

Omega-6 fatty acids and Crohn's disease

1. introduction

- 1.1. Joseph Hibbeln, a prominent researcher on the application of omega-3 fatty acids in psychiatry at the National Institutes of Health, recently wrote, "It is likely that the success and failure of different clinical trials using similar doses of n-3 LCFAs were influenced by differing background intakes of the n-6 fatty acids LA (linoleic acid) and AA (arachidonic acid)." {Hibbeln et al., 2006, Am J Clin Nutr, 83, 1483S-1493S} What is the basis for this statement? Briefly, the long-chain polyunsaturated fatty acids (FAs) important in humans are the omega-3 (n-3) FAs DHA and EPA, and the omega-6 (n-6) AA. The AA in tissues is largely derived from dietary LA (the shorter chain n-6 linoleic acid) which currently provides about 9% of all calories in the typical US diet. The conversion of these large quantities of LA to AA "hogs" the enzymatic conversion pathway used by both n-3 and n-6 FAs, meaning that only a small percentage of shorter-chain n-3 ALA (alpha-linolenic acid) is able to be converted to EPA and DHA. AA is the precursor for pro-inflammatory prostaglandins and leukotrienes, while EPA and DHA are precursors for anti-inflammatory substances. Thus, higher intakes of LA in the diet result in higher tissue concentrations of AA and thus promote inflammatory processes, while higher intakes of EPA and DHA tend to reduce the availability of AA for conversion to inflammatory substances, while at the same time producing anti-inflammatory effects. Whether LA wins out over EPA + DHA and produces more inflammatory disease likely depends on a number of factors, but dietary intakes seem important, both absolute amounts and relative quantities. It seems clear, though, that the absolute amounts of LA and ALA in the diet, and not the ratio, affect the efficiency of conversion of ALA to EPA and DHA {Griffin, 2008, Curr Opin Lipidol, 19, 57-62}.
- 1.2. It is important to keep in mind that the balance between pro-inflammatory and anti-inflammatory activities is not the only factor involved in illness or health. For example, long-chain PUFAs are an important structural element of cells involved in electrical signaling, including the retina, the brain, and conductive tissue in the heart. Thus a particular dietary component may worsen some diseases (eg linoleic acid in age-related macular degeneration) while at the same time be beneficial in heart disease (see below).
- 1.3. Another caveat has to do with extrapolating from animal studies to humans. While humans of many cultures have traditionally had high dietary intakes of long-chain n-3s from fatty fish, some animal species such as mice whose diet contains a high proportion of LA from seeds and grains, may do better on diets supplemented with n-6 FAs than when supplemented with n-3s.
- 1.4. Given the role of omega-6 FAs in the promotion of inflammation, it is curious that many studies of the effects of n-3 FAs have used safflower oil as a control. Far from being inert, safflower oil can contain over 70% LA, according to the USDA National Nutrient Database (<http://www.nal.usda.gov/fnic/foodcomp/search/>), and can be expected to influence the control groups in those studies so as to accentuate the differences between treated and control,

an important bias which is usually not explicitly stated, e.g. {Adams et al., 2006, J Int Soc Sports Nutr, 3, 28-36; Barre et al., 2008, J Oleo Sci, 57, 269-73; Othman et al., 2008, Am J Physiol Heart Circ Physiol, 294, H1452-8}, although in other cases the n-6 content of the control safflower oil diet is reported, e.g. {de Silva et al., 2005, Nutr Metab (Lond), 2, 8; Paschos et al., 2007, Eur J Clin Nutr; Riediger et al., 2008, Eur J Nutr, 47, 153-60}.

- 1.5. Finally, studies of dietary intake frequently make use of food-frequency questionnaires and are dependent on recall. While sources of DHA and EPA such as fatty fish may be remembered relatively easily, including the number of servings, the types and amounts of vegetable oils (principal source of LA) may be impossible to recall accurately, especially since such oils are often "hidden" as ingredients in baked goods and snack foods. To compound the difficulty, the proportions of LA in various vegetable oils varies enormously, and can vary greatly even in one type of oil.
 - 1.6. With the above considerations in mind, let's review some of the research touching on the health effects of dietary n-6 FAs, especially LA. Since this effort was stimulated by a research report on relapse prevention of Crohn's disease by n-3 supplements, we will begin with the role of n-6 in inflammatory bowel disease.
 - 1.7. According to Philip Calder, "The established role of arachidonic acid-derived eicosanoids in the pathophysiology of inflammatory bowel diseases suggests that a high dietary intake of n-6 PUFAs may play a part in establishing or perpetuating the disease" {Calder, 2006, Am J Clin Nutr, 83, 1505S-1519S}.
2. omega-6 in Crohn's
 - 2.1. for example, a recently published Canadian study {Amre et al., 2007, Am J Gastroenterol, 102, 2016-25} looked at diets of children one year prior to being diagnosed with Crohn's and found that higher levels of dietary omega-3s were negatively associated with Crohn's, while higher ratios of omega-3 to omega-6 fatty acids were associated with a lower risk for Crohn's. An earlier Japanese study {Shoda et al., 1996, Am J Clin Nutr, 63, 741-5} had looked for correlations in dietary changes between 1966 and 1985 and the increased incidence of Crohn's disease and found that the most important independent factor was increased dietary protein, with an increased ratio of omega-6 to omega-3 fatty acids being the second strongest factor.
 - 2.2. Although not as directly related, the effect of seal oil (rich in n-3) vs soy oil (rich in n-6) on joint pain in IBD patients has been studied. One group {Bjorkkjaer et al., 2004, Scand J Gastroenterol, 39, 1088-94} found that seal oil led to longlasting improvements, while soy oil showed a (non-statistically-significant) tendency to worsen joint pain in a group of 19 patients, 9 with Crohn's and 10 with ulcerative colitis.
 - 2.3. The effects of n-3 and n-6 FAs on pro-inflammatory and anti-inflammatory cytokines was studied by Nielsen et al {Nielsen et al., 2005, Aliment Pharmacol Ther, 22, 1121-8}. While oral supplementation with n-3 did not lead to significant changes in cytokine levels in 31 patients with active Crohn's disease over 9 weeks of treatment, n-6 supplements induced significant

- increases of the pro-inflammatory cytokines IL-1beta, IFN-gamma, and MCP-1, as well as decreases of the anti-inflammatory cytokines IL-4 and IL-5.
- 2.4. A case-control study of hospitalized Japanese IBD patients looked at the relative risks for ulcerative colitis or Crohn's disease for intakes of various dietary constituents, based on a food-frequency recall questionnaire. Interestingly, higher intakes of both n-3 and n-6 FAs were associated with significantly higher risk of Crohn's disease, but not UC {Sakamoto et al., 2005, *Inflamm Bowel Dis*, 11, 154-63}.
 - 2.5. Another Japanese study which followed 76 patients with CD for one year {Tanaka et al., 2007, *Gastroenterol Nurs*, 30, 202-10} found that a higher risk for relapse with a lower n-6/n-3 ratio for dietary fats, in contrast to other studies. The authors suggested that the older patients with more stable disease were more likely to have eased up on fatty food avoidance in comparison with younger patients with unstable disease who would be more likely to follow a stricter diet.
3. omega-6 in animal studies
 - 3.1. inflammatory bowel disease
 - 3.1.1. The experimental induction of inflammatory bowel disease in rats is affected by diet. For example, in an experiment {Vilaseca et al., 1990, *Gut*, 31, 539-44} where rats were fed diets enriched in either cod liver oil (n-3 rich) or sunflower oil (n-6 rich) for 4 weeks, inflammatory colitis was then induced by intracolonic administration of trinitrobenzene sulphonic acid. In the cod liver oil rats, the damage score was markedly reduced 30 days later, and inflammation and ulceration were almost absent by day 50.
 - 3.1.2. The effect of maternal dietary fat composition on responsiveness to experimental colitis in suckling rat pups was studied {Jacobson et al., 2005, *Am J Physiol Gastrointest Liver Physiol*, 289, G13-20}. A high LA maternal diet led to more severe histological damage than either the high ALA or high oleic acid diet.
 - 3.2. other conditions
 - 3.2.1. In rats, diets high in n-6 polyunsaturates led to severe insulin resistance, whereas substituting 11% of FA in the n-6 diet with long-chain n-3s from fish oils normalized insulin action {Storlien et al., 1991, *Diabetes*, 40, 280-9}.
 - 3.2.2. The impaired learning behaviour in rats fed an n-3 deficient diet over two generations can be reversed by supplementing with n-3s after weaning, but only when the n-6s in diet are limited {Ikemoto et al., 2001, *J Lipid Res*, 42, 1655-63}.
 - 3.2.3. Topically applied n-6 fatty acids improve wound healing in surgically induced wounds in mice, whereas n-3 FAs may retard healing {Cardoso et al., 2004, *Wound Repair Regen*, 12, 235-43}
 - 3.2.4. A study in mice whose diet was supplemented with sucrose or fed safflower oil showed a 1.7-fold or 2.2-fold increase in liver triglyceride content (fatty liver) compared to control mice fed a high-starch diet. Fish oil added to the sucrose-supplemented rats completely prevented the

- fatty liver, but actually worsened the fatty liver for the safflower-oil fed mice {Yamazaki et al., 2007, *Hepatology*, 46, 1779-90}.
- 3.2.5. Middle-aged male rats fed diets containing high LA (safflower oil) lost more bone mineral content (BMC) than those fed a mixture of n-3 and n-6 fats. A third group fed a high n-3 diet (menhaden oil) had the least BMC loss {Shen et al., 2006, *Br J Nutr*, 95, 462-8}.
 - 3.2.6. Bone mineral content and density losses were significantly less in ovariectomized sexually mature rats with diets having a n-6/n-3 ratio of 5:1 than for a ratio of 10:1. Graphs in the article show a trend to greater losses with higher linoleic acid content {Watkins et al., 2006, *J Nutr Biochem*, 17, 282-9}.
 - 3.2.7. Geriatric mice fed safflower oil had a greater decrease (nonsignificant) in measures of immune competence than a control group, whereas those fed flaxseed oil (ALA-rich) had significantly better measures than the safflower oil group, and not significantly different from young mice {Hillyer et al., 2006, *Br J Nutr*, 95, 230-3}.
 - 3.2.8. The influence of dietary n-6/n-3 balance on the tendency to arterial thrombosis and atherosclerosis progress was evaluated in apoE^{-/-}/LDLR^{-/-} double knockout mice. Four groups were fed diets containing varying proportions of flaxseed and safflower oils to give n-6/n-3 ratios of 0.29, 1.43, 5.00, and 8.00. After 16 weeks, the first group had significantly lower plasma triglycerides and LDL, higher HDL, less atherosclerosis, and lower thrombus volume. Most of these parameters appeared to be related to the n-6/n-3 ratio in a dose-dependent manner {Yamashita et al., 2005, *Thromb Res*, 116, 393-401}.
 - 3.2.9. Mice fed diets rich in sunflower oil produced more skin cancer tumors under the influence of daily ultraviolet light, compared to mice receiving saturated fat {Reeve et al., 1996, *Cancer Lett*, 108, 271-9}.
4. omega-6 in other illnesses
 - 4.1. omega-6 and dementia
 - 4.1.1. The Three-City cohort study in France {Barberger-Gateau et al., 2007, *Neurology*, 69, 1921-30} followed over 8000 nondemented individuals over 4 years and found 281 incident cases of dementia. Regular consumption of n-6 rich oils was associated with an increased risk of dementia among apoE epsilon 4 noncarriers, but only when the n-6 was not compensated by consumption of n-3 rich oils or fish.
 - 4.1.2. In the Zutphen Elderly Study, high dietary LA was associated with cognitive impairment after adjustment for age, education, smoking, alcohol, and energy intake {Kalmijn et al., 1997, *Am J Epidemiol*, 145, 33-41}.
 - 4.1.3. Clinical evaluations were performed on a stratified random sample of 815 community residents 65 or over unaffected by Alzheimer disease at baseline. After a mean followup of 3.9 years, 131 developed Alzheimer's. Results from food-frequency questionnaires completed 2.3 years on average before clinical evaluation found that n-6 FA intakes were significantly and inversely associated with Alzheimer's, but this

association was no longer significant when adjusted for consumption of other fats {Morris et al., 2003, Arch Neurol, 60, 194-200}.

4.1.4. The relation between individual fatty acid intakes, lung function, and self-reported respiratory symptoms was studied in a sample of more than 13,000 Dutch adults. High intakes of n-3 fatty acids did not appear to protect against COPD or asthma, but a high intake of several n-6 fatty acids was associated with a significant reduction in FEV1 (forced expiratory volume in 1 s), particularly in smokers {McKeever et al., 2008, Thorax, 63, 208-14}.

4.2. omega-6 in cardiovascular disease

4.2.1. A recent review {Stanley et al., 2007, Br J Nutr, 98, 1305-10} concluded that use of the n-6:n-3 fatty acid ratio to estimate CVD risk should be abandoned, but that absolute amounts of individual n-6 and n-3 FAs on risk factors and CVD endpoints should be further investigated.

4.2.2. Two randomized, double-blind, placebo-controlled parallel studies have shown that the ratio of n-3 to n-6 does not affect the improvement seen in cardiovascular risk factors from fish oil supplementation {Hwang et al., 1997, Am J Clin Nutr, 66, 89-96}. However, the dietary n-6/n-3 ratios studied varied between 1.25:1 and 3.3:1, which is considerably lower than the 15:1 ratio in the typical western diet.

4.2.3. There is good evidence that higher levels of dietary linoleic acid reduce coronary heart disease and type 2 diabetes risk {Willett, 2007, J Cardiovasc Med (Hagerstown), 8 Suppl 1, S42-5}.

4.2.4. In a study of 64 healthy men given either olive oil or fish oil, together with a low-LA or high-LA supplement, plasma triacylglycerol decreased by 51% with fish oil compared to the olive oil placebo, for the low-LA group, but decreased by only 19% for the high-LA group {Damsgaard et al., 2008, J Nutr, 138, 1061-6}.

4.2.5. A 24-week randomized study involving 124 subjects assigned to a control group or one of four dietary interventions showed that two portions of oily fish per week led to significant reductions in serum triacylglycerols, compared to two portions of white fish per week. These changes were maximized when combined with a reduction in LA:ALA (substituting rapeseed oil for sunflower oil) {Moore et al., 2006, Nutrition, 22, 1012-24}.

4.2.6. In 21 moderately overweight, hypercholesterolemic subjects put on experimental isoenergetic diets enriched with plant sterol preparations of sterols esterified to either olive oil, fish oil, or safflower oil. Each experimental diet was given for 4 weeks and separated with 4-week washout periods in a crossover design. The fish oil preparation resulted in markedly lower triglyceride levels. The safflower oil preparation increased concentrations of Plasminogen Activator Inhibitor 1, a marker of endothelial function, compared to fish oil and also to olive oil, although the difference to olive oil was not statistically significant {Jones et al., 2007, Lipids Health Dis, 6, 28}.

4.3. parenteral lipids in sepsis

- 4.3.1. A small study in which 21 critical care patients with sepsis, requiring parenteral nutrition, were randomized to either the conventional lipid emulsion (high in n-6) or an n-3 emulsion rich in DHA and EPA. Cytokine synthesis by isolated mononuclear leukocyte was elicited by endotoxin. Generation of proinflammatory cytokines by mononuclear leukocytes was markedly amplified during n-6 and was suppressed during n-3 lipid application {Mayer et al., 2003, Am J Respir Crit Care Med, 167, 1321-8}.
- 4.4. omega-6 in cancer
 - 4.4.1. Serum samples from 19 prostate cancer patients and 21 age-matched male controls were tested for Fatty acid concentrations {Yang et al., 1999, Clin Biochem, 32, 405-9}. The n-3 PUFA levels were significantly decreased in the cancer patients while n-6 levels were increased.
 - 4.4.2. "Whereas saturated fats have generally been unrelated to tumorigenesis, increasing amounts of essential fatty acids, primarily linoleic acid (the major polyunsaturated fat in the Western diet), increase the incidence and likelihood of metastasis of chemically induced and transplanted mammary tumors.3-5"
- 4.5. omega-6 in depression
 - 4.5.1. In 43 older adults, six individuals with depression had higher n-6:n-3 ratios on blood testing than those who did not meet major depression criteria {Kiecolt-Glaser et al., 2007, Psychosom Med, 69, 217-24}.
 - 4.5.2. The levels of adipose tissue DGLA (dihomo-gammalinolenic acid, an n-6 fatty acid) in 90 healthy adolescents was found to correlate positively with the CES-D (Centre for Epidemiologic Studies Depression Scale) {Mamalakis et al., 2006, Pharmacol Biochem Behav, 85, 474-9}.
 - 4.5.3. A study which compared the fatty acid composition of serum cholesteryl esters and phospholipids between 36 major depressed, 14 minor depressed, and 24 normal subjects, found a significantly increased n-6/n-3 ratio in major depressed vs normal and minor depressed subjects {Maes et al., 1996, J Affect Disord, 38, 35-46}.
- 4.6. omega-6 and osteoporotic fractures
 - 4.6.1. semiquantitative food frequency questionnaire was used in a hospital-based case control study involving 167 patients aged 65 or over with a low-energy fracture, matched to controls by sex and age. Higher intakes of n-6 fatty acids were associated with an elevated risk of fracture {Martinez-Ramirez et al., 2007, Eur J Clin Nutr, 61, 1114-20}.
- 4.7. omega-6 in ophthalmology
 - 4.7.1. The US Twin Study of Age-related Macular Degeneration {Seddon et al., 2006, Arch Ophthalmol, 124, 995-1001} looked at 681 elderly male twins, all WW2 veterans, and found 222 with intermediate or late stage AMD. Increased fish intake reduced AMD risk; the OR for n-3 FA intake was 0.55 comparing highest vs. lowest quartile. However, this effect was seen primarily among subjects with low levels (below median) of linoleic acid intake.
 - 4.7.2. An earlier study by the same group {Seddon et al., 2001, Arch Ophthalmol, 119, 1191-9} compared 349 individuals with advanced AMD

to controls without AMD but with other ocular diseases. Higher linoleic acid intake was significantly associated with AMD in a multivariate analysis controlling for a number of variables including age, sex, blood pressure, BMI, calories, smoking, and alcohol. The OR was 2.0 for the highest quintile of linoleic acid intake compared to the lowest.

- 4.7.3. 32470 women in the Women's Health Study who had provided information on Dry Eye Syndrome (DES) and diet were studied. Of the sample, 1546 (4.7%) reported DES. After adjustment for demographic factors, hormone therapy, and total fat intake, a higher ratio of n-6 to n-3 FA consumption was associated with a significantly increased risk of DES (OR: 2.51) {Miljanovic et al., 2005, Am J Clin Nutr, 82, 887-93}.