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FATTY ACIDS AS MODULATORS OF THE IMMUNE RESPONSE

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Key Words fish oil, omega-3, cytokines, arthritis, asthma

■ **Abstract** Research describing fatty acids as modulators of inflammation and immune responses abounds. Many of these studies have focused on one particular group of fatty acids, omega-3. The data from animal studies have shown that these fatty acids can have powerful anti-inflammatory and immunomodulatory activities in a wide array of diseases (e.g., autoimmunity, arthritis, and infection). However, the evidence from human trials is more equivocal. In this review, a historical framework for understanding how and why fatty acids may affect the immune system is provided. Second, highlights of two recent landmark reports from the Agency for Healthcare Research and Quality are presented. These reports critically evaluate the evidence from human clinical trials of omega-3 fatty acids and rheumatoid arthritis, asthma, and a few other immune-mediated diseases. Third, the data from human clinical trials investigating the impact of various bioactive fatty acids on ex vivo and in vivo immune response are reviewed. Limitations in experimental design and immune assays commonly used are discussed. The discordance between expectation and evidence in this field has been a disappointment. Recommendations for improving both animal-based and human studies are provided.

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INTRODUCTION

Fatty Acids

Fatty acids are a kind of fat or lipid. The term "lipid" is rather broad and can refer to a variety of compounds that share similar properties of hydrophobicity (i.e., poor solubility in water). Dietary lipids include tri-, di-, and monoglycerides, sphingolipids, free fatty acids, cholesterol, plant sterols, various pigments, and fat-soluble vitamins. This review focuses on immunomodulation by fatty acids. The ability of certain fatty acids to influence the immune system and the function of its various cellular components have been recognized for nearly 30 years. The first review of this field emerged in 1978 (56), and the number of reviews has grown steadily over time. In fact, more than 20 reviews have been published on this topic since 2000. Most of the research on this topic has focused on one class of fatty acids, omega-3 polyunsaturated fatty acids (n-3 PUFAs). Many people are aware of the cardio-protective effects of fish oil–derived n-3 PUFAs. These fatty acids, however, also purportedly possess anti-inflammatory and immunosuppressive activity. This has fueled interest in the use of n-3 PUFAs to prevent and/or treat a variety of inflammatory and autoimmune diseases in humans.

Historical Overview

A brief history of the research into the impact of fatty acids on the immune system can be separated into three periods that roughly coincide with the last three decades of the twentieth century. In the 1970s, researchers reported on the essential nature of linoleic acid, an n-6 PUFA, for the development and proper functioning of the immune system. Since essential fatty acid deficiency is uncommon, this area of research received little attention. Much of these data, however, have been summarized by Harbige (33). During this same period, several researchers demonstrated that consuming diets high in fat tends to suppress immune responses, such as phagocytosis and infectious disease resistance (reviewed in 62). Another hypothesis that emerged during this early period was that certain dietary fats induced changes in immune cell membranes. Changes in membrane-dependent functions, such as phagocytosis and cell signaling, were thought to be a direct consequence of alterations in membrane composition and "fluidity" (63). In time, this membrane fluidity theory lost favor, in part because it failed to explain why n-6 and

n-3 PUFAs have contradictory actions on the immune system (64). However, this theory has re-emerged recently because of the discovery of discrete lipid domains, sometimes referred to as "lipid rafts," in cellular membranes (5). Others have demonstrated that PUFAs can remodel the composition of lipid rafts in T-cells and that this is associated with diminished signaling through the T-cell receptor (26, 75). Whether such changes in lipid raft composition and cell signaling are both necessary and sufficient to explain dietary PUFA modulation of immune responses remains unknown.

In the second period, researchers focused primarily on defining the role of certain fatty acid metabolites, collectively known as eicosanoids, as mediators of inflammation and immune cell function. Eicosanoids are primarily derived from arachidonic acid (AA), typically the most abundant PUFA in immune cell membranes. Many researchers have demonstrated that dietary fat source affects both cellular fatty acid composition and subsequent eicosanoid biosynthesis. Nearly every review on the topic of fatty acid modulation of immunity includes some discussion of this subject, with the most comprehensive published in 1990 (45).

In the 1990s, many nutrition-immunology researchers turned their attention to cytokines. Cytokines are protein-based regulators of inflammation and immune cell function (36). During this period, many reports in the literature described associations between fatty acid modulation of inflammatory/immune responses with changes in cytokine production. The evidence in this area has been reviewed previously (11, 13, 24). Since publication of those reviews, considerable effort has gone into defining the molecular mechanism(s) underlying fatty acid modulation of cytokine production. Readers interested in this topic should refer to one of these more recent reviews for additional details (15, 75, 87). Despite these advances, several inconsistencies in the existing data remain unresolved. For example, it is unclear why n-3 PUFA-induced changes in ex vivo and in vivo cytokine production do not always coincide. Nor have valid explanations been provided for why dietary n-3 PUFAs affect cytokine production in humans and mice in opposite directions on occasion. Although some questions remain, existing evidence indicates that dietary fatty acids modulate immune responses through one or more of three major molecular mechanisms: (*a*) altered membrane composition and function, (*b*) modified eicosanoid production, and (*c*) changed cytokine biosynthesis.

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Emerging Areas

Over the past decade, a novel molecular mechanism for fatty acid modulation of the immune system has emerged. Researchers discovered that fatty acids, and various fatty acid–derived metabolites (e.g., 15-deoxy-PGJ_2), can affect gene expression via binding to and activating a novel family of nuclear receptors known as peroxisomal proliferator-activated receptors (PPARs) (46). The three members in the PPAR family are PPARα, PPARδ, and PPARγ. All three PPARs act as transcription factors. Treatment of cells with PPAR ligands can significantly affect cellular differentiation and functional properties. PPARα is the predominate form

expressed in T- and B-cells, whereas PPARγ dominates in cells of myeloid lineage (e.g., monocytes and macrophages). It is too early to know whether these nuclear receptors play a significant role in fatty acid modulation of inflammatory/immune responses in vivo. PUFAs from both n-3 and n-6 families reportedly bind equally well to PPARs (86). Such data have been interpreted to mean that it is unlikely that differential immune modulation by various PUFAs is mediated through PPAR. Yet, it is possible that differences in routing and cellular metabolism of various PUFAs in vivo could result in unique patterns of PPAR activation for n-3 and n-6 PUFAs. Several leading researchers have reviewed the evidence in this area (18, 37, 87). Discovery of this novel molecular mechanism has created considerable excitement in this field, but many practical questions remain unanswered. Importantly, direct evidence that fatty acid modulation of immune response occurs via a PPAR-dependent mechanism is lacking. Feeding trials in conjunction with in vivo immune challenge of mice carrying gene deletions for each PPAR would provide critically needed evidence. Unfortunately, global deletion of PPARγ is lethal. Therefore, mice with an immune cell-specific gene deletion of PPARγ, such as those described by Akiyama and coworkers (3), will need to be used to address the role of this particular PPAR subtype on fatty acid modulation of the immune system.

Finally, advances in our understanding of eicosanoid biology have fueled a renewed interest in these lipid mediators. The two most significant advances are (*a*) the discovery of isoforms of the prostaglandin (PG)-producing enzyme cyclooxygenase (i.e., COX-1 and COX-2) and (*b*) a recognition of the essential role for AA metabolites in the resolution of inflammatory responses (69, 71). Although it has been some time since the first anti-inflammatory eicosanoid was described, the importance of lipoxins and other lipid mediators that promote resolution of acute inflammation has only recently been fully characterized (8). For example, PGE_2 treatment of human peripheral blood neutrophils causes them to switch eicosanoid production from leukotriene (LT) B_4 via the 5-lipoxygenase (LO) pathway to lipoxin (LX) A_4 via the 15-LO pathway. LXA_4 is a potent inhibitor of neutrophil infiltration and function. At first glance, it may seem paradoxical that COX-2 could both promote inflammation and its resolution. However, it is the temporal nature of COX-2 expression and the changing cell populations that create the microenvironment needed for this circuit of inflammation and resolution to occur.

The potential for n-3 PUFA to influence the resolution program has also been explored (71). The reduction in AA-derived eicosanoids and the diminished activity of the alternative products generated from n-3 PUFAs serve as the foundation for explaining some of the beneficial effects of greater n-3 PUFA intake (7). Some of these novel eicosanoids derived from eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been given names, such as resolvins and neuroprotectins, to reflect their potent anti-inflammatory and proresolving biological activity (70). However, it is surprising that more is known about the structure and molecular mechanisms through which these n-3 PUFA-derived eicosanoids operate than about the dose-response relationship between dietary n-3 PUFAs and their

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in vivo generation. Whether the generation of resolvins and/or neuroprotectins is both necessary and sufficient for dietary n-3 PUFA to have a beneficial effect on an inflammatory or immune-mediated disease remains unanswered. Yet if this proves so, then measuring these particular eicosanoids may serve as a reliable surrogate to clinical assessment for determining efficacy for n-3 PUFA treatment.

In summary, during the past few years it appears that we have entered a new period in this field. Considerable progress has been made in our understanding of the cellular and molecular mechanisms through which fatty acid may affect inflammation and immune cell function. These discoveries have opened up many new avenues of research. Despite the apparent advances in our fundamental understanding of fatty acid nutrition, clinical application for the benefit of patients suffering from inflammatory or immune-mediated disease has yielded disappointing results to date.

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IMPACT OF n-3 PUFA ON INFLAMMATION/IMMUNE-MEDIATED DISEASE IN HUMANS

Agency for Healthcare Research and Quality Reports

The Agency for Healthcare Research and Quality (AHRQ) recently published two thorough assessments of the evidence regarding the impact of omega-3 fatty acids on immune-mediated disease in humans. One report considered the data on inflammatory bowel disease (IBD), rheumatoid arthritis (RA), renal disease, and systemic lupus erythemosus (SLE) (54); a separate report on asthma was also published (68). These two reports focused primarily on data from randomized controlled trials (RCTs), the gold standard in clinical medicine research. A brief description of what these reports contained is provided below.

n-3 Polyunsaturated Fatty Acid and Rheumatoid Arthritis

The following clinical parameters were assessed in the RCTs on RA and n-3 PUFA: pain, swollen joints, disease activity, and a patient's global assessment. Of the 19 RCTs that measured pain, three reported that n-3 PUFA significantly reduced pain over a placebo (59, 60, 77), four reported improvements from baseline values, and there was no significant improvement in pain in 12 studies. Regarding swollen joints, n-3 PUFA was reported to improve this outcome in two studies in comparison with placebo (48, 77), and in four studies in comparison with baseline; no significant benefit was reported in nine studies. One study even reported that n-3 PUFA worsened swollen joint count relative to placebo (19). A similar pattern emerged for the other clinical outcomes of RA mentioned above. Meta-analysis of the nine eligible studies indicated that n-3 PUFA had no significant beneficial effects on RA patients. These findings are consistent with a previous meta-analysis

(28). However, in that previous analysis, n-3 PUFA was reported to reduce tender joint count compared with placebo. Overall, these studies tended to have a relative small number of subjects (i.e., typically no more than 25 to 50 in placebo and n-3 PUFA groups). As indicated in the AHRQ report, few of these RA studies examined more than a single dose, source, or duration of n-3 PUFA treatment, and there were insufficient data across studies to perform pooled analyses of dose, duration, and source effects. Taking into account only those studies providing greater than or equal to 3 g/d [∼1.4 energy percent (en%)] of LC n-3 PUFA, generally considered a high-dose treatment, failed to improve the outlook for efficacy of n-3 PUFA treatment. For example, the dosage of LC n-3 PUFA in those RA trials reporting significant reduction in pain or swollen joints was 1.5, 1.5, and 2.4 en%, whereas a total of five trials with the same or higher dosage (i.e., 1.5, 1.5, 2, 2.5, 2.5 en%) reported no benefits. Surprisingly, the trial that reported a worsening of joint stiffness used the highest LC n-3 PUFA dosage (i.e., 3.5 en%) (19).

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Contradictory Opinions

Several leading researchers in the field appear to disagree with the AHRQ findings. In a review of 14 clinical trials of fish oil treatment in RA patients, Calder stated that "all reported improvements" (14). He concluded that the evidence supporting the efficacy of LC n-3 PUFA supplementation in combination with gamma linolenic acid (GLA) (18:3n-6) was sufficient to warrant routine inclusion for all RA patients (16). A review published in 2002 by Artemis Simopoulos (72), a leading advocate for greater n-3 PUFA intake, stated that many of the placebo-controlled trials of fish oil reveal significant benefit to patients with chronic inflammatory disease, including RA. In 2003, Adam (2) reviewed 12 RCTs of fish oil treatment of RA that fulfilled criteria for good clinical practice (GCP). GCP is an FDA-defined standard for the design, conduct, performance, monitoring, auditing, recording, analysis, and reporting of clinical trials. Adam concluded that intake of 2.6 g/d of fish oil or 1.6 g EPA/d (0.7 en%) resulted in "a moderate but consistent improvement in clinical findings and laboratory parameters." Another expert in this field suggested that a minimum daily dose of 3 g (∼1.4 en%) of LC n-3 PUFA for at least 12 weeks is required for consistent clinical benefit to occur (47). In a recent review of various dietary interventions for the treatment of RA, Stamp et al. (74) concluded that dietary n-3 PUFA was the only nutritional approach for which there was "consistent evidence for symptomatic improvement." The disagreement between the AHRQ and the various experts may be a result of "benefit" sometimes being defined too broadly, such as including surrogate markers of inflammation. For example, reductions in circulating proinflammatory cytokines (e.g., IL-1β) have been reported in RA patients following fish oil consumption (25). The AHRQ review only considered the more direct measures of clinical benefit (e.g., morning stiffness, pain, and joint tenderness). Furthermore, AHRQ experts strictly interpret treatment benefit to mean that clinical outcomes are statistically different from placebo. Unintentional bias is probably a more important contributing

factor for these discordant positions on the efficacy of n-3 PUFA (1, 61). Impartiality of the experts is one of the major strengths of the AHRQ evidence-based reviews.

Animal-Based Evidence

That consumption of n-3 PUFA fails to or only modestly benefits RA patients is in sharp contrast to several reports of significant beneficial effects of fish oil supplementation on the development and progression of various experimental models of arthritis in mice (17, 50) and rats (66, 81). Interestingly, it has been reported that fish oil can have a detrimental effect on RA in some animal models. For example, mice fed fish oil showed a higher incidence of arthritis than did those fed diets with PUFA-poor beef tallow. However, there was no difference in the severity of swollen joints between treatment groups (66). Relative to the severity of adjuvant-induced arthritis, fish oil treatment reportedly benefits one strain of rats whereas it harms another (55). Thus, maybe we should not be surprised at the inconsistency of response that has been observed relative to n-3 PUFA and RA in humans. Evidence does exist for differential responsiveness to n-3 PUFA being linked to genotypic variation in humans (32). Researchers are just beginning to appreciate and understand the impact of genetic polymorphism on the responsiveness of humans to various drugs and nutrients. This is an exciting and important new area of research.

n-3 Polyunsaturated Fatty Acid and Asthma

The potential effect of n-3 PUFA on asthma was the subject of a separate report from the AHRQ (68). The hypothesis that n-3 PUFAs have a beneficial effect on asthma is based primarily on the evidence that these fatty acids influence the production of various inflammatory mediators believed to play a role in the development of this disease. Such mediators include leukotrienes B_4, C_4, and D_4, along with the proinflammatory cytokines, interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ. A systematic review of 26 studies was conducted, with the greatest weight given to the 11 RCTs in comparison with 15 studies using designs other than RCT. The AHRQ review (68) concluded that the evidence for a beneficial effect of n-3 PUFA on asthma was weak at best. This conclusion is in agreement with a more recent review (84).

Shortcomings

Deficiencies in the existing human trials were described in some detail in both AHRQ reports (53, 66). For example, most RCTs were statistically underpowered. Many failed to provide adequate details about a number of critical experimental design issues or to control key confounders (i.e., subject/patient profile, nature of the intervention, and cointerventions). Little effort was made to define or control background diets. Incomplete or inadequate blinding of subjects to treatments was

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common. The net effect of these deficiencies is that meaningful interpretation of the data is undermined. In the end, the major conclusion that emerged from these two reports was the need for more and better data on how and whether dietary n-3 PUFA may affect treatment of immune-mediated diseases in both adults and children.

Conclusions

These AHRQ reports (53, 66) stand apart from previously published meta-analyses and reviews on this topic for a number of reasons, including thoroughness, transparency, and impartiality. Yet it was surprising that the data failed to show n-3 PUFA having consistent benefits for patients suffering from RA or other immune-mediated disorders. The RCTs evaluated in the AHRQ reports were mostly the same clinical trials that several experts in the field interpreted as supporting the opposite conclusion (i.e., LC n-3 PUFAs reduce inflammation and immune-mediated disease in humans). Importantly, the authors of the AHRQ reports stopped short of concluding that these fatty acids were without effect in humans. Instead, the authors recommended strengthening future clinical trials so that this important health issue could be appropriately resolved.

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DIETARY POLYUNSATURATED FATTY ACID AND EX VIVO HUMAN IMMUNE CELL FUNCTION

Background

The immune system is remarkably complex, possessing numerous overlapping elements that function in a coordinated fashion in three-dimensional space and in time (i.e., four dimensions). Yet in the field of nutrition-immunology, researchers frequently investigate the impact of a given nutrient on the immune system under ex vivo conditions. Such an approach typically involves providing human subjects an experimental diet or supplement to consume. After a certain period, blood samples are taken and immune cells are isolated. The functional properties of these peripheral blood immune cells are then studied in vitro. Common functional assays include proliferation, phagocytosis, cytotoxicity, and cytokine production.

Most in vitro immune assays involve treating cells with some sort of challenge or stimulus. Stimulation is used when little or no measurable activity exists in unstimulated cells, as is generally the case in lymphocyte proliferation and cytokine biosynthesis investigations. The nature of the stimulus and the amount used are important experimental parameters to be determined by the investigator. Some stimuli mimic what an immune cell might encounter naturally (e.g., opsonized yeast and bacteria), whereas others have little or no physiologic basis other than that they work (e.g., concanavalin A, phytohemagluttin, and ionomycin). There are numerous examples in the literature of different immune stimuli yielding different

results and conclusions, even within the same study. Of course, such variability in the data makes interpretation of the evidence as a whole quite problematic, as described below.

Ex Vivo and In Vivo Evidence

Tables 1–3 display data from human clinical trials that investigated the impact of various dietary fatty acids on ex vivo immune cell function. Not surprisingly, most of the trials focus on n-3 PUFA. Trials concerning other types of fatty acids, such as AA, GLA, and conjugated linoleic acid (CLA), are described separately (Table 3). However, when investigators included n-3 PUFA and some of these other fatty acids in their study, these trials are included with the n-3 PUFA studies. The n-3 PUFA trials that involved substantially large numbers of subjects are presented separately from the more typical underpowered trials (Table 2). Within each table, data are organized such that trials are listed in descending order based on the amount of PUFA provided by the investigators.

Ex vivo lymphocyte proliferation is a popular method for assessing immunomodulation by PUFA as well as by many other nutrients. This is understandable since proliferation and subsequent differentiation of T- and B-cells is a necessary feature of the adaptive immune response. Data from in vitro and animal-based studies have led most researchers in this field to conclude that LC n-3 PUFAs reduce T-lymphocyte proliferation. Yet, of the five trials that examined this immune response, not even one reported a significant reduction in ex vivo proliferation following LC n-3 PUFA supplementation (see Tables 1 and 2). Interestingly, the only study to report an "effect" of n-3 PUFA on proliferation reported that LC n-3 PUFA enhanced proliferation (76). However, the other four trials that examined lymphocyte proliferation had used relatively modest levels of n-3 PUFA (i.e., ranging from 1.4 to 0.7 en% LC n-3 PUFA). This might explain, in part, why "no effect" was observed. Several of these same trials also investigated the impact of other PUFAs, including plant-derived n-3 PUFA (α-linolenic acid and stearidonic acid) and GLA, an n-6 PUFA. When tested, these other PUFAs were also without effect on ex vivo lymphocyte proliferation.

Traditional proliferation assays involve coculturing a lymphocyte population with a polyclonal stimulus (e.g., PHA, Con A, or anti-CD3/anti-CD28 antibodies) in an artificial culture medium supplemented with 10% fetal bovine sera. Fetal serum provides cells with important growth factors and hormones, yet is typically devoid of LC PUFA. Diet-induced changes in immune cell PUFA may be reversed during in vitro culturing (51, 88). Inclusion of autologous (same subject) or homologous (same treatment) sera/plasma to the medium prevents this. Data in the tables suggest inclusion of such sera or plasma has become routine whenever ex vivo assessment of immune cell function is conducted. In another effort to maintain the "in vivo" environment during ex vivo assays, some researchers use whole blood preparations. In this approach, blood is collected with an anticoagulant and then diluted with cell culture medium without sera. Because dietary treatments may

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TABLE 1 Impact of dietary n-3 PUFA on in vivo and ex vivo immune responses in healthy humans[1]

Subjects	Duration	Intervention	Treatment groups and dosage	Cells studied	Immune stimuli	Key findings	Reference
10 men (n = 5)	4 wk	25 g/d fish oil Placebo: sunflower seed oil	(*a*) 3.2 en% n-3 PUFA (4.3 g EPA + 2.8 g DHA/d) (*b*) placebo, 9 en% oleic acid (20.7 g/d)	Monocytes, PMN, and whole blood	Opsonized *E. coli*	EX VIVO: •No effect on phagocytic activity of monocytes and PMN, but did increase in both placebo and n-3 PUFA groups following supplementation. •No effect on oxidative burst activity of whole blood preps.	(78)
9 males and females	6 wk	30 mL cod liver oil/d No placebo	(*a*) 2.7 en% n-3 (3.6 g EPA + 2.4 g DHA/d)	Blood monocytes	Latex particles; Opsonized zymosan A	IN VIVO: •Changed PBMC AA content (23% to 15%); EPA (<0.1% to 2%); DHA (0.7% to 2.2%). EX VIVO: •N-3 PUFA reduced superoxide production (~60%) and chemiluminescence (~55%) by challenged monocytes.	(27)
11 males (n = 4–7)	13 wk	DHASCO^{TM} oil No placebo, but subjects lived in a metabolic unit	(*a*) 2.7 en% DHA (6 g/d) (*b*) placebo, 2.7 en% LA	PBMC (10% autoserum)	LPS (1 mg/L)	IN VIVO: •DHA decreased AA content of PBMC from 20% to 11% of total fatty acids; increased DHA from 2% to 7.5%; without changing EPA levels. EX VIVO: •DHA reduced PGE_2 and LTB_4 production by PBMC (60%–75%). •DHA decreased NK cell activity (20%); •Decreased production of IL-1β and TNF-α by PBMC (~45%).	(41)

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42 adults (n = 10–15)	4 wk	9 × 1 g capsules/d Placebo: olive oil	(*a*) 2.1 en% EPA (4.7 g/d) (*b*) 2.2 en% DHA (4.9 g/d) (*c*) placebo, 2.2 en% oleic acid	PBMC, PMN, and whole blood (diluted w/RPMI)	LPS (15 mg/L) Con A (25 mg/L)	EX VIVO: No effect of EPA nor DHA on: •Monocyte or PMN phagocytosis; •Cytokine production (i.e., TNF-α, IL-1β, IL-6, IL-8, IL-10 in response to LPS; IL-2, IFNγ, IL-4, IL-5, IL-10, TNF-α in response to Con A); •Adhesion molecule expression (CD11b, CD18, CD49, CD54). EX VIVO: •DHA, but not EPA, reduced expression of CD69 (a T-cell activation marker) 24 h post-Con A stimulation (~10%).	(44)
5 males	12 wk	0, 3, 6, or 12 SuperEPA™ capsule/d (progressive) No placebo	(*a*) 2 en% n-3 PUFA (2:1) (3.6 g EPA + 1.8 g DHA/d) (*b*) 1 en% n-3 PUFA (*c*) 0.5 en% n-3 PUFA	PMN	*Staphylococcus aureus*	IN VIVO: •Dose-dependent changes in serum lipids noted. EX VIVO: •No effect on capacity of PMN to kill *S. aureus.*	(12)

(*Continued*)

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TABLE 1 (*Continued*)

Subjects	Duration	Intervention	Treatment groups and dosage	Cells studied	Immune stimuli	Key findings	Reference
58 monks (n = 14–15)	52 wk	EPA-rich fish oil capsules Placebo: olive and palm oil mixture	(*a*) 1.5 en% n-3 PUFA (3:1) (2.4 g EPA + 0.8 g DHA/d) (*b*) 0.75 en% n-3 (*c*) 0.37 en% n-3 (*d*) placebo	Whole-blood cell mixtures	LPS (10 mg/L)	IN VIVO: •No effect on basal circulating IL-1β, TNF-α, IL-1Ra. EX VIVO: •No effect on LPS-stimulated whole-blood cytokine production.	(10)
74 males (n = 8–12)	12 wk	9 × 1 g capsules/d Placebo: palm and sunflower oil mixture	(*a*) 1.4 en% n-3 (2:1) (2.1 g EPA + 0.9 g DHA/d) (*b*) 0.9 en% GLA (*c*) 1.2 en% SDA (*d*) 0.7 en% LC n-3 + 0.5 en% GLA (*e*) 0.5 en% LC n-3 + 0.6 en% SDA (*f*) 0.4 en% LC n-3 + 0.3 en% SDA + 0.3 en% GLA (*g*) Placebo	Whole blood; PBMC in media w/5% autoplasma	*E. coli* PMA Con A (25 mg/L) LPS (15 mg/L)	IN VIVO: •Plasma IgG_2 increased by ~15%; •NK cell # declined by 20%–25% in subject supplemented with LC n-3 or GLA alone. •However, final IgG_2 conc. and NK # were NOT different among diet groups at study end. No diet-induced changes in: •DTH; •Proportion or # of $CD4^+$ and $CD8^+$ T-cells, B-cells, monocytes; •ICAM expression on T-, B-cells, and monocytes. EX VIVO: No effect on: •PMN and monocyte phagocytosis or oxidative burst; •TNF-α and IL-1β by LPS-stimulated PBMC; •IL-2, IFNγ, IL-4, IL-10 by Con A-stimulated PBMC; •Con A-stimulated lymphocyte proliferation.	(58)

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31 males and females (n = 6–8)	6–8 wk	9 × 0.5 g capsules/d	(*a*) 1.1 en% n-3 PUFA (3:2) (1.5 g EPA + 0.9 g DHA/d)	Venous blood incubated directly with LPS	LPS (1 pg/mL to 1×10^9 pg/mL in $\log_{10}$ increments)	IN VIVO: •N-3 PUFA decreased the tachycardia and attenuated the maximal increases in oral temperature and metabolic rate following typhoid vaccine. EX VIVO: •Products of IL-1β and IL-6 by LPS-stimulated whole blood cells were decreased by n-3 PUFA, but only when LPS <1000 pg/mL (1 ng/mL); •No effect on TNF-α biosynthesis at any LPS conc.	(21)
40 adults (n = 8)	12 wk	9 × 1 g capsules/d Placebo: palm and soybean oil mixture (80:20 w/w)	(*a*) 0.8 en% n-3 PUFA (1:3) (0.5 g EPA + 1.4 g DHA/d) (*b*) 0.4 en% n-3 PUFA (*c*) 0.2 en% n-3 PUFA (*d*) 1.6 en% LNA (3.5 g LNA/d) flaxseed oil (*e*) Placebo	PBMC (5% autoplasma)	LPS (15 mg/L) Con A (7.5–75 mg/L)	EX VIVO: Two higher doses of FO resulted in a significant decrease in IL-6 production (medium FO declined by 64%; high FO by 40%). No effect on: •Production of TNF-α, IL-1β, IL-2, IL-10, and IFNγ by LPS-stimulated PBMC; •Production of IL-2, IL-10, IL-4, and IFNγ by Con A-stimulated PBMC; •Con A-stimulated PBMC proliferation.	(82)

(*Continued*)

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TABLE 1 (*Continued*)

Subjects	Duration	Intervention	Treatment groups and dosage	Cells studied	Immune stimuli	Key findings	Reference
16 men	4, 8, 12 wk	1 × 1 g capsules/d (0–4 wk); 3/d (5–8 wk) 6/d (9–12 wk)	(*a*) 0.8 en% n-3 PUFA (2:1) (1.2 g EPA + 0.6 g DHA/d) (*b*) 0.4 en% n-3 PUFA (*c*) 0.2 en% n-3 PUFA	PBMC (5% autoplasma)	LPS (15 mg/L) Con A (25 mg/L)	EX VIVO: Production of PGE_2 by unstimulated and LPS-stimulated PBMC significantly ($p < 0.001$) decreased by 75%–80% at low-dose n-3 PUFA; by >95% at two higher doses. •Proliferation of unstimulated and ConA-stimulated PBMC increased up to twofold at the highest dose of n-3 PUFA ($p < 0.05$). Yet, when these data were expressed as "stimulation index," no effect was observed. •The IFNγ production in response to Con A increased (~threefold) at the highest dose of n-3 PUFA ($p < 0.05$). IL-4 production was increased (twofold) at two high doses, but effect was not significant ($p > 0.15$). •Co-supplementation with antioxidants did NOT affect cytokine production or PBMC proliferation independently nor the impact of n-3 PUFA on these parameters.	(76)
21 children (8–12 years old, with n = 7 ctl; n = 14 trt)	12 wk	1 g chocolate spread/d Placebo: canola oil	(*a*) 0.14 en% n-3 PUFA (3:2) (0.18 g EPA + 0.12 DHA/d) (*b*) Placebo	PBMC (no sera)	LPS (10 mg/L)	EX VIVO: Production and release of TNF-α, IL-1β, IL-6, IL-10, and IL-1RA by unstimulated and LPS-stimulated PBMC was enhanced by n-3 PUFA feeding (~3- to 5-fold; $p < 0.05$).	(79)

[1]Abbreviations: AA, arachidonic acid; auto, autologous; BCSO, blank currant seed oil; CLA, conjugated linoleic acid; Con A, concanavalin A; DHA, docosahexaenoic acid; DTH, delayed-type hypersensitivity; en%, energy percent; EPA, eicosapentaenoic acid; GLA, gamma-linolenic acid; ICAM, intracellular adhesion molecule; IFNγ, interferon-gamma; IL, interleukin; IL-1RA, interleukin-1 receptor antagonist; LC, long chain; α-LNA, alpha-linolenic acid; LPS, lipopolysaccharide; LTB, leukotriene B; NK, natural killer; PBMC, peripheral blood mononuclear cell; PG, prostaglandin; PHA, phytohemagluttin; PMA, phorbol myristate acetate; PMN, polymorphonuclear cells; PUFA, polyunsaturated fatty acid; TNF-α, tumor necrosis factor-alpha.

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TABLE 2 Impact of dietary n-3 PUFA on in vivo and ex vivo immune responses in healthy humans in clinical trials with larger-than-normal subjects per treatment group[1]

Subjects	Duration	Intervention	Treatment groups and dosage	Cells studied	Immune stimuli	Key findings	Reference
169 young and older men (n = 23 and 15, respectively)	12 wk	9 × 1 g capsules/d Placebo: corn oil	(*a*) 2.2 en% n-3 PUFA (4:1) (4 g EPA + 0.9 g DHA/d) (*b*) 1.5 en% n-3 PUFA (*c*) 0.7 en% n-3 PUFA (*d*) Placebo	PBMC (5% auto-plasma) Whole blood	LPS (15 mg/L) PMA Opsinized *E. coli*	EX VIVO: n-3 PUFA at any dose did NOT significantly affect: •Production of TNF-α, IL-1β, IL-6 by LPS-stimulated PBMC; •Neutrophil or monocyte phagocytosis; •Monocyte oxidative burst; •Monocyte ICAM (CD54) or MAC-1 (CD11b) expression. •However, highest dose of n-3 PUFA reduced % of neutrophils undergoing oxidative burst by ~12% ($p < 0.05$) in the older subjects; PGE_2 biosynthesis by ~50% in young and older subjects.	(67)
150 men and women (n = 30)	12 and 25 wk	25 g fat spread plus 3 × 1 g capsules/d Placebo: LA-rich margarine plus capsules w/fatty acids of average intake in the United Kingdom	(*a*) 0.7 en% n-3 PUFA(2:3) (0.66 g EPA + 1 g DHA/d) (*b*) 0.34 en% n-3 PUFA (*c*) 4 en% LNA (9.5 g/d) (*d*) 2 en% LNA (4.5 g/d)	PBMC (5% auto-plasma)	LPS (15 mg/L) Con A (25 mg/L)	IN VIVO: •No effect on DTH response to tetanus and/or tuberculin antigens (n = 16 to 19). EX VIVO: n-3 PUFA did NOT significantly affect: •Neutrophils or monocytes phagocytosis or oxidative burst;	(43)

(*Continued*)

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TABLE 2 (*Continued*)

Subjects	Duration	Intervention	Treatment groups and dosage	Cells studied	Immune stimuli	Key findings	Reference
			(*e*) Placebo			•Proliferation of lymphocytes in response to T cell mitogen; •Production of IL-2, IL-4, IFNγ by Con A-stimulated PBMC; •Production of TNF-α, IL-1β, IL-6, and IL-10 by LPS-stimulated PBMC.	
111 men (n = 111)	12 wk	6 × 1 g MaxEPA™ capsules/d No placebo	0.8 en% n-3 PUFA (~3:1) (1.3 g EPA + 0.5 g DHA/d)	PBMC (5% auto-plasma)	LPS (15 mg/L)	EX VIVO: •No overall effect of n-3 PUFA on TNF-α production by PBMC following LPS stimulation. •However, n-3 PUFA increased by >twofold TNF-α production in inherently low TNF-α producers, yet decreased it ~40% in high TNF-α producers.	(31)

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						•Evidence was presented for an interaction between TNF-α genotype and inherent TNF-α production on the impact of n-3 PUFA on ex vivo TNF-α production by PBMC.	
120 pregnant women	Day 3 postpartum through twelfth wk postpartum	4 × 0.5 g tuna oil capsules/d Placebo: sunflower seed oil	(*a*) 0.36 en% n-3 (1:4) (0.14 g EPA + 0.6 g DHA/d) (*b*) 0.17 en% n-3 PUFA (*c*) Placebo	HMC; PBMC (5% FBS)	LPS (0.2 or 0.5 mg/L)	IN VIVO: •N-3 PUFA had no effect on AA levels in PBMC, but modestly increased EPA (0.4% to 0.6% of total fatty acids) and DHA (2.1% to 3.3%). Similar fatty acid changes were evident in HMC. EX VIVO: •No effect on proinflammatory cytokine (TNF-α, IL-1β, IL-6) production by LPS-stimulated HMC and PBMC.	(35)

[1]Abbreviations: AA, arachidonic acid; auto, autologous; BCSO, blank currant seed oil; CLA, conjugated linoleic acid; Con A, concanavalin A; DHA, docosahexaenoic acid; DTH, delayed-type hypersensitivity; en%, energy percent; EPA, eicosapentaenoic acid; GLA, gamma-linolenic acid; HMC, human milk cell; ICAM, intracellular adhesion molecule; IFNγ, interferon-gamma; IL, interleukin; LC, long chain; α-LNA, alpha-linolenic acid; LPS, lipopolysaccharide; LTB, leukotriene B; NK, natural killer; PBMC, peripheral blood mononuclear cells; PG, prostaglandin; PHA, phytohemagluttin; PMA, phorbol myristate acetate; PMN, polymorphonuclear cells; PUFA, polyunsaturated fatty acids; TNF-α, tumor necrosis factor-alpha.

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TABLE 3 Impact of dietary PUFA, other than n-3 PUFA, on in vivo and ex vivo immune responses in healthy humans[1]

Subjects	Duration	Intervention	Treatment groups and dosage	Cells studied	Immune stimuli	Key findings	Reference
17 women (n = 7–10)	9 wk	CLA capsules (Tonalin™) Placebo: sunflower oil	(*a*) 1.5 en% CLA (mixed isomers) (*b*) Placebo	PBMC (10% autoserum)	PHA (1, 2, 5, 10, 20 mg/mL) Influenza vaccine (5 doses)	IN VIVO: CLA did NOT affect: •Number or differential counts of WBC; •DTH response to 6 recall Ag; •Anti-influenza Ab titers 4 wk after vacc. w/3 strains. EX VIVO: •No effect on PBMC proliferation in response to 5 doses of PHA or 5 conc. of influenza antigen.	(42)
28 men and women (n = 14)	6 and 12 wk	6 × 0.5 g CLA capsules Placebo: high oleic acid sunflower oil	(*a*) 1.4 en% CLA (1:1) (c9,t11: t10,c12 isomers) (*b*) Placebo	Whole blood; PBMC (10% autoplasma)	PPD (10 mg/L) LPS (10 mg/L)	IN VIVO: •After supplementation modest changes (<10%) in plasma IgA, IgM, and IgE conc. observed, but CLA was NOT different from placebo. •No effect on % $CD4^+$ and $CD8^+$ T-cells, NK, ICAM-1^+ monocytes, $CD62^+$ lymphocytes. EX VIVO: Little or no effect (<10%) on: •LPS-stimulated TNF-α, IL-1β, IL-10 biosynthesis by PBMC; •Modest decrease in Ag recall (DTH-like) response to PPD in vitro (~20% lower than placebo).	(73)
71 men (n = 22–25)	12 wk	CLA capsules Placebo: sunflower oil	(*a*) 0.8 en% CLA (1:1) (c9,t11: t10,c12 isomers) (*b*) 0.8 en% CLA (4:1) (*c*) Placebo, 0.8 en% LA	PBMC (10% autoplasma)	LPS (75 mg/L) PHA (10 mg/L) Hepatitis B vaccine; CMI multitest™	IN VIVO: •CLA tended to increase the number of subjects that responded with protective anti-HepB antibody titers than placebo (62% versus 33%, $p < 0.08$) •No effect on DTH response to seven recall antigens.	(4)

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						EX VIVO: No effect of CLA on: •PBMC proliferation in response to PHA or LPS; •PHA-stimulated cytokines (IL-2, IL-4, IFNγ); •LPS-stimulated TNF-α, IL-1β, IL-6, or PGE_2 biosynthesis.	
10 men (n = 4–6)	7 wk	ARASCO™ oil (crossover design) Placebo: low-AA diet	(*a*) 0.7 en% AA (1.5 g/d)	PBMC (10% autoserum)	LPS (0.5 mg/L) PHA (10 mg/L) Influenza vaccine	IN VIVO: No effect on the number of circulating lymphocytes or serum Ab titers against influenza vaccine. EX VIVO: •AA increased PG (50%–100%) and LTB_4 (200%–400%) production by PBMC. •AA did NOT alter the ex vivo production of TNF-α, IL-1β, IL-2, IL-6, and IL-2 receptor expression.	(40)
40 elderly men and women (n = 20)	8 wk	BCSO capsules Placebo: soybean oil	(*a*) 0.4 en% GLA + α-LNA (0.7 g/d each of 18:3 n-6 and 18:3 n-3) (*b*) Placebo	PBMC (1%, 5%, or 9% autoplasma)	LPS (1 mg/L) Heat-killed *S. aureus* PHA/Con A (0.5, 5, 50 mg/L)	IN VIVO: BCSO increased DTH response to 1 of 7 recall antigens (tetanus toxoid). EX VIVO: PHA-induced PGE_2 biosynthesis reduced (∼45%). No effect of BCSO on ex vivo: •PBMC proliferation in response to PHA or Con A; •PHA-stimulated IL-2; •LPS-stimulated IL-1β.	(85)

[1]Abbreviations: AA, arachidonic acid; auto, autologous; BCSO, black currant seed oil; CLA, conjugated linoleic acid; Con A, concanavalin A; DHA, docosahexaenoic acid; DTH, delayed-type hypersensitivity; en%, energy percent; EPA, eicosapentaenoic acid; GLA, gamma-linolenic acid; ICAM, intracellular adhesion molecule; IFNγ, interferon-gamma; IL, interleukin; LC, long chain; α-LNA, alpha-linolenic acid; LPS, lipopolysaccharide; LTB, leukotriene B; NK, natural killer; PBMC, peripheral blood mononuclear cells; PG, prostaglandin; PHA, phytohemagluttin; PMA, phorbol myristate acetate; PMN, polymorphonuclear cells; PUFA, polyunsaturated fatty acid; TNF-α, tumor necrosis factor-alpha.

affect the concentration of blood constituents (e.g., TGF-β and lipid peroxides) that may modify in vitro immune cell response independent of the fatty acid–mediated effects, such an approach may more closely mimic how these dietary PUFAs affect in vivo immune response. Validation and acceptance of this approach have been almost entirely based on comparison with the response of isolated peripheral blood mononuclear cell preparations. However, it would be preferable to validate whole blood immune assays by comparison with in vivo responses.

One way to avoid any concern about culture conditions is to assess the immune response in vivo. The delayed-type hypersensitivity (DTH) skin test is a commonly used method for assessing cell-mediated immunity in vivo. DTH responses reflect the migration and proliferation of memory T-cells to a recall antigen(s). Some researchers mistakenly believe that the DTH test is solely a measure of in vivo T-cell proliferation; however, it is much more than that (9). Other immune cells (e.g., antigen-presenting cells) as well as various cytokines, chemokines, and eicosanoids participate in DTH responses. Thus, PUFA may affect DTH response through a variety of mechanisms. However, in the six human trials (detailed in Tables 1–3) in which DTH was assessed, little or no effect of PUFA supplementation was noted. In one of these studies, DTH response to one of seven recall antigens was increased upon supplementation with GLA (0.2 en% from black currant seed oil) (85). In another study, intake of 1.4 en% CLA reportedly reduced a DTH-like response to a single recall antigen, PPD (73). In the latter study, the reported effect was modest (i.e., ∼20%) and the response was ex vivo, not in vivo as in a traditional DTH test.

We have reason to question the reliability of ex vivo lymphocyte proliferation. Recently, T-cell receptor (TCR) transgenic mice were used to investigate the impact of dietary LC n-3 PUFA on ex vivo and in vivo T-cell proliferation. As expected, ex vivo T-cell proliferation was diminished by n-3 PUFA (65), yet no such effect on in vivo T-cell proliferation was observed (6). Similar results were obtained using a well-characterized infection-driven model of in vivo T-cell proliferation (38). That study assessed the in vivo expansion and differentiation of both $CD4^+$ and $CD8^+$ T-cell subpopulations over time by flow cytometry. It is unlikely that the absence of an effect was a consequence of inadequate n-3 PUFA intake. Mice in both of these studies were consuming diets that provided ∼5 en% LC n-3 PUFA, a level of LC n-3 intake that has been found to be more than sufficient to maximize changes in immune cell PUFA content and function.

Because it has been concluded so many times in the literature that LC n-3 PUFAs diminish proinflammatory cytokine production (e.g., IL-1β, IL-6, and TNF-α), such statements have become part of the n-3 PUFA dogma. Yet as shown in Tables 1 and 2, a surprising number of studies fail, in part or on the whole, to provide evidence to support this conclusion. In total, 15 of 20 human trials investigated the impact of a PUFA on ex vivo cytokine production. Four of these trials reported some reduction of ex vivo biosynthesis of IL-1β, IL-6, or TNF-α from LC n-3 PUFA treatment, whereas six trials failed to observe a significant impact of n-3 PUFA supplementation. Surprisingly, one trial reported that n-3 PUFA supplementation significantly enhanced ex vivo, basal, and LPS-stimulated, production of several

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pro- and anti-inflammatory cytokines (79). Interestingly, this latter study involved a very modest level of n-3 PUFA supplementation (0.18 g EPA and 0.12 g DHA/d; 0.14 en%) in healthy children. The two CLA trials that measured ex vivo cytokine production found no effect of this fatty acid on this immune parameter. In addition, the data suggest that AA, GLA, and linolenic acid do not alter ex vivo cytokine biosynthesis by human peripheral blood mononuclear cells. There exist many possible explanations for, or interpretations of, these findings.

Two issues are critical to the interpretation of these data. First, conditions for most immune assays are "optimized" by immunologists to obtain maximal in vitro response(s). Yet nutrition-immunology researchers should consider using immune stimuli at several dosages when investigating nutrient modulation of immune responses. And at least one dose of stimulus should be suboptimal (i.e., yields less than the maximal response). The importance of using such an approach is best illustrated by the data from Cooper et al. (20). They reported that the impact of LC n-3 PUFA on ex vivo IL-1β and IL-6 biosynthesis was observed only with low to moderate, but not high, doses of lipopolysaccharide. The lesson is that modest changes in the immune system are best discerned by using modest levels of immune stimuli. This appears to be true in vivo as well as in vitro. For example, the impact of LC n-3 PUFA on murine resistance to infection from *Listeria monocytogenes* that has been reported (22, 29) is not observed when the infectious challenge is very high or very low.

The second issue, one that affects all human trials, is related to the potential impact of genotype on immune responsiveness. This is best illustrated by the findings of Grimble and coworkers (32), who investigated the impact of LC n-3 PUFA on ex vivo TNF-α biosynthesis. They found that their failure to observe an overall significant effect of n-3 PUFA on ex vivo TNF-α biosynthesis was related to genetic polymorphism within the lymphotoxin gene (i.e., *TNFB*) in their subject population. Subjects in the lowest tertile of TNF-α production responded to n-3 PUFA supplementation with a 43% reduction in production. In the other tertiles, n-3 PUFA had no effect or increased production (i.e., 160% increase in the highest tertile). Thus, genetic variability in the human population may make it quite difficult to understand how biologically active nutrients, such as PUFA, affect the immune system and subsequently human health. Clearly, there is a great need to explore and define the impact of genotypic heterogeneity on nutrient metabolism and human nutrient requirements.

Experimental Design Issues: Human Versus Animal Studies

Important differences exist between human and animal-based studies in this field. Space limitations prohibit a comparison here of the findings from animal-based and human research, though such comparisons exist (11, 14, 33, 87). Instead, key experimental design issues that might contribute to the discordant results between human and animal experimentation are discussed briefly. First, as mentioned above, genetic heterogeneity is much greater in the human population than in commonly

used laboratory animals. This clearly contributes to greater variability in response to fatty acids and in the immune response data. As described above, recent data have illustrated how human genotype can significantly influence the extent to which individuals respond to dietary n-3 PUFA. It is hoped that advances in technology will make it possible (i.e., cost-effective) in the future to combine genotyping with nutrition intervention studies.

Second, in most human studies, little to no control of background diet occurs, whereas in most animal-based studies, researchers typically control all aspects of diet composition and nutrient intake. For PUFA researchers, this may be a significant problem. Current estimates of LC PUFA intake are of questionable reliability, a point made abundantly clear by Whelan & Rust (83) in a chapter in this volume. Naturally occurring variations in AA intake may significantly impact responsiveness to n-3 PUFA supplementation. The complete absence of long-chain PUFA from most experimental rodent diets may explain, in part, the greater responsiveness of rodents to n-3 PUFA supplementation relative to humans. These PUFAs are commonly found in the human diet from meat, poultry, and fish (57). Inclusion of modest amounts of AA, EPA, and DHA to the background diets in animal-based experiments prior to adding supplemental PUFA would more closely mimic treatment conditions of most human clinical trials. The extraordinary levels of n-3 PUFA incorporated in many rodent diets (e.g, up to 8 en% EPA and DHA combined) are problematic. Such diets provide many-fold greater levels of n-3 PUFA intake than what is achievable in humans even with supplementation and are more than a magnitude higher than current recommendations (49).

Third, most human studies of fatty acid modulation of immune-mediated disease involve providing n-3 PUFA after the subject has the disease. Thus, human studies primarily investigate n-3 PUFA for efficacy in the treatment of inflammation or immune-mediated disease. In contrast, most—if not all—animal studies are designed such that n-3 PUFAs are started before initiation of disease. Such an approach affords n-3 PUFA the opportunity to affect disease in many more ways (i.e., initiation, progression, and resolution). It seems reasonable to suggest that modest diet effects that occur early (i.e., during disease development) are likely to have a greater impact than are effects from the same treatment once disease is well established. In contrast, studies (such as all of those included in Table 1) that explore n-3 PUFA effects on ex vivo immune cell function and in vivo immune response in healthy human subjects are designed to elicit information about future susceptibility to infection or immune-mediated disease. As described above, the major limitation of these studies is the relatively poor predictability of most ex vivo immune assays to assess actual changes in host resistance to infection or cancer. Surprisingly, little progress has been made in defining the impact of higher n-3 PUFA intake on preventing inflammatory or immune-mediated disease in humans. One notable exception is a recent trial in which n-3 PUFAs were supplemented in maternal diets during pregnancy to assess their impact on the neonatal immune system in infants at risk for developing atopy (e.g., allergic dermatitis and

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asthma) (23). LC n-3 PUFA supplementation of the maternal diet had only a modest impact on immunological parameters, and there was some indication that the infants may have received some immunological benefit. Additional studies of this type are needed.

Just as in the RCT of fish oil treatment of RA and other immune-mediated diseases, most human studies of fatty acid modulation of ex vivo immune cell function have been grossly underpowered. One of the earliest studies to report the immunosuppressive effect of n-3 PUFA actually published data from a single volunteer (80). Low subject number has been one of the most frequently cited explanations provided by authors in this field when numerical differences between treatment groups fail to reach statistical significance. Yet a few studies that have been published in the past few years were designed, in part, to overcome this common experimental design deficiency. These studies are included in Table 2. Unfortunately, the statistical power of these trials was diminished in other ways, either by the incorporation of too many treatment groups (43) or by the use of too low a level of PUFA supplementation (34).

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CONCLUSIONS

In vitro and ex vivo experimental approaches have contributed greatly to the rapid advance of immunology as a discipline. Such studies have helped in identifying many of the factors, cells, and signaling pathways involved in the making of an immune response. Yet discrepancies between the results from in vivo and in vitro assessment of immune responses are common and sometimes profound (89). Researchers in the immunotoxicology and immunology fields have recognized this for many years (39, 52). The sensitivity and predictability of such assessments of immune cell function remains a contentious issue in both of these fields (30, 53). Short of overt deficiency, most nutrients affect the immune system in ways that are likely to be subtle, making them difficult to detect. The limitations of ex vivo assays are most apparent when treatment effects are modest. Thus, the nutrition-immunology field should interpret data from in vitro and ex vivo immune assays with caution. Advances in the immunology field continue to provide new and better tools for assessing the immune response in vivo. Yet the use of surrogates to assess nutrition-mediated changes to in vivo immune response(s) will continue to be a necessity into the near future.

RESEARCH OPPORTUNITIES AND FUTURE DIRECTIONS

There is a strongly held belief that dietary PUFAs significantly affect human health, in part by modulating the immune system. The evidence underpinning this belief has been derived primarily from in vitro, ex vivo, and animal-based studies. This review provided a brief overview, within a historical context, of how fatty acids are

thought to be able to affect inflammation and the immune response. Surprisingly, the data from human clinical trials with patients suffering from inflammatory or immune-mediated disease fail to provide compelling evidence that PUFAs are efficacious at altering the course of disease. Similarly, the PUFA supplementation studies with healthy human subjects did not provide consistent or compelling evidence to support the conclusion that dietary PUFAs affect immune/inflammatory responses in a manner that was likely to have clinical significance.

One of the goals of this review was to describe some of the most important reasons for the discrepancies in this field. Part of the problem relates to the immune system itself. The immune system is remarkably complex and consists of a network of cells and soluble factors with both unique and overlapping functions. A substantial suppression or elimination of one or more elements within this network can usually be detected as a discernable decline in host resistance to infection or cancer, the primary functions of this system. However, alterations in host immune response in vivo cannot be reliably predicted with smaller, more subtle changes in immune parameters. If one accepts the hypothesis that dietary PUFAs affect the immune system, then it becomes necessary to explain the relatively weak supporting evidence from existing human trials. There appear to be three major factors for the failure to observe significant treatment effects in most PUFA studies. First, many human studies in this area of research have been greatly underpowered. Second, more effort should be invested in selecting and validating ex vivo immune assays used in future studies. The goal should be to use approaches that are sensitive and that have predictive power for in vivo changes. Third, there is good reason to believe that inherent (genetic) variation exists in individual responsiveness to nutrient modulation of immune and inflammatory responses. Accounting for genetic variation in humans in the maintenance of health and the management of disease is the Holy Grail of modern medicine. Thus, a major effort in future studies in this field should be to identify and clarify genotype-PUFA response interactions at the level of the immune system.

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