IMPACT OF N-3 STATUS ON PERIODONTITIS: A LITERATURE REVIEW

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Abstract

This paper reviews etiology and clinical manifestations of periodontitis. Biosynthesis of omega-3 fatty acids and their effect on immunity and inflammatory processes in the host is also discussed. In periodontitis, bacteria and their byproducts initiate an inflammatory response.[1] Details follow on the mechanisms of the onset and resolution phase of inflammation. Specialized Pro-Resolving Mediators (SPMs) are lipid mediators enzymatically derived from essential omega-3 (n-3) and omega-6 (n-6) fatty acids that have been found to resolve inflammation, enhance host defense, and stimulate tissue regeneration.[2] There is a growing body of research on the impact of n-3 status on periodontitis, including investigations on the correlation of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) to periodontitis,[3,4] effect of n-3 supplementation from a clinical perspective,[5,6] and treatment of periodontitis with n-3 supplementation and aspirin.[7,8] There is a need for expanded research to confirm the effects of n-3 status on clinical outcomes of periodontitis and regenerative periodontal tissues, in particular studies with larger sample sizes and longer durations.[9] Consensus on the relationship between periodontitis and dietary intake of omega-3 fatty acids will improve understanding of this field of research. Dental professionals should educate patients on increasing n-3 intake to manage periodontitis and overall health.[10]

Key words: omega-3, fatty acids, n-3 polyunsaturated fatty acids (PUFAs), nutrition, diet, oral health, periodontitis, and inflammation.

Introduction

Periodontitis is a chronic multifactorial inflammatory disease characterized by the destruction of periodontal tissue due to excess bacterial matrix at the gum line.[11] The Center for Disease Control and Prevention (CDC) reports that approximately 64.7 million Americans have periodontal disease.[1] The proposed primary etiology of periodontal disease is an interaction between a susceptible host and bacteria in the host's oral cavity. *Aggregatibacter actinomycetemcomitans* (A.a)*, Porphyromonas gingivalis* (P.g)*,* and *Tannerella forsythia* have been found to be of the most common bacteria which play a role in the onset and progression of periodontitis.[1] Endotoxins released by these bacteria initiate an immune response in the host's periodontal connective tissue.[1] A cascade of events follow and pro-inflammatory cells and cytokine-mediated markers of inflammation increase, which are associated with irreversible destruction of periodontal tissue.[1] Omega-3 fatty acids have been reported to have profound actions as pro-resolving, anti-inflammatory chemical mediators, facilitating the resolution of inflammation in the body.[12] A field of research is emerging on omega-3's and their impact on the progression and treatment of periodontal disease. Neiva et al.[13] has hypothesized that intake of specific nutrients, in combination with periodontal therapy, could be a safe method to enhance periodontal treatment.[13] Current research studies have involved looking at the direct correlation of EPA and DHA to periodontitis,[3,4] effect of n-3 supplementation on periodontitis from a clinical perspective,[5,6] and the possible treatment of periodontitis with a combination of n-3 supplementation and aspirin.[7,8]

This paper elucidates the literature available in an attempt to answer the question, “To what extent does n-3 status impact periodontitis?”

Periodontitis

Periodontitis is a chronic inflammatory disease characterized by the destruction of periodontal tissue due to excess bacterial matrix at the gum line. Mild periodontitis affects an estimated 47.2% of adults in the United States above the age of 30, while 10-15% of the American adult population has severe forms of the disease.[11] Prevalence of periodontitis increases to 70.1% of adults in the United States above the age of 65. Men are reported to have higher incidence rates of periodontitis at 56.4%, compared to women at 38.4%.[14] Individuals with periodontitis are at increased risk for other oral health complications such as root caries, tooth mobility, and tooth loss.[15] Clinical measures of periodontitis comprise Gingival Index (GI), Clinical Attachment Loss (CAL), Alveolar Bone Loss (BL), Bleeding On Probing (BOP), Papillary Bleeding Index (PBI), Periodontal Probing Depth (PBD), and Periodontal Pocketing, as well as tooth drifting, movement, and exfoliation.[16]

The proposed primary etiology of periodontal disease is due to bacteria residing in a susceptible host.[1] The early stage of periodontal disease is Gingivitis, in which plaque first begins to accumulate on teeth and gums become inflamed. Inflammation of periodontal tissue in gingivitis is reversible. Over time, plaque and inflammation spread

deeper into periodontal connective tissue causing pockets and moderate bone loss, transforming to periodontitis. Characteristics of the next stage, advanced periodontitis, are deep pockets and severe bone and tooth loss.[15] Approximately thirty species of pathogens have been identified to reside in the oral cavity and secrete endotoxins, contributing to systemic disease. Common inhabitants of the oral cavity consist of Gram-negative Treponema, Bacteroides, Porphyromonas, Prevotella, Capnocytophaga, Peptostreptococcus, Fusobacterium, Actinobacillus, and Eikenella.[1] When bacteria reside in the susceptible host, they secrete matrix-degrading enzymes and other molecules.[17] Several bacteria have been reported as key players in the onset and progression of periodontitis, primarily *Aggregatibacter actinomycetemcomitans (A.a), Porphyromonas gingivalis (P.g),* and *Tannerella forsythia.*[17] A study conducted by Umeda et al.[18] concluded H. *pylori* to be at higher levels in patients with periodontitis. However, no significant correlation was reported between H. *pylori* and the state of periodontitis.[18]

Emerging research has identified conditions which amplify the host's response to bacterial plaque.[19] These conditions include use of steroid hormones, systemic diseases, nutrient deficiencies, use of several drugs, diabetes, and smoking.[19] Sugar in the diet is another important component to consider. Sugar is metabolized into organic acids by dental plaque, a biofilm of microorganisms. The organic acid byproducts reduce pH and promote demineralization of teeth. Breakage of blood vessels, whether from tissue

trauma, flossing, or oral procedures, provide a passageway for bacteria to enter the systemic blood stream.[1]

The presence and metabolic byproducts of periodontal pathogens promote oral and systemic inflammation. Lipopolysaccharide products of bacteria elicit an immune response in the human host's periodontal connective tissue.[1] When the immune response is initiated, leucocytes and lymphocytes are recruited, and interleukin and tumor necrosis factor-alpha (TNF-a) production increases.[16] Secondary mediators amplify the immune response, and the cytokines produced interfere with the tissue repair process. In addition, osteoclastogenesis causes alveolar bone destruction (BL).[16]

Periodontitis has also been associated with numerous non-oral and chronic systemic diseases including: cardiovascular disease, diabetes, obesity, respiratory tract infections, cancer, metabolic syndrome, and neurocognitive diseases and impairment.[1,20] Pro-inflammatory cell and cytokine-mediated markers of inflammation, including interleukin-1 (IL-1), IL-6, and TNF-a, are reported to play a pivotal role in the development and advancement of periodontitis. These inflammatory markers are also common in many systemic diseases, therefore; management of inflammation associated with periodontitis can positively assist in the progression, morbidity, mortality, and controlling of non-oral systemic diseases.[21]

Host modulatory therapy (HMT) is a form of treatment of periodontitis that focuses on treatment of the host in the host-bacteria interaction. The objective of this therapy is to modify the host response in order to reduce tissue destruction associated with periodontitis, and potentially regenerate lost periodontium.[22] This is achieved through the downregulation of destructive processes of the host inflammatory response and reduction of inflammation, with up-regulation of regenerative processes, and will aid in periodontal stability.[22] Agents used in this therapy are derived locally or systemically and are prescribed to patients in combination with conventional treatments. Common agents feature non-steroidal anti-inflammatory drugs, bisphosphate, sub antimicrobial doxycycline, enamel matrix protein, growth factors, and bone morphogenic proteins.[23] Emerging research[24] on the powerful anti- inflammatory properties of omega-3 fatty acids have raised question as to whether or not omega- 3 fatty acids could act as a potential HMT agent to treat inflammation associated with periodontal disease.[24]

Omega-3 Fatty Acids

Essential fatty acids can be divided into two classes: omega-6 and omega–3. Food sources rich in omega-3 include fatty fish, walnuts, and flaxseeds, while sources rich in omega-6 include vegetable oils (corn, safflower, sunflower, soybean) and animal products.[25] Over 10,000 years ago, agriculture and the science of breeding farm

animals emerged, and since then profound changes have evolved in the human environment, most notably diet and lifestyle factors. The modern western diet is reported to have had a 6-8% increase in dietary n-6 content, a 40% decrease in n-3 PUFAs, and an increased ratio of omega-6 to omega-3 from 5:1 to 10:1.[25] Excessive intake of omega-6 long-chain PUFAs (LC-PUFAs) increases arachidonic acid (AA) levels, and thus AA derived pro-inflammatory eicosanoids and biomarkers, including prostaglandins, leukotrienes, and Leukotriene B4 (LTB4).[12] This leads to local and systemic inflammation -a catalyst for numerous negative health effects and human diseases. The negative impact of omega-3 fatty acid deficiency on behavior patterns is supported by animal and human studies.[12]

Polyunsaturated fatty acids are synthesized from dietary intake of essential fatty acids and contain two or more double bonds in their chemical structure. The parent to omega-6 fatty acid, linoleic acid (LA; 18:2n-6), can be synthesized in the body to produce long-chain omega-6 fatty acids, such as dihomo-γ-linolenic acid (DGLA; C20:3n-6) and arachidonic acid (AA; C20:4n-6).[26] The parent to omega-3 fatty acids is alpha-linolenic acid (ALA; C18:3n-3), which is synthesized in the body to produce long-chain omega-3 fatty acids, such as eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3).[26] When ALA and LA are synthesized in the body, they undergo desaturation, with Fatty Acid Desaturase 1 (FADS1) and FADS2, followed by elongation, with Elongation of very long chain fatty acids protein 2 (ELOVL2) and ELOVL5.[27] Both ALA

and LA synthesis pathways have numerous steps in common, resulting in competition amongst their metabolic intermediates. Competition between n-3 and n-6 substrates has led to increased synthesis of n-6 LC-PUFAs, and decreased synthesis of n-3 LC-PUFAs.[27,28] Some of the omega-6 fatty acids in cell membranes of erythrocytes, platelets, endothelial cells, monocytes, and fibroblasts can be replaced by omega-3's by adhering to a diet rich in omega-3 fatty acid containing foods.[29] The metabolites produced from n-6 LC-PUFAs have different effects on immunity and inflammatory processes than those produced by n-3 LC-PUFAs. AA produced from n-6 LC-PUFAs is a pro-inflammatory chemical mediator. When present for an extended period of time, it contributes to chronic inflammation.[30]

In contrast, eicosapentaenoic acid (EPA), docosahexaenoic acid

(DHA), and DHA derived from n-3 LC-PUFAs act as pro-resolving anti-inflammatory chemical mediators that actively work to facilitate the clearance of inflammatory cell components in order to resolve inflammation and return the body's tissues back to homeostasis.[30] Of the omega-3 fatty acids, DHA and EPA are more biologically potent in comparison with alpha-linolenic acid (ALA). DHA has been found to be more effective than EPA in dose-response studies.[31] Further, diets composed of DHA and EPA are synergistic.[31] DHA derived specialized pro-resolving mediators, termed D-series resolvins, protectins, and maresins, have been found to not only resolve tissue inflammation, but also enhance host defense, and stimulate tissue regeneration.[2] In

contrast, research has found reduction in DHA is associated with conditions including Alzheimer's disease, schizophrenia, autism, and depression.[30] Evidence points to n-3 LC-PUFAs in reducing cholesterol, inflammation, platelet formation, and mortality.[30]

The World Health Organization (WHO)[32] and Dietary Guidelines of the US 2010[33] recommends adults consume 250-500mg/d of EPA and DHA. The Food and Agriculture Organization (FAO)[34] advocates for 500mg/d of EPA and DHA for prevention of coronary heart disease. It is recommended by the American Heart Association (AHA)[35] to incorporate fish into an individual's diet a minimum of two times per week. A serving of fish constitutes around 3.5 ounces cooked or ¾ cup flaked.[35] Recommendations by the National Heart Foundation of Australia released in 2008,[36] advised Australian adults to consume an average of 250-500mg of omega-3 EPA/DHA from marine sources, and 100mg of ALA from plant sources every day.[36] Omega-3 supplementation is recommended to those who are currently living with heart disease, are at high risk for heart disease, or do not consume fish or seafood products.[36]

Acute and Chronic Inflammation

Inflammation is the body's protective mechanism against injury, infection, and surgery.[37] It is an immune response characterized by vasodilation, increased vessel permeability, secretion of pro-inflammatory cytokines, and enhanced migration of white blood cells from blood vessels to target, inflamed tissues.[37] In periodontitis, bacteria and their byproducts pose a challenge to the body, and the body attempts to eradicate the pathogens through initiation of the inflammatory response.[21]

Inflammation can be classified into two categories, which are interconnected; acute and chronic. A prominent difference between acute and chronic inflammation is the duration of the inflammation. Acute inflammation is immediate, lasting several days. In contrast, chronic inflammation is an ongoing response lasting for months to years.[37] In periodontitis, if inflammation is not resolved, it develops into chronic inflammation and the irreversible destruction of periodontal tissue.[21] The primary goal of the body's inflammatory response is to return the body back to homeostasis, and it does this by detecting and eliminating factors which disrupt homeostasis. The inflammatory response is made up of three phases; the initiation phase, the onset phase, and the resolution phase.[37,38]

The initiation phase of inflammation begins when a challenge, such as trauma or an infection, is present in the body.[38,39] This is the go signal for the onset phase of acute inflammation, in which an inflammatory response takes place. Production of pro-

inflammatory chemical mediators, such as prostaglandins and leukotrienes, which are synthesized from AA, LTB4, and cytokines, such as TNF-a, IL-6, and IL-1B, are increased.[38,39,40]. Prostaglandins and leukotrienes increase membrane permeability, permitting movement of white blood cells from blood vessels to the challenge site[39,40]. Prostaglandins and leukotrienes are important players in protecting the body against invaders, however, when present for an extended period of time, they contribute to chronic inflammation.[37] In periodontitis, the abundance of pro-inflammatory cytokines released outweighs the anti-inflammatory cytokines. Pro-inflammatory cytokines released during the inflammatory response that play a central role in periodontitis are IL-1, IL-6, and TNF-a.[21] Further, in the event of tissue injury, polymorphonuclear neutrophils (PMNs) are recruited to the site, as well as activated macrophages for phagocytosis of invaders.[41]

During the resolution phase of acute inflammation, a pro-resolving response takes place. Lipid mediator class switching activates lipoxin and resolvin production. Lipoxins, such as LXA4, stimulates non-phlogistic monocyte recruitment. When these monocytes undergo phagocytosis, pro-inflammatory mediators are not released.[38] Specialized pro-resolving mediators (SPMs), for instance resolvins, protectins, lipoxins, and maresins, are anti-inflammatory mediators that actively work to facilitate the clearance of inflammatory cell components in order to resolve inflammation and return the body's tissues back to homeostasis.[42] Lipoxins and resolvins act to limit PMN infiltration and

promote efferocytosis, a process in which resolving macrophages engulf apoptotic neutrophils in addition to removing other cellular debris.[43] SPMs further stimulate and enhance efferocytosis, a process carried out by phagocytic cells to remove dead and dying cells.[42]

SPMs are lipid mediators enzymatically derived from essential omega-3 and omega-6 fatty acids, including AA, EPA, and DHA.[44] SPMs have been found to not only resolve tissue inflammation, but enhance host defense, and stimulate tissue regeneration. Once synthesized from PUFAs, SPMs are released from cell membranes by phospholipase A2 (PLA2) for secondary conversion by particular enzymes. SPMs appearance is enhanced during the time when macrophages are actively working to diminish apoptotic PMNs.[2] The core SPM families include lipoxins, derived from AA, E-series resolvins, derived from EPA, and D-series resolvins, protectins, and maresins, derived from DHA.[42]

Research Supporting n-3 Status and Periodontitis

A growing body of research is being conducted investigating the impact of n-3 status on periodontitis. Published studies have examined the direct correlation of EPA and DHA to periodontitis,[3,4] the effect of n-3 supplementation on clinical measures of periodontitis,[5,6] and the treatment of periodontitis with a combination of n-3 supplementation and aspirin. [7,8]

Table 1: Summary of studies included in literature review					
Author; year	**Participants ; age**	**Type of Study; Study Duration;**	**Dietary Intervention/ Experimental Treatments/ Exposures**	**Key Measured Outcomes**	**Main Results**
Masanori Iwasaki; 2010[3]	55 Niigata participants from Japan;74 years of age	Longitudinal study; five years	Participants randomly selected from a longitudinal interdisciplinary study of aging to evaluate correlation between EPA, DHA, and periodontitis	IRR of periodontal disease events	When compared to a high EPA diet, low EPA intake was approached significance with increased periodontal disease events (IRR 1.47, 95% CI 0.97-2.21, p=0.067).
Asghar Naqvi; 2010[4]	9,182 NHANES survey participant from the USA;>=20 years of age	Cross- sectional study; data received from NHANES survey from 1999 to 2004	Dietary intake of FA per day was assessed by 24- hour dietary recall	Adjusted OR of Periodontitis	Inverse association between DHA intake (0mg/d-0.04mg/) and OR of periodontitis. No association when DHA intake equal to or above 0.04gm/d. Modest inverse association between EPA and OR. No association when EPA

					was equal to or greater than 0.04gm/d.
Phillippe Campan; 1997[5]	37 volunteers; mean age 22.9+-2.1 years of age	Double- blind RCT; 35 days, with 14 days of intense oral hygiene	Participants refrained from oral hygiene for 29 days and received fish oil (30% n-3 PUFA) supplementation or placebo (olive oil) pills on day 18.	PI, GI, PBI Gingival levels of AA, EPA, DHA, DPA, PGE2, and LTB4 (10 volunteers)	EPA significantly increased (p=0.04) and GI significantly declined (p=0.008) in fish oil group. PBI significantly declined in fish oil and placebo groups (p=0.002). LTB4 declined in fish oil group.
Rosenstein ED; 2003[6]	30 participants with periodontitis; 18-60 years of age	RCT; 12 weeks	Participants randomized to consume n-3 PUFA EPA in the form of fish oil, or n-6 PUFA Gamma-linoleic acid (GLA) in the form of borage oil, both, or placebo (olive and corn oil mixture).	MGI, PI, BOP, PPD, CAL	Borage oil group significant improvement in MGI (p=0.016). Supplementation with fish oil or borage oil alone showed trends in improvements in PPD. Borage oil demonstrated significant improvements compared to placebo (p=0.044).

Moustafa Elkhouli; 2011[7]	40 participants with grade II furcation defect; 35-60 years of age	RCT double blind; 6 months	Participants randomized to a test group, given a combination of decalcified freeze- dried bone allograft (DFDBA), omega-3 PUFAs, and low- dose aspirin, or a control group, given DFDBA and placebo.	PPD, CAL, IL-1B, IL-10	Compared to control group at 6 months, test group displayed significant greater reduction in mean PPD ($p<0.001$) and significant increase in CAL ($p<0.05$). Intervention demonstrated significant modulatory effects on IL-1b and IL-10 compared to placebo.
Asghar Naqvi; 2014[8]	46 participants with moderate periodontitis; adults	RCT double blind; 3 months	Participants randomized to either a DHA group, receiving 2,000mg of DHA, or the placebo group, receiving soy/ corn oil capsules. Both groups also given 81mg of aspirin.	Mean pocket depth, GI, hsCRP, IL-B, IL-6	Significant decrease in mean pocket depth ($p=0.03$), and GI ($p=0.04$) in DHA group compared to baseline. Significant difference between DHA and placebo group in high sensitivity C- reactive protein (hsCRP) in gingival crevicular fluid (GCF) (−5.3 ± 2.4 ng/mL, $p = 0.03$), and IL-1B (−20.1 ± 8.2 pg/mL, $p = 0.02$).

Martinez; 2014[45]	15 participants with chronic periodontitis; test group 43.1+-6, placebo group 46.1+-11.6 years of age	RCT double blind; 12 months	Participants randomized into two groups test group and receiving SRP and three 300mg capsules (180-mg EPA and 120-mg DHA), or control group receiving SRP and placebo capsule.	%BOP, PPD, CAL, VPI	No significant effect on clinical outcomes of periodontitis
Keskiner; 2017[9]	30 participants with chronic periodontitis; placebo group 40.87+-9.7, test group 42.54+-5.82 years of age	RCT; 6 months	Participants randomized into two groups, control group receiving SRP and placebo, or test group, receiving SRP and 6.25mg EPA and 19.19mg DHA.	PI, GI, BOP, PPD, CAL, SOD, TNF-a	No significant effect of SOD or clinical measures of chronic periodontitis

Iwasaki et al.[3] conducted a longitudinal study evaluating the correlation between EPA and DHA, and periodontitis over time in community-dwelling elderly. A total of fifty-five

patients 74 years of age were randomly selected. Participants' were trained by dieticians on how to record food intake. Specific nutrient intakes of DHA and EPA per day were calculated using the Standard Food Composition Tables in Japan. Participants attended intraoral dental examinations at baseline and continued receiving examinations once per year for five years. A periodontal disease event was defined as a minimum 3mm change in clinical attachment loss (CAL). Statistical analysis, for example negative binomial regression analysis, was utilized to estimate the influence of DHA and EPA on periodontal disease events. When compared to a high EPA diet, low EPA intake approached significance with increased periodontal disease events (incidence rate ratio (IRR) 47, 95% confidence interval (CI) 0.97-2.21, p=0.067). No significant correlation was found between EPA intake and periodontal disease outcomes, however, the low EPA intake group tended to have more periodontal disease events (IRR 1.47, 95% CI 0.97–2.21, P= 0.067). The number of teeth present in the low DHA group was significantly lower when compared to the high DHA intake group (15.2 +- 9.6 and 22.3+-6.9 teeth present, respectively; p=0.013), as noted in figure 1. This significant difference was also found in regard to EPA intake. Low EPA intake individuals had significantly fewer teeth, versus the high EPA intake group, (14.7 +-9.6 and 22.1+-7.5 teeth present, respectively; p=0.013), also noted in figure 1.[3]

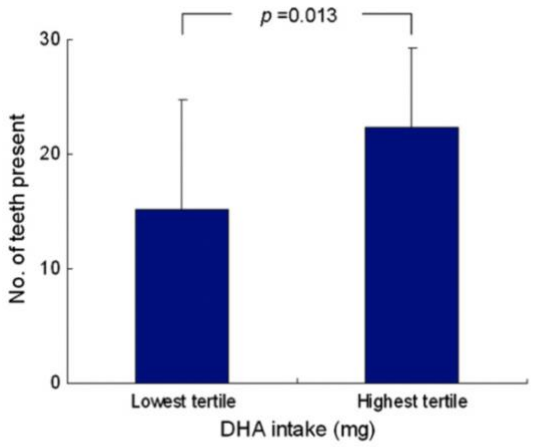


Fig. 1. Relation between DHA intake and number of teeth present at baseline. DHA, docosahexaenoic acid.

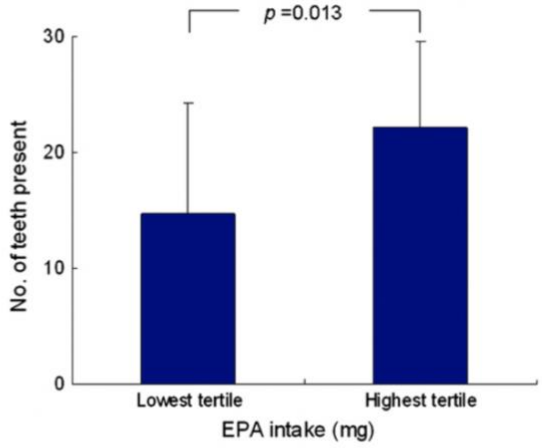


Fig. 2. Relation between EPA intake and number of teeth present at baseline. EPA, eicosapentaenoic acid.

Figure 1: Iwasaki study results[3]

A limitation to this study is socio-behavioral factors may act as confounding variables due to the influence of diet and tooth loss on health behavior. It should be noted nineteen participants withdrew from the study before the 5-year period was over, and their data was not contained in the statistical analysis. The long duration of the study assisted in drawing conclusions on the correlation between EPA, DHA, and periodontitis over time in community-dwelling elderly.[3]

A cross-sectional study[4] investigating n-3 and prevalence of periodontitis provides similar supportive data. Participant data was obtained from the National Health and Nutrition Examination Survey (NHANES) that was released from 1999 to 2004. Information from 9,182 adults 20 years of age or older was used in the study. Periodontitis was evaluated by dental professionals and defined as >= 4mm pocket

depth and >= 3mm CAL. A total of 1024 participants were identified to have periodontitis, a weighted prevalence of 8.2% (95% CI 7.0-9.4). Dietary fatty acid intake per day was determined utilizing a 24-hour dietary recall. The study concluded that increased intake of DHA between 0mg/d and <0.04mg/d is associated with decreased prevalence (OR) of periodontitis. There was no association when DHA intake was equal to or above 0.04gm/d. This finding merely suggests there is no extra benefit beyond modest intake, but additional studies would need to be carried out to test this suggestion as there was no statistical significance. A modest inverse association was found between EPA and OR. There was no association between OR and EPA when EPA was equal to or greater than 0.04gm/d. Further, no statistically significant association between OR of periodontitis and ALA was found (p=0.11). A limitation to the study is the cross-sectional study design. Causal or temporal relationships cannot be concluded, only associations. Further, diet could have been affected by tooth loss related to periodontitis. A 24-hour recall was utilized to collect dietary data, and thus is not an ideal representation of usual dietary intake. However, these data do give reliable insight into recent intake, which can be used to estimate the typical intake of a group. This study has a large sample size that is representative of US adults, and elaborate control procedures were carried out when conducting periodontal assessments.[4]

Research[5] has also looked at the effect of omega-3 supplementation on clinical measures of periodontitis. One of the earliest studies, published by Campan et al.[5], was a double-blind, randomized pilot study. Thirty-seven volunteers, with a mean age of

22.9 years (+-2.1), underwent strict oral hygiene regimens for 14 days, and then completely refrained from any form of oral hygiene for the next 21 days. On day 28, eighteen participants were randomized to receive 6g/day fish oil (30%n-3PUFA), and the remaining nineteen participants received a placebo (olive oil with 1% of n-3 PUFA). EPA, docosapentaenoic acid (DPA), and DHA assays showed an increase in mean levels in the fish oil group on day 35, and a specific statistically significant increase in EPA (p=0.04). The participants' scores on the Papillary Bleeding Index (PBI) were significantly decreased in the fish oil and placebo groups (p=0.002), however, only the fish oil group showed significant decreases in Gingival Index scores (GI; p=0.008), which did not change significantly in the placebo group. Decline in gingival bleeding is suspected to be due to a strengthened host inflammatory response, perhaps due to n-3 PUFAs. Although no significant benefits of n-3's can be concluded, n-3 PUFA supplementation did overall show trends of reduced inflammation in the host.[5]

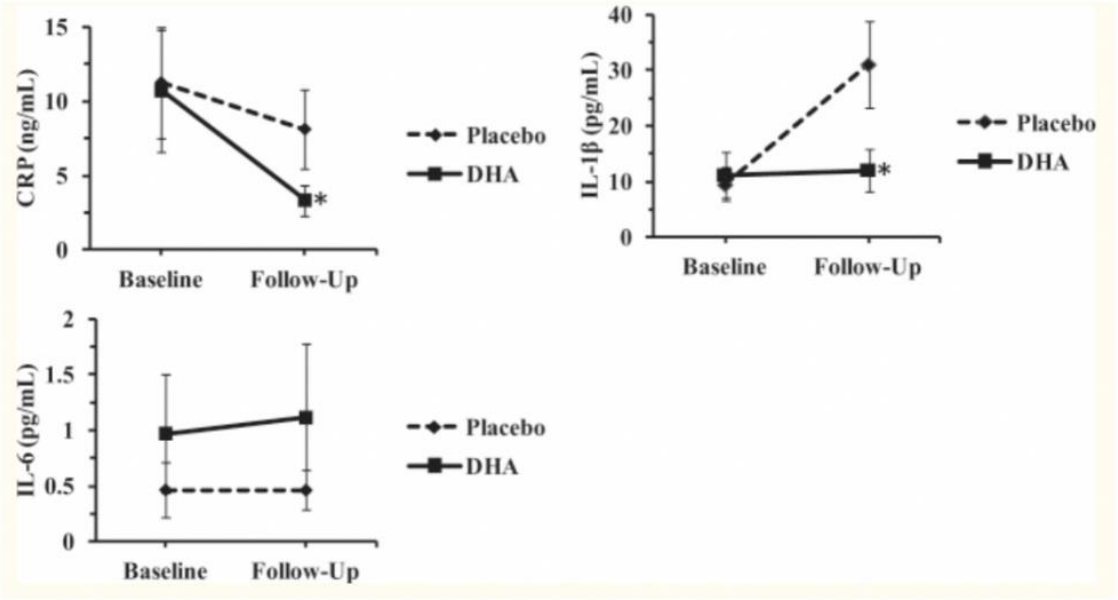
The effect of omega-3 supplementation on clinical measures of periodontitis was investigated further in a 12-week randomized placebo-control pilot study[6] in which 30 adult participants with periodontitis were randomized to consume n-3 PUFA EPA in the form of fish oil, or n-6 PUFA gamma-linoleic acid (GLA) in the form of borage oil. The supplement serving size was 3000mg/day. Broken into four, the groups were fish oil, borage oil, half fish oil and half borage oil, and placebo (olive oil and corn oil mixture). Data was obtained at baseline and 12 weeks later, and participants in the borage oil

group showed significant improvement in modified gingival inflammation (MGI) (1.04 vs. 0.68, p=0.016). Although the remaining groups (fish oil and the 2 combination groups) showed no statistically significant improvement, results show a trend toward improvement. Borage oil showed significant improvements in PPD when compared to placebo (-0.50 vs. 0.02, $p=0.044$). Although not statistically significant, fish oil or borage oil when consumed alone did show improvements in PPD. Lack of statistical significance could be due to the short duration of the study, small sample size, and lack of compliance.[6]

Research[7] has looked at host modulation therapy, omega-3 supplementation in combination with low-dose aspirin, as an adjunctive treatment option for chronic periodontitis. In a randomized, double-blind, placebo-controlled study[7], forty participants with a grade II furcation defect were recruited. The participants were between 35-60 years of age, with a mean age of 42.6+-9.7 years. Participants were randomized into a test group or control group. The test group received a combination of decalcified freeze-dried bone allograft (DFDBA), omega-3 PUFAs, and low-dose aspirin. The control group was given DFDBA and a placebo. Compared to the control group, at 6 months the test group showed a statistically significant greater reduction in mean PPD ($p<0.001$) and significant increase in CAL ($p<0.05$). The intervention in the test group demonstrated significant modulatory effects on IL-1b and IL-10 compared to placebo ($p<0.05$). This study suggests omega-3 supplementation in combination with low dose

aspirin is an adjunctive treatment option that could be efficient for patients with chronic periodontitis. A limitation to this study was the lack of assessment of compliance with taking the fish oil. No erythrocyte fatty acid composition measurements were taken; thus, the beneficial outcomes cannot be connected to a specific fatty acid. Some of the benefit could potentially be linked to the DFDBA regenerative therapy, however, clinical studies on DFDBA have suggested limitation on its effectiveness as a treatment alone.[7]

Naqvi et al.[8] further investigated the effect of omega-3 supplementation and aspirin, but without any periodontal treatment. In a 3-month, double blind, placebo-controlled trial, a total of fifty-five individuals noted to have moderate periodontitis were randomized to either the DHA group, receiving 2,000mg of DHA/day, or the placebo group, receiving identical soy/corn oil capsules as often. Both groups were also given 81mg of aspirin/day. From the original fifty-five, a total of forty-six participants completed the study. Measurements were conducted at baseline and at the end of the study; the DHA group showed a significant decrease in mean pocket depth (-0.29 ± 0.13;p=0.03) and GI (–0.26 ± 0.13; p=0.04) compared to baseline. Furthermore, specific clinical measures showed significant difference between the DHA and placebo groups, specifically high sensitivity C-reactive protein (hsCRP) in gingival crevicular fluid (GCF) (−5.3 ± 2.4 ng/mL, p = 0.03), and IL-1B (−20.1 ± 8.2 pg/mL, p = 0.02) (figure 2). IL-6 and systemic hsCRP showed no significant change.[8]



Effect of intervention on mean (SE) levels of GCF hsCRP, IL-1β, and IL-6 at baseline and follow-up. SE, standard error; GCF, gingival crevicular fluid; hsCRP, high-sensitivity C-reactive Protein; IL-1β, interleukin 1β; IL-6, interleukin-6; DHA, docosahexaenoic acid.

*$p < .05$.

Figure 2: Naqvi Study Results[8]

The significant decrease in local inflammation raises a question as to whether systemic inflammation would also show a significant decrease over a longer period of time[45]. The small sample size limits the ability to note minute differences amongst treatment effects, and the short duration limits the potential to investigate long-term outcomes. No radiographs were utilized when classifying periodontitis, therefore, there is possibility for misclassification. On the other hand, the study design, blinding, adherence, and elaborate quality control procedures are all strengths to the study. This research provides evidence that DHA supplementation when taken in combination with

aspirin significantly improves periodontal outcomes in adults with moderate chronic periodontitis.[8]

In contrast, studies[9,45] have also been published which show no effect between n-3 status and periodontitis. Martinez et al.[45] investigated the effect of n-3 supplementation in combination with nonsurgical periodontitis treatment on EPA, DHA, DPA, and AA in a 12-month study[46]. Fifteen individuals were enrolled in the studied, all of which had chronic periodontitis. Seven participants were in the test group, mean age 43.1+-6, were treated with Scaling and Roots Planning (SRP) and received three 300mg capsules/day composed of 180-mg EPA and 120-mg DHA. The remaining eight participants, mean age 46.1+-11.6 years, received SRP treatment and a placebo capsule. Data was recorded at baseline, 4 months, and 12 months later. Clinical measures incorporated percent of sites BOP, PPD, and CAL. The test group had a significant increase in serum EPA at 4 months ($p<0.05$). Additionally, a significant reduction in AA:EPA and AA:DHA ratios was observed in the test group ($p<0.05$) in contrast to a significant increase in AA:EPA and AA:DHA ratio in the placebo group in this same period ($p<0.05$). The change in EPA in the test group was significantly higher compared to the placebo group from baseline to 12 months (p=-0.02), while no significant difference was reported for change in DHA, DPA, or AA amongst the test and placebo groups. In regard to clinical parameters of periodontitis, after receiving nonsurgical periodontal treatment, there were significant improvements in PD and CAL in both groups, and a significant decline in BOP-positive

sites from 4 to 12 months in the placebo group. This study concludes that although EPA levels significantly increased following 12 months of omega-3 dietary supplementation combined with nonsurgical periodontal treatment, there was no significant effect on clinical outcomes of periodontitis. A significant limitation to this study is the small sample size, which impedes on the ability to collect consistent evidence.[45]

A very recent, randomized, controlled clinical study[9] further investigated the impact of n-3 PUFAs in combination with SRP on clinical parameters of chronic periodontitis patients. Inclusion criteria for the study were diagnosis of chronic periodontitis, minimum of nine posterior teeth, 507mm PPD, and minimum 6mm PA. The main exclusions were individuals who have received any form of periodontal therapy over the past year, SRP in the past 6 months, and/or use of aspirin or non-steroidal anti-inflammatory drugs in the past 6 months. Thirty participants were randomized into two groups, with the control group receiving SRP and placebo and the test group receiving SRP and 6.25mg EPA and 19.19mg DHA/day. Between the placebo and test group, there was no significant difference in the change in GI and BOP scores ($p>0.05$), although the test group did show trends in increased reduction of GI and BOP. Observable in figure 3, TNF-a levels significantly declined in the both the test group (-26.32pg/mL) and control group (-11.8pg/mL) compared to baseline values ($p<0.015$) at 6 months. Notably, the test group's decrease in TNF-a was much larger than the placebo group ($p=0.007$) in this timeframe. Noted in figure 4, Superoxide dismutase (SOD) levels significantly improved

in both groups compared to baseline (p=0.003, p=0.015), however, there was no significant difference between the two groups. A limitation to the study is the small sample size, yet it still provides important insights, setting the foundation for larger and longer-term studies. Although results demonstrated n-3 supplementation after SRP reduces TNF-a levels, no benefit was shown in regard to SOD and clinical parameters of chronic periodontitis.[9]

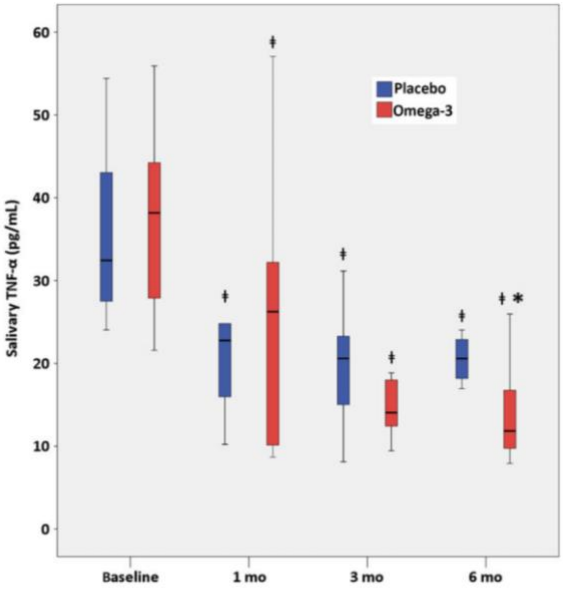


Fig. 1. Salivary TNF-α levels in placebo and omega-3 group at different time intervals. *Significant difference between the two groups at $p < 0.05$. ǂSignificant difference compared with the baseline measurement at $p < 0.05$. TNF, tumor necrosis factor.

Figure 3: Keskiner study results[9]

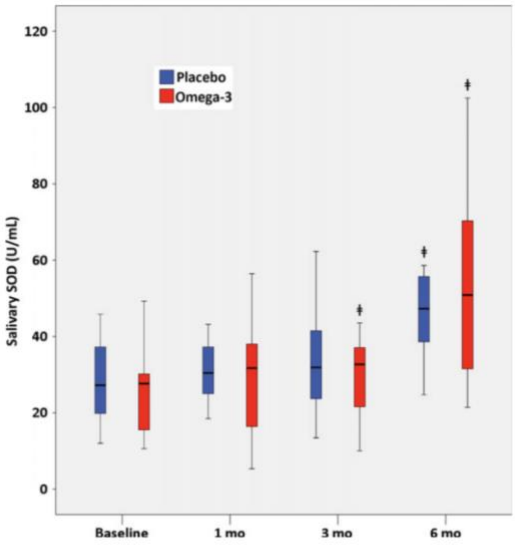


Fig. 2. Salivary SOD levels in placebo and omega-3 group at different time intervals. ‡Significant difference compared with the baseline measurement at $p < 0.05$. SOD, superoxide dismutase.

Figure 4: Keskiner study results[9]

Conclusion

Overall, a large body of evidence[3,4,5,6,7,8] points to increased dietary and supplemental intake of n-3 as having a positive effect on periodontal disease outcomes. Increase in n-3 status may be a highly beneficial, inexpensive, and safe adjunctive therapy option for prevention, management, and/or treatment of chronic periodontitis. Management of chronic periodontitis may further lead to reduction in prevalence of non-oral systemic disease states.

Of the current research reported in this literature review, statistically significant findings were consumption of fish oil significantly reduced GI ($p=0.008$) compared to baseline,[5] improved MGI ($p=0.016$) compared to baseline,[6] improve PPD compared to placebo ($p=0.044$),[6] exhibited modulatory effects on IL-1b and IL-10 compared to placebo,[7] reduced mean PPD ($p<0.001$) and increased CAL ($p<0.05$)[7] compared to control, and decreased mean pocket depth ($p=0.03$) and GI ($p=0.04$) compared to baseline.[8]

Evidenced-based findings can be used in dental practice to guide decisions, as well as an education tool to not only assist the dentist but also inform the client. The findings[3,4,5,6,7,8] in this literature review which point to positive outcomes of increased intake of n-3 on periodontitis are in agreement with the statement posted by the 2011 European workshop of periodontology.[10] It is stated professionals in the dental field should advice their patients to increase intake of fish oil, fruit, vegetables, and fiber in their daily diet, and reduce refined grains and sugars, in order to prevent and/or treat periodontal disease, as well as promote health and wellness.[10]

After analysis of current research, additional studies are needed to further validate n-3 PUFAs as a therapeutic modality, integrating larger sample sizes, longer durations, and a wider range of age groups and physiological situations. This will aid in the ability to generalize findings to larger populations, as currently a majority of the studies analyzed were carried out with participants of very specific ages or who had specific disease

conditions. No one dosage was proven to be more effective than another, however, one study[4] found no association between DHA and OR of periodontitis above 0.04gm/d, suggesting no extra benefit beyond modest intake, a speculation that could be further investigated in future research. A consensus amongst researchers and professionals on periodontitis diagnosis as well as a gold standard dietary intake method may further benefit generalizability and general understanding.

Abbreviations

AA	Arachidonic Acid
ALA	a-linolenic acid
BOP	bleeding on probing
CAL	clinical attachment loss
CDC	Center for Disease Control and Prevention
CI	confidence interval
DFDBA	decalcified freeze- dried bone allograft
DGLA	dihomo-γ-linolenic acid
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
EVOLV2	Elongation of very long fatty acids protein 2
FADS1	Fatty Acid Desaturase 1
GCF	gingivocrevicular fluid
GI	Gingival Index
GLA	Gamma-linoleic acid
HMT	Host modulatory therapy
hsCRP	high sensitivity C-reactive protein
IL-1	Interleukin- 1
IRR	incidence rate ratio
LA	Linoleic Acid
LC-PUFA	Long chain-polyunsaturated fatty acid
LDL	low density lipoprotein

MGI	Modified Gingival Index
NHANES	National Health and Nutrition Survey
OR	odds ratio
PBI	Papillary Bleeding Index
PLA2	phospholipase A2
PPD	Periodontal probing depth
PUFA	polyunsaturated fatty acid
RCT	randomized-controlled trial
SFA	saturated fatty acids
SOD	Superoxide Dismutase
SPMs	Specialized Pro- Resolving Mediators
SRP	scaling and root planning
TNF-a	tumor necrosis factor- a
VPI	Visible Plaque Index

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