ELSEVIER

Nutrition 22 (2006) 512–519

NUTRITION

www.elsevier.com/locate/nut

Applied nutritional investigation

# Limited effect of eicosapentaenoic acid on T-lymphocyte and natural killer cell numbers and functions in healthy young males

Elizabeth A. Miles, Ph.D.*, Tapati Banerjee, B.Sc., Solenne J. Wells, B.Sc., and Philip C. Calder, Ph.D., D.Phil.

*Institute of Human Nutrition, School of Medicine, University of Southampton, Southampton, United Kingdom*

Manuscript received June 20, 2005; accepted November 22, 2005.

**Abstract**

**Objective:** Greatly increasing the amount of long-chain ω-3 polyunsaturated fatty acids in the diet has been reported in some studies to decrease T-lymphocyte and natural killer functions. However, dose-response relations have not been identified. The objective of this study was to determine the effect of supplementing the diet of young male subjects with different amounts of an oil rich in eicosapentaenoic acid (EPA) on T-lymphocyte proliferation, cytokine production by T lymphocytes, and natural killer cell activity.

**Methods:** In a placebo-controlled, double-blind, parallel study, healthy young (18 to 42 y) males were randomized to one of four supplements. These were placebo (no additional ω-3 polyunsaturated fatty acids) or different amounts of an EPA-rich oil that provided 1.35, 2.7, or 4.05 g/d of EPA for 12 wk. Blood samples were taken at baseline and after 12 wk.

**Results:** Eicosapentaenoic acid was incorporated in a linear dose-response fashion into mononuclear cell phospholipids. EPA did not alter the proportions of T lymphocytes, helper T lymphocytes, cytotoxic T lymphocytes, B lymphocytes, or natural killer cells in the bloodstream. T-lymphocyte proliferation in response to concanavalin A and the production of the cytokines interleukin-2, interferon-γ, and interleukin-10 were not affected by the different treatments. However, interleukin-4 production was increased with increasing intake of EPA. Natural killer cell activity was little affected by the treatments, although there was a trend for EPA to increase activity at a low effector-to-target cell ratio.

**Conclusion:** T-lymphocyte and natural killer cell numbers and function in healthy young males are little affected by supplemental EPA intakes up to 4 g/d. 

*Keywords:* Fish oil; ω-3 Polyunsaturated fatty acids; T cell; Immunity; Natural killer cell; Cytokine

## Introduction

There is continuing interest in the effects of long-chain ω-3 polyunsaturated fatty acids (PUFAs) on human immune function [1–4]. Oily fish and fish oil contain the long-chain ω-3 PUFAs eicosapentaenoic acid (EPA; 20:5ω-3) and docosahexaenoic acid (DHA; 22:6ω-3). A dietary intervention with oily fish altered circulating T-lymphocyte numbers [5], whereas some dietary supplementation studies with fish oil have reported decreased T-lymphocyte proliferation [6,7], decreased production of interleukin (IL)-2 [6,8] or interferon-γ (IFN-γ) [8], and decreased natural killer cell activity [9]. A high intake of DHA also decreased natural killer cell activity [10]. Some of these studies were not controlled [6,8,10] or used very high doses (>5 g/d) of ω-3 PUFAs [8,10]. The effect of lower intakes of long-chain ω-3 PUFAs on T-lymphocyte and natural killer cell functions remains unclear, with a variety of effects being reported [5–7,9,11–16]. A decrease in the activity of T lymphocytes and natural killer cells could compromise host defense. It is important to ensure that there is no adverse immunologic effect of increases in the consumption of these PUFAs because there are recommendations to increase their intake

This research was supported by a grant (NUHEAL) to Philip C. Calder, Ph.D., D.Phil., from the European Commission under Framework 6 (QLK1-CT-199-00888). Pronova Biocare ASA provided the oil capsules.

* Corresponding author. Tel.: +44-0-23-8059-4689; fax: +44-0-23-8059-4379.

*E-mail address:* eam@soton.ac.uk (E.A. Miles).

0899-9007/06/$ – see front matter 
doi:10.1016/j.nut.2005.11.011

[17–20]. There is also a need to identify dose-dependent effects on immune function because these remain unclear. Therefore, the present study investigated the effects of three intakes of long-chain ω-3 PUFAs, mainly in the form of EPA, on T-lymphocyte and natural killer cell activities in a group of young males.

## Materials and methods

### *Materials*

Phosphate buffered saline tablets were obtained from Unipath (Basingstoke, UK). Histopaque, HEPES-buffered RPMI medium, glutamine, antibiotics (penicillin and streptomycin), concanavalin A (Con A), boron trifluoride, butylated hydroxytoluene, formaldehyde, solvents, and standard chemicals were purchased from Sigma (Poole, UK). Dual-labeled, combined anti-human CD3/CD4, CD3/CD8, CD3/CD16, and CD19/λ were purchased from Serotec (Kidlington, UK). Kits for measurement of natural killer cell activity (NKTEST, ORPEGEN Pharma, Heidelberg, Germany) and Lysing Buffer, Cell Wash, and Cell Fix were purchased from BD Biosciences (Oxford, UK). Cytokine EASIA enzyme-linked immunosorbent assay kits were obtained from BioSource (Nivelles, Belgium). [$^3$H]Thymidine was purchased from Amersham International (Amersham, UK).

### *Subjects and study design*

Ethical permission for all procedures involving human volunteers was obtained from the Southampton and South West Hampshire research ethics committee. Healthy adult males 18 to 42 y of age were invited to participate in the study. All volunteers completed a health and lifestyle questionnaire before entering the study. Volunteers were excluded if they were taking any prescribed medication; were vegetarian; consumed fish oil, evening primrose oil, or vitamin supplements; smoked more than 10 cigarettes/d; drank more than 10 U of alcohol/week; had a body mass index higher than 32 kg/m$^2$; or consumed more than two portions of oily fish per week. One hundred subjects were recruited to the study and 93 subjects completed the study.

Subjects were randomly allocated in a double-blind fashion to one of four intervention groups: placebo, low EPA, moderate EPA, or high EPA ($n$ = 25/group). Subjects' mean age and body mass index did not differ across treatment groups (Table 1).

Table 1
Age and BMI of subjects in the different treatment groups at baseline*

| Treatment | $n$ | Age (y) | BMI (kg/m$^2$) |
|---|---|---|---|
| Placebo | 25 | 25.3 ± 1.3 | 23.8 ± 0.6 |
| Low EPA | 25 | 24.0 ± 1.1 | 25.1 ± 0.7 |
| Moderate EPA | 25 | 24.6 ± 1.3 | 23.4 ± 0.4 |
| High EPA | 25 | 23.6 ± 1.2 | 24.1 ± 0.6 |

BMI, body mass index; EPA, eicosapentaenoic acid
* Data are mean ± SEM.

Subjects consumed nine 1-g capsules/d for 12 wk. Capsules were provided by Pronova Biocare AS (Lysaker, Norway). The placebo group consumed 9 × 1-g corn oil capsules/d that contained approximately 5 g/d of linoleic acid in addition to habitual consumption. Subjects in the low EPA group consumed 6 g of corn oil plus 3 g of EPA-rich oil in capsules (9 × 1 g) per day. Subjects in the moderate EPA group consumed 3 g of corn oil plus 6 g of EPA-rich oil in capsules (9 × 1 g) per day. Subjects in the high EPA group consumed 9 g of EPA-rich oil in capsules (9 × 1 g) per day. Capsules were provided to subjects in small plastic pots, with each pot containing the daily allocation (i.e., nine capsules), with instructions to take three capsules three times daily. The EPA-rich oil (EPAX4510TG) contained 45% EPA and 10% DHA, mainly (>90%) in triacylglycerol form. Thus intakes of EPA from the capsules were 1.35, 2.7, and 4.05 g/d in the low, moderate, and high EPA groups, respectively, and intakes of DHA were 0.3, 0.6, and 0.9 g/day. Habitual intake of EPA was not measured but was expected to be less than 0.2 g/d [18,20]. All capsules contained 3.6 mg of α-tocopherol. Thus all subjects consumed an additional 32 mg/d of α-tocopherol from the capsules (average habitual intake was expected to be ~5 to 20 mg/d, mean ~10 mg/d) [21].

Blood was collected immediately before beginning the interventions and at 12 wk. Heparinized Vacutainer tubes were used for blood collection, which was 7:00 to 10:00 AM after a fast of at least 10 h.

### *Preparation of peripheral blood mononuclear cells*

Blood was layered onto Histopaque (density 1.077 g/L, ratio of blood to Histopaque 1:1) and centrifuged at 800$g$ for 15 min at 20°C. Peripheral blood mononuclear cells (PBMCs; a mixture of lymphocytes and monocytes) were collected from the interphase and washed once with RPMI medium that contained 0.75 mmol/L of glutamine and antibiotics (penicillin and streptomycin; culture medium). After resuspension in 4 mL of culture medium, cells were layered onto 4 mL of Histopaque. These were centrifuged once more (800$g$ for 15 min at 20°C) to achieve a lower degree of erythrocyte contamination, washed with culture medium, and resuspended and counted on a Coulter Z1 Cell Counter (Coulter Electronics, Luton, UK).

### *Analysis of PBMC subsets*

For determination of PBMC subsets, whole blood (100 μL) was incubated with various combinations of fluorescently labeled monoclonal antibodies (10 μL of each antibody) for 30 min at 4°C. Monoclonal antibody combinations used were anti-CD3/anti-CD4 (to distinguish T lymphocytes as CD3$^+$ and T-helper lymphocytes as