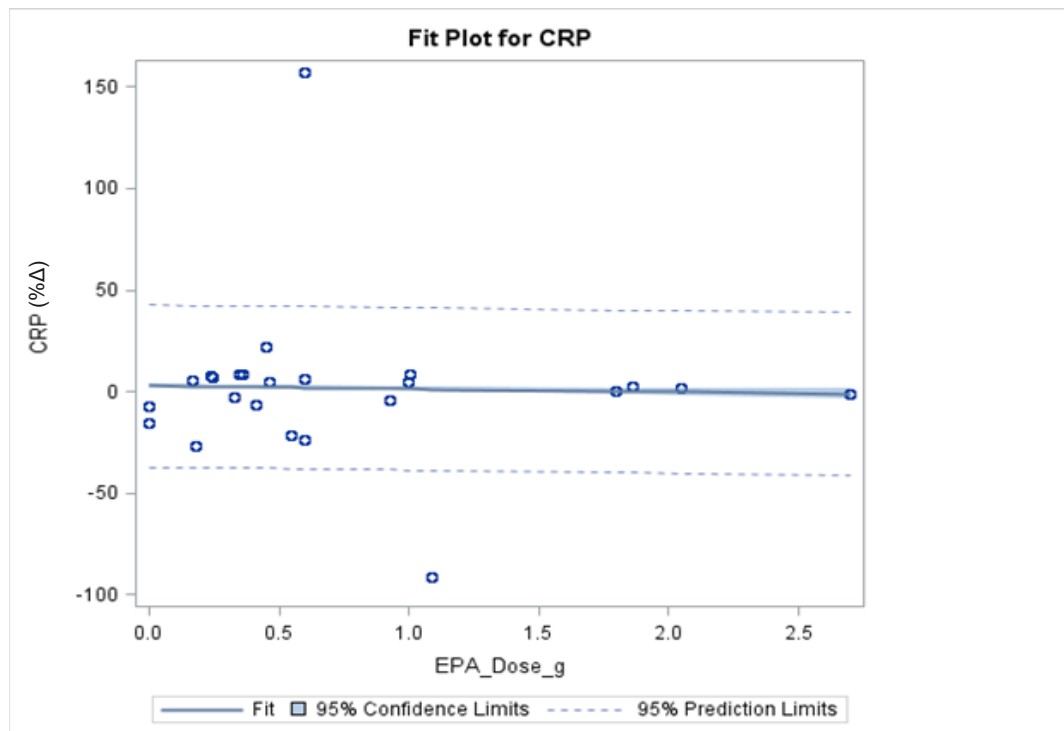


Figure 5. EPA Supplementation Fit Plot for CRP (Assumed Independence)

The figure above provides a linear model of the effect of EPA supplementation on CRP levels based on the 17 aforementioned studies used for this section of the meta-analysis, assuming EPA holds independent effects. Each point on the graph represents a different study outcome. CRP (% Δ) represents the average percentage change in population CRP levels after EPA supplementation, whereas EPA_Dose_g represents the daily EPA dose in grams given to the subjects. All studies in the above graph were weighed by the number of observations for the purpose of this regression. Assuming independent effects from DHA, EPA had a statistically significant effect on CRP levels in the study populations via F-testing ($p=0.0140$). Based on the graph above, supplementation with EPA appears to slightly decrease circulating levels of CRP, assuming independent effects from DHA. However, it is unlikely to make any physiological difference.

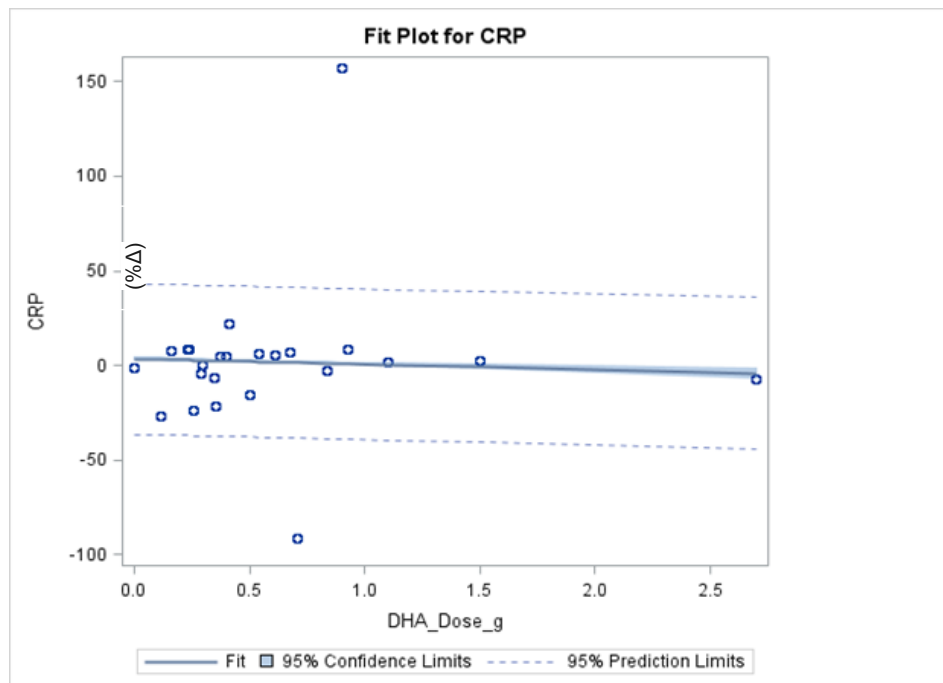


Figure 6. DHA Supplementation Fit Plot for CRP (Assumed Independence).

The figure above provides a linear model of the effect of DHA supplementation on CRP levels based on the 17 aforementioned studies used for this section of the meta-analysis, assuming DHA holds independent effects. Each point on the graph represents a different study outcome. CRP (% Δ) represents the average percentage change in population CRP levels after DHA supplementation, whereas DHA_Dose represents the daily DHA dose in grams given to each of the subjects. All studies in the above graph were weighed by the number of observations for the purpose of this regression. Assuming independent effects from EPA, DHA had a statistically significant effect on CRP levels in the study populations via F-testing ($p < 0.0001$). Based on the graph above, supplementation with DHA appears to slightly decrease circulating levels of CRP, assuming independent effects from EPA. However, it is unlikely to make any physiological difference.

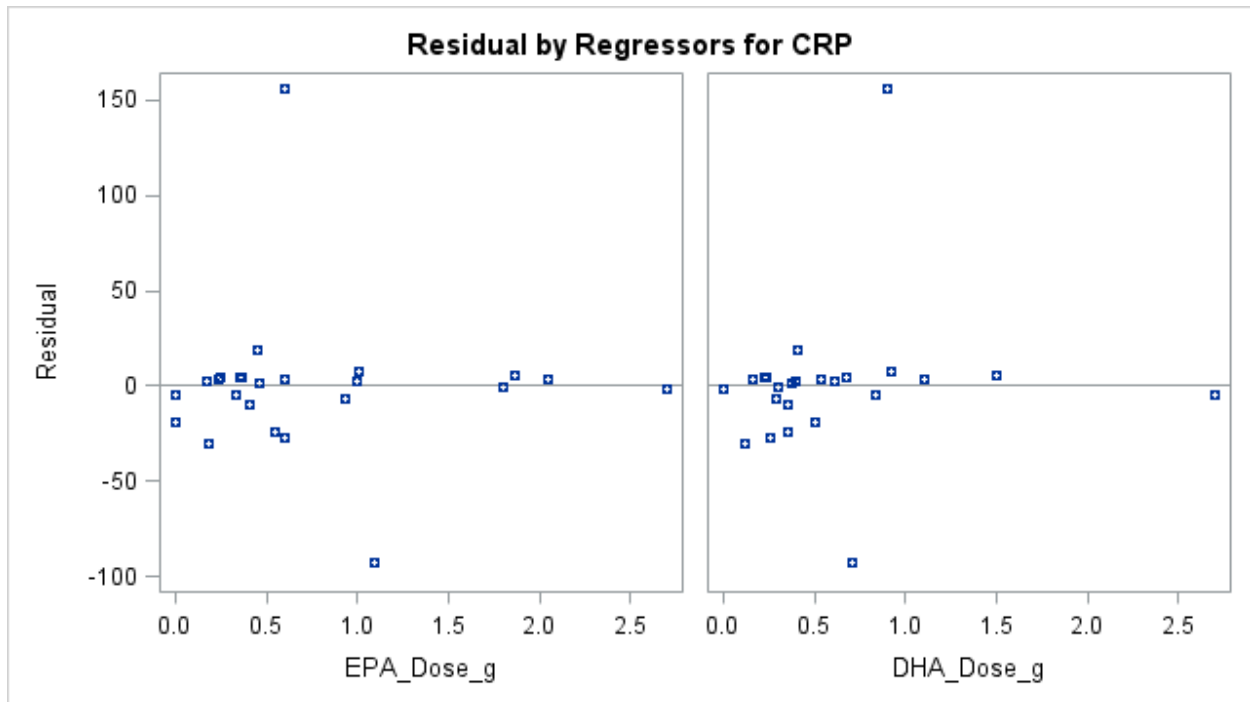


Figure 7. Residual by Regressors for CRP

The figure above provides a residual by regressors plot for CRP, using data from the 17 aforementioned studies. The relatively random dispersion above and below the x-axis suggests that the aforementioned models assuming EPA/DHA independence provide reasonable fits. A visible pattern in the residual by regressors plot, such as a sloped line or parabola, would suggest an unexplained variable not accounted for in the linear plots. EPA_Dose_g and DHA_Dose_g represent the EPA and DHA data used from the studies, respectively.

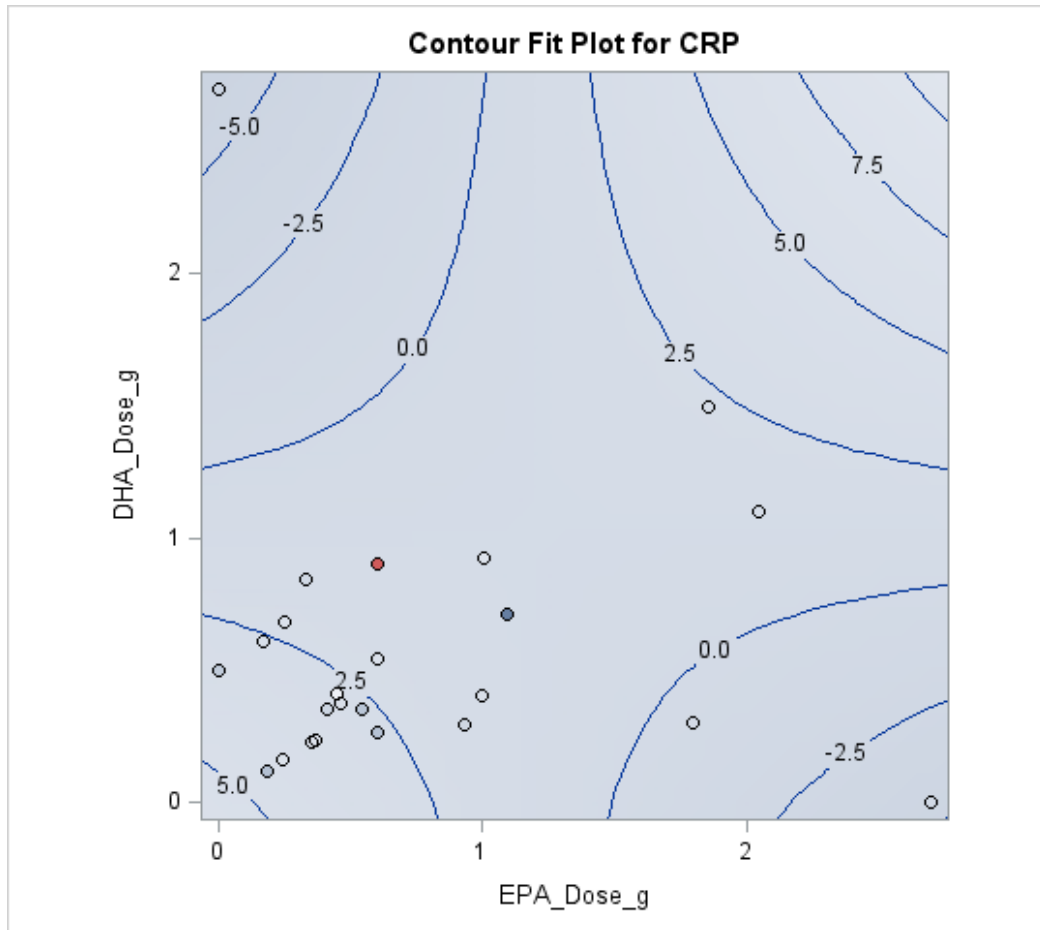


Figure 8. Contour Fit Plot for CRP

The figure above provides a potentially easier way to visualize the 17 aforementioned studies used for this section of the meta-analysis, as well as providing a predictive data plot based upon study data. The plot above is a projection based upon study data from CRP. The y-axis represents daily DHA dosage, while the x-axis represents daily EPA dosage. The labeled lines throughout the chart represent the predicted percentage change in CRP values, while the dots throughout the graph represent individual study outcomes, weighted by number of observations. The red and blue dots represent the recorded maximum and minimum observations from the study data, respectively. As the dots represent individual study outcomes separate from

the graph itself, they may be considered extraneous to the data chart, but they do provide insight regarding individual study outcomes, relative to one another.

Based on this projection modeled from study data and the resulting F-test data, it is likely an interaction between EPA and DHA exists. In addition, the previously used linear models assuming EPA/DHA independence are unable to account for the projections from study data observed in this graph, further suggesting a potential interaction. When assuming independent effects, both compounds were observed to slightly decrease circulating CRP levels based on study data. However, when both EPA and DHA are taken account in one unifying model, it is observed from the graph that whether the level of CRP increases or decreases as the supplementation with one compound increases is dependent upon the supplemented dose of the alternative compound based upon study data and projections. This suggests a potential interaction.

Based on this resulting contour plot, it appears that EPA/DHA supplementation reduces circulating CRP levels best when the compounds are taken individually and in relatively large doses. Notably, DHA appears to have a stronger overall effect gram per gram on CRP levels than EPA. Supplementation of the respective compounds appears to generally have a null effect or increase CRP levels when taken in similar doses. However, at the noted percentage differences, such supplementation is unlikely to produce any physiological change, and supplementation will likely have no noticeable effect.

TNF- α

A total of 19 studies [18, 20, 52, 55, 57, 116, 118-124, 126, 128-132] were used for analysis of TNF- α levels, yielding 1231 individuals, and 1378 observations. The average subject age was 39 years with a BMI of <26.8. Paired t-testing, which was used to assess for a statistically significant change in mean inflammatory marker levels before and after EPA and DHA supplementation, revealed a significant impact of EPA/DHA supplementation on TNF- α levels ($p=.0068$). An F-test was run afterwards to assess for potential individual or combined effects of EPA/DHA supplementation on marker levels. The F-test assuming EPA independence resulted in a p-value of $p=.5550$, indicating no statistically significant correlation with TNF- α levels, assuming EPA held independent effects. A linear model was found adequate in modeling the EPA non-interaction. F-test data assuming DHA independence resulted in a statistically significant p-value ($p<.0001$). Running a final F-test assuming EPA/DHA dependence resulted in a statistically significant p-value ($p<.0001$), indicating EPA/DHA may influence each other's overall effect. A resulting contour plot derived from the study data was then generated, which indicated that the prior linear models assuming effect independence were no longer adequate predictors of the effect of EPA/DHA on inflammatory marker levels when both compounds were examined for dependent effects. Individual effect sizes and graphs depicting the data spread have been provided on the following pages.

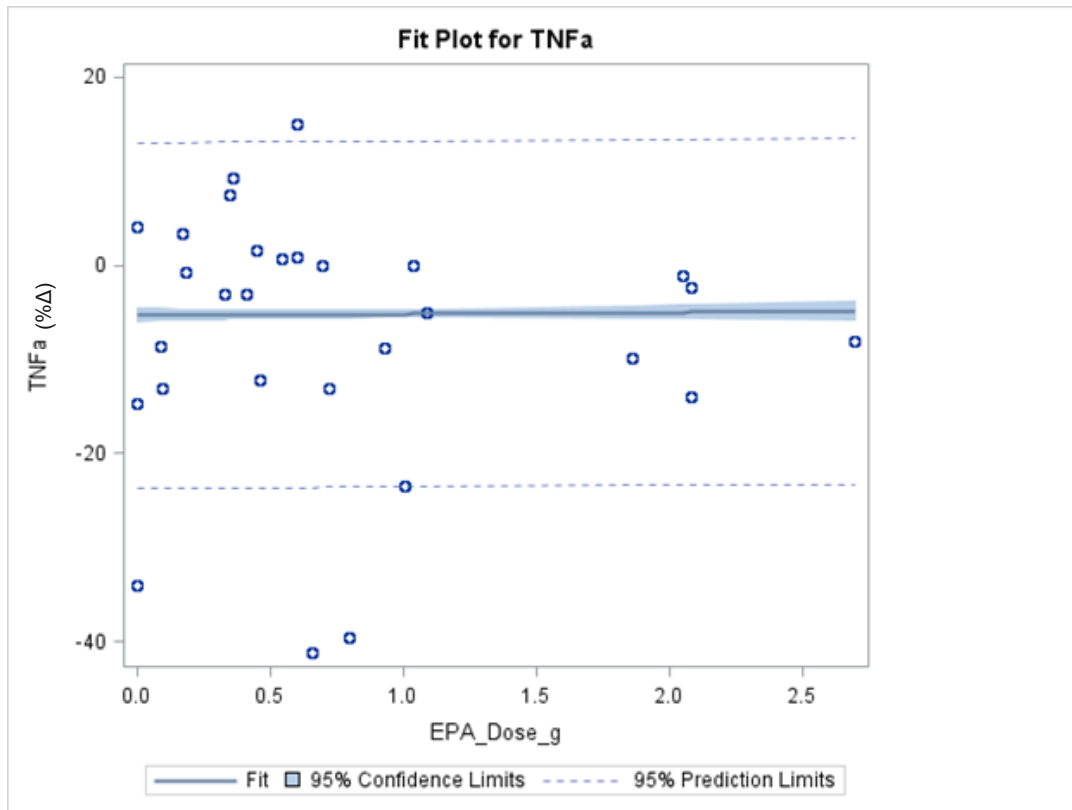


Figure 9. EPA Supplementation Fit Plot for TNF- α (Assumed Independence)

The figure above provides a linear model of the effect EPA supplementation has on TNF- α levels based on the 19 aforementioned studies used for this section of the meta-analysis, assuming EPA holds independent effects. Each point on the graph represents a different study outcome. TNFa (% Δ) represents the average percentage change in population TNF- α levels after EPA supplementation, whereas EPA_Dose_g represents the daily EPA dose in grams given to the subjects. All studies in the above graph were weighed by the number of observations for the purpose of this regression. Assuming independent effects from DHA, EPA did not have a statistically significant effect on TNF- α levels in the study populations via F-testing ($p=.5550$). Based on the graph above, supplementation with EPA appears to have no effect on circulating levels of TNF- α , assuming independent effects from DHA.

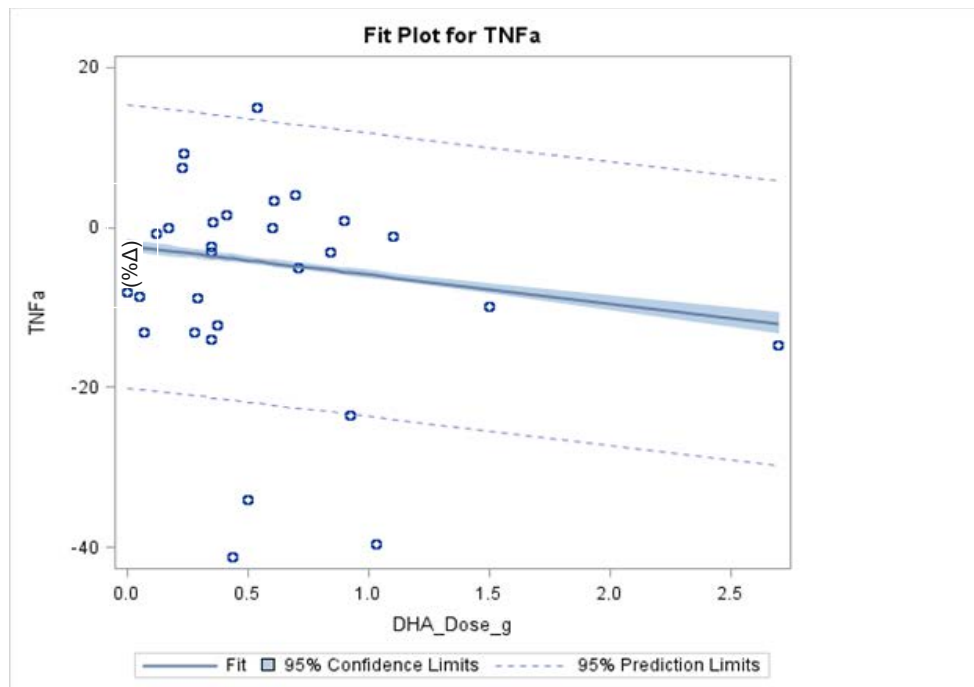


Figure 10. DHA Supplementation Fit Plot for TNF- α (Assumed Independence)

The figure above provides a linear model of the effect DHA supplementation has on TNF- α levels based on the 19 aforementioned studies used for this section of the meta-analysis, assuming DHA holds independent effects. Each point on the graph represents a different study outcome. TNF α (% Δ) represents the average percentage change in population TNF- α levels after DHA supplementation, whereas DHA_Dose_g represents the daily DHA dose in grams given to the subjects. All studies in the above graph were weighed by the number of observations for the purpose of this regression. Assuming independent effects from EPA, DHA had a statistically significant effect on TNF- α levels in the study populations ($p < .0001$). Based on the graph above, supplementation with DHA appears to reduce circulating levels of TNF- α , assuming independent effects from EPA.

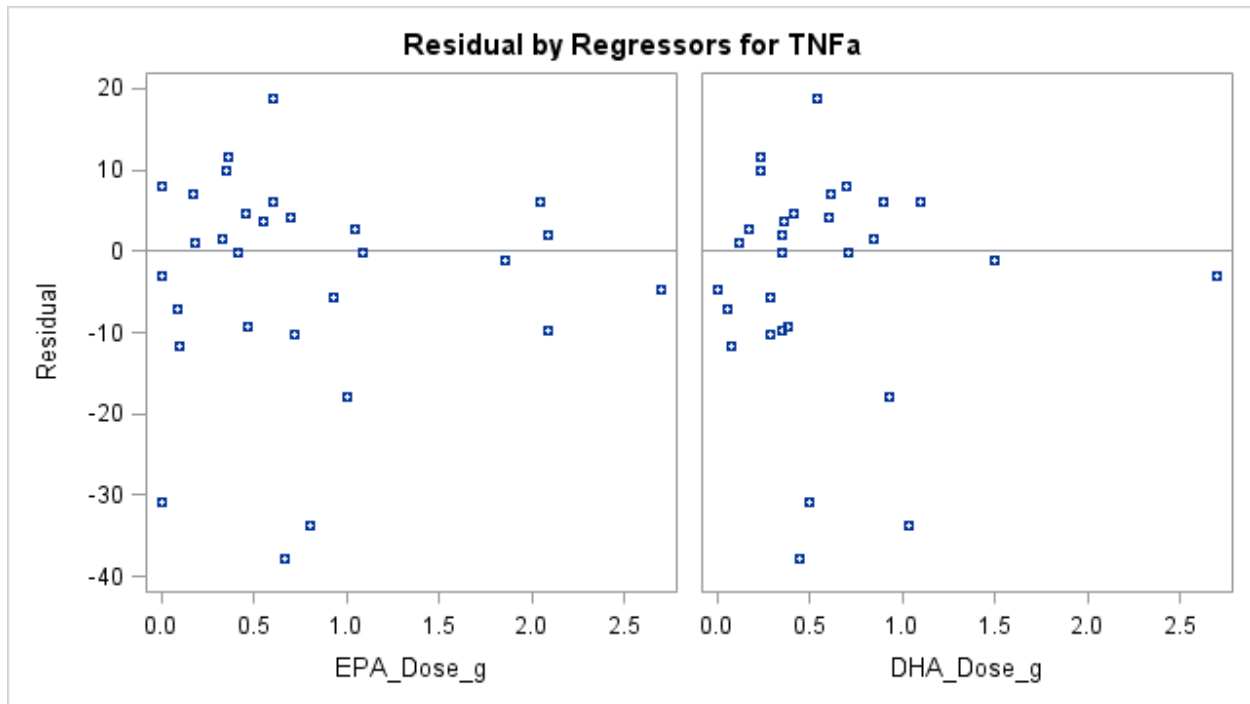


Figure 11. Residual by Regressors Plot for TNF- α

The figure above provides a residual by regressors plot for TNF- α , using data from the 19 aforementioned studies. The relatively random dispersion above and below the x-axis suggests that the aforementioned models assuming EPA/DHA independence provide reasonable fits. A visible pattern in the residual by regressors plot, such as a sloped line or parabola, would suggest an unexplained variable not accounted for in the linear plots. EPA_Dose_g and DHA_Dose_g represent the EPA and DHA data used from the studies, respectively.

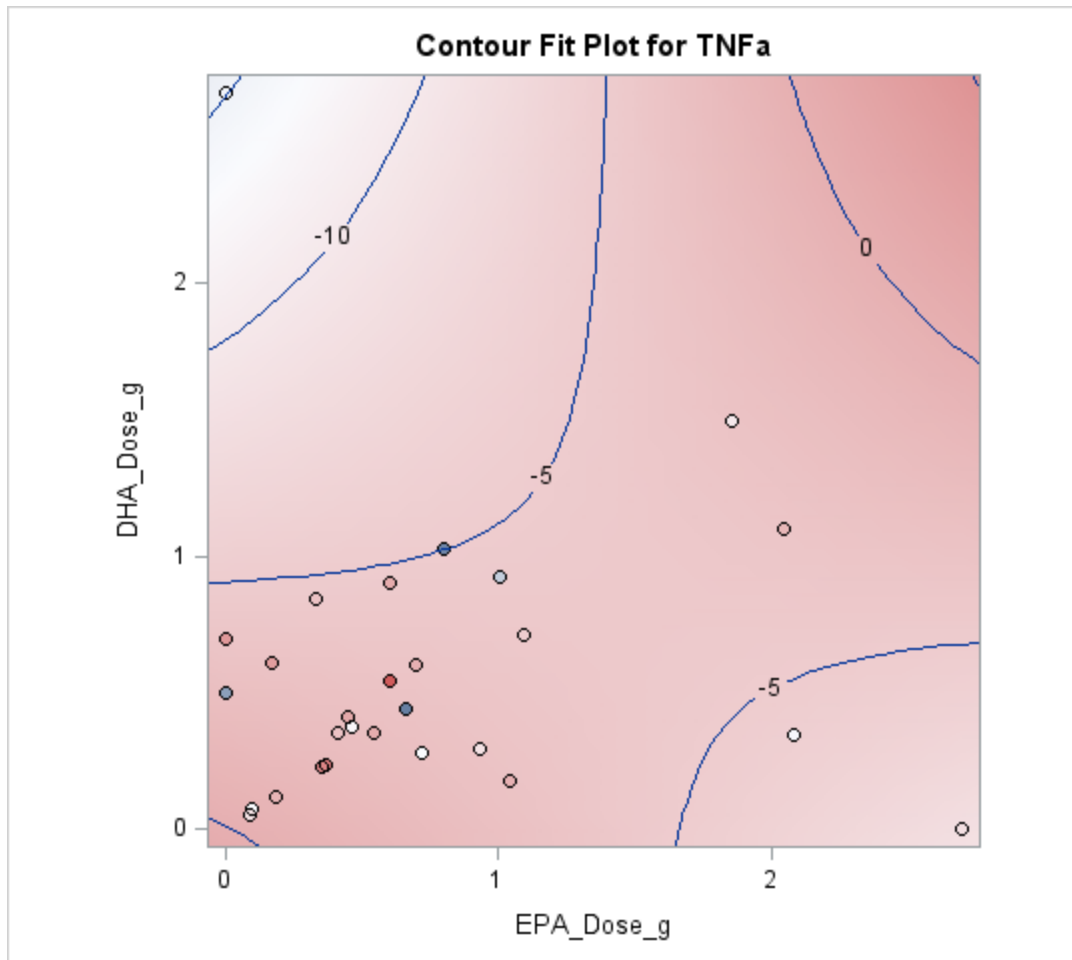


Figure 12. Contour Fit Plot for TNF- α

The figure above provides a potentially easier way to visualize the 19 aforementioned studies used for this section of the meta-analysis, as well as providing a predictive data plot based upon study data. The plot above is a projection based upon study data from TNF- α . The y-axis represents daily DHA dosage, while the x-axis represents daily EPA dosage. The labeled lines throughout the chart represent the predicted percentage change in TNF- α values, while the dots throughout the graph represent individual study outcomes, weighted by number of observations. The red and blue dots represent the recorded maximum and minimum observations from the study data, respectively. As the dots represent individual study outcomes separate from

the graph itself, they may be considered extraneous to the data chart, but they do provide insight regarding individual study outcomes, relative to one another.

Based on this projection based upon study data, it is likely an interaction between EPA and DHA exist as the previously used linear models which were found adequate assuming EPA/DHA independence are unable to account for the projections from study data observed in this graph. Notably, assuming independence, increases of DHA supplementation are observed to decrease circulating TNF- α levels, while supplementation of EPA was found to have a neutral effect. However, when both EPA and DHA are taken account into one unifying model, it can be observed from the graph that the decreases or relatively neutral effect of DHA and EPA respectively do not follow a linear, predictable pattern with increases; the curvature of the contour lines suggests interplay between EPA and DHA, and potentially other compounds.

For TNF- α , this resultant contour plot suggests that supplementation of EPA/DHA generally decreases circulating levels of TNF- α in the bloodstream. Interestingly, EPA had no effect when independently tested for impact on TNF- α , but when DHA was included in the statistical model, a dependent effect was observed, suggesting EPA may require co-supplementation with DHA to exhibit effects. DHA also appeared to have a stronger anti-inflammatory effect than EPA when compared gram per gram, although supplementation in reasonable doses tends to yield an overall net decrease in circulating TNF- α levels. As TNF- α has been implicated in the development of a number of chronic diseases, this could be a potential benefit of EPA/DHA supplementation.

IL-6

A total of 20 studies [18, 20, 52, 55, 57, 58, 116, 118-126, 128, 131] were used for analysis of IL-6 levels, yielding 1290 individuals, and 1437 observations. The average subject age was 39 years with a BMI of <26.9. Paired t-testing, which was used to assess for a statistically significant change in mean inflammatory marker levels before and after EPA and DHA supplementation, revealed no significant impact of EPA/DHA supplementation on IL-6 levels ($p=.5030$). An F-test was run afterwards to assess for potential individual or combined effects of EPA/DHA supplementation on marker levels. The F-test assuming EPA independence resulted in a statistically significant p-value ($p>.0001$), indicating an effect on TNF- α levels assuming EPA held independent effects. A linear model was found adequate in modeling an independent EPA interaction. F-test data assuming DHA independence resulted in a statistically significant p-value ($p<.0001$). As with EPA, a linear model was found adequate in modeling an independent DHA interaction. Running a final F-test for EPA/DHA dependence resulted in a statistically significant p-value ($p<.0001$), indicating EPA/DHA may influence each other's overall effect. A resulting contour plot derived from the study data was generated, which indicated that the prior linear models were no longer adequate predictors of the effect of EPA/DHA on inflammatory marker levels when both compounds were examined for dependent effects. Individual effect sizes and graphs depicting the data spread have been provided on the following pages.

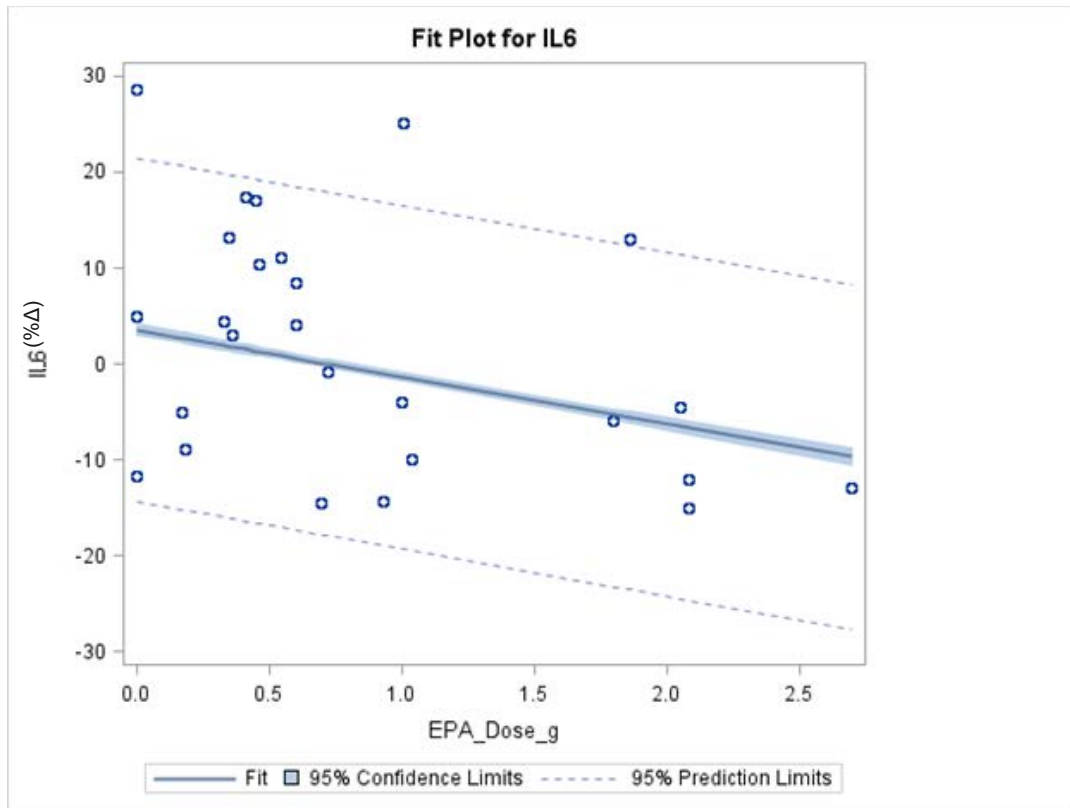


Figure 13. EPA Supplementation Fit Plot for IL-6 (Assumed Independence)

The figure above provides a linear model of the effect EPA supplementation has on IL-6 levels based on the 20 aforementioned studies used for this section of the meta-analysis, assuming EPA holds independent effects. Each point on the graph represents a different study outcome. IL6 (% Δ) represents the average percentage change in population IL-6 levels after EPA supplementation, whereas EPA_Dose_g represents the daily EPA dose in grams given to the subjects. All studies in the above graph were weighed by the number of observations for the purpose of this regression. Assuming independent effects from DHA, EPA had a statistically significant effect on IL-6 levels in the study populations via F-testing ($p < .0001$). Based on the graph above, supplementation with EPA appears to significantly reduce circulating levels of IL-6, assuming independent effects from DHA.

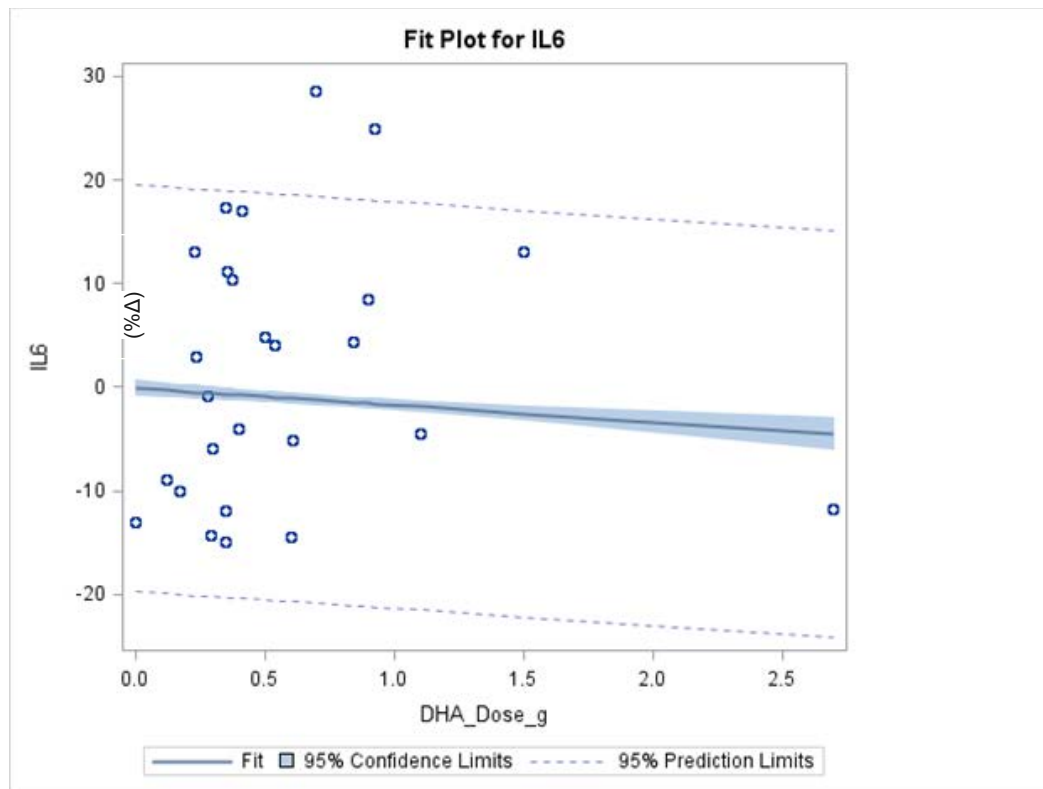


Figure 14. DHA Supplementation Fit Plot for IL-6 (Assumed Independence)

The figure above provides a linear model of the effect DHA supplementation has on IL-6 levels based on the 20 aforementioned studies used for this section of the meta-analysis, assuming DHA holds independent effects. Each point on the graph represents a different study outcome. IL6 (% Δ) represents the average percentage change in population IL-6 levels after DHA supplementation, whereas DHA_Dose_g represents the daily DHA dose in grams given to the subjects. All studies in the above graph were weighed by the number of observations for the purpose of this regression. Assuming independent effects from EPA, DHA had a statistically significant effect on IL-6 levels in the study populations via F-testing ($p < .0001$). Based on the graph above, supplementation with DHA appears to slightly reduce circulating levels of IL-6, assuming independent effects from EPA.

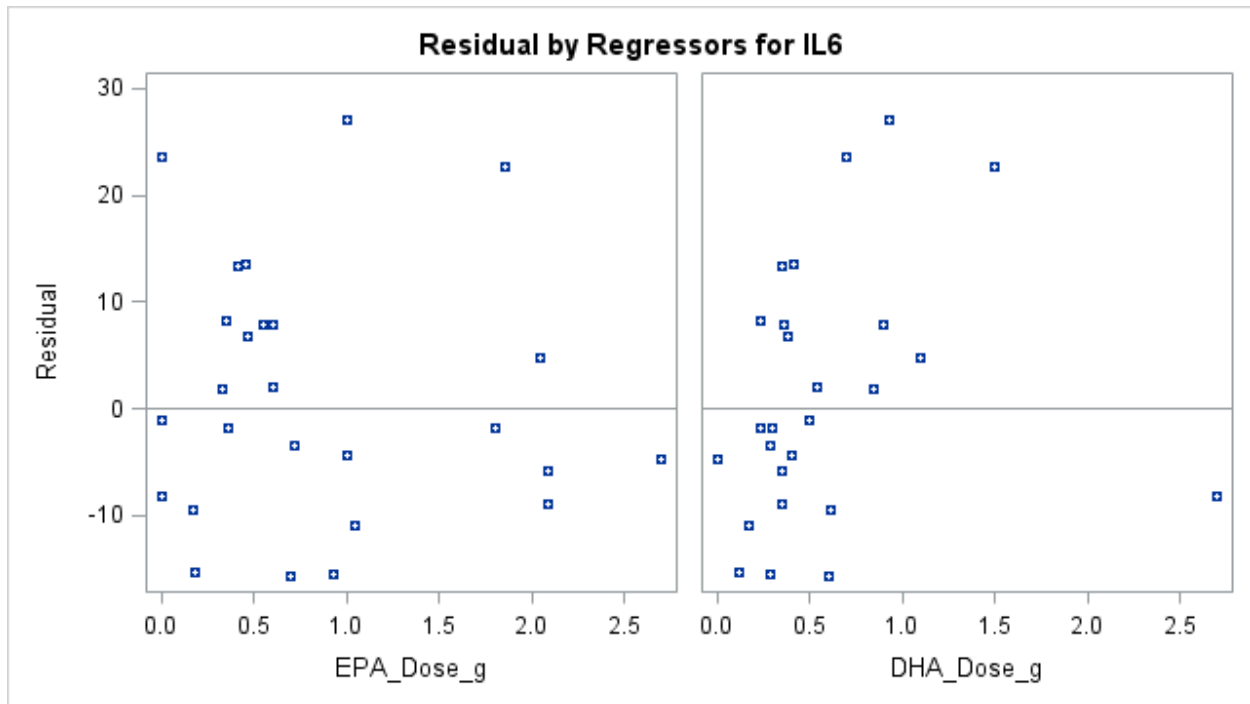


Figure 15. Residual by Regressors Plot for IL-6

The figure above provides a residual by regressors plot for IL-6, using data from the 20 aforementioned studies. The relatively random dispersion above and below the x-axis suggests that the aforementioned models assuming EPA/DHA independence provide reasonable fits. A visible pattern in the residual by regressors plot, such as a sloped line or parabola, would suggest an unexplained variable not accounted for in the linear plots. EPA_Dose_g and DHA_Dose_g represent the EPA and DHA data used from the studies, respectively.

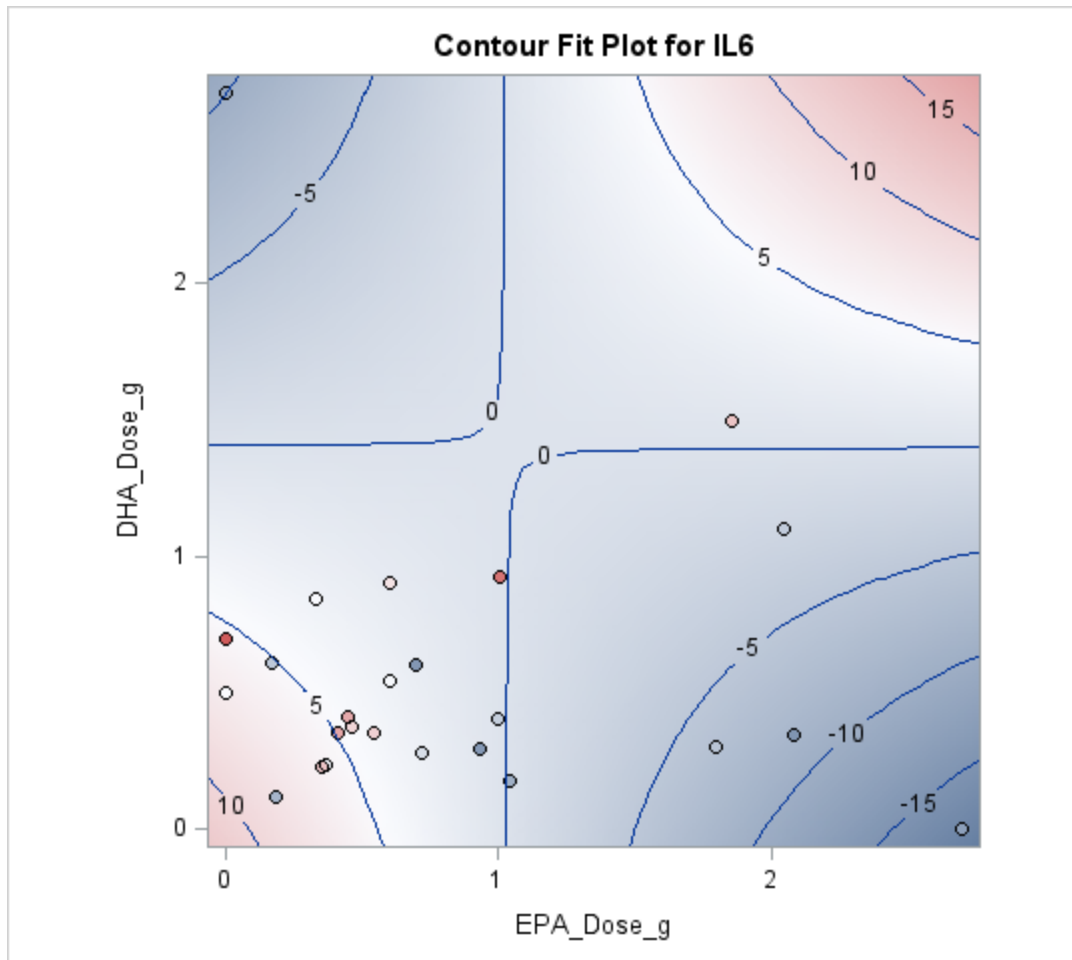


Figure 16. Contour Fit Plot for IL-6

The figure above provides a potentially easier way to visualize the 19 aforementioned studies used for this section of the meta-analysis, as well as providing a predictive data plot based upon study data. The plot above is a projection based upon study data from IL-6. The y-axis represents daily DHA dosage, while the x-axis represents daily EPA dosage. The labeled lines throughout the chart represent the predicted percentage change in TNF- α values, while the dots throughout the graph represent individual study outcomes, weighted by number of observations. The red and blue dots represent the recorded maximum and minimum observations from the study data, respectively. As the dots represent individual study outcomes separate from

the graph itself, they may be considered extraneous to the data chart, but they do provide insight regarding individual study outcomes, relative to one another.

Based on this projection based upon study data, it is likely an interaction between EPA and DHA exist as the previously used linear models which were found adequate assuming EPA/DHA independence are unable to account for the projections from study data observed in this graph. Notably, assuming independence, both compounds are observed to slightly decrease circulating IL-6 levels based on study data. However, when both EPA and DHA are taken account into one unifying model, it can be observed from the graph that whether the level of IL-6 increases or decreases as the supplementation with one compound increases is dependent upon the supplemented dose of the alternative compound based upon study data and projections.

This contour plot generated for IL-6 suggests that decreases in IL-6 levels are best obtained when EPA or DHA are taken individually and in relatively large doses. Notably, EPA appears to have a stronger overall effect on IL-6 levels than DHA when taken on a gram per gram basis. Supplementation with the respective compounds appears to generally increase IL-6 levels when taken in similar doses, and decrease IL-6 levels when supplementation amounts are more offset.

IL-1 β

A total of 6 studies [18, 55, 57, 120, 122, 128] were used for analysis of IL-1 β levels, yielding 150 individuals, and 176 observations. The average subject age was 46 years old, with a BMI of 30.8. Paired t-testing, which was used to assess for a statistically significant change in mean inflammatory marker levels before and after EPA and DHA supplementation, revealed no significant impact of EPA/DHA supplementation on IL-1 β levels ($p=.1756$). An F-test was run afterwards to assess for potential individual or combined effects of EPA/DHA supplementation on marker levels. The F-test assuming EPA independence resulted in a statistically significant p-value ($p=.0116$), indicating an effect on IL-1 β levels assuming EPA held independent effects. A linear model was found adequate in modeling an independent EPA interaction. F-test data assuming DHA independence resulted in a statistically significant p-value ($p<.0001$). Running a final F-test for EPA/DHA dependence resulted in a statistically significant p-value ($p=.0015$), indicating EPA/DHA may influence each other's overall effect. A resulting contour plot derived from the study data was generated, which indicated that the prior linear models were no longer adequate predictors of the effect of EPA/DHA on inflammatory marker levels when both compounds were examined for dependent effects. Individual effect sizes and graphs depicting the data spread have been provided on the following pages.

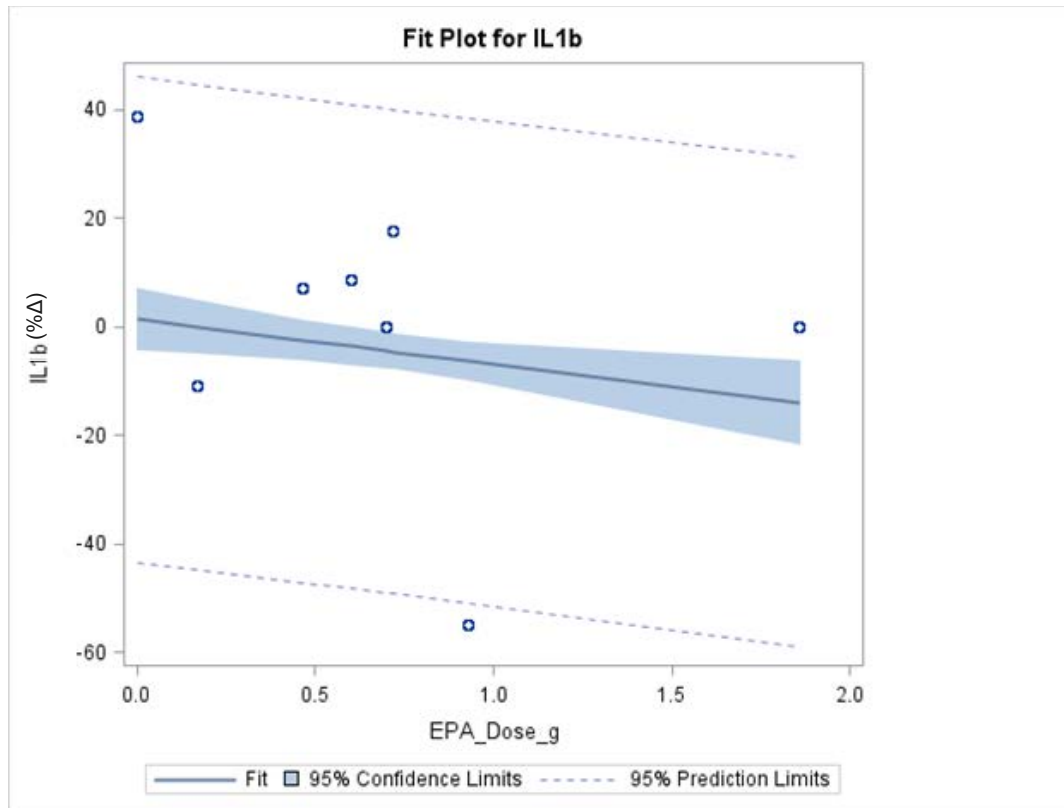


Figure 17. EPA Supplementation Fit Plot for IL-1 β (Assumed Independence)

The figure above provides a linear model of the effect EPA supplementation has on IL-1 β levels based on the 6 aforementioned studies used for this section of the meta-analysis, assuming EPA holds independent effects. Each point on the graph represents a different study outcome. IL1b (% Δ) represents the average percentage change in population IL-1 β levels after EPA supplementation, whereas EPA_Dose_g represents the daily EPA dose in grams given to the subjects. All studies in the above graph were weighed by the number of observations for the purpose of this regression. Assuming independent effects from DHA, EPA had a statistically significant effect on IL-1 β levels in the study populations via F-testing ($p=.0116$). Based on the graph above, supplementation with EPA appears to reduce circulating levels of IL-1 β , assuming independent effects from DHA.

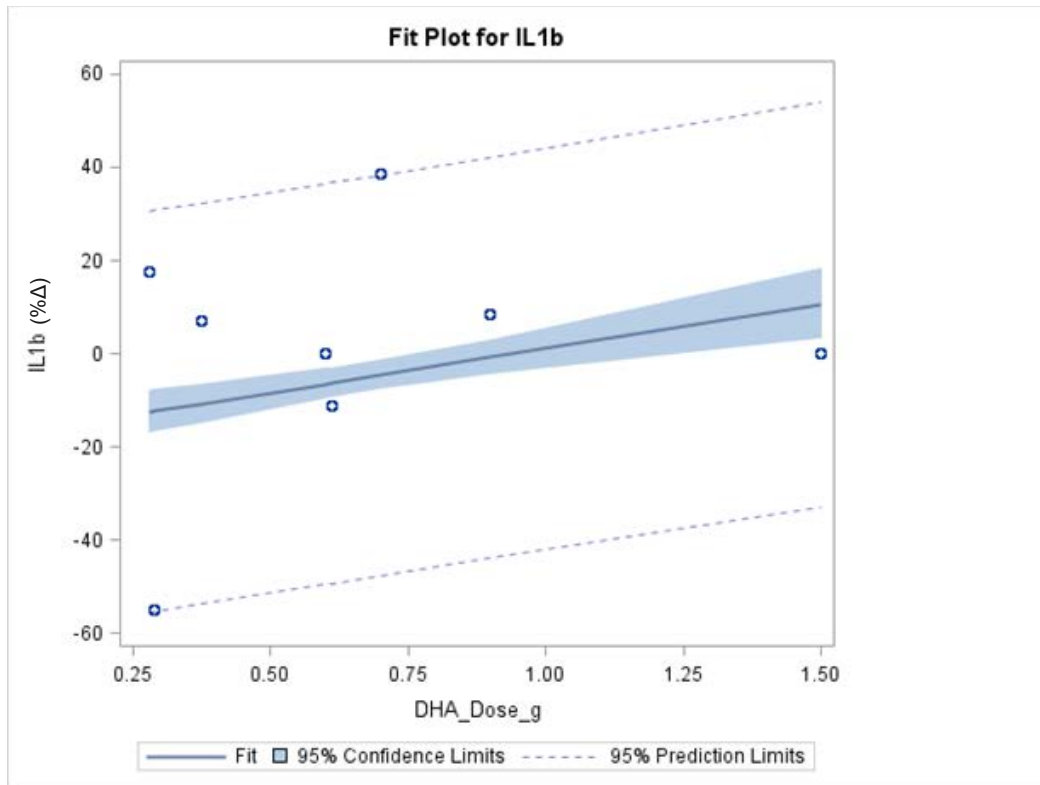


Figure 18. DHA Supplementation Fit Plot for IL-1 β (Assumed Independence)

The figure above provides a linear model of the effect DHA supplementation has on IL-1 β levels based on the 20 aforementioned studies used for this section of the meta-analysis, assuming DHA holds independent effects. Each point on the graph represents a different study outcome. IL1b (% Δ) represents the average percentage change in population IL-1 β levels after DHA supplementation, whereas DHA_Dose_g represents the daily DHA dose in grams given to the subjects. All studies in the above graph were weighed by the number of observations for the purpose of this regression. Assuming independent effects from EPA, DHA had a statistically significant effect on IL-1 β levels in the study populations via F-testing ($p < .0001$). Based on the graph above, supplementation with EPA appears to increase circulating levels of IL-1 β , assuming independent effects from DHA.

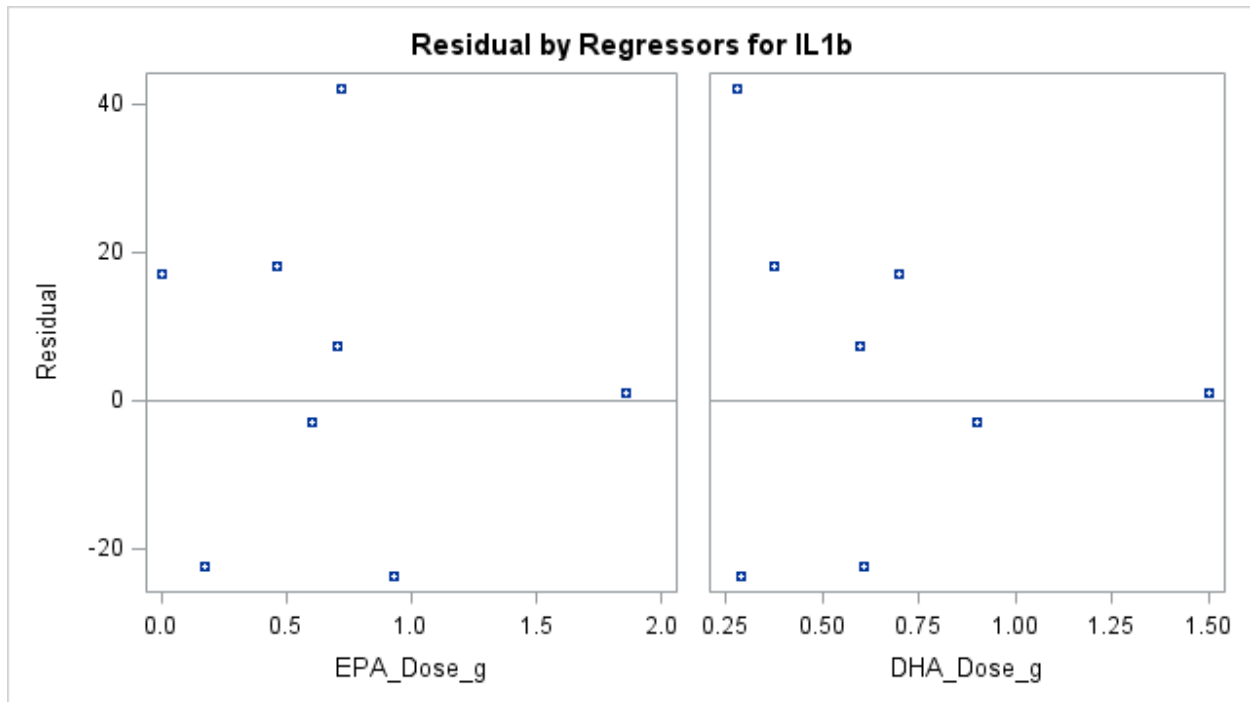


Figure 19. Residual by Regressors Plot for IL-1 β

The figure above provides a residual by regressors plot for IL-1 β , using data from the 20 aforementioned studies. The relatively random dispersion above and below the x-axis suggests that the aforementioned models assuming EPA/DHA independence provide reasonable fits. A visible pattern in the residual by regressors plot, such as a sloped line or parabola, would suggest an unexplained variable not accounted for in the linear plots. EPA_Dose_g and DHA_Dose_g represent the EPA and DHA data used from the studies, respectively.

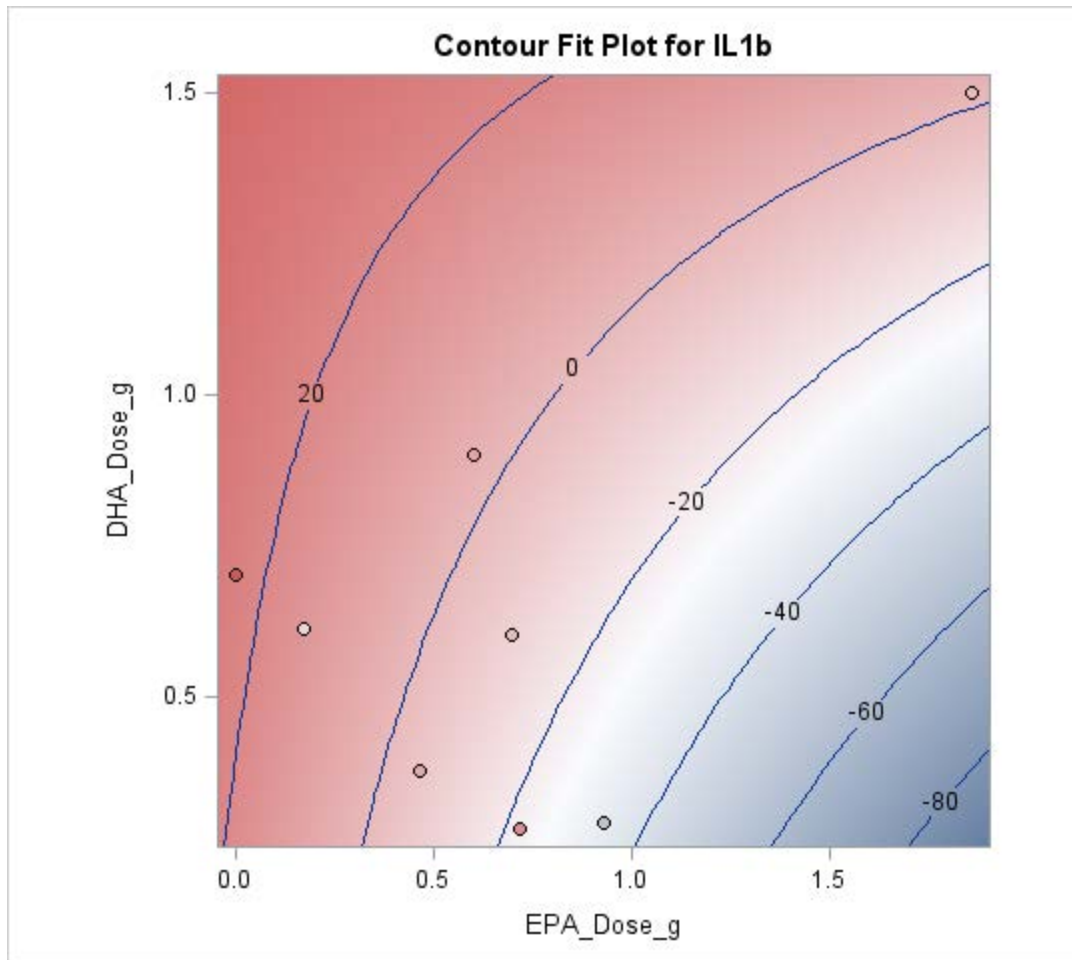


Figure 20. Contour Fit Plot of IL-1 β

The figure above provides a potentially easier way to visualize the 6 aforementioned studies used for this section of the meta-analysis, as well as providing a predictive data plot based upon study data. The plot above is a projection based upon study data from IL-1 β . The y-axis represents daily DHA dosage, while the x-axis represents daily EPA dosage. The labeled lines throughout the chart represent the predicted percentage change in IL-1 β values, while the dots throughout the graph represent individual study outcomes, weighted by number of observations. The red and blue dots represent the recorded maximum and minimum observations from the study data, respectively. As the dots represent individual study outcomes separate from

the graph itself, they may be considered extraneous to the data chart, but they do provide insight regarding individual study outcomes, relative to one another.

Based on this projection based upon study data, it is likely an interaction between EPA and DHA exist as the previously used linear models which were found adequate assuming EPA/DHA independence are unable to account for the projections from study data observed in this graph. Notably, assuming independence, increases of DHA supplementation are observed to increase circulating IL-1 β levels, while supplementation of EPA was found to decrease circulating IL-1 β levels. However, when both EPA and DHA are taken account into one unifying model, it can be observed from the graph that increased supplemented amounts of EPA and DHA do not decrease or increase the levels of circulating IL-1 β respectively, in a linear, predictable matter. As may be seen from the contour plot, the curvature of the contour lines suggests that interplay may be present between EPA and DHA, and potentially other compounds.

The contour plot for IL-1 β suggests that EPA supplementation is effective in IL-1 β reduction when taken in moderate to large doses. However, in contrast, DHA appears to increase IL-1 β circulatory levels regardless of dosage used. At similar doses, the overall effect on circulating IL-1 β doses tends to be relatively neutral.

In summary, no statistically significant differences resulting from EPA/DHA supplementation by paired t-test were found, with the exception of TNF- α . Linear models assuming EPA/DHA independence indicated EPA and DHA could generate statistically significant effects for the measured inflammatory markers, which was validated by F-tests. However, the contour plots extrapolated from study data also indicated the potential for dependent effects, which was also validated by F-tests. Thus, based on an assumption of

dependence and the generated contour plots, it is likely that EPA/DHA exhibit dose-dependent effects on the measured circulating inflammatory marker levels in this study. When strictly utilizing before and after data alone, the paired t-tests suggest there is no effect, but when stratified by dosage and response, the contour plots suggest that an effect exists. The contour plots likely override the t-test data.

DISCUSSION

With higher EPA/DHA consumption associated with lower-all cause mortality, and with molecular pathways since discovered regarding how EPA/DHA may reduce inflammation, it is possible for the observed decrease in all-cause mortality to be due to a decrease of circulating inflammatory factors via EPA/DHA consumption. If true, it would be of interest for many to supplement with EPA/DHA to combat a general level of inflammation and to reduce the risk of all-cause mortality.

Based upon the paired t-tests and F-tests, the collected studies strongly suggest that co-supplementation of EPA/DHA across otherwise healthy individuals does not generally affect the circulating levels of CRP, IL-6, or IL-1 β over time in a statistically significant way, although individual, dependent effects may exist on circulating factor levels. Based on the data, it is likely that if EPA/DHA supplementation reduces inflammation in the body, it is generally not through the inflammatory markers or dosages measured in this study. With the exception of TNF- α , the impact of EPA/DHA supplementation on the circulating inflammatory factors was mixed and largely dependent upon supplemented dosages of EPA/DHA in diet (Figures 8, 16, and 20). Further research may be necessary to determine the mechanisms by which EPA/DHA may interact to affect these levels of circulating inflammatory markers.

At the start of the study, it was hypothesized that EPA/DHA would work synergistically to reduce the measured inflammatory markers given the similarities between the compound classes and their targets, but after further analysis it appears that this does not occur in human studies. It should be noted that despite these findings, an overall anti-inflammatory impact may still exist through supplementation, such as through the immunosuppression of cellular adhesion

factors or alternative inflammatory markers (e.g. ICAM-1, MCP-1) [16, 133]. The markers picked for this study were based on the most widely available data for which marker information was available, and thus their relative decrease or constancy may not be representative of the entire inflammatory process as a whole. As stated previously, this means that overall inflammatory status may still decrease throughout the body after supplementation with EPA and DHA. However, if it does, it is likely not through a decrease in markers CRP, IL-6, or IL-1 β through the populations used in this study.

When examining the impact of EPA/DHA supplementation assuming the compounds had independent effects, statistically significant differences were found; however, the overall changes discovered are likely too small to make a physiologically significant difference in regards to the inflammatory markers CRP, IL-6, and IL-1 β . In addition, further analysis revealed a likely EPA/DHA interaction in co-supplementation, potentially nullifying the utility of the previously performed F-tests that assumed independent effects of both EPA and DHA. Both tests assuming independent effects had initially been run prior to testing for an EPA/DHA interaction, as it was unknown whether or not an interaction was present. The analyses and models assuming EPA and DHA independence were since included to provide a more holistic view of the project, as well as to provide models for comparison, from which the study conclusions were drawn

Given the mixed impact of EPA/DHA on the aforementioned inflammatory markers, it is difficult to propose dietary recommendations for EPA/DHA dosage, especially when it appears from the data that overall decreases in inflammatory markers are generally not associated with EPA/DHA supplementation in healthy populations. Although a relatively high intake of ~1g of EPA and DHA daily appears to yield a somewhat favorable balance across all inflammatory

markers, this relatively high dosage may lead to anticoagulation issues in certain populations, such as those taking blood thinners [134, 135]. Notably, some of the figures suggest pro-inflammatory potential of EPA/DHA supplementation in some doses, which, although unlikely, has been observed in some studies based on age and dose [16, 133]. This may warrant further study.

As EPA/DHA are essential fatty acids for diet, perhaps the biggest overall impact the general public may see will be observed in regards to inflammatory factor TNF- α . TNF- α has been implicated in affecting both lipid and glucose metabolism in the cell, leading to metabolic syndrome conditions in individuals [136]. While TNF- α provides a number of acute benefits for the body, such as increased nutrient availability, chronically elevated TNF- α has been observed to promote atherosclerotic lipid changes and the development of insulin resistance [6, 136, 137]. Given that TNF- α plays a key role in the development a number of chronic inflammatory conditions (e.g., obesity, non-insulin-dependent diabetes mellitus), the dietary consumption of EPA/DHA may indirectly prolong the onset of such diseases in healthy populations by affecting circulating levels of TNF- α , assuming minimal impact on other inflammatory factors [6, 7].

Notably, for a majority of the studies in this analysis, EPA and DHA tended to be co-supplemented in similar dosages, which may have been intentionally reflective of the EPA/DHA concentrations in dietary seafood and fish [138]. As EPA and DHA are not naturally found isolated from one another, it is in this sense that the study data may more accurately reflect a general dietary scenario. Given the mixed impact of EPA/DHA on circulating inflammatory markers, it is possible that alternative factors, such as an improved sense of well-being, as

measured in previous studies (via the Profile of Mood States test), may be more important in terms of contributing towards an extended lifespan (Table 2) [26-28].

Disagreement in general literature may likely arise given the dependent effect EPA and DHA have upon one another, as well as the study populations used. In particular, it is possible for inflammatory factor levels to be more or less sensitive to EPA/DHA in populations afflicted by chronic inflammatory disease, as well as through general diet, affecting interpretations from one study to the next [139, 140].

A potential weakness not accounted for in this study is the effect n-6 PUFA consumption may have on individual inflammatory status in context of individual diet. Not all studies used within this analysis provided an average n-6:n-3 ratio in regards to participant fatty acid consumption, due to practical reasons. Knowledge of being under observation may also have affected the diets of subjects in a significant way. As excess consumption n-6 fatty acids is suggested to compete with n-3 fatty acids for similar rate-limiting enzymes, a balanced consumption of n-6 PUFAs and n-3 PUFAs could lead to a decrease in overall circulating inflammatory factors [141]. Notably, for this study, a majority (~85%) of the subjects came from North America, where estimates of average n-6:n-3 ratio lie around 15:1 to 17:1, so these results may be somewhat more applicable for those living in North America [142, 143].

In conclusion, EPA/DHA supplementation was not found to affect the levels of circulating inflammatory markers CRP, IL-6, or IL-1 β in a statistically significant way, although further analysis suggests that they may impact inflammatory marker levels independently. With the exception of TNF- α , the overall effect on all other inflammatory markers was largely variable and dependent upon the specific dosages used across studies. Due to this data spread, it is

difficult to make a general dietary recommendation in regards to reducing levels of inflammatory markers in the body. It is similarly difficult to say that EPA/DHA supplementation reduces inflammatory factors in a way that reduces the baseline levels of inflammation in the body. When assessed for effects of supplementation assuming EPA/DHA independence, statistically significant changes were found but the changes were unlikely to make any clinically noticeable difference, especially in light of the paired t-test results and the observation that the overall effect of EPA/DHA supplementation is likely dependent on the dosages of EPA/DHA used. Finally, as previously mentioned, it is still possible for overall inflammatory status to decrease throughout the body after supplementation with EPA and DHA; however, if it does, it is likely not through a decrease in markers CRP, IL-6, or IL-1 β , based on the populations used in this study. Given these findings, potential studies for the future include the impact of the noted decrease in TNF- α , as well as the mechanisms behind which EPA/DHA may influence each other's relative impact in the body.

REFERENCES

1. Serhan, C.N., N. Chiang, and J. Dalli, *Review: The resolution code of acute inflammation: Novel pro-resolving lipid mediators in resolution*. *Seminars in Immunology*, 2015. **27**: p. 200-215.
2. Sansbury, B.E. and M. Spite, *Resolution of Acute Inflammation and the Role of Resolvins in Immunity, Thrombosis, and Vascular Biology*. *Circulation Research*, 2016. **119**(1): p. 113-130.
3. *Researchers at Mayo Clinic Release New Data on Inflammation (Immunosuppression associated with chronic inflammation in the tumor microenvironment)*. 2015, NewsRX LLC.
4. Naylor, A.J., A. Filer, and C.D. Buckley, *The role of stromal cells in the persistence of chronic inflammation*. *CLINICAL AND EXPERIMENTAL IMMUNOLOGY*, 2013. **171**(1): p. 30-35.
5. Howcroft, T.K., et al., *The role of inflammation in age-related disease*. *AGING-US*, 2013. **5**(1): p. 84-93.
6. Hotamisligil, G.S., N.S. Shargill, and B.M. Spiegelman, *Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance*. *Science*, 1993(5091): p. 87.
7. Paolisso, G., M.R. Rizzo, and C. Mazziotti, *Advancing age and insulin resistance: role of plasma tumor necrosis factor- α* . *American Journal of Physiology*, 1998. **275**(2): p. E294-E299.
8. Bruunsgaard, H., *Physical activity and modulation of systemic low-level inflammation*. *Journal Of Leukocyte Biology*, 2005. **78**(4): p. 819-835.
9. Febbraio, M.A. and B.K. Pedersen, *Muscle-derived interleukin-6: mechanisms for activation and possible biological roles*. *FASEB Journal: Official Publication Of The Federation Of American Societies For Experimental Biology*, 2002. **16**(11): p. 1335-1347.
10. Febbraio, M.A. and B.K. Pedersen, *Contraction-induced myokine production and release: is skeletal muscle an endocrine organ?* *Exercise And Sport Sciences Reviews*, 2005. **33**(3): p. 114-119.

11. Trayhurn, P. and I.S. Wood, *Adipokines: inflammation and the pleiotropic role of white adipose tissue*. The British Journal Of Nutrition, 2004. **92**(3): p. 347-355.
12. Bastard, J.-P., et al., *Recent advances in the relationship between obesity, inflammation, and insulin resistance*. European Cytokine Network, 2006. **17**(1): p. 4-12.
13. Jonasson, T., et al., *Plasma homocysteine and markers for oxidative stress and inflammation in patients with coronary artery disease--a prospective randomized study of vitamin supplementation*. Clinical Chemistry And Laboratory Medicine, 2005. **43**(6): p. 628-634.
14. Liu, S., et al., *A HIGH GLYCEMIC DIET IN RELATION TO PLASMA LEVELS OF HIGH-SENSITIVITY C-REACTIVE PROTEIN IN MIDDLE-AGED WOMEN*. 2001, Johns Hopkins University Press.
15. Song, Y., et al., *Magnesium intake, C-reactive protein, and the prevalence of metabolic syndrome in middle-aged and older U.S. women*. Diabetes Care, 2005. **28**(6): p. 1438-1444.
16. Cazzola, R., et al., *Age- and dose-dependent effects of an eicosapentaenoic acid-rich oil on cardiovascular risk factors in healthy male subjects*. Atherosclerosis, 2007. **193**: p. 159-167.
17. Ciubotaru, I., Y.S. Lee, and R.C. Wander, *Dietary fish oil decreases C-reactive protein, interleukin-6, and triacylglycerol to HDL-cholesterol ratio in postmenopausal women on HRT*. The Journal of nutritional biochemistry, 2003. **14**(9): p. 513-521.
18. Vega-López, S., et al., *Supplementation with omega3 polyunsaturated fatty acids and all-rac alpha-tocopherol alone and in combination failed to exert an anti-inflammatory effect in human volunteers*. Metabolism: clinical and experimental, 2004. **53**(2): p. 236-240.
19. Centre for, R. and Dissemination, *A systematic review of fish-oil supplements for the prevention and treatment of hypertension (Structured abstract)*, F. Campbell, et al., Editors. 2013. p. 107-120.

20. Rizza, S., et al., *Fish oil supplementation improves endothelial function in normoglycemic offspring of patients with type 2 diabetes*. *Atherosclerosis*, 2009. **206**: p. 569-574.
21. Michaeli, B., et al., *ORIGINAL ARTICLE: Effects of fish oil on the neuro-endocrine responses to an endotoxin challenge in healthy volunteers*. *Clinical Nutrition*, 2007. **26**: p. 70-77.
22. Pittet, Y.K., et al., *Blunting the response to endotoxin in healthy subjects: effects of various doses of intravenous fish oil*. *INTENSIVE CARE MEDICINE*, 2010. **36**(2): p. 289-295.
23. Gray, P., et al., *Fish oil supplementation augments post-exercise immune function in young males*. *Brain Behavior and Immunity*, 2012. **26**: p. 1265-1272.
24. Hashimoto, M., et al., *Beneficial effects of dietary docosahexaenoic acid intervention on cognitive function and mental health of the oldest elderly in Japanese care facilities and nursing homes*. *Geriatrics & Gerontology International*, 2017. **17**(2): p. 330-337.
25. Chew, E.Y., et al., *Effect of omega-3 fatty acids, lutein/zeaxanthin, or other nutrient supplementation on cognitive function*. *JAMA, The Journal of the American Medical Association*, 2015(8): p. 791.
26. Fontani, G., et al., *Cognitive and physiological effects of Omega-3 polyunsaturated fatty acid supplementation in healthy subjects*. *European Journal of Clinical Investigation*, 2005. **35**(11): p. 691-699.
27. Antypa, N., et al., *Omega-3 fatty acids (fish-oil) and depression-related cognition in healthy volunteers*. *Journal of psychopharmacology (Oxford, England)*, 2009. **23**(7): p. 831-840.
28. Fontani, G., et al., *Blood profiles, body fat and mood state in healthy subjects on different diets supplemented with Omega-3 polyunsaturated fatty acids*. *European Journal of Clinical Investigation*, 2005. **35**(8): p. 499-507.

29. Kuriki, K., et al., *Plasma concentrations of (n-3) highly unsaturated fatty acids are good biomarkers of relative dietary fatty acid intakes: a cross-sectional study*. The Journal of Nutrition, 2003(11): p. 3643.
30. Gupta, V., et al., *Oily fish, coffee and walnuts: Dietary treatment for nonalcoholic fatty liver disease*. World journal of gastroenterology, 2015. **21**(37): p. 10621-10635.
31. Doughman, S.D., S. Krupanidhi, and C.B. Sanjeevi, *Omega-3 fatty acids for nutrition and medicine: considering microalgae oil as a vegetarian source of EPA and DHA*. Current Diabetes Reviews, 2007. **3**(3): p. 198-203.
32. Anderson, B.M. and D.W.L. Ma, *Are all n-3 polyunsaturated fatty acids created equal? LIPIDS IN HEALTH AND DISEASE*, 2009. **8**.
33. Wijendran, V. and K.C. Hayes, *DIETARY n-6 AND n-3 FATTY ACID BALANCE AND CARDIOVASCULAR HEALTH*. Annual Review of Nutrition, 2004. **24**(1): p. 597-615.
34. Jolly, C.A., et al., *Dietary (n-3) polyunsaturated fatty acids suppress murine lymphoproliferation, interleukin-2 secretion, and the formation of diacylglycerol and ceramide*. The Journal of Nutrition, 1997(1): p. 37.
35. Ariel, A. and C.N. Serhan, *Review: Resolvins and protectins in the termination program of acute inflammation*. Trends in Immunology, 2007. **28**: p. 176-183.
36. Xu, Z.Z., et al., *Resolvins RvE1 and RvD1 attenuate inflammatory pain via central and peripheral actions*. NATURE MEDICINE, 2010. **16**(5): p. 592-U129.
37. Bessa, A.L., et al., *Exercise intensity and recovery: biomarkers of injury, inflammation, and oxidative stress*. Journal of Strength and Conditioning Research, 2016(2): p. 311.
38. Lee, J.H., et al., *Omega-3 fatty acids: cardiovascular benefits, sources and sustainability*. Nature Reviews Cardiology, 2009(12): p. 753.

39. Pottala, J.V., et al., *Blood Eicosapentaenoic and Docosahexaenoic Acids Predict All-Cause Mortality in Patients With Stable Coronary Heart Disease The Heart and Soul Study*. CIRCULATION-CARDIOVASCULAR QUALITY AND OUTCOMES, 2010. **3**(4): p. 406-412.
40. Marchioli, R., et al., *Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione*. Circulation, 2002. **105**(16): p. 1897-1903.
41. Waters-Banker, C.c.w.u.c., et al., *Investigating the Mechanisms of Massage Efficacy: The Role of Mechanical Immunomodulation*. Journal of Athletic Training (Allen Press), 2014. **49**(2): p. 266-273.
42. McPhee, J.S. and A.P. Lightfoot, *Post-exercise recovery regimes: blowing hot and cold*. Journal of Physiology, 2017. **595**(3): p. 627.
43. Araki, Y. and T. Mimura, *The Mechanisms Underlying Chronic Inflammation in Rheumatoid Arthritis from the Perspective of the Epigenetic Landscape*. Journal of Immunology Research, 2016: p. 1-10.
44. Hardbower, D.M., et al., *EGFR regulates macrophage activation and function in bacterial infection*. Journal of Clinical Investigation, 2016(9): p. 3296.
45. Herder, C., et al., *Associations between inflammation-related biomarkers and depressive symptoms in individuals with recently diagnosed type 1 and type 2 diabetes*. Brain, Behavior, and Immunity, 2017. **61**: p. 137-145.
46. Kaspersen, K.A., et al., *Low-Grade Inflammation Is Associated with Susceptibility to Infection in Healthy Men: Results from the Danish Blood Donor Study (DBDS)*. PLoS ONE, 2016. **11**(10): p. 1-13.

47. Millar, N.L., et al., *Wounds that heal and wounds that don't - The role of the IL-33/ST2 pathway in tissue repair and tumorigenesis*. Seminars in Cell and Developmental Biology, 2017: p. 41.
48. Wick, G. and C. Grundtman, *Inflammation and atherosclerosis*. [electronic resource]. 2012: Wien ; New York : Springer-Verlag/Wien, 2012.
49. Deike, E., et al., *Research Brief: The Effects of Fish Oil Supplementation on Markers of Inflammation in Chronic Kidney Disease Patients*. Journal of Renal Nutrition, 2012. **22**: p. 572-577.
50. Kabir, M., et al., *Treatment for 2 mo with n 3 polyunsaturated fatty acids reduces adiposity and some atherogenic factors but does not improve insulin sensitivity in women with type 2 diabetes: a randomized controlled study*. The American journal of clinical nutrition, 2007. **86**(6): p. 1670-1679.
51. Kelley, D.S., et al., *Docosahexaenoic acid ingestion inhibits natural killer cell activity and production of inflammatory mediators in young healthy men*. Lipids, 1999(4): p. 317.
52. Kiecolt-Glaser, J.K., et al., *Omega-3 supplementation lowers inflammation and anxiety in medical students: A randomized controlled trial*. Brain Behavior and Immunity, 2011. **25**: p. 1725-1734.
53. Mackay, I., et al., *Effect of Omega-3 fatty acid supplementation on markers of platelet and endothelial function in patients with peripheral arterial disease*. Atherosclerosis, 2012. **221**: p. 514-520.
54. Poppitt, S.D., et al., *Effects of moderate-dose omega-3 fish oil on cardiovascular risk factors and mood after ischemic stroke: a randomized, controlled trial*. Stroke; a journal of cerebral circulation, 2009. **40**(11): p. 3485-3492.

55. Pot, G.K., et al., *No effect of fish oil supplementation on serum inflammatory markers and their interrelationships: a randomized controlled trial in healthy, middle-aged individuals.* EUROPEAN JOURNAL OF CLINICAL NUTRITION, 2009. **63**(11): p. 1353-1359.
56. Schirmer, S.H., et al., *Effects of omega-3 fatty acids on postprandial triglycerides and monocyte activation.* Atherosclerosis, 2012. **225**: p. 166-172.
57. Skulas-Ray, A.C., et al., *Dose-response effects of omega-3 fatty acids on triglycerides, inflammation, and endothelial function in healthy persons with moderate hypertriglyceridemia.* The American journal of clinical nutrition, 2011. **93**(2): p. 243-252.
58. Yusof, H.M., E.A. Miles, and P. Calder, *Influence of very long-chain n-3 fatty acids on plasma markers of inflammation in middle-aged men.* Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA), 2008. **78**: p. 219-228.
59. Lorente-Cebrián, S., et al., *Research article: Eicosapentaenoic acid inhibits tumour necrosis factor- α -induced lipolysis in murine cultured adipocytes.* The Journal of Nutritional Biochemistry, 2012. **23**: p. 218-227.
60. Yang, Z.G., et al., *Macrophage alpha 1 AMP-activated Protein Kinase (alpha 1AMPK) Antagonizes Fatty Acid-induced Inflammation through SIRT1.* JOURNAL OF BIOLOGICAL CHEMISTRY, 2010. **285**(25): p. 19051-19059.
61. Bouwens, M., et al., *Fish-oil supplementation induces antiinflammatory gene expression profiles in human blood mononuclear cells.* AMERICAN JOURNAL OF CLINICAL NUTRITION, 2009. **90**(2): p. 415-424.
62. Du Clos, T.W., *Function of C-reactive protein.* ANNALS OF MEDICINE, 2000. **32**(4): p. 274-278.
63. Di Paolo, N.C. and D.M. Shayakhmetov, *Interleukin 1 α and the inflammatory process.* Nature Immunology, 2016. **17**(8): p. 906-913.

64. Dinarello, C.A., *Immunological and Inflammatory Functions of the Interleukin-1 Family*. Annual Review of Immunology, 2009: p. 519.
65. Ren, K. and R. Torres, *Role of interleukin-1beta during pain and inflammation*. Brain Research Reviews, 2009. **60**(1): p. 57-64.
66. Sedger, L.M. and M.F. McDermott, *Mini review: TNF and TNF-receptors: From mediators of cell death and inflammation to therapeutic giants – past, present and future*. Cytokine and Growth Factor Reviews, 2014. **25**: p. 453-472.
67. Hunter, C.A. and S.A. Jones, *IL-6 as a keystone cytokine in health and disease*. Nature Immunology, 2015(5): p. 448.
68. Sullivan, M., *Statistics: Informed Decisions Using Data*. 5th ed. Vol. 5. 2016: Pearson. 960.
69. Tvrzicka, E., et al., *FATTY ACIDS AS BIOCOMPOUNDS: THEIR ROLE IN HUMAN METABOLISM, HEALTH AND DISEASE - A REVIEW. PART 1: CLASSIFICATION, DIETARY SOURCES AND BIOLOGICAL FUNCTIONS*. BIOMEDICAL PAPERS-OLOMOUC, 2011. **155**(2): p. 117-130.
70. Brenna, J.T., et al., *alpha-Linolenic acid supplementation and conversion to n-3 long-chain polyunsaturated fatty acids in humans*. PROSTAGLANDINS LEUKOTRIENES AND ESSENTIAL FATTY ACIDS, 2009. **80**(2-3): p. 85-91.
71. Tsukui, T., et al., *Fucoxanthin and fucoxanthinol enhance the amount of docosahexaenoic acid in the liver of KKAY obese/diabetic mice*. JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY, 2007. **55**(13): p. 5025-5029.
72. Yamazaki, K., et al., *Comparison of the conversion rates of alpha-linolenic acid (18:3(n - 3)) and stearidonic acid (18:4(n - 3)) to longer polyunsaturated fatty acids in rats*. Biochimica Et Biophysica Acta, 1992. **1123**(1): p. 18-26.

73. Rao, J.S., et al., *Dietary n-3 PUFA deprivation alters expression of enzymes of the arachidonic and docosahexaenoic acid cascades in rat frontal cortex*. MOLECULAR PSYCHIATRY, 2007. **12**(2): p. 151-157.
74. Strokina, M., M. Sergeeva, and G. Reiser, *Docosahexaenoic acid and arachidonic acid release in rat brain astrocytes is mediated by two separate isoforms of phospholipase A2 and is differently regulated by cyclic AMP and Ca²⁺*. British Journal Of Pharmacology, 2003. **139**(5): p. 1014-1022.
75. Crecelius, A.R., et al., *Mechanisms of ATP-mediated vasodilation in humans: modest role for nitric oxide and vasodilating prostaglandins*.
76. García-Alonso, V., et al., *Prostaglandin E2 Exerts Multiple Regulatory Actions on Human Obese Adipose Tissue Remodeling, Inflammation, Adaptive Thermogenesis and Lipolysis*. PLoS ONE, 2016. **11**(4): p. 1-17.
77. Woodcock, N.A., C.W. Taylor, and S. Thornton, *Prostaglandin F2alpha increases the sensitivity of the contractile proteins to Ca²⁺ in human myometrium*. American Journal of Obstetrics & Gynecology, 2006. **195**(5): p. 1404-1406.
78. Säfholm, J., et al., *Asthma and lower airway disease: Prostaglandin E2 inhibits mast cell-dependent bronchoconstriction in human small airways through the E prostanoid subtype 2 receptor*. The Journal of Allergy and Clinical Immunology, 2015. **136**: p. 1232-1239.e1.
79. Karaki, S.I. and A. Kuwahara, *Regulation of intestinal secretion involved in the interaction between neurotransmitters and prostaglandin E2*. Neurogastroenterology And Motility: The Official Journal Of The European Gastrointestinal Motility Society, 2004. **16 Suppl 1**: p. 96-99.
80. Dennis, E.A., *Phospholipase A2 mechanism: Inhibition and role in arachidonic acid release*. Drug Development Research, 1987. **10**(4): p. 205.

81. Mulugeta, S., et al., *Identification and absolute configuration of dihydroxy-arachidonic acids formed by oxygenation of 5S-HETE by native and aspirin-acetylated COX-2*.
82. DeMar, J.J.C., et al., *Half-lives of docosahexaenoic acid in rat brain phospholipids are prolonged by 15 weeks of nutritional deprivation of n-3 polyunsaturated fatty acids*. *Journal of Neurochemistry*, 2004. **91**(5): p. 1125-1137.
83. Igarashi, M., et al., *Low liver conversion rate of alpha-linolenic to docosahexaenoic acid in awake rats on a high-docosahexaenoate-containing diet*. *JOURNAL OF LIPID RESEARCH*, 2006. **47**(8): p. 1812-1822.
84. Neuringer, M., et al., *Biochemical and functional effects of prenatal and postnatal omega 3 fatty acid deficiency on retina and brain in rhesus monkeys*. *Proceedings Of The National Academy Of Sciences Of The United States Of America*, 1986. **83**(11): p. 4021-4025.
85. Neuringer, M., et al., *DIETARY OMEGA-3 FATTY ACID DEFICIENCY AND VISUAL LOSS IN INFANT RHESUS MONKEYS*. 1984.
86. Bannenberg, G.L., *Resolvins: Current understanding and future potential in the control of inflammation*. *CURRENT OPINION IN DRUG DISCOVERY & DEVELOPMENT*, 2009. **12**(5): p. 644-658.
87. Mas, E., et al., *Resolvins D1, D2, and Other Mediators of Self-Limited Resolution of Inflammation in Human Blood following n-3 Fatty Acid Supplementation*. *CLINICAL CHEMISTRY*, 2012. **58**(10): p. 1476-1484.
88. Serhan, C.N., et al., *Resolvins, docosatrienes, and neuroprotectins, novel omega-3-derived mediators, and their endogenous aspirin-triggered epimers*. *Lipids*, 2004(11): p. 1125.
89. Hasturk, H., et al., *RvE1 protects from local inflammation and osteoclast-mediated bone destruction in periodontitis*. *FASEB Journal: Official Publication Of The Federation Of American Societies For Experimental Biology*, 2006. **20**(2): p. 401-403.

90. Irimia, D., et al., *Resolvin E2 Formation and Impact in Inflammation Resolution*. Journal of immunology, 2012. **188**(9): p. 4527-4534.
91. recchiuti, a., et al., *Pro-resolving actions and stereoselective biosynthesis of 18S E-series resolvins in human leukocytes and murine inflammation*. Journal of clinical investigation, 2011. **121**(2): p. 569-581.
92. Recchiuti, A., *Review: Resolvin D1 and its GPCRs in resolution circuits of inflammation*. Prostaglandins and Other Lipid Mediators, 2013. **107**: p. 64-76.
93. Serhan, C.N., et al., *Resolvins: A family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals*. JOURNAL OF EXPERIMENTAL MEDICINE, 2002. **196**(8): p. 1025-1037.
94. Xu, M.X., et al., *Resolvin D1, an Endogenous Lipid Mediator for Inactivation of Inflammation-Related Signaling Pathways in Microglial Cells, Prevents Lipopolysaccharide-Induced Inflammatory Responses (Retracted article. See vol. 19, pg. 541, 2013)*. CNS NEUROSCIENCE & THERAPEUTICS, 2013. **19**(4): p. 235-243.
95. Arita, M., et al., *Stereochemical assignment, antiinflammatory properties, and receptor for the omega-3 lipid mediator resolvin E1*. JOURNAL OF EXPERIMENTAL MEDICINE, 2005. **201**(5): p. 713-722.
96. Cash, J.L., et al., *Synthetic chemerin-derived peptides suppress inflammation through ChemR23*. JOURNAL OF EXPERIMENTAL MEDICINE, 2008. **205**(4): p. 767-775.
97. Meder, W., et al., *Characterization of human circulating TIG2 as a ligand for the orphan receptor ChemR23*. FEBS Letters, 2003. **555**: p. 495-499.
98. Sun, G.Y. and Z.Y. Hu, *Stimulation of phospholipase A(2) expression in rat cultured astrocytes by LPS, TNF alpha and IL-1 beta*. GENE EXPRESSION IN THE CENTRAL NERVOUS SYSTEM, 1995. **105**: p. 231-238.

99. Walev, I., et al., *Potassium regulates IL-1 beta processing via calcium-independent phospholipase A(2)*. JOURNAL OF IMMUNOLOGY, 2000. **164**(10): p. 5120-5124.
100. Mukherjee, P.K., et al., *Neuroprotectin D1: A Docosahexaenoic Acid-Derived Docosatriene Protects Human Retinal Pigment Epithelial Cells from Oxidative Stress*. 2004, National Academy of Sciences. p. 8491.
101. Zhao, Y.H., et al., *Docosahexaenoic Acid-Derived Neuroprotectin D1 Induces Neuronal Survival via Secretase- and PPAR gamma-Mediated Mechanisms in Alzheimer's Disease Models*. PLOS ONE, 2011. **6**(1).
102. Dalli, J., et al., *The novel 13S,14S-epoxy-maresin is converted by human macrophages to maresin 1 (MaR1), inhibits leukotriene A(4) hydrolase (LTA(4)H), and shifts macrophage phenotype*. FASEB JOURNAL, 2013. **27**(7): p. 2573-2583.
103. Xue, B.Z., et al., *Omega-3 Polyunsaturated Fatty Acids Antagonize Macrophage Inflammation via Activation of AMPK/SIRT1 Pathway*. PLOS ONE, 2012. **7**(10).
104. Oh, D.Y., et al., *Article: GPR120 Is an Omega-3 Fatty Acid Receptor Mediating Potent Anti-inflammatory and Insulin-Sensitizing Effects*. Cell, 2010. **142**: p. 687-698.
105. Numerof, R.P., F.R. Aronson, and J.W. Mier, *IL-2 stimulates the production of IL-1 alpha and IL-1 beta by human peripheral blood mononuclear cells*. Journal Of Immunology (Baltimore, Md.: 1950), 1988. **141**(12): p. 4250-4257.
106. Schmidt, E.B., et al., *COD LIVER OIL INHIBITS NEUTROPHIL AND MONOCYTE CHEMOTAXIS IN HEALTHY-MALES*. ATHEROSCLEROSIS, 1989. **77**(1): p. 53-57.
107. Sperling, R.I., et al., *Dietary omega-3 polyunsaturated fatty acids inhibit phosphoinositide formation and chemotaxis in neutrophils*. The Journal Of Clinical Investigation, 1993. **91**(2): p. 651-660.

108. Byleveld, M., et al., *Fish oil feeding enhances lymphocyte proliferation but impairs virus-specific T lymphocyte cytotoxicity in mice following challenge with influenza virus*. Clinical & Experimental Immunology, 2000. **119**(2): p. 287.
109. Byleveld, P.M., et al., *Fish oil feeding delays influenza virus clearance and impairs production of interferon-gamma and virus-specific immunoglobulin A in the lungs of mice*. The Journal of Nutrition, 1999(2): p. 328.
110. Schwerbrock, N.M., et al., *Fish oil-fed mice have impaired resistance to influenza infection*. Journal of Nutrition, 2009. **139**(8): p. 1588-1594.
111. Figueras, M., et al., *Effects of Eicosapentaenoic Acid (EPA) Treatment on Insulin Sensitivity in an Animal Model of Diabetes: Improvement of the Inflammatory Status*. Obesity (19307381), 2011. **19**(2): p. 362-369.
112. Pepys, M.B. and G.M. Hirschfield, *C-reactive protein: a critical update*. Journal of Clinical Investigation, 2003. **111**(12): p. 1805.
113. Giulio, K., et al., *Cytokine Levels in the Serum of Healthy Subjects*. Mediators of Inflammation, Vol 2013 (2013), 2013.
114. Lee, S.J., et al., *Serum levels of tumor necrosis factor-alpha and interleukin-6 in ocular cicatricial pemphigoid*. Investigative Ophthalmology & Visual Science, 1993. **34**(13): p. 3522-3525.
115. *Levels of circulating tumor necrosis factor alpha and interleukin-6 in patients with rheumatoid arthritis. Relationship to serum levels of hyaluronan and antigenic keratan sulfate*. 1993, 1993.
116. Cormier, H., et al., *Expression and Sequence Variants of Inflammatory Genes; Effects on Plasma Inflammation Biomarkers Following a 6-Week Supplementation with Fish Oil*. International Journal Of Molecular Sciences, 2016. **17**(3): p. 375-375.

117. Fujioka, S., et al., *The effects of eicosapentaenoic acid-fortified food on inflammatory markers in healthy subjects--A randomized, placebo-controlled, double-blind study*. Journal of nutritional science and vitaminology, 2006. **52**(4): p. 261-265.
118. Flock, M.R., et al., *Effects of supplemental long-chain omega-3 fatty acids and erythrocyte membrane fatty acid content on circulating inflammatory markers in a randomized controlled trial of healthy adults*. Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA), 2014. **91**: p. 161-168.
119. Kirkhus, B., et al., *Effects of similar intakes of marine n-3 fatty acids from enriched food products and fish oil on cardiovascular risk markers in healthy human subjects*. BRITISH JOURNAL OF NUTRITION, 2012. **107**(9): p. 1339-1349.
120. Chiang, Y.-L., et al., *The effect of dietary walnuts compared to fatty fish on eicosanoids, cytokines, soluble endothelial adhesion molecules and lymphocyte subsets: a randomized, controlled crossover trial*. Prostaglandins, Leukotrienes & Essential Fatty Acids, 2012(4-5): p. 111.
121. Root, M., et al., *A randomized trial of fish oil omega-3 fatty acids on arterial health, inflammation, and metabolic syndrome in a young healthy population*. Nutrition Journal, 2013. **12**(1): p. 1-6.
122. Dangardt, F., et al., *Omega-3 fatty acid supplementation improves vascular function and reduces inflammation in obese adolescents*. Atherosclerosis, 2010. **212**: p. 580-585.
123. Allaire, J., et al., *A randomized, crossover, head-to-head comparison of eicosapentaenoic acid and docosahexaenoic acid supplementation to reduce inflammation markers in men and women: the Comparing EPA to DHA (ComparED) Study*. 2016, American Society for Clinical Nutrition, Inc. p. 280.

124. Luis, D., et al., *Effect of DHA supplementation in a very low-calorie ketogenic diet in the treatment of obesity: a randomized clinical trial*. Endocrine, 2016(1): p. 111.
125. Muldoon, M.F., et al., *Fish oil supplementation does not lower C-reactive protein or interleukin-6 levels in healthy adults*. Journal of internal medicine, 2016. **279**(1): p. 98-109.
126. van Bussel, B.C.T., et al., *Fish Consumption in Healthy Adults Is Associated with Decreased Circulating Biomarkers of Endothelial Dysfunction and Inflammation during a 6-Year Follow-Up*. JOURNAL OF NUTRITION, 2011. **141**(9): p. 1719-1725.
127. Hoogeveen, E.K., et al., *No effect of n-3 fatty acids on high-sensitivity C-reactive protein after myocardial infarction: the Alpha Omega Trial*. EUROPEAN JOURNAL OF PREVENTIVE CARDIOLOGY, 2014. **21**(11): p. 1429-1436.
128. Thies, F., et al., *Influence of dietary supplementation with long-chain n-3 or n-6 polyunsaturated fatty acids on blood inflammatory cell populations and functions and on plasma soluble adhesion molecules in healthy adults*. Lipids, 2001. **36**(11): p. 1183-1193.
129. Mantzioris, E., et al., *Biochemical effects of a diet containing foods enriched with n-3 fatty acids*. American Journal of Clinical Nutrition, 2000. **72**(1): p. 42-48.
130. Murphy, K.J., et al., *Low dose supplementation with two different marine oils does not reduce pro-inflammatory eicosanoids and cytokines in vivo*. Asia Pacific journal of clinical nutrition, 2006. **15**(3): p. 418-424.
131. Kiecolt-Glaser, J.K., et al., *Omega-3 supplementation lowers inflammation in healthy middle-aged and older adults: A randomized controlled trial*. Brain Behavior and Immunity, 2012. **26**: p. 988-995.
132. Accinni, R., et al., *Effects of combined dietary supplementation on oxidative and inflammatory status in dyslipidemic subjects*. Nutrition, Metabolism and Cardiovascular Diseases, 2006. **16**: p. 121-127.

133. Paulo, M.C., et al., *Influence of n-3 polyunsaturated fatty acids on soluble cellular adhesion molecules as biomarkers of cardiovascular risk in young healthy subjects*. Nutrition, Metabolism and Cardiovascular Diseases, 2008. **18**: p. 664-670.
134. Buckley, M.S., A.D. Goff, and W.E. Knapp, *Fish oil interaction with warfarin*. Annals of Pharmacotherapy, 2004. **38**(1): p. 50-53.
135. Gross, B.W., et al., *Omega-3 Fatty Acid Supplementation and Warfarin: A Lethal Combination in Traumatic Brain Injury*. Journal of Trauma Nursing, 2017. **24**(1): p. 15-18.
136. Stalenhoef, A.F.H., et al., *The role of TNF-alpha in chronic inflammatory conditions, intermediary metabolism, and cardiovascular risk*. Journal of lipid research, 2007. **48**(4): p. 751-762.
137. Skoog, T., F. Karpe, and W. Dichtl, *Plasma tumour necrosis factor-alpha and early carotid atherosclerosis in healthy middle-aged men*. European heart journal, 2002. **23**(5): p. 376-383.
138. Committee, D.G.A., *Addendum A: EPA and DHA Content of Fish Species*, D.o. Agriculture, Editor. 2005, U.S. Department of Health and Human Services: Washington, D.C. 20201.
139. Sijben, J.W.C. and P.C. Calder, *Differential immunomodulation with long-chain n-3 PUFA in health and chronic disease*. PROCEEDINGS OF THE NUTRITION SOCIETY, 2007. **66**(2): p. 237-259.
140. Wu, D., *Modulation of immune and inflammatory responses by dietary lipids*. Current Opinion In Lipidology, 2004. **15**(1): p. 43-47.
141. Ferrucci, L., et al., *Relationship of plasma polyunsaturated fatty acids to circulating inflammatory markers*. JOURNAL OF CLINICAL ENDOCRINOLOGY & METABOLISM, 2006. **91**(2): p. 439-446.
142. Hibbeln, J.R., et al., *Do plasma polyunsaturates predict hostility and depression?* World Review Of Nutrition And Dietetics, 1997. **82**: p. 175-186.

143. Simopoulos, A.P., *Omega-3 fatty acids in inflammation and autoimmune diseases*. Journal Of The American College Of Nutrition, 2002. **21**(6): p. 495-505.