Fish Oil Feeding Delays Influenza Virus Clearance and Impairs Production of Interferon-γ and Virus-Specific Immunoglobulin A in the Lungs of Mice¹,²

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ABSTRACT Ingestion of fish oil can suppress the inflammatory response to injury and may impair host resistance to infection. To investigate the effect of a diet containing fish oil on immunity to viral infection, 148 BALB/c mice were fed diets containing 3 g/100 g of sunflower oil with either 17 g/100 g of fish oil or beef tallow for 14 d before intranasal challenge with live influenza virus. At d 1 and d 5 after infection, the mice fed fish oil had higher lung viral load and lower body weight (P < 0.05). In addition to the greater viral load and weight loss at d 5 after infection, the fish oil group consumed less food (P < 0.05) while the beef tallow group was clearing the virus, had regained their preinfection weights and was returning to their preinfection food consumption. The fish oil group had impaired production of lung interferon-γ (IFN-γ), serum immunoglobulin (Ig) G and lung IgA-specific antibodies (all P < 0.05) although lung IFN-α/β and the relative proportions of bronchial lymph node CD4⁺ and CD8⁺ T lymphocytes did not differ between groups after infection. The present study demonstrates a delay in virus clearance in mice fed fish oil associated with reduced IFN-γ and antibody production and a greater weight loss and suppression of appetite following influenza virus infection. However, differences observed during the course of infection did not affect the ultimate outcome as both groups cleared the virus and returned to preinfection food consumption and body weight by d 7. J. Nutr. 129: 328–335, 1999.

KEY WORDS: • fish oil • (n-3) polyunsaturated fatty acids • immunity • infection • mice

Evidence exists that fish oil supplementation may reduce the severity of immunologically mediated diseases including rheumatoid arthritis and ulcerative colitis (Kremer et al. 1990, Stenson et al. 1992) and may be beneficial in systemic lupus erythematosus (Walton et al. 1991) and Crohn’s disease (Bellozzi et al. 1996). In humans, dietary fish oil reduces the production of inflammatory mediators such as cytokines which have a role in the development of atherosclerotic lesions (Bevilacqua et al. 1985, Endres et al. 1995) although the macrophages of mice fed (n-3) fatty acids contain more cell-associated tumor necrosis factor (TNF)⁴ and secrete more TNF than those from mice fed (n-6) fatty acids (Hardardottir and Kinsella 1992). Mice fed fish oil also have higher circulating interferon-γ (IFN-γ) during listeriosis than those fed soybean or lard diets (Fritsche et al. 1997a).

Suppression of inflammation is associated with an increased severity of infectious disease (Rubin et al. 1989), and concerns were raised that the use of dietary fish oil or (n-3) polyunsaturated fatty acid supplements may have a similar effect (Meydani et al. 1993). Data from populations that traditionally consume a diet rich in (n-3) fatty acids appear to support this. In the past, Alaskan Inuits experienced a greater incidence of tuberculosis (Comstock et al. 1967), and northern Canadian Indian and Inuit children suffered recurrent persistent upper respiratory tract and chest infections associated with weakened cellular immunity (Hildes and Shaefer 1984). Environmental health problems (Meydani et al. 1993) or exposure of an immunologically naive population to these infections may also have contributed to this burden of disease.

Variable suppression of innate immune function was observed after feeding fish oil in human and animal studies depending on the amount of fat fed, the species and the immune indices being examined (Kelley and Daudu 1993). Although in vitro assays, using cells derived from humans and mice, showed a suppression of some immunological responses including cytokine production and lymphocyte proliferation (Endres et al. 1989, Jolly et al. 1997), one cannot directly translate this into an increased susceptibility to infection. Relatively few studies have investigated the effects of dietary (n-3) fatty acids on the course of infection in vivo.


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Abbreviations used: ELISA, enzyme-linked immunosorbent assay; Ig, immunoglobulin; IFN, interferon; IL, interleukin; MDCK, Madin-Darby canine kidney; MHC Class II, major histocompatibility compatibility Class II molecule; PBS, phosphate buffered saline; PFU, plaque-forming units; TNF, tumor necrosis factor.

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Chang et al. (1992) demonstrated that after challenge with Salmonella typhimurium, mice fed a high-fat diet rich in fish oil (20 g/100 g) had a significantly lower survival rate than low-fat or high-fat hydrogenated coconut oil-fed mice. Spleen cultures were negative for S. typhimurium in surviving fish oil-fed mice, suggesting an impaired ability to clear the bacteria from their blood. D’Ambola et al. (1991) reported that neonatal rabbits fed a high dose of fish or safflower oil in addition to their mothers’ milk had an impaired ability to clear Staphylococcus aureus compared to the controls (which received milk supplemented with the same volume of saline). These high-fat diets did not alter lung neutrophil recruitment or alveolar macrophage bacterial phagocytosis compared to controls although the high-fat fish oil diet reduced macrophage superoxide anion formation. Huang et al. (1992) examined immunological changes in mice, infected with Listeria monocytogenes, which were fed diets including coconut oil (saturated fat), safflower oil [(n-6) polyunsaturated fat] and menhaden fish oil [(n-3) polyunsaturated fat]. After infection, the fish oil group had the highest percentage of B cells but the lowest percentage of T cells, macrophages and peritoneal major histocompatibility (MHC) Class II (reported as la) positive cells, suggesting a reduced capacity for antigen presentation and T cell activation in this group.

The mouse serves as a useful model for the study of respiratory infection in which intranasal challenge with influenza virus causes severe pneumonia and tracheitis (Ramphal et al. 1979). Immunization or challenge with influenza virus induces the production of specific antibodies including serum immunoglobulin (Ig) G and lung IgG and IgA (Chen et al. 1987, Pang et al. 1992). Lung IgA makes the most important contribution to antibody-mediated immunity to influenza in mice (Chen et al. 1987) while serum antibody is protective against lethal pneumonia but not tracheitis associated with the infection (Ramphal et al. 1979). Antibody production may also be regarded as a general measure of antigen presenting cell function (Kiyono and McGhee 1994). The mucosal IgA response is CD4+ T helper cell-dependent, and the proportion of these cells may increase at sites of IgA secretion (Kiyono and McGhee 1994).

Cell-mediated defenses are activated after immunization or infection with influenza. The proportion of antinfluenza CD8+ cytotoxic T lymphocytes may increase in the mouse lung during the course of infection (Bender et al. 1995) while the action of specific “cytotoxic” macrophages and nonspecific NK cells contribute to host protection (Mak et al. 1982). Experimental influenza infection in mice is also accompanied by increased secretion of the cytokines interleukin (IL)-6, TNF-α and interferons (IFN) in the lung (Conn et al. 1995). During infection, IFN-α/β has antiviral properties (Heng et al. 1996) while IFN-γ has a role in the induction of mucosal immune responses including the production of IgA (Panja and Mayer 1994) and is associated with upregulation of MHC expression and increased macrophage-mediated killing of intracellular pathogens (Fritsche et al. 1997a).

The present study was undertaken to determine whether feeding a high-fat fish oil diet would affect innate and adaptive immunity to low-dose influenza infection in mice. To date few investigators have reported the effects of a fish oil diet on viral infection although the findings of several experiments suggest that such a diet has the potential to impair virus clearance by reducing cell-mediated defenses including natural killer cell cytotoxicity (Lumpkin et al. 1993, Meydani et al. 1988).

### MATERIALS AND METHODS

**Diets and animals.** This protocol was approved by the Animal Care and Ethics Committee of the University of Newcastle operating under the guidelines of the Animal Research Act (NSW 1985). Specific pathogen-free male (148) 6-wk-old BALB/c mice (Animal Research Centre, Murdock, WA, Australia) were randomly assigned to diets containing 20 g of fat/100 g of either fish oil or beef tallow blend. The composition of the diets is shown in Table 1. Additional mice were fed the diets and included as noninfected controls (n = 10 group). The mice were housed individually and were fed 5 g/d. New batches of diet were prepared once per week, sealed under nitrogen, and stored at −20°C until use. The lipid was extracted from the diets into chloroform and methanol (2:1) for analysis by gas chromatography as previously described (Folch et al. 1957, Lepage and Roy 1986). The fatty acid composition, in g/100 g of fatty acid, of the beef tallow diet was 44.7 g of saturates, 43.1 g of monounsaturates, 1.1 g of (n-3) and 11.1 g of (n-6) polyunsaturates and of the fish oil diet was 29.1 g of saturates, 26.3 g of monounsaturates, 30.2 g of (n-3) and 14.5 g of (n-6) polyunsaturates. An aliquot of lipid extract (0.5 g) was saponified for tocopherol determination as previously described (Slover et al. 1983) and extracted into hexane (1 mL) containing 0.1% butylated hydroxytoluene by vortex mixing for 2 min. Butylated hydroxyanisole was present as an internal standard at 0.5 g/L. The hexane phase was analyzed on a Hewlett-Packard Series 1100 high pressure liquid chromatograph fitted with a Hypersil silica 100 × 2.1 mm column (Hewlett-Packard, North Ryde, NSW Australia). Samples were eluted at 30°C with initial mobile phase of 99.4% hexane and 0.6% propanol at a flow rate of 0.3 mL/min for 5 min, then 99.70/0.3% hexane/propanol at 1.5 mL/min for 5 min and finally 99.50/0.5% hexane/propanol at 0.3 mL/min for 5 min. All rac-α-tocopherol was detected using a fluorescence detector at an excitation wavelength of 295 nm and an emission wavelength of 330 nm. The all-rac-α-tocopherol content of the fish oil diet was found to be 0.534 g/kg and the beef tallow diet 0.355 g/kg. The higher tocopherol content of the fish oil diet provided extra antioxidant protection against the prooxidant potential of this diet.

**Experimental design.** Mice received the experimental diet for 14 d before being infected with live A/Queensland/672/ (H3N2)

### TABLE 1

**Composition of the experimental diets**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Beef tallow</th>
<th>Fish oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>g/kg</td>
<td>g/kg</td>
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<tr>
<td>Dextrose</td>
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<td>294</td>
</tr>
<tr>
<td>Corn starch</td>
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<td>200</td>
</tr>
<tr>
<td>Casein</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Alphacel</td>
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<td>50</td>
</tr>
<tr>
<td>ALN 76 mineral mixture1</td>
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<td>40</td>
</tr>
<tr>
<td>Vitamin diet fortification mixture2</td>
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<td>12</td>
</tr>
<tr>
<td>α-tocopherine</td>
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<td>0.068</td>
</tr>
<tr>
<td>Sunflower oil</td>
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<td>30</td>
</tr>
<tr>
<td>Beef tallow</td>
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<td>0</td>
</tr>
<tr>
<td>Fish oil3</td>
<td>0</td>
<td>170</td>
</tr>
<tr>
<td>all-rac-α-tocopherol</td>
<td>0.107</td>
<td>0.068</td>
</tr>
</tbody>
</table>

1 American Institute of Nutrition (1977).
2 ICN, Seven Hills, NSW, Australia. The mixture, formulated in dextrose, contributed, in g/kg of diet, 0.0216 g of vitamin A acetate, 0.0015 g of ergocalciferol, 0.24 g of all-rac-α-tocopherol, 0.54 g of ascorbic acid, 0.06 g of inositol, 0.09 g of choline chloride, 0.027 g of menadione, 0.06 g of p-aminobenzoic acid, 0.051 g of niacin, 0.012 g of riboflavin, 0.012 g of pyridoxine hydrochloride, 0.012 g of thiamine hydrochloride, 0.036 g of calcium pantothenate, 0.00024 g of biotin, 0.00108 g of folate acid and 0.00016 g of vitamin B-12.
3 MaxEPA, R.P. Scherer Holdings (Braeside, VIC, Australia), stated all-rac-α-tocopherol content 1.82 g/kg oil.
influenza virus which was supplied kindly by the Discipline of Immunology and Microbiology, the University of Newcastle, Australia. Mice were weighed and food consumption was measured once per week prior to challenge. After challenge, mice were weighed at d 1, 2, 5 and 7 and food consumption at d 2, 5 and 7. Unfed food was discarded, feed bowls were cleaned and fresh food (5 g) was provided every day. Food consumption was measured by weighing the full bowl at time of feeding and then after 24 h. Food spilled by mice was added to the bowls prior to the final weighing. At d 14 the mice were anesthetized with Halothane (Rhône-Merieux, Harlow Essex, United Kingdom) and a dose of log_{10} five plaque-forming units (PFU) of virus suspended in sterile phosphate buffered saline (PBS) (50 μL) was administered using a calibrated pipette into the nostrils of each mouse as described by Pang et al. 1992. An additional 10 mice per diet group received a sham challenge with PBS. Mice were then killed by CO₂ asphyxiation at 3 h (n = 7/diet group), d 1 (n = 3), d 2 (n = 24), d 5 (n = 20) and d 7 (n = 20) after infection.

Lung lavage. A virus stabilization medium was prepared from 1.25 g/L of gelatine and 0.025 g/L of penicillin/streptomycin (Trace Biosciences Pty. Ltd., Castle Hill, NSW, Australia) in deionized water and autoclaved. The trachea was exposed, a transverse incision made and virus stabilization medium (1 mL) introduced using a syringe fitted with a pipette tip. The bronchial-alveolar lavage fluid was removed using the same syringe and stored at −80°C until analysis. Virus load was assayed by plaque assay using Madin-Darby Canine Kidney (MDCK) cell line. Briefly, three 10-fold dilutions of each lung homogenate were prepared in sterile PBS, and these were applied to confluent MDCK cells in six-well plates which were then incubated at room temperature for 45 min before the nutrient overlay was added (Pang et al. 1992). The plates were incubated in a humidified 5% CO₂ atmosphere at 34°C for 4 d before determining the number of plaques in each well which were expressed as mean log_{10} plaque-forming units per liter (PFU/L) ± SEM. The lowest limit of detection for this assay was log_{10} 5 PFU/L, and below this the virus was considered effectively cleared.

Flow cytometry. The percentage of lung lymph node CD4⁺ and CD8⁺ T cells was determined. Briefly, lymph nodes were dissected from the trachea and bronchi of mice and placed into Hanks’ Balanced Salts Solution supplemented with 10 mL penicillin/streptomycin (Trace Biosciences Pty. Ltd., Castle Hill, NSW, Australia) and 1.25 g/L of gelatine. The lymph nodes were fragmented with a scalpel and plastic syringe on a wire-mesh filter to obtain a single-cell suspension. The cells were washed once and a count of viable cells was made by trypan blue exclusion. Cell aliquots (4 x 10⁶) were resuspended in buffer (PBS with 50 g/L of fetal calf serum and 0.2 g/L of NaCl) and washed twice. The cells were stained with 20 μL of L1T4-phycocerythrin (for CD4⁺), LYT-2 (CD8⁺)-biotinylated (for CD8) or OKT9 isotype control and incubated on ice for 30 min (Becton Dickson, Lincoln Park, NJ). The specific antibodies and the isotype control were each diluted to a concentration of 1 μg/mL. The cells were then washed twice in the buffer and streptavidin-conjugated fluorescein isothiocyanate (diluted 1:1000) was added to the LYT-2 stained cells for a further 30-min incubation on ice. The cells were washed twice and suspended in PBS with 10 g/L paraformaldehyde. Five thousand cells per sample were analyzed using a FACScan flow cytometer (Becton Dickson, San Jose, CA).

Antibody measurement. Blood was taken by heart puncture immediately after death, allowed to clot on ice and centrifuged at 8000 x g for 5 min. Phenyl methyl sulfonyl fluoride (Sigma-Aldrich, Castle Hill, NSW, Australia) was added to the serum as a preservative (concentration in serum 0.4 mg/L) before freezing (−80°C). Serum influenza-specific IgG antibodies were determined by enzyme-linked immunosorbent assay (ELISA) as previously described (Pang et al. 1992). The serum was diluted 1:50, 1:100 and 1:200 in PBS/Tween 20 (0.05%). Similarly, lung viral IgG and IgA antibodies were determined using 1:5, 1:10 and 1:20 dilutions of lung lavage. Linearity in absorbance was observed and the antibody concentration was expressed as ELISA units/L of serum or of lung lavage by extrapolating the absorbance plot to the point corresponding to an undiluted sample and multiplying this value by 10⁷.

Lung IFN-α/β. Lung lavage IFN-α/β was titered in a bioassay based on the inhibition of cytopathic effect in L929 cells infected with encephalomyocarditis virus (Lawson et al. 1997). Samples were treated at pH 2 to remove acid-labile IFN and neutralized to pH 7 before assay. Murine IFN-α/β standard was titered as the control (Lee Biomolecular, San Diego, CA).

Lung IFN-γ. IFN-γ was determined in the lung lavage using a commercial ELISA kit in accordance with the manufacturer’s instructions (Endogen, Woburn, MA). Duplicate aliquots of lavage and conjugate solution were applied to the plate (which was supplied precoated with anti-IFN-γ antibody) and incubated (2 h, 4°C). Lavage from d 2 and d 7 postinfection was applied directly to the plate while lavage from d 7 was first diluted 1:1.5 in virus stabilization medium. The plate was washed, 3×, 5.5’ tetramethyl benzidine added, incubated for 30 min at room temperature and color development stopped with H₂SO₄. The plate was read using an ELISA plate reader at dual wavelengths (450 nm, corrected for 550 nm) and IFN-γ concentration determined by reference to a six-point standard curve.

Lung lymph node lymphocyte fatty acid analysis. Lymphocytes from bronchial lymph nodes (1 x 10⁶ cells) were transferred to glass culture tubes and methanol and tolulene (4:1, v/v, 2 mL, containing methyl tridecanoate (13:0) and methyl hexadecanoate (21:0) at 200 mL as internal standards) added. Samples were directly transmethylated by the addition of acetyl chloride (200 μL) while vortex mixing followed by heating for 1 h at 100°C (Lepage and Roy 1986). The samples were cooled, potassium carbonate (60 g/L v/v, 5 mL) added and then centrifuged (1700 x g, 10 min, 4°C). The tolulene phase was analyzed by capillary gas chromatography using a Hewlett-Packard 6890 comamograph (Hewlett-Packard) fitted with a 30-m x 0.25-mm x 0.25-μm propylphenyl column (DB-225; J&W Scientific, Folsom, CA). Fatty acid methyl esters were identified by comparison of their retention times with authentic fatty acid standards (Nu-Chek-Prep Inc., Elyria, MI) and reported in g/100 g of total fatty acids.

Statistical analysis. The data were examined to determine whether the distribution was normal, variances were checked for homogeneity between groups and all analyses were performed using Abacus Concepts, StatView Version 4.5 (Abacus Concepts 1994). Comparisons between dietary groups were made with the nonparametric Mann-Whitney test because the data were not normally distributed. Variations within dietary groups over time were assessed with the Kruskal-Wallace test. The relationship between lung viral load and weight change was assessed by linear regression and the significance of this relationship determined with the F statistic. Differences were considered significant at P < 0.05. Values are means ± SEM.

RESULTS
Effect of influenza virus infection on body weight and food consumption in mice fed fish oil. Prior to infection, no significant differences existed in body weight (d 14, beef tallow 22.6 ± 0.2 g, fish oil 22.7 ± 0.2 g, n = 60) or daily food consumption (d 13 to d 14, beef tallow 4.6 ± 0.1 g, fish oil 4.3 ± 0.1 g, n = 30) between dietary groups. At d 1 after infection, the fish oil-fed mice exhibited a significant reduction in body weight (P < 0.05) (Fig. 1). By d 2 after infection, both infected groups demonstrated a significant loss in weight and reduction in food consumption relative to preinfection values and to noninfected controls (P < 0.05) (Figs. 1 and 2). At d 5 following infection, a significantly lower (P < 0.05) body weight existed in mice fed fish oil compared with the beef tallow group which had already returned to preinfection weight. By d 7, the infected fish oil-fed mice had regained their preinfection body weight. However, food consumption was significantly lower (P < 0.05) in the infected, fish oil-fed group at d 5 and d 7 after infection while the infected beef tallow group had resumed their preinfection consumption. Weights and food consumption within each noninfected control group did not vary over time. At d 2, weight change in each infected
group was greater than in noninfected controls ($P < 0.05$). By d 7 the noninfected beef tallow-fed mice had gained more weight than all other groups ($P < 0.05$). At d 2 and d 5 each infected group was consuming significantly less food than noninfected controls ($P < 0.05$) while at d 7 the infected fish oil group was still consuming less food than all other groups ($P < 0.05$). A t d 2 the noninfected fish oil-fed mice were eating less food than noninfected mice fed beef tallow ($P < 0.05$).

**Lung virus load.** To determine whether the weight loss and the change in food consumption were due to the difference in host response to infection, the virus load in the lungs was measured. In both dietary groups a significant increase in virus load was observed from 3 h to d 1 following infection (Fig. 3). Virus titers were higher in mice fed fish oil than in those fed beef tallow at d 1 and d 5 after infection ($P < 0.05$) (Fig. 3). At this time an inverse correlation ($r = 0.66$, $P < 0.001$, $n = 20$/group) was present between lung virus titer and change in body weight, indicating that mice carrying a greater virus load also experienced the greatest weight loss, particularly in the fish oil group (Fig. 4). Two distinct responses were present in the group fed fish oil, with many mice exhibiting a small reduction in weight while some experienced dramatic weight loss. This difference was associated with food consumption. At d 5 after infection, food consumption was significantly inversely correlated with weight change ($r = 0.82$, $P < 0.005$, $n = 10$/diet group) and lung virus load ($r = 0.67$, $P < 0.05$, $n = 10$/diet group) in the mice fed fish oil but not in those fed beef tallow. The proportions of lung lymph node helper CD4+ T cells (fish oil 47.1 ± 2.0%, $n = 27$; beef tallow 47.7 ± 1.4%, $n = 29$) and suppressor/cytotoxic CD8+ T cells (fish oil 25.2 ± 1.4%, $n = 27$; beef tallow 27.1 ± 1.4%, $n = 29$) were not significantly different between diet groups at that time point ($P > 0.05$).
Within each group there was a significant reduction in IFN-γ in fish oil-fed mice to have higher concentrations (Table 2). At d 5 postinfection, influenza-specific serum IgG and lung IFN-γ production were significantly higher in the fish oil group relative to d 2 postinfection. At d 7, IFN-γ was significantly higher in the fish oil group than in the beef tallow group (P < 0.05). At d 5 and d 7, IFN-γ was significantly higher in the fish oil group than in the beef tallow group (P < 0.05).

**Lung IFN-α/β production after infection.** No significant differences existed in lung IFN-α/β between diet groups at any time point after infection nor did the CD4+ to CD8+ ratio differ. In both groups, virus was cleared from the lungs by d 7 after infection.

**Virus-specific antibodies.** The clearance of virus was associated with an increase in influenza virus-specific serum IgG levels in the fish oil group at d 5 and d 7 after infection compared to d 2 (P < 0.05) (Fig. 5A). At d 7 serum IgG was higher in the fish oil group relative to the beef tallow group. Serum IgG did not increase significantly in the fish oil group although at d 5 after infection this group had more serum IgG (P < 0.05) than the beef tallow group. The two diet groups had similar lung IgG responses on d 7 when this antibody was significantly increased (P < 0.05) (Fig. 5B). Lung IgA within the fish oil group was significantly increased at d 7, and this concentration was higher than that of the fish oil group (P < 0.05) (Fig. 5C). There was no corresponding increase in lung IgG in the fish oil group.

**Lung lymphocyte fatty acids.** The fatty acid composition of lymphocytes from bronchial lymph nodes reflected the dietary intake (Table 3). The fish oil group had significantly more myristic (tetradecanoic), palmitic (hexadecanoic), stearic (octadecanoic), vaccenic (11-octadecenoic), eicosapentaenoic, docosapentaenoic and docosahexaenoic acids while the beef tallow group had significantly more oleic (octadecenoic), α-linolenic (Δ9,12,15 octadecatrienoic) and arachidonic (eicosatetraenoic) acids (P < 0.05).

**DISCUSSION**

We demonstrated in a murine model of acute influenza virus infection that feeding a diet high in fish oil resulted in greater weight loss, lower food consumption and higher virus load during the course of infection. However, the fish oil diet did not impair the ability to ultimately clear virus from the lung but did alter the ability to mount normal IFN-γ and primary antibody responses to the infection.

Weight loss and reduction in food consumption form part of the acute phase response to influenza virus infection in mice (Conn et al. 1995). In the present experiment a dichotomy was observed in weight change at d 5 after infection among mice with similar lung viral loads fed fish oil but not in those fed beef tallow (Fig. 4). Within the fish oil group, the differences in weight were significantly inversely correlated with differences in food consumption. Weight loss may also relate to individual differences in the production of, or response to, proinflammatory cytokines. The acute phase response to influenza in mice is associated with increases in lung interleukin (IL)-1, IL-6, tumor necrosis factor and IFN (Conn et al. 1995). The relationship between diet, cytokine production and weight loss following infection warrants further investigation.

The fish oil diet appeared to be less palatable to mice. Before infection, mice fed fish oil were eating slightly, but not significantly, less food than those fed beef tallow. At d 2 the noninfected, fish oil-fed controls were eating significantly less food than those fed beef tallow. Possibly the fish oil group took longer to recover from the stress of anesthesia and sham challenge than those fed beef tallow. A reduction in food intake was reported in weanling rats fed a fish oil diet when compared to those fed sesame oil (Dominguez and Bosch 1994).

By d 7 postinfection influenza-specific serum IgG and lung secretory IgA antibody concentrations in the fish oil-fed group were significantly lower than in the beef tallow group. Although lower secretory IgA production was demonstrated in malnourished humans (Chandra 1975), the short-term reduction in food intake and weight loss in the present experiment is unlikely to account for the differences. The induction of an IgA response is highly dependent on efficient uptake, processing and presentation of antigen by macrophages in mucosal tissue (Kiyono and McGhee 1994). The lower antibody response may relate to the decreased ability of macrophages to present antigen as indicated by studies which showed that feeding a diet rich in fish oil to rats or mice reduced the expression of MHC Class II molecules on the surface of macrophages (Mosquera et al. 1990, Sherrington et al. 1995). Differences in IFN-γ may affect the class of antibody produced, upregulation of MHC expression and macrophage-mediated killing of intracellular pathogens (Fritsche et al. 1997A).

In addition to weight loss and decreased food intake, influenza virus infection in mice is accompanied by increased secretion of IFN-γ into the lung (Conn et al. 1995). In human studies, a diet supplemented with fish or fish oil results in decreased mitogen-induced peripheral blood mononuclear
cell-proliferative response and lower IFN-γ production by cultured mononuclear cells (Gallai et al. 1995, Meydani et al. 1993). The present study demonstrated that mice fed fish oil produced less IFN-γ but not IFN-α/β in the lung following influenza infection. This suggests that diet has no effect on cells secreting IFN-α/β which form the first line of host defense before antibody or cell-mediated responses are activated, but can affect IFN-γ which is produced after T-cells are sensitized against the antigen. The high IFN-α/β levels at d 2 in the mice fed fish oil may reflect the high virus replication rate in these animals.

Lung macrophages recovered from mice 5 d after influenza A or sendai virus infection are cytotoxic against virus-infected target cells (Mak et al. 1982) while monocyte (a macrophage precursor) superoxide production is reduced by diets containing (n-3) polyunsaturated fatty acids (D’ambola et al. 1991, Weiner 1989). The delayed virus clearance from the lung in fish oil-fed mice may be due to a down-regulatory effect on macrophage cytotoxic function or the failure to activate macrophages for effector function due to reduced production of IFN-γ. Dietary fish oil was found to reduce natural killer cell activity in mice (Meydani et al. 1988), although further experiments are necessary to determine specific effects of diet on natural killer cells and macrophage cytotoxicity in this model.

In contrast to the effect on IFN-γ secreted into the lung in the present experiment, a fish oil diet was associated with an increase in circulating IFN-γ in mice during systemic infection induced by L. monocytogenes (Fritsche et al. 1997a). The difference in response between experiments may relate to differences in pathogen and in the nature of the challenge (local vs. systemic).

D’ambola et al. (1991) demonstrated that neonatal rabbits receiving a high-fat fish oil diet have an impaired ability to clear a pulmonary bacterial challenge. Increased mortality to systemic challenge with S. typhimurium (Chang et al. 1992) and reduced immune response to L. monocytogenes (Huang et
TABLE 2
Lung interferon (IFN) α/β concentration in mice fed beef tallow or fish oil diets for 2 wk prior to challenge with influenza.

<table>
<thead>
<tr>
<th>Day after infection</th>
<th>2</th>
<th>5</th>
<th>7</th>
</tr>
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<tbody>
<tr>
<td>Diet</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Beef Tallow</td>
<td>a14.1 ± 125.0</td>
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<tr>
<td>Fish oil</td>
<td>a655.8 ± 314.7</td>
<td>b20.9 ± 2.7</td>
<td>b9.9 ± 1.3</td>
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</tbody>
</table>

1 Data are mean ± SEM, beef tallow n = 15 at each day, fish oil d 2 and 7 n = 13, n = 14 at d 5. There were no significant differences between diet groups at each time point. Means within a diet group not sharing a superscript are significantly different (P < 0.05).
2 The beef tallow diet contained 3% sunflower oil and 17% beef tallow.
3 The fish oil diet contained 3% sunflower oil and 17% fish oil.

FIGURE 6 Interferon (IFN)-γ concentration in lung lavage fluid after infection in mice fed diets containing fish oil or beef tallow for 14 d before intranasal challenge with influenza virus. IFN-γ was determined by enzyme-linked immunosorbent assay kit. Values are means ± SEM, fish oil d 2 n = 13, beef tallow d 7 n = 14, all other time points n = 15. Different superscript letters denote significant changes in antibody concentration within a diet group over time (P < 0.05). Asterisks denote significant difference between diet groups at that time point (P < 0.05).

TABLE 3
Effect of beef tallow or fish oil diets on bronchial lymph node lymphocyte fatty acid composition in mice infected with influenza virus.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Beef tallow</th>
<th>Fish oil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/100 g total fatty acids</td>
<td></td>
</tr>
<tr>
<td>14:0</td>
<td>2.01 ± 0.07</td>
<td>2.12 ± 0.26</td>
</tr>
<tr>
<td>16:0</td>
<td>22.27 ± 0.20</td>
<td>24.63 ± 0.34</td>
</tr>
<tr>
<td>16:1n-7</td>
<td>2.32 ± 0.10</td>
<td>2.33 ± 0.13</td>
</tr>
<tr>
<td>18:0</td>
<td>18.10 ± 0.57</td>
<td>20.30 ± 0.71</td>
</tr>
<tr>
<td>18:1n-9</td>
<td>28.00 ± 0.93</td>
<td>19.54 ± 0.56</td>
</tr>
<tr>
<td>18:1n-7</td>
<td>2.50 ± 0.07</td>
<td>2.85 ± 0.07</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>6.95 ± 0.29</td>
<td>6.95 ± 0.43</td>
</tr>
<tr>
<td>18:3n-3</td>
<td>0.94 ± 0.03</td>
<td>0.66 ± 0.05</td>
</tr>
<tr>
<td>20:4n-6</td>
<td>5.85 ± 0.29</td>
<td>2.90 ± 0.14</td>
</tr>
<tr>
<td>20:5n-3</td>
<td>0.24 ± 0.08</td>
<td>2.42 ± 0.11</td>
</tr>
<tr>
<td>22:5n-3</td>
<td>0.44 ± 0.03</td>
<td>1.69 ± 0.08</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>1.67 ± 0.18</td>
<td>4.91 ± 0.31</td>
</tr>
</tbody>
</table>

1 Data are mean ± SEM, n = 15. Mice were fed diets containing fish oil or beef tallow for 14 d before infection. Asterisk indicates a significantly higher concentration of fatty acid (P < 0.05).
2 The beef tallow diet contained 3% sunflower oil and 17% beef tallow.
3 The fish oil diet contained 3% sunflower oil and 17% fish oil.

The variation in immunological effects attributed to fish oil or (n-3) fatty acids may reflect differences in the amount of fatty and fatty acid composition of diets used in different studies. Although the diets used in the present experiment differed in their proportions of saturated and (n-6) polyunsaturated fatty acids, the effects observed are likely to be related to the (n-3) polyunsaturated fatty acid content which differed markedly. Diets rich in saturated fatty acids, such as those found in the beef tallow diet, have little effect on immune function (Rondono 1995). Fritsche et al. (1997b), using fish oil and lard diets with the same fat concentration (20 g/100 g) and a very similar fatty acid composition to the present experiment, demonstrated that a fish oil diet impaired immunity of mice infected with L. monocytogenes.

ACKNOWLEDGMENT
The authors would like to thank Cassandra Lawson, Department of Microbiology, University of Western Australia for her advice and for performing the IFN-α/β assays and Robert Blake, Ma Cong, Helen Haynes, Brett Hill, Andrew Milbourne and Kylie Smith for their expert technical assistance. We also thank R.P. Scherer Holdings Pty. Ltd. (Braeside, VIC, Australia) for donating the MaxEPA fish oil.

LITERATURE CITED
Fish Oil Delays Influenza Clearance


