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Docosahexaenoic Acid and Periodontitis in Adults: A Randomized Controlled Trial

ABSTRACT

Periodontitis is a common chronic inflammatory disease initiated by bacteria, resulting in bone resorption, tooth loss, and systemic inflammation. Long-chain omega-3 fatty acids such as docosahexaenoic acid (DHA) reduce periodontitis in animals. We aimed to determine whether DHA supplementation with low-dose aspirin would reduce periodontitis in humans. We conducted a double-blind placebo-controlled parallel trial lasting 3 mo. Fifty-five adults with moderate periodontitis were randomized to 2,000 mg of DHA or identical soy/corn oil capsules. All participants received 81 mg of aspirin but received no other treatments. We analyzed the primary outcome of per-pocket change in pocket depth using mixed models among teeth with pocket depth ≥5 mm. Secondary outcomes assessed with generalized estimating equations included gingival index, plaque index, and bleeding on probing. Gingival crevicular fluid samples were analyzed for changes in high-sensitivity C-reactive protein (hsCRP) and interleukins 6 and 1β (IL-6 and IL-1β). Plasma was analyzed for changes in systemic inflammatory markers, including hsCRP. We confirmed adherence with erythrocyte fatty acid measurement. Forty-six participants completed the trial. While similar at baseline, the proportion of DHA in red blood cell plasma membranes increased from 3.6% ± 0.9% to 6.2% ± 1.6% in the intervention group but did not change among controls. DHA supplementation decreased mean pocket depth (–0.29 ± 0.13; p = .03) and gingival index (–0.26 ± 0.13; p = .04). Plaque index and bleeding on probing did not change. Significant adjusted differences were found between DHA and control for both gingival crevicular fluid hsCRP (–5.3 ng/mL, standard error [SE] = 2.4, p = .03) and IL-1β (–20.1 pg/mL, SE = 8.2, p = .02) but not IL-6 (0.02 pg/mL, SE = 0.71, p = .98) or systemic hsCRP (–1.19 mg/L, SE = 0.90, p = .20). In this randomized controlled trial, aspirin-triggered DHA supplementation significantly improved periodontal outcomes in people with periodontitis, indicating its potential therapeutic efficacy (clinicaltrials.gov NTC01976806).

KEY WORDS: gingivitis, omega-3, fatty acid, inflammation, DHA, clinical study.

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INTRODUCTION

Periodontitis is a common chronic disease caused by bacteria at the gum line (Lehner *et al.*, 1974). It is characterized by gingival separation from the tooth, which forms a periodontal pocket and can lead to bone resorption, tooth loss, and systemic inflammation. Traditional therapies for periodontitis focus on the bacterial infection. More recent therapeutic strategies have targeted the host response, which may play a more crucial role in the pathogenesis and its associated systemic effects (Jain *et al.*, 2003; Serhan *et al.*, 2003; Serhan *et al.*, 2008).

Omega-3 fatty acids (N-3s), such as eicosapentaenoic acid and docosahexaenoic acid (DHA), have anti-inflammatory properties (Campan *et al.*, 1997; Eberhard *et al.*, 2002; Zhao *et al.*, 2007). Indeed, topical application of bioactive products derived from N-3s in the presence of aspirin (ASA), which reduces enzymatic inactivation via acetylation (Sun *et al.*, 2007), confers dramatic protection against inflammation-induced tissue and bone loss from periodontitis in experimental models (Hasturk *et al.*, 2006).

In humans, a nationally representative cross-sectional study in U.S. adults and a small prospective cohort found higher dietary intakes of DHA to be associated with less periodontitis (Iwasaki *et al.*, 2010; Naqvi *et al.*, 2010). In 2 randomized controlled trials, DHA augmented with low-dose ASA enhanced the effect of surgical therapies (regenerative therapy with decalcified freeze-dried bone allograft; Elkhouli, 2011) and nonsurgical therapies (scaling and root planning) when compared with surgical and nonsurgical therapies alone (El-Sharkawy *et al.*, 2010).

It is currently unknown if DHA (with low-dose ASA) can reduce periodontitis alone. Given a 30% prevalence of moderate periodontitis in Americans (Eke *et al.*, 2012), lowering costs and morbidity associated with current therapies for periodontitis is urgently needed. This randomized controlled trial aimed to examine the impact of DHA and low-dose ASA supplementation on adults with moderate periodontitis.

MATERIALS & METHODS

Subjects

Adults 40 yr and older with moderate periodontitis (American Academy of Periodontology criteria; Armitage, 1999) were recruited from the Boston area

from June 2009 to December 2011. Inclusion criteria for subjects included age ≥40 yr, ≥20 natural teeth (excluding third molars), and periodontitis defined as ≥4 teeth with pocket probing depths ≥5 mm. Exclusion criteria included pregnancy; diabetes; severe chronic diseases; gastrointestinal bleeding; autoimmune disorders; conditions requiring antibiotics, warfarin, clopidogrel, antimicrobial therapy within 30 d, or chronic use of nonsteroidal anti-inflammatory drugs (other than ASA); supplemental N-3s within 6 mo; carious lesions requiring immediate treatment; stage 4 periodontitis (American Dental Association classification); periodontal therapy within the past 2 yr; orthodontic appliances; or allergies to study interventions.

Potential subjects who responded to advertisements underwent a prescreening telephone interview and—if eligible by history and if likely to have chronic periodontitis based on a four-item questionnaire (Genco *et al.*, 2007)—underwent a screening appointment within 2 wk.

The trial was approved and monitored by the Committee on Clinical Investigations at Beth Israel Deaconess Medical Center, Boston, Massachusetts. An Institutional Review Board–approved written informed consent was obtained from all individuals prior to study procedures. The study protocol was also approved by the Institutional Review Boards of Boston University and the Forsyth Institute for sample analysis.

Intervention and Study Procedures

Eligibility was determined at the baseline appointment on the basis of medical and dental history and examination. Eligible participants underwent randomization stratified by smoking status and had additional dental measurements made, including collection of gingival crevicular fluid (GCF), blood, urine, anthropometric measurements, and a 24-hr dietary recall. Blood pressure was measured by selecting the appropriate blood pressure cuff size and allowing a brief resting period before systolic and diastolic blood pressure measurement from the right arm (brachial artery).

Participants were randomized into 2 arms in a parallel double-blinded design. The DHA and placebo capsules were masked for color and flavor. The control arm received a three-month supply of 4 daily placebo capsules each with 950 mg of oil (50% corn oil and 50% soybean oil). The intervention arm received a three-month supply of 4 daily DHA capsules. Each DHA capsule contained 950 mg of oil (53.6% DHA) from *Crypthecodinium cohnii*, for a total daily dose of approximately 2,000 mg of DHA. All participants received 81-mg ASA. Placebo and DHA capsules were supplied by DSM Nutritional Products. All participants received standard oral hygiene instructions but were asked not to undergo periodontal treatment for the duration of the study.

Participants received telephone calls every 1 to 2 wk to assess compliance and potential adverse effects. Participants returned after 3 mo for repeat periodontal assessment, collection of follow-up GCF, blood, urine, anthropometric measurements, and questionnaires assessing diet, tolerability of the intervention, and adequacy of blinding.

Primary Endpoints

Periodontitis was assessed by calibrated dental hygienists. Inter- and intraexaminer calibration took place prior to study initiation and was repeated annually. The interexaminer agreement was measured as 85.6% for agreement within 1.0 mm based on interclass correlation coefficient analysis, while intraexaminer agreement was 98.2% for pocket depth (PD) and clinical attachment–level measurements. Briefly, periodontal examinations were conducted at 6 points (midbuccal, mesiobuccal, distobuccal, midlingual, mesiolingual, distolingual) for each tooth in all quadrants. Periodontal measurements were rounded to the lowest whole millimeter and were made with a millimetric periodontal probe (PCPUNC15 with 1-mm increments). Clinical measurements at 6 sites per tooth included PD, modified gingival index (Kabir-ud-Din *et al.*, 2012), and bleeding on probing scores (dichotomous). For plaque index (Elkhouli, 2011), only 4 sites per tooth were measured: distobuccal, midbuccal, mesiobuccal, and the entire lingual surface (Silness and Loe, 1964).

Laboratory Methods

Blood samples were collected after an overnight fast at baseline and three-month follow-up, for tests including complete blood count, fasting lipid panel, and high-sensitivity C-reactive protein (hsCRP), all conducted by Laboratory Corporation of America (Raritan, NJ, USA). Additional tests of serum interleukin 6 (IL-6) and soluble vascular cell adhesion molecule (VCAM) were performed at the Harvard Catalyst Central Laboratory (Brigham and Women's Hospital, Boston, MA, USA). Red blood cell (RBC) phospholipid fatty acid analysis (percentage of DHA incorporated into RBC plasma membranes relative to total RBC plasma membrane fatty acids by weight) was conducted at the Department of Nutrition, Harvard School of Public Health, Boston, MA.

Complete blood count was measured by using Sysmex X-series analyzer (Sysmex Corporation, Kobe, Japan). Serum concentrations of total cholesterol, high-density lipoprotein, low-density lipoprotein, and triglyceride were measured by enzymatic assays on a Hitachi analyzer (Roche Diagnostics, Mannheim, Germany); the intra-assay coefficients of variation were <2% for all measurements. Plasma concentration of hsCRP was assessed by a particle-enhanced turbidimetric assay on a COBAS INTEGRA system (Roche Diagnostics). Serum concentration of IL-6 was measured with access chemiluminescent immunoassay (Beckman Coulter, Fullerton, CA, USA), serum concentration of VCAM with enzyme-linked immunosorbent assay (R & D Systems, Minneapolis, MN, USA), and urine concentration of N-terminal telopeptides (NTx) with enzyme-linked immunosorbent assay (Wampole Laboratories, Princeton, NJ, USA). RBC fatty acids were quantified by gas-liquid chromatography on a fused silica capillary cis/trans column SP2560 (Supelco, Bellefonte, PA, USA) with peak retentions analyzed via Agilent Technologies ChemStation A.08.03 software. The intra-assay coefficients of variation for CRP, IL-6, VCAM, and NTx were 1.3%, 1.7%-4.6%, 0.9%-3.4%, and 4.2%-5.2%, respectively.

Oral GCF samples were collected at baseline and three-month follow-up visits from the mesiobuccal aspect of first molar teeth at each quadrant (total of 4 samples) by using sterile PerioPaper strips (Oraflow, Inc., Smithtown, NY, USA) until slight resistance was felt for 30 s. Following collection, the sample was kept on ice for transport to the laboratory and stored at –80°C. The frozen GCF samples were then thawed at room temperature, and proteins were eluted through 2 centrifugations at 13,000 × *g* at 4°C for 8 min in a total of 110 µL of sterile phosphate buffered saline (pH 7.4). Upon completing elution, samples were used immediately for analyses of proinflammatory cytokines interleukin 1β (IL-1β), IL-6, and acute phase protein CRP via high-sensitivity kits. Multiplexed sandwich immunoassays, based on flowmetric Luminex xMAP technology, were conducted at the Forsyth Institute (Cambridge, MA, USA). Assays were carried out on a Luminex 100 Bio-Plex Platform (Bio-Rad Laboratories, Inc., Hercules, CA, USA), and data were read with Bio-Plex Manager 6.1 (Bio-Rad Laboratories, Inc.). Immediately prior to the initiation of study measurements, the Bio-Plex platform underwent a complete on-site maintenance cycle and and were deemed operational by qualified Luminex field engineers. Daily and weekly performance qualification was continuously verified by Forsyth Institute technicians during the analytic period.

Assay kits provided by the commercial vendors consisted of 3 panels: (1) single-plex of high-sensitivity IL-1β (EMD Millipore Corporation, Billerica, MA, USA), (2) human neurodegenerative disease panel single-plex hsCRP (EMD Millipore Corporation), and (3), hsIL-6 ELISA kit (R&D Systems, Minneapolis, MN, USA).

Each panel for biomarker analysis was performed according to manufacturer's protocols. Single lot numbers of each kit were used to minimize analytic variability. Reagents provided in these kits included magnetic beads precoated with capture antibodies, standards, assay diluents, biotin-conjugated secondary antibodies, biotin diluent, streptavidin conjugated to the fluorescent protein R-phycoerythrin (streptavidin-RPE), streptavidin-RPE diluent, washing buffer concentrates, and assay buffers, as well as the 96-well filter or magnetic plates. The quality control measurements and coefficient variations were calculated as described previously by the same laboratory (Browne *et al.*, 2013). The detection limits of the kits were 0.023 pg/mL for IL-6, 0.03 pg/mL for IL-1β, and 0.01 ng/mL for hsCRP.

Data Handling and Statistical Analyses

Data were collected and managed with the Research Electronic Data Capture platform (Harris *et al.*, 2009) and analyzed with SAS 9.3 (SAS Institute Inc, Cary, NC, USA) on an intent-to-treat basis, with a type I error rate of 0.05. We confirmed the effectiveness of the intervention with paired *t* tests comparing RBC DHA levels at baseline and follow-up visits within each intervention group. Dental outcomes were *a priori* studied among sites with periodontitis at baseline, defined as PD ≥5 mm. The primary outcome, follow-up PD, was assessed in linear mixed effects models with a compound-symmetry covariance structure—with age, sex, body mass index, race, baseline PD, baseline RBC DHA level (dichotomized at median), and intervention group as fixed effect variables. In prespecified subgroup analyses, we dichotomized by sex, median age, and baseline median RBC DHA level and tested their respective multiplicative interactions. To evaluate the effect of DHA on the severity and extent of periodontitis, we employed generalized estimating equations with a logit link and exchangeable correlation structure to evaluate clinically relevant binary outcomes of PD reduction of ≥2 mm and number of sites with PD ≥5 mm, respectively.

We similarly analyzed the outcome of the intervention on gingival index and plaque index, which were both scaled as 0-3, and we used identity link and Poisson and Gaussian distributions, respectively, to maximize model convergence. We analyzed bleeding on probing via generalized estimating equations with a binomial distribution and logit link. We assessed the impact of the intervention on (1) GCF levels of hsCRP, IL-6, and IL-1β and (2) systemic levels of hsCRP, IL-6, VCAM, and NTx. For each marker, we conducted (1) two-sample *t* tests comparing mean changes from baseline to three-month follow-up in the DHA and placebo groups and (2) regression models with dependent variables of end-of-follow-up levels adjusted for age, sex, race, intervention, and corresponding baseline measurements. IL-6 was log-transformed to maximize normality. Other outcome variables were normally distributed.

To ensure the robustness of our findings to missing data from dropouts, we repeated our primary analyses including all randomized participants using multiple imputation. We performed 5 imputations using monotone regression methods, then performed generalized estimating equations or mixed model analyses as in our original analyses, accounting for within- and between-imputation variance.

RESULTS

A total of 58 participants were randomized, three of whom did not receive the study capsules due to previously undisclosed uncontrolled hypertension, diabetes, and DHA supplementation (Figure 1). Among the 55 participants who received study capsules, 27 were randomly assigned to DHA with low-dose ASA and 28 to placebo with ASA. The study retention rates were 89% for DHA + ASA and 79% for placebo + ASA, leaving 46 participants who completed the primary outcome assessment. Baseline characteristics are presented in Table 1.

RBC-phospholipid-DHA (percentage of total fatty acids by weight) increased from 3.6% ± 0.9% to 6.2% ± 1.6% in the intervention group ($p < .01$) but did not change significantly among controls, from 3.2% (1.0%) at baseline to 3.1% (0.9%) at follow-up ($p = .20$).

As Table 2 shows, DHA + ASA supplementation significantly decreased mean PD compared with placebo + ASA. In secondary outcomes, DHA supplementation also significantly decreased mean gingival index, but there were no significant changes in plaque index. There were also no significant changes in bleeding on probing (odds ratio [OR] = 0.71; 95% confidence interval [CI] = 0.36-1.38; $p = .31$). In sensitivity analyses, a clinically significant improvement of 2 mm in PD was more common in the DHA group (adjusted OR = 2.05; 95% CI = 1.16-3.63; $p = .01$). DHA also reduced the number of sites with PD ≥5 mm (OR = 0.62, 95% CI = 0.43-0.90, $p = .01$).

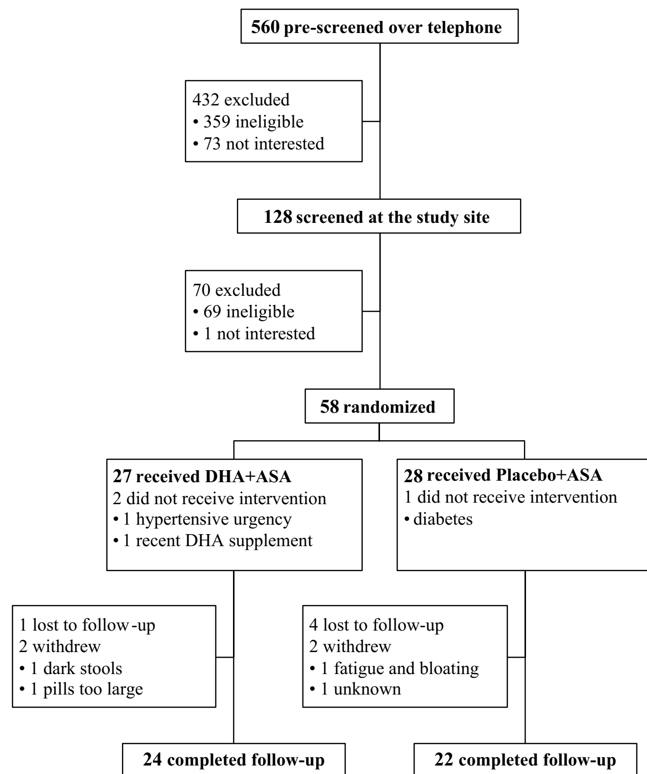

Figure 1. Flow of participants through the trial. DHA, docosahexaenoic acid, 2 g daily; ASA, aspirin, 81 mg daily.

We observed significant local anti-inflammatory actions of DHA in analyses of GCF. As Figure 2 shows, there were significant adjusted differences between DHA and control for both GCF hsCRP (–5.3 ng/mL, standard error [SE] = 2.4, p = .03) and IL-1β (–20.1 pg/mL, SE = 8.2, p = .02) but not IL-6 (0.02 pg/mL, SE = 0.71, p = .98).

In contrast, we did not observe significant changes in systemic inflammatory markers or a marker of bone metabolism, NTx. Adjusted differences between DHA and control were –1.19 mg/L (SE = 0.90, p = .20) for hsCRP; 0.08 mcg/mL (SE = 0.16, p = .62) for IL-6; –26 (SE = 32, p = .42) for VCAM; and 1.7 nM BCE (SE = 103.5, p = .99) for NTx. A total of 15 subjects experienced adverse events, none of which were serious. Seven were in the intervention group and 8 in the control group. There were no differences in perception of which intervention was received among subjects (p = .82) or dental assessors (p = .84; Appendix Tables 1 and 2). There were no material differences in effects of intervention *vs.* placebo on PD and gingival index when multiple imputations were used for missing data from all dropouts (p = .02 and p = .04, respectively; Appendix Table 3 for baseline characteristics of dropouts).

DISCUSSION

In this three-month randomized controlled trial, 2,000 mg of daily DHA with 81-mg ASA significantly improved periodontitis and gingivitis when compared to placebo with low-dose ASA. Although the intervention had no significant effects on markers of systemic inflammation, it did decrease local inflammation. Although the changes in effect sizes of dental measures and local inflammation were modest, the inverse effect on periodontitis severity was clinically significant and was not modified by baseline dietary DHA, age, or sex (Appendix Figure).

Our findings expand on the only reported randomized clinical trial of polyunsaturated fatty acids for the treatment of periodontitis (Rosenstein *et al.*, 2003). The trial randomized 30 subjects with periodontitis to receive 12 wk of systemic therapy of eicosapentaenoic acid, gamma-linolenic acid (GLA, an omega-6 fatty acid), both eicosapentaenoic acid and GLA, or olive oil (placebo; Rosenstein *et al.*, 2003). They found a significant decrease in PD and gingival index in patients supplemented with 3,000 mg of daily GLA alone compared to placebo. In the current study, we found significant improvements in PD, gingival index, and local inflammation with 2,000 mg of daily supplemental DHA in subjects receiving low-dose ASA for 3 mo. In addition, we found that clinically significant improvements in PD were more common in the DHA group.

Our findings accord well with a prospective observational study (Iwasaki *et al.*, 2010) and a cross-sectional nationally representative study (Naqvi *et al.*, 2010) of N-3 fatty acid intake and periodontitis. The latter study also showed an inverse relation between dietary DHA intake and serum hsCRP. In the current study, DHA + ASA treatment for 3 mo had no significant effects on systemic hsCRP, IL-6, VCAM, or GCF IL-6. However, DHA + ASA significantly decreased local inflammation, indicated by decreased GCF CRP and IL1-β levels, suggesting that systemic inflammation might also decrease with longer treatment.

N-3s have been found to be substrates for neutrophil production of novel eicosinoids called resolvins, protectins, and maresins, which appear central to the resolution of inflammation (Serhan and Savill, 2005; Levy *et al.*, 2007). For example, resolvins minimize excessive polymorphonuclear cell infiltration into inflamed tissues and promote phagocytosis and clearance of apoptotic cells and microbes (Spite and Serhan, 2010). In the presence of ASA, acetylated COX-2 converts DHA into 17-hydroxydocosahexaenoic acid, in which the hydroxyl group is in the R configuration, which leads to ASA-triggered resolvins with enhanced proresolution effects. Clinically, neither N-3 supplementation (eicosapentaenoic acid, 3,000 mg daily; Rosenstein *et al.*, 2003) nor ASA (Faizuddin *et al.*, 2012) by itself appears to significantly affect periodontitis.

Limitations of our study include the small sample size, which limits the power to detect small differences in treatment effects, such as those on serum inflammatory biomarkers. Also, the relatively short duration may limit the ability to determine long-term effects. In addition, it is unknown whether DHA supplementation without low-dose ASA augmentation reduces periodontitis. Also, our results may not be generalizable to other N-3s. In addition, it is possible that there was confounding by smoking due to misclassification of smoking status. This does not seem likely given the degree to which most reported and measured demographic and clinical measurements were evenly distributed between the 2 treatment arms. Misclassification of

Table 1. Baseline Demographic and Clinical Characteristics by Intervention

	DHA + ASA (*n* = 24)	Placebo + ASA (*n* = 22)
Demographics		
Age, yr	53 ± 8	57 ± 8
Sex, female	11 (45.8)	10 (45.5)
Race		
White	12 (50.0)	12 (54.6)
African American	7 (29.2)	8 (36.4)
Asian	4 (16.7)	1 (4.6)
Multiracial	0 (0)	1 (4.6)
Other	1 (4.2)	0 (0)
Cardiovascular risk factors		
Smoking status		
Former	7 (29.2)	6 (27.3)
Current	1 (4.2)	1 (4.6)
Body mass index, kg/m^2	28.7 ± 5.4	28.9 ± 4.5
Overweight, 25-30	4 (16.7)	10 (45.5)
Obese, >30	11 (45.8)	7 (31.8)
Blood pressure, mm Hg	132 ± 12 / 79 ± 10	140 ± 17 / 82 ± 10
Triglycerides, mg/dL	105 ± 47	114 ± 55[a]
High-density lipoprotein, mg/dL	60 ± 19	59 ± 19[a]
Low-density lipoprotein DL, mg/dL	113 ± 30	126 ± 62[a]
White blood cell count, K/μL	6.4 ± 1.9	6.6 ± 1.9[b]
Plasma high-sensitivity C-reactive protein, mg/L	2.31 ± 3.19	2.31 ± 2.63[b]
Serum interleukin 6, pg/mL	2.63 ± 1.41[b]	2.84 ± 1.89
Serum VCAM, ng/mL	771 ± 429[b]	791 ± 390
Urine N-telopeptides, nM BCE	278 ± 283[b]	340 ± 288[b]
Hypertension	4 (16.7)	6 (27.3)
Hyperlipidemia	5 (20.8)	4 (18.2)
Periodontal measurements		
Pocket depth, mm	2.5 ± 0.4	2.6 ± 0.4
Attachment loss, mm	2.0 ± 0.9	1.9 ± 0.5
Gingival index, 0-3	1.6 ± 0.4	1.6 ± 0.4
Plaque index, 0-3	0.5 ± 0.4	0.7 ± 0.5
Bleeding on probing,[c] yes/no	1,042 (30)	1,455 (42)

Data are mean ± SD or number (%). None of the baseline characteristics were significantly different between intervention groups.
DHA: docosahexaenoic acid (2 g/d); ASA, aspirin (81 mg/d); VCAM, vascular cell adhesion molecule.
[a] *n* = 20.
[b] *n* = 21.
[c] *n* = 3,455 for DHA + ASA, *n* = 3,426 for placebo + ASA.

Table 2. DHA and Periodontal Disease Outcomes among Dental Sites with Baseline Pocket Depths ≥5 mm

Outcome	*n*	Δ_{DHA}	$\Delta_{placebo}$	$\Delta_{DHA} - \Delta_{placebo}$	β_{DHA}	β_{DHA}, *p*
Pocket depth	533	–0.71 (0.07)	–0.54 (0.07)	–0.17 (0.10)	–0.29 (0.13)	.03
Gingival index	532	–0.26 (0.04)	–0.07 (0.04)	–0.19 (0.06)	–0.26 (0.13)	.04
Plaque index	244	–0.10 (0.06)	0.07 (0.07)	–0.17 (0.09)	0.04 (0.07)	.57

Results are based on linear mixed effects regression models. Standard errors in parentheses.
DHA, docosahexaenoic acid; SE, standard error.

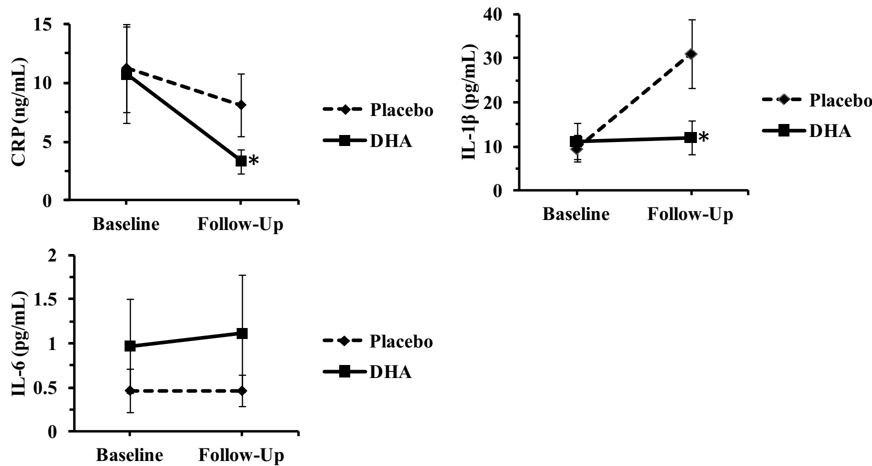

Figure 2. Effect of intervention on mean (SE) levels of GCF hsCRP, IL-1β, and IL-6 at baseline and follow-up. SE, standard error; GCF, gingival crevicular fluid; hsCRP, high-sensitivity C-reactive Protein; IL-1β, interleukin 1β; IL-6, interleukin-6; DHA, docosahexaenoic acid. $^{*}p < .05$.

periodontitis was also possible, since no radiographs were taken to further support the diagnosis. Also, changes in GCF analytes may have been more substantial had we limited GCF sample collection to sites with ≥5mm PD at baseline. Nonetheless, the standardized approach that we used to collect GCF samples without taking PD into account was sufficient to detect significant differences in GCF hsCRP and GCF IL1-β. Similarly, excluding severe periodontitis may have limited our ability to detect greater effects of the intervention.

Strengths of our study include the randomized controlled design, effective blinding, verified adherence, and consistent impact on complementary outcomes, including measures of periodontitis, gingivitis, and local inflammation both clinically and biochemically. Also, detailed periodontal assessments were conducted with a number of quality control procedures, including calibration of dental assessors prior to and biannually throughout the study period.

In summary, DHA supplementation with low-dose ASA significantly improved moderate periodontitis and gingival inflammation in this randomized trial. To date, the treatment of periodontitis has primarily involved mechanical cleaning and local antibiotic application. This nutritional therapy, if confirmed, has promise as a less expensive and safer method for the prevention and treatment of periodontitis. Both these questions warrant further investigation in large prospective cohort and clinical trials.

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