

Important Differences Exist in the Dose–Response Relationship between Diet and Immune Cell Fatty Acids in Humans and Rodents

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Abstract Omega-3 polyunsaturated fatty acids (n-3 PUFA) are noted for their ability to diminish inflammatory and immune responses in vitro and in a variety of animal-based models of autoimmunity and inflammation. Yet, recent systematic reviews suggest that the evidence for these fatty acids having beneficial effects on inflammation or autoimmunity in humans is equivocal. A possible explanation for these disappointing and somewhat paradoxical findings emerged from the analyses described in this review. The available data on the changes in immune cell fatty acid profiles in mice, rats and humans, fed various forms and amounts of n-3 PUFA are summarized and displayed graphically. The dose–response curves generated provide new insights into the relationship between dietary n-3 PUFA and immune cell fatty acid profiles. The author suggests that the poor predictive value of most in vitro as well as many animal trials may, in part, be a consequence of the frequent adoption of experimental conditions that create differences in immune cell fatty acid profiles that far exceed what is possible in free-living humans through dietary intervention. Recommendations for improving the preclinical value of future in vitro and animal-based studies with n-3 PUFA are provided.

Keywords Fatty acid metabolism · Metabolism, n-3 fatty acids · Nutrition, immunology · Physiology, arachidonic acid · Specific lipids, fish oil · Specific lipids, n-3 fatty acids · Specific lipids

Abbreviations

AA	Arachidonic acid
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
en%	Energy percent
EPA	Eicosapentaenoic acid
HUFA	Highly unsaturated fatty acids
ALA	Alpha-linolenic acid
M ϕ	Macrophages
PBMC	Peripheral blood mononuclear cells
PUFA	Polyunsaturated fatty acids
n-3	omega-3
n-6	omega-6
SDA	Stearidonic acid

Introduction

The ability of certain fatty acids to influence the immune system and the function of its various cellular components has been recognized for nearly 30 years [1]. Most of the research on this topic has focused on polyunsaturated fatty acids (PUFA). There are two major families of PUFA, the omega-6 (n-6) and the omega-3 (n-3). These fatty acids cannot be synthesized de novo in animals, thus they must be provided in the diet. Evidence for the essentiality of these PUFA is unequivocal [2]. In most Western societies dietary intake of n-6 PUFA greatly exceeds that of PUFA from the n-3 family [3, 4]. This imbalance between n-6 and n-3 PUFA intake has been blamed for the high rate of numerous chronic diseases, including inflammatory and autoimmune diseases [5, 6].

The principle mechanism underlying this diet-disease paradigm is thought to be the accumulation of tissue

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arachidonic acid (AA), an n-6 PUFA, at the expense of unsaturated fatty acids of the n-3 family, particularly docosahexaenoic acid (DHA). AA serves as a precursor to cellular biosynthesis of eicosanoids, a large family of lipid mediators, many of which have pro-inflammatory and immuno-regulatory activity [7]. Lipid mediators derived from n-3 PUFA, however, tend to have biological activities that differ from those derived from AA [8]. Referred to as “resolvins” and “protectins”, some n-3 PUFA-derived mediators have been shown to promote resolution of inflammation and to protect cells from oxidant-induced cell death [9, 10].

The amount and type of fat people consume affects the balance between n-6 and n-3 PUFA in their tissues. Specifically, as diet n-3 PUFA intake increases, tissue AA declines and n-3 PUFA accumulate. Measuring cellular fatty acids is a relatively simple and reliable means to discern exposure to n-3 PUFA, whether through diet or experimental *in vitro* manipulation. More than a decade ago a leading researcher in the field developed a series of equations for predicting this diet-tissue PUFA relationship, first in rats [11], then in humans [12]. These equations are based on fatty acid profiles of circulating lipids (i.e., triglycerides and phospholipids) and may be particularly useful in predicting short-term PUFA intake in free-living humans. However, the dose–response relationship between dietary PUFA and immune cell fatty acid profiles has not previously been examined in a comprehensive manner. Thus, the primary objective of this review is to describe the quantitative relationship between diet and immune cell PUFA content and compare and contrast this relationship in humans, rats, and mice. Such analyses should address the question of whether rodent and human immune cells respond to dietary n-3 PUFA in a quantitatively similar manner.

The Evidence

What is described in this review is a cross-study meta-regression dose–response analyses of the effect of dietary n-3 highly unsaturated fatty acids (HUFA, fatty acids with greater than three double bonds) on the arachidonic acid (AA) content of human, murine, and rat immune cells. Studies that met the following criteria were included in the analyses: (1) dietary n-3 HUFA (i.e., EPA and/or DHA) or AA intake was a dependent variable in the study design; (2) the fatty acid profile of an identifiable immune cell population was reported; (3) data were published and identified in PUBMED (National Library of Medicine, Bethesda, MD, USA) through December of 2006.

To accomplish the primary objective of this review, fatty acid intake in human and animal-based studies must

be expressed in a way that allows for direct comparison. In most human trials, n-3 PUFA intake is most often expressed as grams consumed per day, or occasionally, on a body weight basis (i.e., mg/kg/day). In contrast, most animal studies provide information about total fat content and the relative amount of various individual fatty acids (i.e., g/100 g of total fatty acids) in the fat/diet. Information about food intake is rarely provided and difficult to measure accurately in rodent studies. Expressing n-3 PUFA intake as a percentage of energy (en%) in the diet obviates the need to measure food intake in rodent studies and allows for meaningful comparisons between human and animal-based studies in this field. Therefore, in this review, dietary n-3 PUFA will be expressed as a percentage of energy (en%) consumed (humans) or provided by the diet (rodents).

The data in Tables 1 and 2 are organized according to species and/or dominant immune cell type/source. This review focuses on diet-induced changes to immune cell highly unsaturated fatty acids (i.e., those with four or more double bonds; HUFA). HUFA are much more potent modulators of inflammation and immune cell function than their 18-carbon PUFA precursors (i.e., linoleic and α -linolenic acids, n-6 and n-3 PUFA, respectively). Since mice and rats are the most frequently used animal models for studying the impact of dietary fats on the immune response, the data in these tables are derived from these two rodent species as well as from humans. Fatty acid data from other animal species exist, but were not included in these tables because of the limited nature of these data sets and space considerations. To simplify comparisons and clarify trends across studies most of the fatty acid data presented in Tables 1 and 2 have been rounded off to the nearest whole integer. In situations where meaningful information may have been lost by such rounding off, values were reported to the nearest single decimal.

The spleen and peritoneal cavity have been the source of immune cells most frequently used in rodent studies. In contrast, the peripheral blood has been the sole source of immune cells in human studies. Generally, immune organ or whole blood leukocyte preparations are fractionated via density gradient centrifugation prior to analyses. With blood samples this procedure separates the erythrocytes and polymorphonuclear cells (e.g., neutrophils) from peripheral blood mononuclear cells (PBMC). PBMC consist of T- and B-lymphocytes primarily, and lesser numbers of natural killer cells and monocytes.

Marine n-3 HUFA and Lymphocyte HUFA Content

Baseline fatty acid profiles are remarkably similar between rodent splenocytes and human peripheral blood

mononuclear cells (PBMC). For example, these lymphocyte-rich immune cell preparations are rich in arachidonic acid (AA, 20:4n-6). AA typically accounts for 19 to 23% of total fatty acids in these cells. Furthermore, these immune cells generally contain 2–3% n-3 HUFA, primarily docosahexaenoic acid (DHA, 22:6n-3). The presence of other n-3 HUFA, such as eicosapentaenoic acid (EPA, 20:5n-3) and docosapentaenoic acid (DPA, 22:5n-3), in immune cells is more variable and typically is lower than DHA content. The impact of varying dietary n-3 PUFA on the HUFA content of lymphocyte-rich immune cell preparations from human blood and rodent spleens is summarized in Table 1. Data from human subjects are provided first, followed by data from mice, and finally rats. On some occasions the researcher did not isolate immune cells (i.e., splenocytes) prior to conducting fatty acid analysis of the spleen. The spleen is rich in erythrocytes, thus these data reflect a combination of lymphocyte and erythrocyte fatty acid profiles. However, the presence of erythrocytes did not seem to significantly affect the ability to detect diet-induced changes in fatty acid profiles. In fact, diet-induced changes in erythrocyte and PBMC appear to be quite similar in magnitude [13]. Furthermore, omitting the data from studies using whole spleen (1 of 6 rat studies; 3 of 7 mouse studies) did not significantly change the shape of the rodent lymphocyte fatty acid profile curves.

As n-3 HUFA content of the diet increases, lymphocyte AA content declines in a curvilinear fashion (Fig. 1). The data displayed in this figure are derived from 17 of the 18 studies in Table 1. One study (i.e., Jensi) was omitted from Fig. 1, because the initial AA content of the mouse splenocytes was inexplicably low (i.e., 4%). Also, the absence of DHA in the splenocytes of control mice reported in this study suggests that the authors may have experienced some technical problems with their fatty acid analyses. Note that in human studies n-3 HUFA in the diet never exceeded three percent of total energy (i.e., 3 en%) intake, while intake in most of the rodent studies was considerably higher. Importantly, these data suggest that at lower levels of n-3 HUFA intake, rodent lymphocytes appear to be much more responsive than human to the effects of n-3 HUFA on lymphocyte AA content. This rodent-only response is best described by a two-phase exponential decay curve ($R^2 = 0.95$) and is depicted in Fig. 1 by the line with alternating dots and dashes.

One possible explanation for this differential responsiveness is that human diets routinely contain some n-3 and n-6 HUFA, while experimental rodent diets are typically devoid of all HUFA. Thus, it may be that a small amount of n-3 HUFA has a greater impact on immune cell AA when incorporated into a diet devoid of n-3 and n-6 HUFA (as was the situation in all rodent studies), than when some n-3 PUFA are already present in the diet, as was the case with

most free-living human subjects. A second, alternative, explanation for this differential responsiveness between rodents and humans may relate to immune cell source, in other words peripheral blood lymphocytes maybe less responsive than tissue lymphocytes to dietary n-3 HUFA. Surprisingly, not a single study exists for which fatty acid data are reported for immune cells isolated from blood and a tissue in the same animal or human subject following n-3 PUFA intake. A third possible explanation relates to the differential predominance of T- versus B-lymphocytes in PBMC and rodent spleen, respectively. Evidence from one study suggests that diet-induced changes in fatty acid profiles of various immune cell subtypes found in the blood may not be similar [14]. Their data indicate that high-dose fish oil supplementation failed to reduce human monocyte AA content, but led to a 10, 20, and over 40% reduction in neutrophils, B-cells, and T-cells, respectively. Finally, it is possible that human immune cells are simply more resistant to n-3 HUFA-mediated changes in AA content compared to rodent immune cells.

Lymphocyte EPA content in free-living human subjects and in the rodents fed low n-3 PUFA “control” diets is generally quite low (e.g., typically undetectable to less than 1% of total fatty acids). Because of these very low and variable initial levels, expressing diet-induced changes in EPA content as a “fold-change” or “percent change” is problematic, and is best avoided. The actual amount of EPA in the cell is likely to have more biological relevance than the fold-change. Therefore, data for EPA and other N-3 HUFA in tables and figures are presented simply as a percentage of total cellular fatty acids.

Providing a source of pre-formed EPA in the diet results in its rapid accumulation in human and rodent lymphocytes (Table 1). The relationship between dietary EPA and lymphocyte EPA appears to be curvilinear for EPA intake ranging from 0 to over 4 en% in the diet (Fig. 2). This relationship is best represented by the following equation: $Y = 0.27 + 4.28X - 0.453X^2$ ($R^2 = 0.89$). Note that EPA accumulation in human lymphocytes never exceeded 4% of total lymphocyte fatty acids. In contrast, it appears that rodent lymphocytes can accumulate considerably more EPA (e.g., up to 12% of total fatty acids). However, one possible explanation of this observation is that dietary EPA intake was considerably lower in the human studies compared to the rodent studies. For example, three of four doses of EPA provided in the human trials were less than 1 en% EPA, with the highest intake less than 2 en%. In contrast, the majority of studies with rodent lymphocytes provided EPA at levels exceeding 2 en% of the diet.

Following EPA consumption, its elongation product, DPA generally increased in the lymphocyte enriched immune cell preparations (Table 1). These changes as well as the relative content of lymphocyte DPA were greater in

Table 1 Summary of data demonstrating the effect of dietary polyunsaturated fatty acids (PUFA) to alter arachidonic acid (AA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) content of human versus rodent lymphocyte-rich immune cells/tissues

Tissue/cell type ^a	Species	n-3 source ^b	n-3 intake ^c	Duration (weeks)	%AA ^d 20:4(n-6)	%EPA ^d 20:5(n-3)	%DPA ^d 22:5(n-3)	%DHA ^d 22:6(n-3)	Comments	Citation
PBMC	Human	Tuna oil	0.15 en% 0.33 en% (1 EPA: 4 DHA)	4	23 → 22 23 → 22	0.4 → 0.5 0.4 → 0.6	NR	2 → 3 2 → 3	Subjects were breast-feeding women, 4 week post-partum (<i>n</i> = 12–15/ treatment group)	[54]
PBMC	Human (healthy males)	FO capsules (corn oil)	0.6 en% 1.2 en% 1.8 en% (4 g EPA/day) (4 EPA: 1 DHA)	12	19 → 16 17 → 15 18 → 15	0.5 → 1.4 0.4 → 2.2 0.6 → 2.9	2.5 → 3.6 2.0 → 3.8 2.2 → 4.4	2.5 → 2.3 2.2 → 2.2 2.1 → 2.3	Older subjects (~60 year old) tended to incorporate more EPA compared to young subjects (~25 year old) reported here	[55]
PBMC	Human (healthy males, 21–44 year old)	FO capsules (palm and hi oleic sunflower oil) borage oil <i>echium oil</i>	0.9 en% (3 EPA: 1 DHA) (b) 0.8 en% GLA (c) 0.4 en% SDA (1 g/day)	12	(a) 17 → 16 (-5%) (b) 21 → 20 (c) 21 → 20	0.7 → 1.6	NR	2.3 → 2.2	Data are reported as pre- and post-trt means within each group of subjects. (<i>n</i> = 8–10)	[25]
PBMC	Human	FO capsules <i>ALA-spread</i>	(a) 0.8 en% (b) 0.4 en% (2 EPA: 3 DHA) (c) 4.5 en% ALA (d) 2.2 en% ALA (5 g/day)	25	(a) 19 → 15 (-21%) (b) 19 → 16 (-16%) (c) 18 → 15 (-17%) (d) 19 → 16 (-16%)	0.6 → 1.1 0.5 → 0.6 0.6 → 0.8 0.5 → 0.5	3 → 3 3 → 3 3 → 3 4 → 4	2.6 → 3.3 2.9 → 2.7 2.5 → 2.0 3.2 → 2.3	<i>n</i> = 29–31 pre treatment group. Data from week 12 was similar to week 25 values shown here. Basal intake of ALA, AA, EPA, DHA was 1.5, 0.2, 0.2, 0.3 g/day	[20]
PBMC	Human (Crohn's patients)	MaxEPA™	1.2 en% (2 EPA: 1 DHA)	(a) 8 (b) 16 (c) 24	19 → 15 (-21%) 18 → 16 (-11%) 21 → 18 (-14%)	0.5 → 2 0.8 → 2 0.7 → 3	2 → 4 2 → 3 3 → 4	3 → 5 3 → 5 2 → 3.5	Kinetics indicate that changes in PBMC are mostly complete within first 8 week, only EPA seems to accumulate through the study; Plasma PL reflected similar changes, but could not reliably predict changes in PBMC	[56]
(a) T-cells (b) B-cells	Human	MaxEPA	2.7 en% (2 EPA: 1 DHA)	12	(a) 16 → 12 (-25%) (b) 11 → 8 (-27%)	1 → 3 1 → 3	NR	4 → 4 2 → 4	Data from 2 week stowed similar changes in fatty acids	[14]

Table 1 continued

Tissue/cell type ^a	Species	n-3 source ^b	n-3 intake ^c	Duration (weeks)	%AA ^d 20:4(n-6)	%EPA ^d 20:5(n-3)	%DPA ^d 22:5(n-3)	%DHA ^d 22:6(n-3)	Comments	Citation
PBMC	Human	DHA only (LA)	2.7 en% (6 g DHA/day)	12	20 → 11 (-45%)	NR	NR	2 → 7	DHA was incorporated into foods in place of LA; small study (n = 7)	[57]
Spleen	Mouse	n-3 ethyl esters (olive oil)	(a) 0.3 en% EPA (b) 0.7 en% DHA (c) 0.8 en% ALA	1.5	15 → 11 (-27%) 15 → 8 (-47%) 15 → 13 (-13%)	0 → 4 0 → 1 0 → 2	0.3 → 4 0.3 → 1 0.3 → 3	2 → 7 2 → 9 2 → 6	Similar reduction in ex vivo PG and leukotriene production regardless of n-3 source	[37]
Splenocytes	Mouse	MaxEPA (olive oil: SAF mix)	2 en% 4 8 12 en% (4 EPA: 3 DHA)	4	21 → 13 (-38%) 21 → 10 (-52%) 21 → 8 (-62%) 21 → 7 (-67%)	0.1 → 4 0.1 → 7 0.1 → 8.5 0.1 → 9	2 → 4 2 → 5 2 → 5 2 → 5	5 → 12 5 → 12 5 → 13 5 → 13	Curvilinear dose response suggests that changes in DPA and DHA saturate at lower n-3 PUFA intake than AA and EPA	[58]
Splenocytes	Mouse	MFO (corn oil) <i>linseed oil</i>	4 en% (3 EPA: 1 DHA) 11 en% ALA	10	19 → 12 (-37%) 19 → 13 (-32%)	0.1 → 12 0.1 → 4	0.8 → 8 0.8 → 5	3 → 11 3 → 6	Ex vivo PG production was reduce 70–80% with either n-3 source. Source differentially affected NK activity	[59]
Splenocytes	Mouse	MFO (hydrogenated coconut oil)	4 en% (3 EPA: 2 DHA)	34	23 → 8 (-65%)	0 → 8	0 → 4	4 → 9	Balb/c mice were 9 months old at start and 18 months at end of study	[60]
Splenocytes	Mouse	MFO (corn oil)	1 en% 2 en% 4 en% (3 EPA: 2 DHA)	3	4 → 2 (-50%) 4 → 3.5 4 → 3.6	0 → 3 0 → 4 0 → 4	0 → 1 0 → 3 0 → 2	0 → 3 0 → 6 0 → 6	Unclear how many mice were used or the # of samples that were analyzed for fatty acids; no statistical analyses were reported	[61]
Spleen	Mouse	EPA-EE	4 en% EPA only	4	20 → 7 (-65%)	0 → 7	1 → 7	3 → 3	Similar results with C3H and C57 strains of mice	[16]
Spleen	Mouse	MFO (SAF)	6 en% (2 EPA: 1 DHA)	4	19 → 8 (-58%)	0 → 9	0 → 6	6 → 11	Detailed info on PL subclass content and fatty acid profiles as well as PG and leukotriene biosynthesis in vitro	[62]
Splenocytes	Rat	MaxEPA (corn oil) <i>linseed oil</i>	1 en% (3 EPA: 2 DHA) 2 en% ALA	6	19 → 11 (-42%) 19 → 15 (-21%)	0.5 → 3 0.5 → 1	1 → 2 1 → 1	2 → 2 2 → 2		[63]

Table 1 continued

Tissue/cell type ^a	Species	n-3 source ^b	n-3 intake ^c	Duration (weeks)	%AA ^d 20:4(n-6)	%EPA ^d 20:5(n-3)	%DPA ^d 22:5(n-3)	%DHA ^d 22:6(n-3)	Comments	Citation
Splenocytes	Rat	EPA-sTG	2 en% (3 EPA; 1 DHA)	6	18 → 14 (-22%)	0.3 → 2	1 → 2	3 → 5	Position of n-3 HUFA within structured TG did not significantly affect diet-induced changes in lymphocyte fatty acid profiles	[20]
		DHA-sTG (mix of palm, canola, soy oils)	1.5 en% DHA		18 → 13 (-26%)	0.3 → 0.5	1 → 1	3 → 6		
Splenocytes	Rat (5 strains)	MaxEPA (olive oil)	5.5 en% (3 EPA; 2 DHA)	4	19 → 9 (-53%)	0 → 11	0.9 → 5	1 → 5	These data suggest that diet is a more important determinant of membrane fatty acid composition than genetics	[39]
			2 en%	23 → 10 (-57%)	0 → 10	0.4 → 5	2 → 5			
			13 en%	22 → 9 (-59%)	0 → 11	0.4 → 5	1 → 5			
			20 en% (ALA only)	21 → 10 (-52%)	0 → 10	0.5 → 5	1 → 5			
			9 en% ALA	21 → 10 (-52%)	0 → 10	0.5 → 5	2 → 6			
Splenocytes	Rat	<i>linseed oil</i> <i>Linseed oil</i> (sunflower oil)	1 en%	6	19–23 to 12–16 (-30%)	0 to 3–5	0.4–1 to 4	1–2 to 1–2	Data should be viewed with caution due to the complete absence of detectable DHA in these lymphocyte samples	[64]
			2 en%	12 → 14	NR	NR	NR			
			13 en%	12 → 7 (-40%)						
			20 en% (ALA only)	12 → 8 (-33%)						
Splenocytes	Rat (male, Lewis)	MFO (coconut oil) olive oil safflower oil primrose oil	7 en% (2 EPA; 1 DHA)	10	10 → 2 (-80%)	2 → 4	NR	1 → 3	Data for PL fraction similar to that shown here for total lipids	[65]
				10 → 7 (-30%)	2 → 1		1 → 1			
				10 → 5 (-50%)	2 → 1		1 → 1			
				10 → 6 (-40%)	2 → 1		1 → 2			
Spleen	BB rat Diabetes-prone	MFO <i>Flaxseed oil</i>	3.5 en% (2 EPA; 1 DHA)	3	15 → 8 (-50%)	0.8 → 5	3 → 4	3.5 → 5.4	Ratio of IFN γ /IL-10 mRNA in GALT reduced by MFO, ALA had opposite impact; no effect in pancreas or on insulinitis	[66]
			8 en% ALA		15 → 9 (-40%)	0.8 → 1	3 → 3	3.5 → 2		

en% % of energy, ALA α -linolenic acid, MFO menhaden fish oil, M ϕ macrophages, NR not reported, PBMC peripheral blood mononuclear cell, PL phospholipids, PG prostaglandin, SAF safflower oil

^a All M ϕ were from the peritoneum, unless indicated otherwise

^b Fat source in parentheses designates the placebo/control oil/fatty acid; data from plant sources of (n-3) PUFA are shown in italics

^c Energy % (en%) calculations for humans are based on a 2,000 kcal/day intake

^d Fatty acid data are presented as the value before and after or without and with dietary treatment with n-3 PUFA, expressed as a % of total fatty acids present. For AA only, the relative change, pre- versus post-treatment, is indicated as a percentage within the parenthesis. Most values have been rounded off to the nearest integer

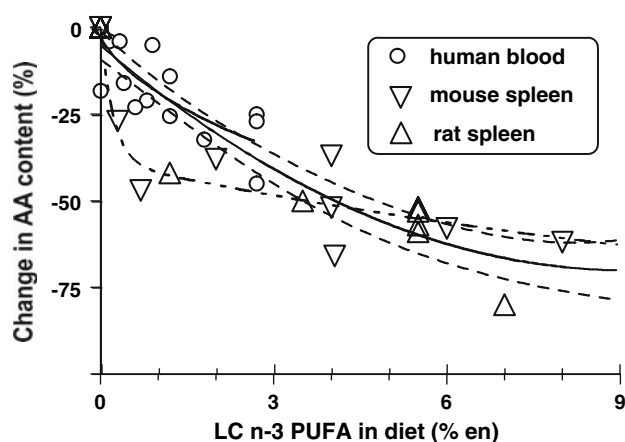


Fig. 1 Cross-study meta-regression dose–response analyses of the effect of dietary n-3 highly unsaturated fatty acids (HUFA) on the arachidonic acid (AA) content of human, murine, and rat lymphocytes. Studies that met the following criteria were included in the analyses: (1) dietary n-3 HUFA (i.e., EPA and/or DHA) intake was a dependent variable in the study design; (2) the fatty acid profile of an identifiable immune cell population was reported; (3) data were published and identified in PUBMED (National Library of Medicine, Bethesda, MD, USA) through December of 2006. N-3 PUFA intake is expressed as a percentage of total energy consumed (i.e., en%). In most studies, daily caloric intake was not reported. Thus, the following assumptions were made: (1) human subjects consumed 2,000 kcal/day; (2) rodents consumed the same calories across diet treatment groups. AA data are expressed as a percent change from basal or “control” (i.e., lowest n-3 PUFA treatment group). Best-fit lines/curves and the 95% confidence limits (dotted lines) were generated using Prism software (v. 4.0b, GraphPad, San Diego, CA, USA). The equation for the AA curve in this figure is as follows: $y = -4.87 + -14.2x + 0.776x^2$, $r^2 = 0.85$. At lower levels of n-3 HUFA intake, rodent lymphocytes appear to be much more responsive than human to the effects of diet n-3 HUFA on lymphocyte AA content. This rodent-only response is best described by a two-phase exponential decay curve ($R^2 = 0.95$) and is depicted in Fig. 1 by the line with alternating dots and dashes

rodent lymphocyte preparations compared to those from human subjects. To date direct evidence that DPA affects immune cell function is lacking. Yet, it is present in immune tissues in significant amounts particularly when EPA is provided in the diet. Recent findings of the potent anti-inflammatory activities of EPA- and DHA-derived metabolites suggest that DPA might also be metabolized to biologically active agents. Investigating the potential function of DPA in these and other cells where it is found in significant quantities seems warranted.

Only two studies investigated the impact of EPA by itself on immune cells DPA and DHA content. In one study [15], mice were fed a diet containing 0.3 en% EPA, as an ethyl ester, for 10 days prior to immune cell isolation and analyses. EPA, DPA and DHA content of murine splenocytes increased significantly. In contrast to these findings, Fujikawa et al. [16] reported that feeding mice 4 en% EPA

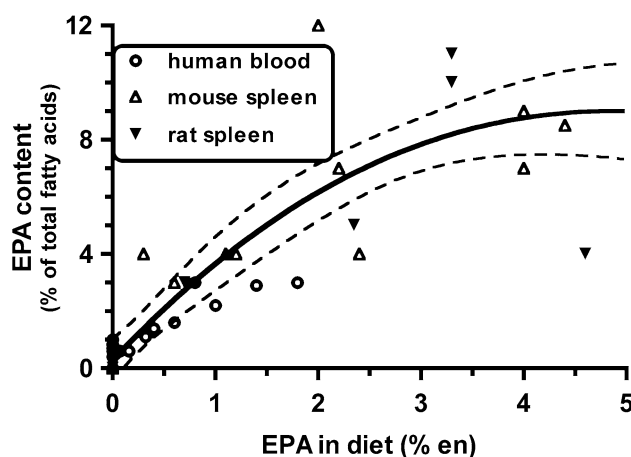


Fig. 2 Cross-study meta-regression dose–response analyses of the effect of dietary eicosapentaenoic acid (EPA) on the EPA content of human, murine, and rat lymphocytes. Refer to the legend in Fig. 1 for criteria for study inclusion and a description of how the data in the figure were analyzed. EPA data are expressed as a percentage of total fatty acids (i.e., g/100 g) present in the cells. The relationship between dietary EPA and lymphocyte EPA appears to be curvilinear for EPA intake ranging from 0 to over 4 en% in the diet. The best-fit equation for the EPA curve depicted is as follows: $y = 0.27 + 4.28x - 0.453x^2$, $r^2 = 0.89$. Note that EPA accumulation in human lymphocytes never exceeded 4% of total lymphocyte fatty acids. In contrast, it appears that rodent lymphocytes can accumulate considerably more EPA (e.g., up to 12% of total fatty acids)

as the sole n-3 PUFA in the diet increased splenocyte EPA and DPA, but left DHA content unchanged. Feeding mice only DHA (0.7 en%) greatly enriches splenocyte DHA, but is much less effective than a smaller amount of dietary EPA at elevating immune cell EPA and DPA.

The relationship between dietary DHA on lymphocyte DHA content is shown in Fig. 3. Consuming more DHA results in an increase in immune cell DHA content, but the effects in rats and humans were not as pronounced as those frequently noted for immune cell EPA or DPA. This may be due, in part, to the relatively higher initial DHA content of these cells. Rat splenocytes and human PBMC typically contained between 2 and 3% DHA. This is true even when rats are fed diets devoid of n-3 PUFA. In human trials, DHA intake prior to supplementation was rarely measured. However, it is estimated that n-3 HUFA intake by individuals in Western societies is approximately 200 mg/day (i.e., 0.1 en%) [4, 17].

The data in Fig. 3 illustrates how DHA content of lymphocytes-rich cell preparations from both rats and humans modestly increased in a linear fashion as DHA in the diet increases. This relationship is represented by the following equation: $Y = 2.2 + 1.43X$ ($r^2 = 0.75$). In contrast, the response in mice is quite different to that observed in rats and people. This relationship is curvilinear with the best-fit line and 95% confidence intervals depicted on

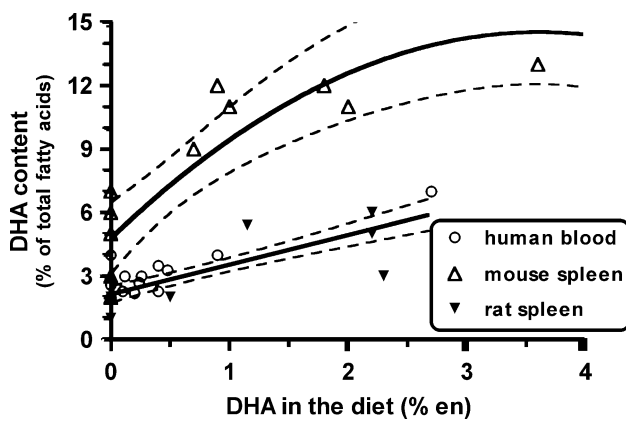


Fig. 3 Cross-study meta-regression dose–response analyses of the effect of dietary docosahexaenoic acid (*DHA*) on the DHA content of human, murine, and rat lymphocytes. Refer to the legend in Fig. 1 for criteria for study inclusion and a description of how the data in the figure were analyzed. DHA data are expressed as a percentage of total fatty acids (i.e., g/100 g) present in the cells. The best-fit equation for the diet-lymphocyte DHA response in humans and rats was linear with a best-fit equation represented as follows: $y = 2.2 + 1.43x$ ($r^2 = 0.75$). In contrast, the diet-lymphocyte DHA response in mice was curvi-linear, with the best-fit equation as follows: $y = 5.13 + 5.1x + -0.7x^2$, $r^2 = 0.80$

Fig. 3. This best-fit line is represented by a second order polynomial equation (i.e., $Y = 5.13 + 5.1X + -0.7X^2$; $R^2 = 0.80$).

Interestingly, the initial DHA content of murine lymphocytes averages more than twofold greater than lymphocytes from rats or human subjects. DHA content of murine lymphocytes appears to plateau at $\sim 12\%$ of total fatty acids, while only occasionally reaching 6% of the total fatty acids in rat or human lymphocytes. This differential response of murine immune cells to become more enriched with DHA relative to rats and humans is evident even at comparable levels of DHA in the diet. These data are consistent with the observations of Hulbert et al. [18] They reported that DHA content of phospholipids from various tissues (i.e., heart, liver, kidney and skeletal muscle) is significantly and inversely correlated with body mass. For example, DHA content of kidney microsomes in mice and rats was reported to vary by ~ 6 -fold (i.e., 29 vs. 5% of total fatty acids). This large difference in membrane DHA content appeared to have a genetic basis, since both rodent species were being fed the same diet in this study [19].

Plant-based n-3 PUFA Affect Lymphocyte HUFA

Current estimates suggest that the average U.S. citizen consumes 0.6 en% n-3 PUFA and that $>90\%$ of this is in the form of alpha-linolenic acid (ALA, 18:3n-3) from plant

sources, such as soybean and canola oils [17]. Direct comparisons between plant-based sources of n-3 PUFA with preformed EPA and DHA to affect immune cell HUFA content were conducted in humans and rodent studies. The data in Table 1 suggest that mice are more capable of converting ALA into n-3 HUFA than rats or humans. In humans, ALA is approximately one-tenth as effective as n-3 HUFA at lowering AA and raising EPA, but fails to elevate DHA content of PBMC [20]. This species difference may be a consequence of the higher desaturase activity in rodents compared with humans [21]. Another contributing factor to this observed difference is that the rodents in these studies were “young” relative to the human subjects. It is well known that desaturase activity declines substantially with age [22]. Another factor that may contribute to the relative inefficient conversion of ALA to HUFA in humans is the presence of HUFA in the typical human diet. Dietary HUFA are quite effective at depressing hepatic desaturase activity [23].

A novel plant-based n-3 PUFA, stearidonic acid (SDA; 18:4n-3) may be considerably more effective than ALA at enriching tissue n-3 HUFA [24]. When healthy male subjects consumed 0.4 en% of SDA (i.e., 1 g/day) from Echium oil there was a modest increase in both EPA and DHA content of PBMC, from 0.4 to 0.9% and 1.8 to 3% of total fatty acids, respectively [25]. SDA had no impact on PBMC AA content. Studies in which the impact of both ALA- and SDA-rich oils are directly compared relative to modulation of immune cell HUFA profiles do not exist presently. Although limited in scope, these data suggest that SDA is more effective than ALA at affecting immune cell HUFA content.

Monocyte/M ϕ

In addition to lymphocytes, monocyte/M ϕ are the other immune cell type for which considerable data exist regarding the impact of dietary PUFA on their fatty acid composition. Monocytes/M ϕ are part of the innate immune system [26]. These cells are responsible for engulfing bacterial pathogens, dead cells and processing and presenting foreign antigens to T- and B-lymphocytes. By producing pro- and anti-inflammatory eicosanoids and cytokines, these cells play a central role in initiating and resolving inflammation and tissue injury [27].

In rodent models the peritoneum has served as the primary source of M ϕ , which typically make up approximately 50% of the resident cells. After collection, in vitro adherence followed by vigorous washing is frequently employed to increase the purity of this cell preparation (e.g., typically $\sim 90\%$ M ϕ). Also, it is common for researchers to inject a sterile inflammatory agent (e.g., thioglycolate broth,

proteose peptone, or glycogen) into the peritoneum of rodents prior to cell collection. These agents greatly enhance immune cell yield by tenfold or more. Another benefit of eliciting immune cells is the increase in homogeneity within the immune cell population harvested. For example, when cells are collected within the first 12–24 h post-elicitation, neutrophils make up greater than 85% of the total cells. [28] In contrast, M ϕ are the predominant cell type present when collection occurs 3–4 days later.

These “elicited” M ϕ are derived primarily from blood monocytes that migrate to the inflammatory site (i.e., peritoneum). In fact, it is widely thought that tissue M ϕ are primarily derived from peripheral blood monocytes [29]. This relationship, as well as functional similarities between monocytes and tissue M ϕ , served as the primary justification for grouping these immune cell populations together in Table 2. Elicited M ϕ differ from resident peritoneal M ϕ in several important ways, including: cytokine and eicosanoid production [30–32]. Therefore, fatty acid data for resident and elicited M ϕ were presented separately. Interestingly, the fatty acid data suggest that these two immune cell populations also differ in responsiveness to dietary HUFA treatment.

Marine n-3 PUFA and Monocyte/M ϕ HUFA

AA and DHA content of monocyte/M ϕ -rich cell preparations tended to be much more variable than lymphocyte-rich immune cell preparations. For example, AA content of M ϕ from mice and rats fed control diets across the 10 studies included in Table 2 ranged from 8 to 28% of total fatty acids. Rodent M ϕ DHA content varied from 0 to 7% of total fatty acids in these same studies. Some of the factors that may have contributed to this variability in basal HUFA, include: genetic/species differences, differences in dietary PUFA content of background/control diets, differences in cell purity, and differences in analytical procedures used by the various investigators. Whatever the underlying cause(s), this variation makes direct comparisons between studies problematic. Thus, diet-induced changes in fatty acid AA and DHA content are described in the context of fold-change as well as in terms of actual percentage of total fatty acids. However, caution should be exercised in the interpretation of these data. For example, it is unclear whether a doubling of immune cell DHA from an initial low value (e.g., from 0.5 up to 1%) is functionally equivalent to the same fold increase in cells containing substantially more initial DHA (e.g., from 3 up to 6%). Additionally, it is uncertain whether a 50% decline in AA will have the same physiologic consequence if cells start out with vastly different initial AA content.

Similar to lymphocyte-rich immune cell populations, the inclusion of n-3 HUFA in the diet of rodents, as well as human subjects, can have a significant effect on the AA content of monocytes/M ϕ . As n-3 HUFA intake increases, M ϕ AA levels decline in a curvilinear fashion (Fig. 4). The decline appears to plateau at reduction in AA content of ~50% of the original content, once n-3 HUFA intake reaches 3 en%. The best-fit line and 95% confidence intervals is depicted on the figure and are represented by a one-phase exponential decay equation: $Y = 50.9 \exp(-1.1X) + -50.1$ ($R^2 = 0.72$). The data from human subjects suggest that peripheral blood monocytes may be less responsive to n-3 HUFA-mediated changes in AA content than rodent M ϕ . However, the human monocyte data are too limited to analyze separately.

The dose–response relationships between EPA intake and monocyte/M ϕ EPA accumulation is illustrated in Fig. 5. EPA accumulation in human monocytes and elicited rodent peritoneal M ϕ increases in a curvilinear fashion with increasing dietary EPA. This relationship is represented by the following equation: $Y = 0.29 + 2.58X + -0.436X^2$ ($R^2 = 0.86$). Accumulation of EPA in monocyte/M ϕ plateaus at ~4% of total fatty acids, which is only half as much observed in lymphocyte-rich cell preparations. In contrast to human monocytes and elicited peritoneal M ϕ , the relationship between dietary EPA and EPA content of resident peritoneal M ϕ is linear (i.e., $Y = 0.4 + 3.3X$; $r^2 = 0.89$). Interestingly, resident M ϕ from the peritoneum of rodents accumulate more EPA than human monocytes and elicited M ϕ rodents at each level of dietary EPA intake. Yet, only a single research group directly compared the response of resident and elicited M ϕ to diet n-3 PUFA treatment [33]. Those data, however, follow the same general pattern illustrated in Fig. 5 in that the dose–response relationship between EPA intake and cellular EPA is steeper in resident than elicited M ϕ .

DHA content of monocyte/M ϕ increases in a linear fashion in response to increasing DHA intake (Fig. 6). However, these data are quite variable (i.e., $Y = 3.8 + 1.7X$; $r^2 = 0.42$). The “fit” was only slightly improved when non-linear functions were used to represent these data. Unlike lymphocytes, a “species effect” in the dose–response relationship between dietary DHA and DHA in the monocyte/M ϕ -rich populations is not readily apparent. Yet, human blood monocytes seem relatively resistant to the accumulation of DHA. Figure 6 shows that DHA content of rodent M ϕ , elicited and resident alike, exceed that found in human monocytes at every level of dietary DHA intake. Surprisingly, direct evidence that blood monocytes and tissue M ϕ are differentially responsive under identical experimental conditions (i.e., same subject on the same dietary treatment) is lacking.

Table 2 Summary of data demonstrating the effect of dietary polyunsaturated fatty acids (PUFA) to alter arachidonic acid (AA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) content of human versus rodent innate immune cells/tissues

Tissue/cell type ^a	Species	n-3 source ^b	n-3 intake ^c	Duration (weeks)	%AA ^d 20:4(n-6)	%EPA ^d 20:5(n-3)	%DPA ^d 22:5(n-3)	%DHA ^d 22:6(n-3)	Comments	Citation
Monocytes	Human	EPA + DHA ethyl esters	1 en% (3 EPA; 2 DHA)	12	19 → 15 (-20%)	0.2 → 1.7	1 → 3	2 → 3.5	Fatty acid data deduced from figures; data from 18 week washout indicated rapid drop in EPA, slower response with DHA	[13]
Monocytes	Human	Cod liver-oil	2.7 en% (3 EPA; 2 DHA)	6	23 → 15 (-35%)	0.04 → 2	NR	0.7 → 2	Individual values (<i>n</i> = 9) for AA and EPA illustrate variation in responsiveness	[67]
Monocytes	Human	MaxEPA TM	2.7 en% (2 EPA; 1 DHA)	12	13 → 12 (-8%)	1 → 3	NR	4 → 4	Most change (>90%) occurred within 2 week	[14]
Resident Mφ	Mouse	Sardine oil (SAF mix)	0.26 en% 0.54 en% 2.8 en% (2 EPA; 1 DHA)	2	8 → 7 (-13%) 8 → 5 (-38%) 8 → 4 (-50%)	0.1 → 1 0.1 → 2 0.1 → 5	0.1 → 3 0.1 → 3 0.1 → 4	1 → 4 1 → 5 1 → 7	50% reduction in <i>in vitro</i> Mφ cytotoxicity maxed out at middle dose of n-3 PUFA	[33]
Resident Mφ	Mouse	Fish oil (mix of oils) tri-ALA	0.4 en% 1 en% 3 en% (2 EPA; 1 DHA) 0.5 en% 1.1 en% 2.8 en% ALA	2	9 → 5 (-45%) 9 → 5 9 → 4 9 → 9 9 → 7 9 → 6	0 → 2 0 → 3 0 → 5 0 → 0.1 0 → 0.5 0 → 1	0.1 → 3 0.1 → 3 0.1 → 4 0.1 → 1 0.1 → 2 0.1 → 2	1 → 5 1 → 6 1 → 7 1 → 3 1 → 3 1 → 4	LA content of all diets was kept constant at ~3 en%. Data are from PL fraction only	[34]
Resident Mφ	Mouse	Fish oil (corn oil)	2 en% (3 EPA; 2 DHA)	9	24 → 12 (-50%)	0 → 4	NR	6 → 10	Most of the changes in fatty acids occurred within the 1st week according to kinetic data	[42]
Resident Mφ	Mouse	AA ethyl ester (mix of oils and triglycerides)	1.3 en% 3.7 en% (AA only)	2	11 → 19 (+68%) 11 → 18 (+57%)	NR	NR	1 → 1 1 → 1		[36]

Table 2 continued

Tissue/cell type ^a	Species	n-3 source ^b	n-3 intake ^c	Duration (weeks)	%AA ^d 20:4(n-6)	%EPA ^d 20:5(n-3)	%DPA ^d 22:5(n-3)	%DHA ^d 22:6(n-3)	Comments	Citation
Resident M ϕ	Rat	MaxEPA (corn oil) <i>linseed oil</i>	(a) 1.2 en% (2 EPA: 1 DHA) (b) 2 en% ALA	6	(a) 15 \rightarrow 9 (-40%) (b) 15 \rightarrow 10 (-33%)	0.5 \rightarrow 4 0.5 \rightarrow 1	2 \rightarrow 4 2 \rightarrow 3	2 \rightarrow 4 2 \rightarrow 2	Detailed info on PL (PC, PE, PL, PS) content and fatty acid profiles as well as PGE, TXB, LTB biosynthesis in vitro	[35]
Resident M ϕ	Rat	Sardine oil (rapeseed/ peanut oils; 1:1)	2.4 en% (3 EPA: 1 DHA)	16	28 \rightarrow 13 (-54%)	0.2 \rightarrow 8	NR	7 \rightarrow 11	No n-3 effect on adherence or phagocytosis	[68]
Elicited M ϕ	Mouse	Sardine oil (SAF mix)	0.26 en%	2	11 \rightarrow 10 (-9%)	1 \rightarrow 1	0.5 \rightarrow 2	3 \rightarrow 3	50% reduction in in vitro M ϕ cytotoxicity maxed out at middle dose of n-3 PUFA	[33]
Elicited M ϕ	Mouse	Sardine oil (SAF)	0.3 en%	2	15 \rightarrow 12 (-20%)	0 \rightarrow 0.5	0.5 \rightarrow 2	2 \rightarrow 4	Dose response was quite similar in the liver PL. LT production was reduced ~35% at median dose with no additional effect of more n-3 PUFA	[69]
Elicited M ϕ	Mouse	MFO (sunflower oil)	4 en% (1 EPA: 1 DHA)	6	12 \rightarrow 6 (-50%)	0.4 \rightarrow 5	0.5 \rightarrow 5	1 \rightarrow 4	Cells were collected 4 days post-Listeria challenge and were nearly equal numbers of M ϕ and lymphocytes	[48]
Elicited M ϕ	Mouse	MFO (SAF)	4 en% (3 EPA: 2 DHA)	4	13 \rightarrow 7 (-45%)	0 \rightarrow 4	NR	1 \rightarrow 6		[70]
Elicited M ϕ	Mouse	MFO (SAF)	6 en% (3 EPA: 2 DHA)	4	15 \rightarrow 6 (-60%)	0 \rightarrow 4	0 \rightarrow 7	0 \rightarrow 4	Study also included a borage oil and coconut oil-fed groups	[62]
Neutrophils	Human	(a) EPA capsules (b) DHA capsules	(a) 2 en% EPA (b) 2 en% DHA	4	(a) 14 \rightarrow 13 (-7%) (b) 16 \rightarrow 12 (-20%)	1 \rightarrow 3 1 \rightarrow 2	1 \rightarrow 3 1 \rightarrow 2	3 \rightarrow 3 3 \rightarrow 5	EPA group also consumed 0.7 g/day DHA;	[38]
Neutrophils	Human	MaxEPA	2.7 en% (2 EPA: 1 DHA)	12	11 \rightarrow 10 (-9%)	1 \rightarrow 3	NR	2 \rightarrow 3	DHA group also consumed 0.8 g/day EPA	[14]
									Most change (>90%) occurred within 2 week	

Table 2 continued

Tissue/cell type ^a	Species	n-3 source ^b	n-3 intake ^c	Duration (weeks)	%AA ^d 20:4(n-6)	%EPA ^d 20:5(n-3)	%DPA ^d 22:5(n-3)	%DHA ^d 22:6(n-3)	Comments	Citation
Neutrophils	Human	Echium oil	1.9 en% ALA + 0.8 en% SDA	4	9 → 9	0.1 → 0.5	0.6 → 1.2	0.7 → 0.9	Treatment reduced plasma (71) by 25% in subjects with hypertriglyceridemia	[40]
Elicited PMN (30 min post-zymosan i.p.)	Mouse	Ethyl ester of DHA (~10 en% LA in all diets)	0.8 en% 1.7 en% 7.4 en% (DHA only)	2	28 → 18 (-36%) 28 → 12 (-57%) 28 → 8 (-71%)	0 → 0 0 → 0.3 0 → 1.5	0 → 1 0 → 2 0 → 2	2 → 9 2 → 12 2 → 15	Reduction of in vivo PGE max at 0.8 en%; for PGI and LTE max at 1.7 en% DHA. Fatty acid data converted from nmoles/mouse to %	[37]
Elicited PMN	Mouse	Sardine oil (safflower oil)	3 en% (2 EPA; 1 DHA)	3	13 → 6 (-45%)	0 → 4	0 → 2	2 → 7	Frequency of consumption of high n-3 HUFA diet was purposely varied from 1 day/week to daily	[46]
Elicited PMN	Rat	MaxEPA (olive oil; MaxEPA @1:1)	6 en% (3 EPA; 2 DHA)	3	19 → 12 (-30%)	5 → 14	NR	1.5 → 3	"Control" diet contained 3 en% n-3 HUFA	[71]
Elicited PMN	Rat (5 strains)	MaxEPA (olive oil)	5.5 en% (3 EPA; 2 DHA)	4	21 → 14 (-33%) 27 → 13 (-52%) 25 → 13 (-48%) 23 → 14 (-39%) 25 → 15 (-40%)	0 → 14 0 → 14 0 → 16 0 → 12 0 → 13	0.3 → 2 0.2 → 3 0.1 → 3 0.1 → 2 0.1 → 2	1 → 2 1 → 3 1 → 3 1 → 3 1 → 2	These data suggest that diet is a more important determinant of membrane fatty acid composition than genetics	[39]
		linseed oil	9 en% ALA		21 → 27 to 14 → 21 (-25%)	0 to 4.6	0.2 to 1.2	1 to 2 to 3		

en% % of energy, ALA α -linolenic acid, MFO menhaden fish oil, M ϕ macrophages, NR not reported, PBMC peripheral blood mononuclear cell, PL phospholipids, PG prostaglandin, SAF safflower oil

^a All M ϕ were from the peritoneum, unless indicated otherwise

^b Fat source in parentheses designates the placebo/control oil/fatty acid; data from plant sources of (n-3) PUFA are shown in italics

^c Energy % (en%) calculations for humans are based on a 2,000 kcal/day intake

^d Fatty acid data are presented as the value before and after or without and with dietary treatment with n-3 PUFA, expressed as a % of total fatty acids present. For AA only, the relative change, pre- versus post-treatment, is indicated as a percentage within the parenthesis. Most values have been rounded off to the nearest integer

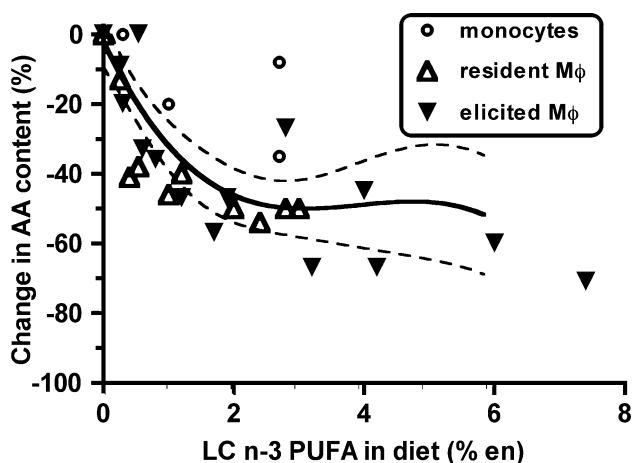


Fig. 4 Cross-study meta-regression dose–response analyses of the effect of dietary n-3 highly unsaturated fatty acids (HUFA) on the arachidonic acid (AA) content of human, murine, and rat monocytes/macrophages (M ϕ). Refer to the legend in Fig. 1 for criteria for study inclusion and a description of how the data in the figure were analyzed. The best-fit line and 95% confidence intervals is depicted on the figure and are represented by a one-phase exponential decay equation: $Y = 50.9 \exp(-1.1X) + -50.1$ ($R^2 = 0.72$). The data from human subjects suggest that peripheral blood monocytes may be less responsive to n-3 HUFA-mediated changes in AA content than rodent M ϕ . However, the human monocyte data are too limited to analyze separately

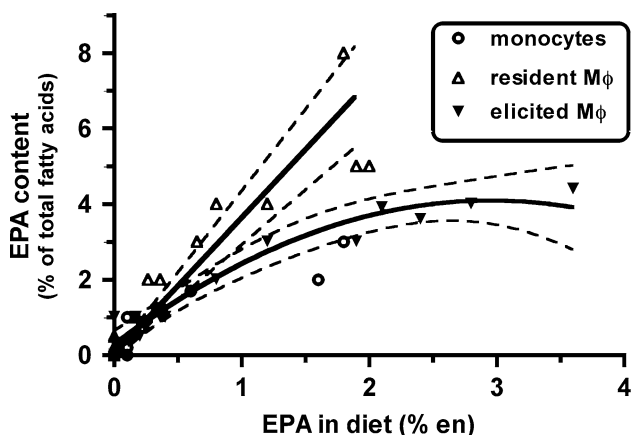


Fig. 5 Cross-study meta-regression dose–response analyses of the effect of dietary eicosapentaenoic acid (EPA) on the EPA content of human monocytes, as well as resident and elicited peritoneal macrophages (M ϕ) from mice and rats. Refer to the legend in Figs. 1 and 2 for criteria for study inclusion and a description of how the data in the figure were analyzed and presented. The equation for the EPA curve for human monocytes and elicited peritoneal M ϕ from rodents was: $y = 0.29 + 2.58x + -0.436x^2$, $r^2 = 0.86$; while the best-fit for EPA in resident peritoneal M ϕ (is linear: $y = 0.4 + 3.3x$; $r^2 = 0.89$)

Plant-based n-3 PUFA Affect Monocyte/M ϕ HUFA

As with lymphocytes, dietary ALA appears to be considerable less effective at reducing AA and elevating n-3 HUFA in M ϕ . For example, Whelan et al. [34] conducted a carefully designed experiment to directly compare the potency of n-3 HUFA from fish oil to the shorter-chain

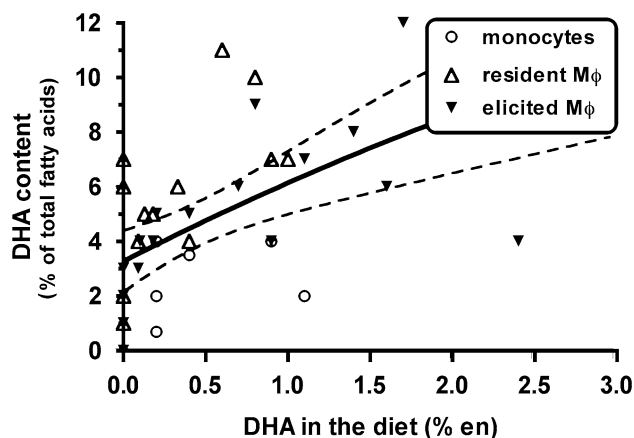


Fig. 6 Cross-study meta-regression dose–response analyses of the effect of dietary docosahexaenoic acid (DHA) on the DHA content of human monocytes, as well as resident and elicited peritoneal macrophages (M ϕ) from mice and rats. Refer to the legend in Figs. 1 and 3 for criteria for study inclusion and a description of how the data in the figure were analyzed and presented. The equation for DHA is: $y = 3.8 + 1.7x$, $r^2 = 0.42$. The “fit” for the DHA data is not significantly improved when non-linear functions are used (data not shown). Human blood monocytes seem relatively resistant to the accumulation of DHA. Furthermore, DHA content of rodent M ϕ , elicited and resident alike, exceed that found in human monocytes at every level of dietary DHA intake

length precursor n-3 PUFA (i.e., ALA) at three different levels in the diet (i.e., ~ 0.5 , 1 and 3 en%). They formulated experimental diets to contain similar amounts of total fat, but variable levels of n-3 HUFA from fish oil or a manufactured tri-ALA. Their data indicate that consuming a diet with 3 en% ALA for two weeks was nearly as effective as 0.4 en% n-3 HUFA at lowering resident murine M ϕ AA and elevating EPA, DPA, and DHA content. Brouard et al. [35] reported that 2 en% ALA from linseed oil was nearly as effective as 1.2 en% n-3 HUFA from fish oil at lowering AA and elevating DPA content in rat resident peritoneal M ϕ . Yet, this plant-derived n-3 PUFA was much less effective at elevating EPA and did not change DHA content of these immune cells.

There is only a single report describing the impact of dietary AA on immune cell fatty acid profiles [36]. As expected, the addition of preformed AA to the diet (i.e., 1.3 or 3.7 en%) enhanced resident murine M ϕ AA content by 57 and 68%, respectively, but had no effect on DHA levels (data on EPA and DPA were not reported). In contrast, when LA intake of mice was increased from ~ 4 to 8% of total calories, neither hepatic lipids or resident peritoneal macrophage phospholipid AA content were significantly increased.

Neutrophils

Neutrophils are the predominant leukocyte in human blood and play a central role in early “innate” host

defense against bacterial infection. Yet, the data on diet-induced changes of their PUFA content are limited to three studies with human neutrophils, one study comparing five different rat strains, and a possibly a single mouse study (Table 2). There is some uncertainty about the identification of the immune cells analyzed by Lokesh et al. [37]. These researchers indicated that they were working with macrophages, yet the cells isolated were from the peritoneum of mice 30 min following the injection of an inflammatory agent (i.e., zymosan). The predominate immune cell that would have been recovered under these circumstances would have been neutrophils, not macrophages. Therefore, this study has been included in this section of the Table 2.

Overall, the data in this section of Table 2 indicate that neutrophil HUFA content can be affected by dietary n-3 PUFA. In fact, the amount of EPA that rat neutrophils incorporated in response to n-3 HUFA intake was striking (i.e., from undetectable levels to greater than 12% of total fatty acids). This response in rats contrasts sharply with the more modest accumulation of EPA observed in human neutrophils, that was never reported to exceed 3% of total fatty acids. Accumulation of DPA and DHA in neutrophils following n-3 HUFA supplementation was similar among rats and humans; was considerably lower than that seen with EPA.

Kew et al. [38] directly compared the impact of EPA to DHA on human neutrophil fatty acid profiles and function. They found that consumption of 2 en% EPA for 4 weeks resulted in only a modest increase in EPA and DPA, but with little detectable change in either AA or DHA. In contrast, intake of a similar amount of DHA decreased AA by 20% and increased DHA content from 3 to 5% of total fatty acids, while EPA and DPA were also modestly increased. These data suggest that DHA-enriched oils may be more effective at changing the fatty acid profiles of some human immune cells compared to EPA-rich oils.

Cleland et al. [39] directly compared the impact of a fish oil source of n-3 PUFA (i.e., MaxEPA) with a plant-based source (i.e., linseed oil) on the fatty acid profiles of neutrophils from five different strains of rats. They demonstrated that compared to 5.5 en% n-3 HUFA, 9 en% ALA resulted in more modest reductions in AA and increases in EPA in these immune cells. However, DPA and DHA accumulation was modest and similar between the fish oil-derived and plant derived sources of n-3 PUFA. Surette et al. [40] reported that ALA and SDA from Echium oil, a novel plant source of n-3 PUFA, were able to modestly affect human blood neutrophils fatty acid profiles. After 4 weeks of consuming 1.9 en% ALA along with 0.8 en% SDA, neutrophil EPA increased from 0.1 to 0.5% and DPA doubled (i.e., 0.6–1.2%), while AA and DHA levels were not significantly affected.

In contrast to these studies with dietary n-3 PUFA, intake of LA over a broad range failed to alter AA content of human neutrophils or plasma lipids [41]. Yet, in rats increasing dietary LA over a range of 0.3–7 en% was associated with a modest increase in the AA content in elicited peritoneal macrophages [41].

Kinetics

Data from time course studies with mice [42] and humans [43] suggest that diet-induced changes in immune cell fatty acid profiles occur rapidly (i.e., within a few weeks). This time frame is quite similar to that observed with other tissues, such as heart, liver and kidney [44]. In recognition of the rapid response of tissues to n-3 HUFA, many researchers using animal models feed experimental diets for 4 weeks or less. This appears to be sufficient to alter rodent immune cell fatty acid composition, and in most cases, *in vitro* functions. In contrast, most human studies use fatty acid treatment periods extending several months, and in one case, a year. Presumably, extended treatments are done to increase the likelihood of observing treatment effects at more modest doses of n-3 PUFA. The data suggest that HUFA incorporate into cell membranes in proportion to their availability (i.e., concentration in the plasma or extracellular milieu), with the length of time they are exposed being much less important. For example, changes in fatty acid profiles for human PBMC were similar at 3 versus 6 months of dietary n-3 PUFA supplementation [45]. Extended treatment periods make subject recruitment more difficult, reduces overall subject compliance, increases dropout rates, and is more costly. For example, the impact of fish oil supplementation on HUFA content of PBMC from 31 Crohn's patients appeared to peak at 8 week, then declined as the supplementation period extended to 16 and 24 week. All of these outcomes would detract from, rather than, strengthen a clinical trial. Clinicians should reconsider the need for using extended treatment periods in clinical trials with fatty acids, unless there are clear and compelling reasons for doing so.

In an interesting and clinically-relevant approach to study kinetics and dose-response, Broughton and Morgan [46] investigated the impact of periodicity of n-3 HUFA intake on tissue fatty acid composition and eicosanoid biosynthesis. For this study, mice were offered diets enriched with EPA (~2 en%) and DHA (~1 en%) for one or more days per week. As the number of days mice consumed the fish oil-containing diet increased there was a frequency-dependent reduction in immune cell AA; elevations in EPA, DPA and DHA. Changes in eicosanoid biosynthesis were statistically different only when the n-3 PUFA enriched diet was consumed at least on an every

other day basis, while tissue fatty acid changes differed with n-3 PUFA intake occurring as infrequently as once every 5 days. This study is intriguing because the experimental feeding approach closely mimics the pattern of n-3 HUFA intake that occur with free-living individuals whom might occasionally consume cold-water, fatty fish (e.g., tuna, salmon, herring) or fish oil supplements.

Discussion and Recommendations

The major objective of this review was to provide readers with a better understanding of the dose–response relationship between diet and subsequent immune cell HUFA content. Inclusion of n-3 HUFA in the diet of humans and laboratory rodents produces a predictable pattern of changes in immune cell fatty acid profiles: AA content diminishes, while n-3 HUFA accumulate. It is clear from these data that preformed n-3 HUFA are significantly more effective at inducing these changes compared to plant sources of n-3 PUFA (e.g., alpha-LNA). Furthermore, consumption of preformed AA does appear to elevate immune cell AA. Unfortunately, the diet AA data are too limited to draw conclusions about dose–response relationships, differential responsiveness among types of immune cells or across species. This is particularly unfortunate since human diets routinely contain AA and understanding the impact of this fatty acid on the accumulation of n-3 HUFA as well as the reduction of immune cell AA may be central to improving the outcome of ongoing human n-3 PUFA clinical trials.

Clear identification of the immune cell population under study was an important consideration in the presentation and interpretation of these data. While the functional differences between various types of immune cell are well recognized, potential biochemical distinctions are less well defined. Collectively the data presented in this review suggest that the nature of the immune cell population under study affect both the initial, as well as diet-induced changes in, fatty acid content. However, changes in PUFA or HUFA intake do not appear to significantly affect immune cell subset distribution in humans or rodents [47, 48]. Therefore, it seems reasonable to conclude that changes in fatty acid profiles within a given study noted in this review are not a consequence of diet-induced shifts in immune cell distribution. While n-3 PUFA intake induces similar patterns of change in fatty acid profiles among the various immune cell populations examined, significant differences existed for individual HUFA. Though limited (i.e., $n = 8$ subjects), data from a single study suggest that human B-cells may contain lower amounts of AA and DHA, than T-cells. The relative proportion of T- and B-cells in human blood [i.e., 6:1] is quite different than that found in the

rodent spleen, which contains one T-cell for every three B-cells. At the present time it is unclear to what extent tissue of origin or species of origin may be contributing to some of the differences observed in humans and mice regarding their response to dietary n-3 PUFA exposure. Furthermore, it is possible that differences in cellular activities for tissue versus blood-borne immune cells may contribute to some of the differences in HUFA incorporation noted in this review. What is needed to resolve this issue are data from blood and tissue immune cells obtained from the same experimental subjects following consumption of diets enriched with n-3 PUFA. Such studies would help discern the independent contribution of cell source on dietary n-3 PUFA-driven changes to immune cell HUFA content.

Importantly, while humans and rodents share similar patterns of changes in immune cell HUFA content, they do not appear to be equally responsive to dietary n-3 HUFA. N-3 HUFA do not appear to lower AA, nor enrich n-3 HUFA, in human blood monocytes as much as in tissue $M\phi$ from either rats or mice. These data may also be interpreted to mean that responsiveness to n-3 PUFA enrichment for circulating immune cells is not the same for tissue immune cells within the same lineage. For example, cells of the monocytic lineage differ in responsiveness as follows: resident $M\phi >$ elicited $M\phi >$ blood monocytes. The absence of fatty acid data from blood monocytes and tissue $M\phi$ from the same human subjects or animals after n-3 PUFA supplementation makes it impossible to distinguish between these two equally plausible explanations. For lymphocytes, there is solid evidence for differential responsiveness to diet n-3 HUFA between species. For example, the reduction in AA content in response to low doses of n-3 HUFA intake (i.e., 0–1 en%) appears to be much greater in rodents than humans. Furthermore, murine lymphocytes start out with, and accumulate more, DHA than human or rat lymphocytes in response to n-3 PUFA intake. The underlying mechanism(s) for, and biological significance of, such differences in immune cell responsiveness to n-3 PUFA are unclear at this time, but warrant further study.

The data presented in this review may help explain, in part, the paradox of equivocal results from human clinical trials with n-3 PUFA to treat inflammatory and immune-mediated diseases [49]. In contrast, there have been many positive outcomes reported for animal(rodent)-based models of such diseases. First, as noted in this review, the relative amount of n-3 PUFA provided to animals in most “immune-focused” studies far exceed what is achievable in free-living humans. This difference in n-3 PUFA intake results in changes in HUFA profiles in rodents immune cells that surpass those achieved in humans. Though unproven, it is widely presumed that changes in immune cell membrane phospholipids fatty acid content are both

Table 3 Recommendations for improving the preclinical value of animal- and cell culture-based experiments with n-3 PUFA

Animal-based n-3 PUFA/HUFA diet studies:

Good: Design experimental diets to provide a level of n-3 PUFA intake that is achievable in humans (e.g., ~1 en% or less)

Better: Only provide an amount and form of dietary n-3 PUFA in animal diets that reproduces the changes in fatty acid HUFA content previously reported in humans

Best: Provide a source of pre-formed AA in the control and n-3 PUFA treatment diets

Cell culture (in vitro) studies:

Good: Introduce fatty acids in a physiological context (i.e., complexed to albumin)

Better: Check fatty acid profiles of cells prior to treatment. It is not uncommon for cells maintained in culture for prolonged periods of time to be depleted of HUFA. Normalization of cell HUFA levels in cell lines will avoid problems associated with essential fatty acid deficiency. The goal of this normalization process should be to reproduce the fatty acid profile found in freshly isolated primary cells prior to in vitro HUFA treatment

Better: Avoid using HUFA concentrations in vitro that exceed those typically observed in vivo (i.e., 10 μ M)

Better: Extend fatty acid treatment beyond 24 h to model in vivo exposure via diet and to avoid acute treatment artifacts (e.g., membrane disruption, triacylglyceride accumulation)

Best: Use treatment conditions that mimic the quantity and quality of fatty acid changes noted in these same cells following dietary n-3 PUFA supplementation. One way to accomplish this outcome is to use of mixtures of various fatty acids, rather than just adding one single fatty acid

necessary and sufficient to affect immune cell function (e.g., eicosanoid production, cytokine biosynthesis, receptor function, and cell signaling). Therefore, if health benefits associated with dietary n-3 PUFA require changes in immune cell function, then the poor outcome of most clinical trials might have been predicted on this basis alone. Another, important and often overlooked, difference between most animal-based studies and human clinical trials is that in the former test subjects are provided high n-3 diets BEFORE and during the disease development. In human clinical trials published to date, subject selection is based on the individual already suffering clinical signs of the “disease” for which n-3 PUFA are supposed to treat.

One way to look at these findings is that for animal-based studies to have greater preclinical value, diets should be designed to include only enough n-3 PUFA to reproduce the changes in immune cell HUFA noted in human clinical trials. However, it is not known if changes in immune cell HUFA content simply correlate with or are necessary for changes in immune cell function and subsequent in vivo inflammation and immune responses.

Another factor that may be contributing to the greater frequency of positive outcomes in rodent-based studies of n-3 PUFA is the fatty acid composition of “control” diets. Most rodent studies of n-3 PUFA bioactivity use vegetable oil (e.g., corn and soybean oils) as the fat source in the diet of the control group animals. These fats contain high levels (i.e., 50%) of linoleic acid (18:2n-6) and no preformed AA or n-3 HUFA. Yet, intake of AA and n-3 HUFA for non-vegetarian individuals is common. The inclusion of small amounts of AA and n-3 HUFA to match current average daily intake in humans to “control” as well as “experimental” rodent diets may significantly improve how well rodent studies extrapolate to human n-3 PUFA

supplementation trials. Similarly, investigators using in vitro fatty acid treatment to explore mechanism by which n-3 PUFA affect immune cell function may wish to establish treatment modalities that mimic the pattern and amount of fatty acid changes described here in Tables 1 and 2. The author’s experience is that it is all too easy to induce changes in cellular fatty acid profiles in vitro that far exceed what can be achieved by dietary intervention.

Finally, the recent generation of a line of transgenic mice (i.e., *fat-1* mice), that are capable of synthesizing n-3 PUFA from endogenous n-6 PUFA, has created many new opportunities to study the biological effects of these essential nutrients [50]. Compared to wild-type mice, *fat-1* mice are protected from dextran sulfate-induced colitis and show diminished growth of implanted B16 melanoma cells [51, 52]. Tissues of *fat-1* mice possess lower n-6 and higher n-3 HUFA compared to their wild-type littermates. These differences occur in the absence of any dietary manipulation. It appears that the changes in n-3 and n-6 HUFA content of some tissues of *fat-1* mice mimic the sort of changes that occur upon feeding wild-type mice diets rich in n-3 PUFA. However, no data on immune cells from these mice have been published to date. Generation of *fat-1* mice, as well as the recently developed *fat-1* pigs [53], provide researchers with a powerful tool to study underlying mechanisms potentially responsible for some of the health benefits associated with increased dietary intake of n-3 PUFA. Yet, results from studies with these novel transgenic animals should be viewed with caution until it is clear that this genetic modification accurately models diet-induced changes in tissue fatty acid profiles and function.

There is nearly universal agreement that current intake levels for n-3 PUFA by most individuals in the USA and other westernized societies are less than optimal. In the

past few years many countries have adopted dietary guidelines that call for increasing intake of n-3 PUFA. Yet, the evidence that such a change will have the expected beneficial outcomes remains equivocal. It remains unclear what form, how often, and how much n-3 PUFA individuals need to consume to obtain a health benefit. On what basis should dietitians and other health professionals advise people on this issue? Evidence from randomized clinical trials (RCT) is the gold standard in clinical medicine. Yet, RCT are very expensive and in many ways impractical for establishing dietary guidelines. Current recommendations for n-3 PUFA are based largely on epidemiological evidence. Thanks largely to advances in micro-encapsulation new food products enriched with n-3 PUFA are entering the marketplace at a rapid pace. Widespread distribution and consumption of such products will undermine the reliability of existing nutrient databases to accurately estimate n-3 PUFA intake patterns in free-living human subjects. The net effect of this could be an undermining of the reliability of epidemiological studies for setting future guidelines for optimal n-3 PUFA intake. Animal-based and in vitro experimental approaches for investigating the health benefits of n-3 PUFA can make significant contributions to this field. However, modest changes in experimental design could substantially enhance the preclinical value of such studies relative to understanding how and when n-3 PUFA may affect human health (Table 3).

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