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Dietary Polyunsaturated Fatty Acids Modulate Resistance to *Mycobacterium tuberculosis* in Guinea Pigs[1,2]

Christine T. McFarland,[3]* Yang-Yi Fan,[4,5] Robert S. Chapkin,[4–6] Bradley R. Weeks,[7] and David N. McMurray[3–5]

[3]Department of Microbial and Molecular Pathogenesis, Texas A&M Health Science Center, College Station, TX 77843 and [4]Center for Environmental and Rural Health, [5]Faculty of Nutrition, [6]Department of Nutrition and Food Science, and [7]Department of Veterinary Pathobiology, Texas A&M University, College Station, TX 77843

Abstract

It is well established that the nutritional status of the host affects resistance to disease. The impact of dietary lipids on experimental pulmonary infection with mycobacteria has not been investigated. Therefore, the purpose of this study was to determine the role of dietary (n-3) and (n-6) fatty acids on immunity and resistance to aerosol infection with virulent *Mycobacterium tuberculosis* in guinea pigs. Weanling guinea pigs were fed purified, isocaloric diets differing only in lipid source, and the effects of diet on specific immune cell functions were evaluated after 3 or 6 wk. Dietary (n-3) fatty acid consumption reduced in vivo skin test and in vitro lympho-proliferative responses ($P < 0.05$) relative to (n-6) fatty acid consumption. The effect of diet on resistance to mycobacterial infection was assessed by enumerating viable mycobacteria in the lungs and spleens of guinea pigs infected with virulent *M. tuberculosis* by the aerosol route. (n-3) Fatty acid-fed guinea pigs had more bacteria in the lungs compared with (n-6) fatty acid-fed guinea pigs at 3 ($P < 0.05$) and 6 wk postinfection ($P < 0.01$). These data document the immunomodulatory effects of (n-3) fatty acid consumption in the context of tuberculosis resistance. The loss of antigen-specific T-cell functions in addition to impaired resistance to mycobacterial disease suggests a susceptible phenotype in (n-3) fatty acid-fed guinea pigs. J. Nutr. 138: 2123–2128, 2008.

Introduction

Tuberculosis (TB)[8] is the leading cause of death worldwide from a bacterial infection (1). Every year, ~8 million new cases of TB are diagnosed and nearly 2 million people die of this disease. The only vaccine currently available for TB, *Mycobacterium bovis* Bacille Calmette-Guérin (BCG), has a variable efficacy rate in humans ranging from 0 to 80% (1,2). We used the well-characterized and biologically relevant guinea pig model of low-dose pulmonary TB to study this important infectious disease (3). Following exposure to very small numbers of virulent bacilli, the guinea pig develops clinical disease that mimics human TB in many important ways, including the development of classic pulmonary granulomas, histologically and macroscopically similar delayed type hypersensitivity (DTH) reactions, and a strong protective immune response following BCG vaccination (3,4).

It is well documented that resistance to TB is affected by the host's nutritional status (5,6). We demonstrated previously that dietary protein deficiency was associated with a dramatic loss of T cell function in BCG-vaccinated and nonvaccinated guinea pigs, including diminished purified protein derivative of tuberculin (PPD) skin test reactivity, mitogen- and antigen-specific T cell lymphoproliferative responses, and alterations in cytokine production compared with normally nourished controls (7–9). Most importantly, chronic protein malnutrition was consistently associated with a significant loss of BCG-induced resistance following respiratory challenge with a low dose of virulent *Mycobacterium tuberculosis* compared with normally nourished guinea pigs (8,10).

Recently, a role for dietary lipids, and in particular PUFA, in determining the host's immune response to infection with *M. tuberculosis* has been suggested (11). Similar to the well-described immunomodulatory effects of dietary protein, lipids also modulate the function(s) of T-cells, macrophages, and their effector molecules (12). The effects of (n-3) PUFA consumption are the best documented and associated with suppression of lymphocyte proliferation, cytotoxic T-lymphocyte activity, DTH, natural killer cell-mediated cytolysis, major histocompatibility complex class II expression, functional antigen presentation, macrophage-mediated cytotoxicity, and proinflammatory cytokine production (11–17).

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[8] Abbreviations used: BCG, Bacille Calmette-Guérin; cfu, colony-forming unit; ConA, concanavalin A; CO, corn oil; DTH, delayed type hypersensitivity; FO, fish oil; IL, interleukin; IFNγ, interferon-γ; PPD, purified protein derivative of tuberculin; SI, stimulation index; TB, tuberculosis.
* To whom correspondence should be addressed. E-mail: cmcfarland@medicine.tamhsc.edu.

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The same antiinflammatory properties of (n-3) PUFA that account for their beneficial, protective effects on cardiovascular, autoimmune, and inflammatory disorders may negatively impact host resistance to infectious diseases, such as TB, where a robust cell-mediated immune response is critical to controlling infection (5,7,11). Evidence in support of this hypothesis comes from epidemiological studies of Greenlanders and Alaskan Eskimos, who despite consuming diets rich in fish oil (FO) containing (n-3) fatty acids, exhibit an unusually low incidence of cardiovascular and other inflammatory diseases but an unusually high frequency of TB infection (18,19). Further evidence consistent with these epidemiological observations is provided by experimental data from (n-3) PUFA-fed guinea pigs infected via the i.m. route with virulent *M. tuberculosis*. Pronounced progression of disease and higher bacterial counts in the spleen were observed in (n-3) PUFA-fed guinea pigs compared with guinea pigs consuming diets enriched in SFA or (n-6) PUFA (20). Increased susceptibility to other intracellular pathogens, including *Listeria* and *Salmonella*, in (n-3) PUFA-fed animals has also been reported (11,21–23).

To determine the impact of dietary fatty acids on infection with pulmonary TB, we evaluated specific in vitro and in vivo immune responses and resistance to aerosol infection with virulent *M. tuberculosis* in (n-3) and (n-6) fatty acid-fed guinea pigs.

Methods

Experimental animals. Weanling, male and female, outbred Hartley strain guinea pigs, weighing ~150 g (Charles River Laboratories) were used for all experiments. Guinea pigs were housed individually in polycarbonate caging with stainless steel grid floors and feeders and maintained at 25°C on a 12-h light:dark cycle. Experimental diet and deionized water, supplemented with vitamin C (0.05%), were consumed ad libitum and provided fresh daily. All animal procedures were reviewed and approved by the Texas A&M Institutional Animal Care and Use Committee (IACUC).

Experimental diets. Both diets were isocaloric, differed only with respect to lipid source (4% of the diet by weight), and were designed to meet NRC nutritional requirements for the guinea pig (24) (**Table 1**). Corn oil (CO) and Menhaden FO were the lipid sources for the (n-6) and (n-3) fatty acid diets, respectively. The (n-3) fatty acid diet included a minimal amount of CO in a 3:1, v:v, FO-CO mixture to prevent essential fatty acid deficiency. Linoleic acid [18:2(n-6)] content was 5.8% for the CO diet and 1.6% for the FO diet and thus met the minimum 0.88–1.04% essential fatty acid requirement for guinea pigs (24). Purified diets were initially prepared in powder form and stored at −20°C. For feeding, a gel form of each diet was prepared with agar (2% of diet by weight) and provided fresh daily to minimize fatty acid oxidation (24). Fatty acid composition of the diet was confirmed by GC (**Table 2**). Immediately upon arrival, weanling guinea pigs were initiated on the (n-6) CO diet for a 2- to 3-wk acclimation/wash-out period. Subsequently, one-half of the guinea pigs consuming the CO diet were transferred to the (n-3) FO diet and feeding was continued for 3 wk. Guinea pigs were weighed weekly throughout the study and diet consumption and body weight did not differ between the 2 groups (data not shown).

BCG vaccination and PPD skin test. As indicated, weanling guinea pigs were subcutaneously vaccinated with 10^3 colony-forming units (cfu) of *M. bovis* BCG 1331 (Statens Serum Institute) injected in the left inguinal region. The freeze-dried vaccine was reconstituted in Sauton SSI diluent immediately prior to vaccination. Guinea pigs were vaccinated 6 wk prior to aerosol challenge.

To measure DTH, guinea pigs were injected with PPD with 1 μg in a 0.1-mL dose (Mycos Research) intradermally on a shaved site 48 h prior to necropsy. Skin test reactions were measured and recorded as the mean of 2 perpendicular measurements of induration (mm) at 24 and 48 h following PPD injection.

TABLE 1 Basic diet composition

Constituents	*g/100 g*
Oil[1]	4
Casein	30
Cornstarch	27.8
Sucrose	10
Cellulose	13
Mineral mix[2]	6
Vitamin mix[3]	4
Agar[4]	2
DL-Methionine	0.2
Potassium acetate	2.5
Magnesium oxide	0.5
Zinc carbonate	0.002

[1] Either CO or FO/CO mix (3:1, v:v) with 0.02% tertiary butylhydroquinone.
[2] Briggs Chick Salts A contained (g/kg): $CaCO_3$, 250; $Ca_3(PO_4)_2$, 233; $CuSO_4 \cdot 5H_20$, 0.33; ferric citrate, 6.67; $MgSO_4 \cdot 7H_20$, 83.3; $MnSO_4$, 5.3; KI, 0.7; K_2HPO_4, 150; NaCl, 146.7; Na_2HPO_4, 121.7; $ZnCO_3$, 0.33; MP Biomedicals.
[3] Vitamin mix provided (g/kg):vitamin A acetate (500,000 IU/g), 1.8; ergocalciferol (850,000 IU/g), 0.125; DL-α-tocopherol acetate (250 IU/g), 22.0; ascorbic acid, 45.0; inositol, 5.0; choline chloride, 75.0, menadione, 2.25; p-aminobenzoic acid, 5.0; niacin, 4.25; riboflavin, 1.0; pyridoxine HCl, 1.0; thiamine HCl, 2.0; calcium pantothenate, 3.0; biotin, 0.02; folic acid, 0.09; vitamin B-12 (0.1% TRIT),1.35; MP Biomedicals.
[4] Difco Labs.

Aerosol challenge, necropsy, and microbial enumeration. Guinea pigs were infected with virulent *M. tuberculosis* H37Rv via the respiratory route using the Madison Aerosol Chamber, as described previously (25). The challenge suspension of *M. tuberculosis* H37Rv was prepared from a frozen single-cell stock stored at −80°C and introduced into the nebulizer at a concentration of 1×10^8 cfu/L. This concentration results in the inhalation and retention of ~10–20 viable organisms per guinea pig.

At necropsy, guinea pigs were killed with an overdose of sodium pentobarbital (Sleepaway, Fort Dodge Animal Health). The right lower lobe of the lung and two-thirds of the spleen were aseptically removed, homogenized in sterile saline, and plated in duplicate onto Middlebrook 7H10 agar plates (BVA Scientific) to determine bacillary levels (26). Colonies were counted following 3-wk incubation at 37°C; cfu data were expressed as a mean log 10 ± SEM per lobe. Detection limits of log 10 values in the lung and spleen were 2.35 and 1.35, respectively. The left lower lung lobe was preserved in 10% neutral buffered formalin,

TABLE 2 Fatty acid composition of the guinea pig diets

Fatty acid[1]	CO	FO
	g/100 g	
14:0	tr[2]	8.16 ± 0.06
16:0	11.56 ± 0.10	20.7 ± 0.06
16:1(n-7)	tr	11.38 ± 0.13
18:0	2.11 ± 0.00	4.16 ± 0.00
18:1[(n-7)+(n-9)]	30.24 ± 0.02	17.26 ± 0.05
18:2(n-6)	55.05 ± 0.13	14.32 ± 0.03
18:3(n-3)	1.06 ± 0.05	2.17 ± 0.03
20:5(n-3) (EPA)	tr	9.55 ± 0.13
22:5(n-3)	tr	1.89 ± 0.03
22:6(n-3) (DHA)	tr	10.38 ± 0.08

[1] Dietary fatty acid composition was analyzed by GC, according to a published protocol (29).
[2] tr, Trace amount (<0.1%).

processed for histopathological analysis, stained with hematoxylin and eosin, and examined by a trained pathologist without knowledge of the treatment.

Spleen cell preparation and T-lymphocyte enrichment. Spleens from BCG-vaccinated and nonvaccinated guinea pigs were removed aseptically and gently homogenized in RPMI 1640 medium (Gibco Invitrogen). A single cell suspension was prepared according to our published standard protocol (27). Culture media were modified to include 2.5% heat-inactivated fetal bovine sera (Atlanta Biologicals) and 2.5% heat-inactivated, homologous sera derived from guinea pigs fed the same [(n-3) or (n-6) fatty acid] diet. Viable splenocytes were enumerated by trypan blue exclusion and adjusted to a final concentration of 2×10^9 cells/L. Cell viability was routinely 90–95%.

Whole splenocytes were enriched for T-cells using nylon wool columns and our standard protocol (28). Nylon wool columns were prepared using 0.5 gm of scrubbed and combed nylon wool fiber (Polysciences) packed into 10-mL syringe barrels. Single cell suspensions of viable splenocytes (10^{10} cells/L) were loaded onto each column and incubated for 1 h at 37°C. Nonadherent T-cells were collected in RPMI-1640, centrifuged at $320 \times g$ for 10 min, and enumerated as described above. Cell viability, based upon trypan blue exclusion, following nylon wool enrichment was >85–90%. T cell purity, based on flow cytometric analysis, ranged from 88 to 100% (28) (unpublished observations).

Lipid analysis of splenocytes. Total lipids in splenocytes from CO- and FO-fed animals (**Table 3**) were extracted by the method of Folch et al. (29). Total phospholipids were separated from other lipid fractions by 1-dimensional TLC on silica gel 60 G plates using chloroform:methanol:acetic acid:water (90:8:1:0.8, v:v) as the developing solvent. Isolated total phospholipids were trans-esterified in the presence of 6% methanolic HCl. FAME were subsequently analyzed by capillary GC as previously described (30).

Lymphocyte proliferation assay. Cells were seeded into 96-well, flat-bottomed tissue culture plates (2×10^5 cells/well) in the presence of concanavalin A (ConA) (Sigma), PPD, or RPMI media for 96 h (31). Cell cultures were labeled with 1.0 μCi of tritiated thymidine (MP Biomedicals) per well for the final 6 h of incubation and harvested onto glass fiber filters using a multiple, automated cell harvester. Filters were counted in liquid scintillation format and the counts were expressed as a stimulation index (SI), calculated from the means obtained with stimulated cells divided by those obtained with unstimulated cells.

RNA isolation and real-time PCR. Following in-vitro stimulation with mitogen, antigen, or media only, splenocytes were lysed in Buffer RLT (Qiagen). Total RNA was isolated using the RNeasy kit (Qiagen) and TaqMan RT reagents (Applied Biosystems) were used to reverse transcribe RNA into cDNA for detection by real-time PCR. Primers for guinea pig cytokines and chemokines were developed in our laboratory and their sequences published previously (31,32). Quantitative real-time PCR was carried out using SYBR Green PCR Supermix and the ABI Prism 7500 Sequence Detector (Applied Biosystems) according to our published protocol (32). Fold induction of mRNA was determined from the threshold cycle values normalized for Hypoxanthine phophoribosyl transferase expression and to the value derived from calibrator samples ('Time 0' or 'media only samples' for each time point at which RNA was collected).

Statistical analysis. Data are expressed as means ± SEM. Where applicable, data were analyzed using either a Student's *t* test or ANOVA followed by Tukey's post hoc test. Differences were considered significant at $P < 0.05$.

Results

Incorporation of dietary PUFA into lymphocyte membrane lipids. Incorporation of (n-3) fatty acids into splenocyte membrane lipids was confirmed by GC of total phospholipids (Table 3). As expected, both 20:5(n-3) and 22:6(n-3) levels in splenocytes from (n-3) PUFA-fed guinea pigs were significantly elevated relative to (n-6) PUFA-fed guinea pigs. Similarly, splenocyte membrane phospholipids from (n-6) PUFA-fed guinea pigs were significantly enriched for 18:2(n-6) and 20:4(n-6) fatty acids compared with (n-3) PUFA-fed guinea pigs. Therefore, the 3-wk feeding period was adequate and effective in producing the desired changes in fatty acid composition of guinea pig splenocyte membrane phospholipids.

Effect of dietary lipids on mitogen- and antigen-induced lymphocyte proliferation. Relative to dietary (n-6) PUFA, (n-3) fatty acid consumption resulted in reductions ($P < 0.05$) in both mitogen- and antigen-induced proliferation in whole splenocyte cultures (**Table 4**). Similarly, mitogen-induced (n-3) PUFA-derived T-cells proliferated at significantly reduced levels compared with (n-6) PUFA-derived T cells; antigen-specific proliferation was also lower in (n-3) PUFA-derived T cells compared with (n-6) PUFA-derived T cells. In general, proliferation of nylon wool-enriched T cell cultures was lower compared with responses observed in whole splenocyte cultures and proliferation

TABLE 3 Fatty acid composition of splenocytes from guinea pigs fed (n-3) or (n-6) fatty acid diets for 3 wk[1]

Fatty acid	(n-6) PUFA	(n-3) PUFA
	g/100 g	
14:0	0.60 ± 0.04	1.96 ± 0.09*
16:0	21.92 ± 0.14	24.36 ± 0.80*
16:1(n-7)	0.83 ± 0.02	3.83 ± 0.10*
18:0	19.39 ± 0.28	20.42 ± 0.23*
18:1[(n-7)+(n-9)]	13.55 ± 0.23	13.97 ± 0.40*
18:2(n-6)	21.07 ± 0.73	10.21 ± 0.39*
20:1(n-9)	0.68 ± 0.02	tr
20:2(n-6)	1.90 ± 0.10	tr
20:3(n-6)	1.16 ± 0.07	1.00 ± 0.13*
20:4(n-6)	11.52 ± 0.41	8.28 ± 0.25*
20:5(n-3)	tr[2]	5.13 ± 0.40*
22:0	0.73 ± 0.03	0.39 ± 0.16*
22:4(n-6)	2.70 ± 0.14	tr
22:5(n-6)	0.85 ± 0.05	tr
22:5(n-3)	0.43 ± 0.02	4.10 ± 0.23*
22:6(n-3)	0.78 ± 0.05	3.29 ± 0.19*
24:0	0.78 ± 0.06	0.90 ± 0.09*
24:1(n-9)	1.12 ± 0.05	1.28 ± 0.31*

[1] Values are means ± SD, $n = 5$. *Different from (n-6) PUFA, $P < 0.05$.
[2] tr, Trace amount (<0.1%).

TABLE 4 Proliferation of splenocytes from guinea pigs fed (n-3) or (n-6) fatty acid diets for 3 wk[1]

	Stimulated with ConA		Stimulated with PPD	
	n-3 PUFA	n-6 PUFA	n-3 PUFA	n-6 PUFA
	SI[2]			
Whole splenocytes	223 ± 27*	374 ± 41	91 ± 13*	157 ± 18
Nylon-enriched T-cells	34 ± 10*	148 ± 39	28 ± 8	53 ± 13

[1] Values are means ± SEM, $n = 8$–10. *Different from corresponding (n-6) PUFA, $P < 0.05$.
[2] SI calculated from the means obtained with stimulated cells divided by those obtained with unstimulated cells.

induced by a specific antigen (PPD) was consistently lower than proliferation induced by mitogen, regardless of dietary treatment.

Effect of dietary lipids on in vivo hypersensitivity to tuberculin. The effect of (n-3) and (n-6) fatty acid consumption on an in-vivo measure of immunity (i.e. DTH) was measured 24 and 48 h following injection of PPD and recorded as a mean diameter of induration (mm). A significant decrease in PPD reactivity in BCG-vaccinated, uninfected (n-3) PUFA-fed guinea pigs compared with (n-6) PUFA fed guinea pigs was consistently observed (**Table 5**). Skin test reactions were also significantly reduced in (n-3) PUFA relative to (n-6) PUFA-fed guinea pigs at both 3 and 6 wk (Table 5) following mycobacterial infection. Similar results were observed 48 h post-PPD injection (data not shown).

Effects of dietary PUFA on cytokine mRNA expression. A reduction in interferon-γ (IFNγ) and interleukin (IL)-10 and an increase in tumor necrosis factor-α and transforming growth factor-β mRNA levels were observed in (n-3) PUFA-fed guinea pigs following PPD stimulation (**Table 6**). Relative to ConA stimulation, PPD stimulation resulted in increased mRNA expression levels of all cytokines, regardless of dietary treatment group.

Effect of dietary lipids on resistance to mycobacterial infection. Given the immunosuppressive effects observed by us and others (11,16,17) following (n-3) PUFA consumption, we evaluated the impact of dietary lipids on host resistance in guinea pigs following a low-dose pulmonary challenge with virulent *M. tuberculosis* H37Rv. At both 3 and 6 wk postinfection, the mean log 10 cfu of *M. tuberculosis* recovered from the lungs of (n-3) PUFA-fed guinea pigs were significantly greater than in (n-6) PUFA-fed guinea pigs (**Table 7**). The effect of dietary lipids on extrapulmonary infection in the spleen was not as apparent. At 3 wk postchallenge, bacterial counts in the spleen were slightly greater ($P = 0.16$) in (n-3) compared with (n-6) PUFA-fed guinea pigs, but by 6 wk postinfection, spleen bacterial levels did not differ between the groups.

Lung lobes from infected guinea pigs were also compared for evidence of diet-induced histopathological differences at 6 wk postinfection. Regardless of dietary treatment, lung sections from *Mtb*-infected guinea pigs were characterized by a mild, diffuse interstitial accumulation of macrophages and lymphocytes, with scattered, discrete moderate-to-dense accumulations of macrophages and lymphocytes (granulomas). The presence of multi-nucleated giant cells was noted within these larger lesions, which tended to coalesce and organize around a central region of caseous necrosis. Regions of extensive inflammation were noted in lung tissues from both diet groups. Tissue scoring on the basis of numbers of granulomas noted per section did not reveal any significant differences between treatment groups (data not shown).

TABLE 6 Cytokine mRNA expression levels in splenocytes from guinea pigs fed (n-3) or (n-6) fatty acid diets for 3 wk[1]

	Stimulated with ConA		Stimulated with PPD	
	(n-3) PUFA	(n-6) PUFA	(n-3) PUFA	(n-6) PUFA
	Fold induction[2]			
IFNγ	165 ± 66.7	337.4 ± 150.3	272 ± 115.4	333 ± 150.1
TNFα[3]	6.2 ± 2.4	3.6 ± 0.6	7.2 ± 1.5	5.9 ± 1
IL-10	4.0 ± 1.3	10.0 ± 5.3	9.5 ± 3.2	23.5 ± 7.5
TGFβ[3]	2.2 ± 0.5	3.9 ± 1.7	5.5 ± 2.8	2.5 ± 0.6

[1] Values are means ± SEM, $n = 8$.
[2] Threshold cycle values normalized to Hypoxanthine phophoribosyl transferase expression and to the mean of unstimulated cell cultures at time 0.
[3] TNFα, tumor necrosis factor-α; TGFβ; transforming growth factor-β.

Discussion

The relationship between diet and infectious disease resistance is well documented (5,6,10,11). Previously, our research focused on chronic dietary protein deficiency and its negative impact on vaccine efficacy and pulmonary TB in the guinea pig model (5–10). More recently, the role of fatty acids, particularly (n-3) PUFA, in TB pathogenesis has been the subject of investigation. Dietary PUFA are incorporated into cell membranes of virtually every cell in the body, including effector cells of the immune system, resulting in functional changes that may translate to alterations in disease resistance or susceptibility (15,16). For example, the nature of endosomal membrane lipid composition modulates phagosome-lysosome fusion, thereby affecting intracellular survival of mycobacteria (33). (n-6) PUFA-enriched membranes fused readily, whereas membranes enriched with (n-3) PUFA did not fuse, resulting in enhancement of intracellular mycobacterial growth (33).

Our study assessed the impact of dietary (n-3) fatty acids on resistance to experimental pulmonary TB using a highly relevant and well-characterized disease model. Isocaloric, purified diets, differing only with respect to fatty acid source [(n-3) vs. (n-6) fatty acids], were fed to weanling guinea pigs. Weanlings were

TABLE 5 PPD-induced skin test responses in BCG-vaccinated, uninfected guinea pigs and in unvaccinated, *M. tuberculosis*-infected guinea pigs at 3 and 6 wk postinfection[1]

	(n-3) PUFA	(n-6) PUFA
	Induration diameter, mm	
Preinfection	3.2 ± 1.7*	11.4 ± 3.2
3 Wk postinfection	0.87 ± 0.87**	11.4 ± 2.8
6 Wk postinfection	15.4 ± 4.1***	34.5 ± 1.7

[1] Values are means ± SEM, $n = 5$–10. Asterisks indicate different from (n-3) PUFA: *$P = 0.03$,**$P = 0.003$, ***$P = 0.0008$. Results shown were obtained 24 h following PPD injection.

TABLE 7 Effect of diet on bacterial burden in the lung and spleen of *M. tuberculosis*-infected guinea pigs fed (n-3) or (n-6) fatty acid diets for 3 and 6 wk [1]

	3 Wk postinfection		6 Wk postinfection	
	Lung[2]	Spleen	Lung	Spleen
	log 10 cfu			
(n-3) PUFA	5.73 ± 0.11*	4.36 ± 0.29	5.7 ± 0.25**	6.07 ± 0.21
(n-6) PUFA	5.19 ± 0.15	3.77 ± 0.26	4.4 ± 0.16	5.97 ± 0.19

[1] Values are group means ± SEM, $n = 5$. Asterisks indicate different from (n-6) PUFA, *$P = 0.01$, **$P = 0.001$.
[2] Minimum detection limit of log10 cfu is 2.35 for the lung and 1.35 for the spleen.

chosen for these experiments because they are more adaptable and willing to accept abrupt changes in form and composition of diet (24). Experimental PUFA diets were well tolerated throughout the study and growth rates did not differ. We established that the fatty acids ingested were incorporated into splenocyte membranes, because these cell populations were predictably modulated within a relatively short time to reflect the primary lipid present in the diet.

The antiinflammatory effects of (n-3) PUFA consumption on lymphocyte function reported previously have often yielded inconsistent findings, perhaps due to suboptimal culture conditions (15). Using culture conditions designed to eliminate masking of dietary treatment effects and to optimize cell viability, we routinely observed significantly suppressed lymphoproliferative responses, in whole splenocyte and nylon wool-enriched T-cell cultures, in (n-3) PUFA-derived compared with (n-6) PUFA-derived cells. Our data are in general agreement with those from previous animal and human studies (12).

Similar results were observed when we assessed the effect of dietary (n-3) PUFA on in vivo hypersensitivity to tuberculin. Despite prior BCG vaccination and even in the presence of active pulmonary and extrapulmonary mycobacterial infection, guinea pigs consuming (n-3) PUFA diets had significantly reduced DTH responses to intradermally injected PPD compared with (n-6) PUFA-fed guinea pigs. The significant inhibition observed in vivo to PPD among (n-3) PUFA-fed guinea pigs supports our in vitro data and establishes an antiinflammatory/immunosuppressive effect of these dietary lipids on T-lymphocyte function. Importantly, these in vitro and in vivo effects were observed using a biologically relevant percentage of fat in the diet (4%) and following a relatively short time period (3 wk) of lipid consumption.

Considering the suppressive effects of dietary PUFA on lymphocyte-mediated immune responses, we anticipated an immunomodulatory effect of dietary lipids on cytokine production by lymphocytes as well. Few studies report the effects of dietary PUFA on lymphocyte-produced cytokines, other than IL-2 (34,35), and the results of these studies are also often inconsistent and inconclusive (36). In general, SFA minimally affect cytokine production, whereas PUFA inhibit production of Th1-type cytokines, including IFNγ and IL-2, and have little effect on Th2-type cytokine (e.g. IL-4) production (36). (n-3) Fatty acids are the most effective and appear to exert their suppressive effects at the level of gene expression (12,36). In contrast to the significant effects by diet on lymphocyte proliferation and DTH responses, no effect by diet on cytokine mRNA expressions levels was observed in our study. Unfortunately, protein levels for guinea pig cytokines cannot be measured as the necessary reagents and assays are not currently available.

Previous human epidemiological data (18,19), experimental data (20,37), and even cell culture data (33) all suggest that (n-3) PUFA consumption may impair host resistance to infection with *M. tuberculosis*, as well as to other intracellular pathogens, including *Salmonella* (22) and *Listeria* (23). The results of this study also established an adverse effect of dietary (n-3) fatty acids on resistance to pulmonary TB in the guinea pig model. At 3 wk postinfection, a modest increase in bacterial burden in the lungs of (n-3) PUFA-fed guinea pigs was observed. By 6 wk postinfection, differences between (n-3) PUFA- and (n-6) PUFA-fed guinea pigs were more pronounced and a significant increase in lung cfu was observed for (n-3) PUFA compared with (n-6) PUFA-fed guinea pigs. The effects of (n-3) PUFA consumption did not extend to extrapulmonary infection, because bacillary burden did not increase in the spleen at either interval postinfection. It was interesting to note that, despite significant differences in lung cfu between (n-3) and (n-6) PUFA-fed guinea pigs, lung histopathology did not differ.

These data contribute to the growing body of literature documenting the immunomodulatory effects of (n-3) PUFA consumption, particularly in the context of infectious disease resistance (11,16,17). The loss of specific T-cell functions (i.e. proliferation and DTH) and the impaired resistance to mycobacterial disease in our studies suggest a susceptible phenotype in (n-3) PUFA-fed guinea pigs. If studies in other animal model systems are any indication, the mechanism(s) responsible most likely involve PUFA-mediated cell membrane alterations leading to changes in receptor-mediated signal transduction and downstream gene expression (12,15,38). Unfortunately, such mechanistic studies in guinea pigs await the development of much-needed immunological reagents which are, as of yet, unavailable.

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