

SHORT COMMUNICATION

Inflammation and intestinal leakiness in older HIV+ individuals with fish oil treatment

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Abstract Fish oil is a natural product that has shown efficacy for managing inflammatory conditions with few side effects. There is emerging evidence that crosstalks between gut epithelial cells and immune cells contribute to chronic infectious diseases. HIV-infected (HIV+) older adults show age-related co-morbidities at a younger age than their uninfected counterparts. Persistent inflammation related to the chronic viral infection and its sequelae is thought to contribute to this disparity. However, little is known about whether fish oil reduces intestinal inflammation in HIV+ patients. We measure inflammation and gut barrier function in HIV+ older adults (median age = 52, N = 33), following 12 weeks of fish oil supplementation (a total daily dose of 1.6 g of omega-3 fatty acids). We showed a reduction in inflammation and gut permeability as measured by CD14, inflammatory cytokines, lipopolysaccharide, and lipopolysaccharide binding protein. The results indicate that older HIV+ adults may benefit from a diet supplemented with the omega-3 fatty acids found in fish oil.

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Introduction

Due to treatment advances, the number of persons who are living with HIV/AIDS in the United States has steadily

increased. One cohort study of older, largely minority HIV+ persons found that 34% had metabolic syndrome and 50% had a Framingham Cardiac Risk score >10%.¹ HIV+ older adults of all races show a premature onset of

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other age-associated co-morbidities, such as osteoporosis, non-AIDS related malignancies, and cardiovascular disease.² These co-morbidities have been linked to persistent inflammation and elevated serum levels of proinflammatory cytokines that mimic an aging phenotype known as “inflamm-aging.”³ In both middle-aged HIV + persons and older HIV seronegative adults, inflamm-aging is associated with more limited T cell repertoires and increased risk for morbidities and mortality.^{3–9}

One possible mechanism that could be responsible for inflamm-aging is HIV-induced disruption of intestinal epithelial integrity.^{3,4} HIV infection induces depletion of gut Th17 CD4+ T cells which maintain intestinal epithelial integrity⁵ and their depletion may allow translocation of gut microflora into the systemic circulation leading to endotoxemia and chronic inflammation.⁴ Disruption of intestinal epithelial integrity in HIV + persons, as measured by indirect markers of gut permeability, are predictive of mortality after adjusting for the number of CD4+ T lymphocytes.⁶ The increased intestinal permeability in HIV infected individuals may result in dysbiosis. Microbiota from both treated and untreated HIV + men over-represent inflammation related microbes and correlate with markers of HIV disease progression.⁷

Treatments that promote regeneration of the intestinal mucosa and normalize dysbiosis have the potential to reduce the HIV-related intestinal injury. Cold water fish are rich in the omega-3 highly unsaturated fatty acids (HUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid,⁸ which stimulate regeneration of intestinal mucosa damaged by methotrexate,⁹ IL-4,¹⁰ experimental ulcerative colitis,¹¹ and LPS.¹² Additionally, in murine models, fish oil increases the representation of beneficial microbes, such as *Lactobacillus* and *Bifidobacteria*,¹³ and reduces dysbiosis.¹⁴ A recent study in women showed the use of omega-3 supplementation to improve the microbiome composition.¹⁵ Yet to date, no studies have been conducted on the ability of fish oil to modulate gut barrier function or dysbiosis in HIV + persons.

In the current study, we explored the efficacy of 12 weeks of fish oil supplementation compared to placebo in older HIV + adults by measuring markers of inflammation and gut barrier function.

Study design, materials and methods

Subjects

As reported in the parental study,¹⁶ the included participants in this study are: HIV positive and negative individuals between the ages of 40 and 70 with an hsCRP level ≥ 2.0 mg/L, a CD4+ T lymphocyte count of at least 250 cells/mm³, a viral load of <75 copies/ml, and a history of stable antiretroviral therapy for at least two months. Participants were excluded from the study if they had a history of coronary artery disease or stroke; a platelet count $<150,000$ cells/mm³; a chronic condition known to affect lipid status; use of medications or herbal therapies reported to influence bleeding parameters; documented active opportunistic infections or malignancies; serum creatinine >2.0 mg/dL or liver

enzyme elevations $>$ three times the upper limit of normal; LDL cholesterol ≥ 120 mg/dL; or use of lipid-lowering medications.

The study used a randomized, controlled, double-blind clinical trial design and was registered in [ClinicalTrials.gov](https://clinicaltrials.gov) (NCT02102724).¹⁶ Institutional review board approval was granted and the ethical standards of the Helsinki Declaration of 1975 were followed. All participants gave written informed consent before enrollment in the study. A demographic and lifestyle behaviors questionnaire that was developed for an earlier study.¹⁷

Intervention

A randomized controlled trial in HIV + older adults is conducted to assess the gut barrier effects of the proposed fish oil condition (1.6 gm/day of omega-3 fatty acids) by comparing it to the proposed control condition at 4 and 12 weeks, controlling for covariates (demographics, lifestyle, medications), on HIV disease parameters (CD4+ T cell counts and HIV RNA levels). The fish oil and placebo were compounded into gel caps by Martin Avenue Pharmacy (Naperville, IL). Participants were randomized 1:1 to either the fish oil or placebo condition, using computer-generated numbers in a random-length permuted block design. Participants randomized to the fish oil condition received 12 weeks of 1.6 g/day of fish oil in gelcaps that contained 800 mg of eicosapentaenoic acid (EPA), 600 mg of Docosahexaenoic acid,⁸ and 200 mg of other omega-3 fatty acids (Carlson® Fish Oil; Arlington Heights, IL, USA). Participants who were randomized to the placebo condition received 1.0 g/day of oleic sunflower oil in gel caps, as reported in the parental study.¹⁶

Procedures

Randomized participants had two screening visits (T1 and T2), one baseline visit (T3), and two visits post-baseline (T4 [4 weeks] and T5 [12 weeks]). Participants who had a plasma hsCRP concentration ≥ 2.0 mg/ml T1, were asked to return for a second screening visit (T2) to measure additional laboratory parameters and determine eligibility. At T3, participants were randomized, provided with written instructions and safety precautions pertinent to the fish oil intervention and began taking fish oil or placebo. At T4 and T5, we obtained follow-up measurements of the safety (T4 and T5) and efficacy outcomes (T5).¹⁶

Specimen collection and storage

All blood specimens from a single participant were obtained within the same 2-h time frame for all study visits. Serum samples were separated by centrifugation of specimens (1200 rpm for 15 min) at room temperature and then stored at -80°C until analysis. Serum samples were subjected to no more than one freeze–thaw cycle.

Human serum CD14

Serum CD14 was measured by using ELISA (EHCD14, Grand Island, NY, USA) with a lower limit of detection of 6 pg/m.

Serum was diluted 1:100 in assay diluent and analyses performed according to the manufacturer's instructions.

Serum zonulin

Serum zonulin concentrations were measured by zonulin ELISA Kit that only detects the active (uncleaved) form of zonulin (K5600, Immundiagnostik AG, Bensheim, Germany). Serum samples were diluted 1:2 and assayed according to the manufacturer's instructions. Intra- and inter-assay coefficients of variation for these determinations were between 3–7% and 5–12%, respectively.

LPS

LPS in blood samples was measured by the limulus amoebocyte lysate (LAL) chromogenic endpoint assay (HIT302, Hycult Biotech, Plymouth Meeting, PA, USA) according to manufacturer's indications. Samples were diluted 1:4 with endotoxin-free water and then heated at 75 °C for 5 min in a warm plate in order to denature protein before reaction. A standard curve was generated and used to calculate concentrations, expressed as EU/ml, in blood samples.

Human LBP

Serum LBP concentration was measured by using ELISA (CKH113, Cell Sciences, Canton, MA, USA). Samples were diluted 1:800 in dilution buffer and analyses performed according to the manufacturer's instructions.

Human serum cytokines

The human 25-Plex cytokine panel (LHC0009, Invitrogen, Grand Island, NY, USA) was used to quantify serum cytokine concentrations. The Invitrogen Human Cytokine 25-Plex panel consists of the following cytokines and chemokines: eotaxin, GM-CSF, IFN- α , IFN- γ , IL-1 β , IL-1RA, IL-2, IL-2R, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p40/p70), IL-13, IL-15, IL-17, IP-10, MCP-1, MIG, MIP-1 α , MIP-1 β , RANTES, and TNF- α . To generate a standard curve, 3-fold serial dilutions of multiplex standard, provided in the 25 cytokine panel assay kit, were prepared in assay diluent and pipetted in duplicate. Participants' serum was diluted 1:1 in assay diluent and according to the manufacturer's instructions. Cytokines were quantified with the Luminex detection system (PerkinElmer CS1000 Autoplex Analyzer).

Statistical analysis

Descriptive statistics for continuous variables were expressed as mean $\pm$ standard deviation¹⁶ or median as indicated; categorical variables are presented as frequency and proportion. All statistical tests were two-sided except those indicated. P-values of less than 0.05 were considered to be statistically significant. For measures of serum biomarkers, inflammation markers, gut permeability, and serum cytokines, first the change values from baseline to week 12 were calculated; next change values were visualized via a boxplot; then the group differences of the change were tested by the Wilcoxon rank sum test. All statistical analyses were conducted by SAS version 9.3 (SAS Institute Inc., Cary, NC, USA).

Results

Changes serum cytokines

Soluble CD40 ligand (sCD40L) levels are significantly higher in elderly patients and in HIV-infected patients with dementia and impaired DC responses in the periphery. Presumably, the levels of sCD40L are higher in these groups because they have more gut permeability than younger individuals or HIV negative subjects. Since we were studying older HIV positive individuals, we expected to see high sCD40L concentrations; our goal here was to see if treatment with omega 3 fatty acids could reduce the concentrations of sCD40L. Even though we did see elevated sCD40L values in the pretreatment HIV + group, there was no significant difference between these groups with or without treatment.

Serum CD14 was determined by using ELISA. We found that the treatment group ($n = 18$) showed a significant decrease in soluble CD14 concentration in serum, compared to the placebo group ($n = 15$) (Fig. 1A). Cytokines IP-10 and IL-1RA were reduced in the fish oil group, but did not have a statistical significance (Fig. 1B–C). The treatment group also showed a decrease trend in inflammation markers in IFN- α , IL-8, and IL-1 β in serum compared to the placebo group (Fig. 1D–F). There is no difference of serum IL-10 with or without fish oil (data not shown).

Gut permeability

The change of intestinal permeability is associated with the serum LBP and LPS. We compared the profiles of LPS and LBP in serum in groups with or without treatment. Assessment of serum LBP and LPS concentrations was performed using a commercially available ELISA kit (Fig. 2). The treatment group showed a reduced LBP in serum, compared to the placebo group. Circulating zonulin is a protein that regulates intestinal permeability by modulating intestinal tight junctions (TJs).¹⁸ Zonulin is known to regulate intestinal permeability reversibly by modulating intercellular TJs.¹⁹ Serum Zonulin concentrations were measured by zonulin ELISA Kit ELISA in our studies. There is no difference of serum zonulin with or without fish oil (data not shown).

Discussion

To our knowledge, this is the first study to explore the efficacy of fish oil to modulate parameters of gut barrier function in HIV + older adults. We found that a total daily dose of 1.6 g of omega-3 fatty acids for 12 weeks was associated with improvements in indirect markers of gut permeability, including CD14 and LBP in HIV + older adults. We report a potential anti-inflammatory effect of fish oil for reducing HIV-induced intestinal permeability in older adults with HIV infection.

Omega-3 fatty acids and inflammation

Cold water fish are rich in the omega-3 highly unsaturated fatty acids EPA and DHA, which have anti-inflammatory

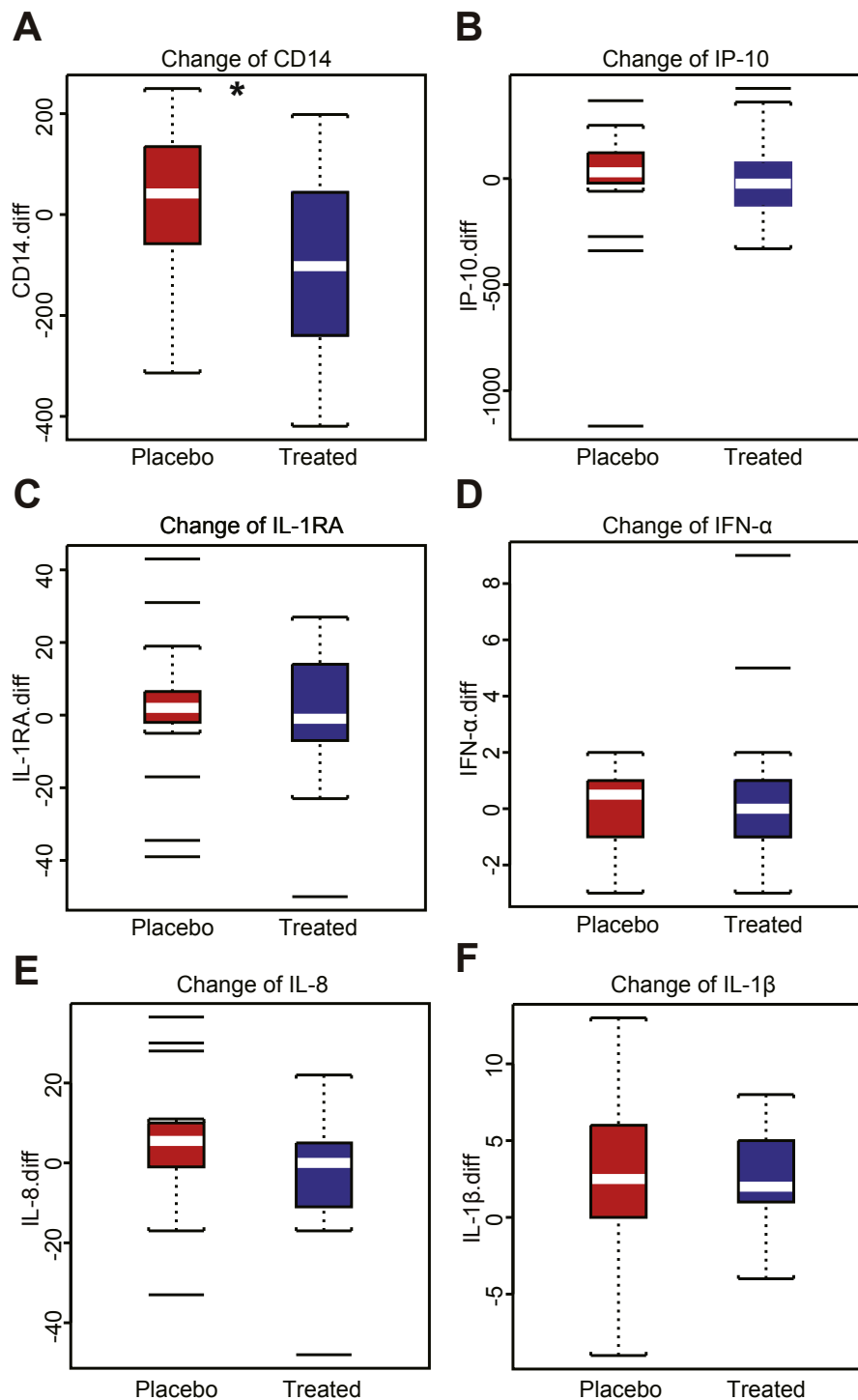


Figure 1 Serum biomarkers (CD14, IP-10, IL-1RA, IFN- α) and inflammation markers (IL-8, IL-1 β) in 12 weeks of fish oil supplementation ($n = 18$) vs. placebo ($n = 15$) HIV + older adults. The treatment group showed a significant decrease in soluble CD14 in serum, compared to the placebo group. The treatment group also showed a decrease trend in inflammation marker IL-8 compared to the placebo group. Y axis indicates the serum biomarkers and X axis shows the Group Assignment. * $p < 0.05$ by one-sided test.

effects.²⁰ When consumed as fish or fish oil supplements, EPA and DHA replace arachidonic acid in cell membranes and inhibit the synthesis of proinflammatory arachidonic acid metabolites.^{21,22} In clinical trials, fish oil supplementation has been associated with symptom relief and reductions in serum levels of proinflammatory cytokines in

patients with asthma, Crohn's disease, pancreatitis, and rheumatoid arthritis.^{23–29} In a murine model of AIDS, mice fed a fish oil diet produced lower concentrations of soluble inflammatory mediators than mice fed a corn oil diet.³⁰ Only a few fish oil trials have been conducted in HIV + persons.

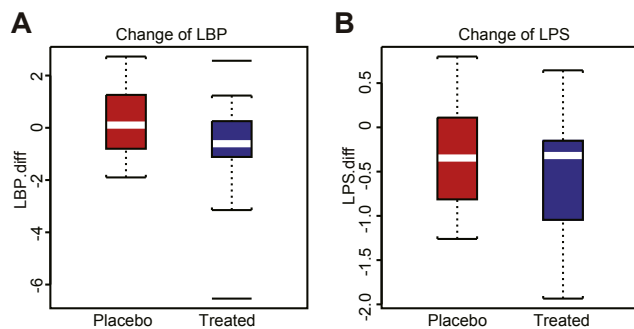


Figure 2 Serum LBP and LPS in 12 weeks of fish oil supplementation ($n = 18$) vs. placebo ($n = 15$) HIV + older adults. LBP showed a decrease trend in treatment group. Y axis indicates the serum LBP or LPS and X axis shows the Group Assignment.

HIV infection and inflammation

HIV infection is associated with chronic inflammation. Elevated levels of IL-1 β , IL-6, IFN, and TNF- α have been detected in the serum, cerebrospinal fluid, and cell culture supernatants of HIV + persons.^{31–34} In our current study, serum IFN- α , IL-8, and IL-1 β are somewhat lower in individuals treated with fish oil; these data did not reach statistical significance, perhaps due to the sample size and treatment duration. Previous studies have shown that HAART does not completely eliminate HIV-associated inflammation,^{33,35} possibly due to ongoing low level replication of HIV and co-pathogens.²⁷ Therefore, therapies that target inflammatory pathways are still needed.

HIV-induced disruption of intestinal epithelial integrity

Our current study showed promising data that fish oil is able to protect intestinal integrity. The intestinal epithelium is constantly exposed to microbes and plays a critical role in barrier functions, host defense, and inflammation.^{30,36–41} Intestinal TJs seal the space between adjacent epithelial cells, which serve as a barrier, provide structure, and play a role in host defense. One postulated mechanism for inflammation is HIV-induced disruption of intestinal epithelial integrity with subsequent translocation of microbial products into the systemic circulation.^{3,4,42,43} HIV infection depletes gut Th17 CD4⁺ T cells which maintain intestinal epithelial integrity¹¹ and their depletion may allow translocation of gut microbiota into the systemic circulation leading to endotoxemia and chronic inflammation.^{11,44,45}

In our current study, LBP in serum was reduced in fish oil group (Fig. 2). These data indicate the favorable effects of fish oil on gut barrier function. In middle aged and elderly women, Omega-3 supplementation improved the microbiome composition, particularly with bacteria of the *Lachnospiraceae* family.¹⁵ The link of Omega-3 circulating levels/intake and microbiome composition was independent of dietary fibre intake.¹⁵

In the future study, we will study the potential use of omega-3 supplementation in regulating the gut microbiome

in HIV patients and also consider increasing our sample size and extending the time for treatment.

Conflict of interest

The authors have no conflict of interest.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.jendis.2018.07.001>.

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