The Effect of Highly Purified Eicosapentaenoic and Docosahexaenoic Acids on Monocyte Phagocytosis in Man

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ABSTRACT: The n-3 fatty acids (FA) from marine sources are known to exert antiinflammatory effects on monocyte function. There is still controversy whether n-3 FA may increase the susceptibility to infections. The present study was designed to assess the effect of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on monocyte phagocytosis and respiratory burst activity. Fifty-eight healthy men were randomized to take a daily supplement of 3.8 g highly purified EPA (n = 20), 3.6 g DHA (n = 19), or corn oil (n = 19) for 7 wk. Mononuclear leukocytes were collected, isolated, and cryopreserved prior to and after dietary supplementation. Paired samples were analyzed in the presence of autologous serum in a crossover design. Monocyte phagocytosis and respiratory burst activity were measured by flow cytometry after ingestion of Escherichia coli. Monocytes retained their phagocytic ability and respiratory burst activity after supplementation. No reduction in internalization of bacteria was registered. Dietary n-3 FA and particularly EPA improved bacterial adherence to the monocyte surface. In the crossover experiments, there was an adverse effect of serum enriched with n-3 FA on bacterial adherence. We conclude that monocytes retain their phagocytic potential after supplementation with purified EPA and DHA.


Monocytes play an important part in the defense against infections. They act as antigen-presenting cells, as producers of cytokines, and as phagocytes. In addition, they have protective functions by ingesting and killing invading microorganisms. Monocytes are particularly involved in chronic infections, such as tuberculosis and malaria, and in viral infections.

The n-3 fatty acids (FA) have been reported to decrease leukocyte superoxide generation (7,8), modify eicosanoid production, and decrease cytokine synthesis (9,10). Thus, n-3 FA may influence inflammatory and immune reactions which are involved in the development of disease. Populations who consume diets rich in n-3 FA have low incidence of inflammatory diseases (11), and fish oil enriched with eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) seems beneficial to patients suffering from such disorders (12,13). On the other hand, it has been suggested that polyunsaturated FA may encourage infection via attenuated inflammatory response and immunosuppression (14). Fish oil has been reported both to decrease and to improve survival rate of animals after experimentally induced infections (15–17).

Leukocyte phagocytosis is an important part of the host defense against infections, and few studies have examined the effect of n-3 FA on phagocytosis. In human neutrophils, the phagocytic process diminished (18) or remained unchanged (19) following n-3 FA supplementation. Both improved and decreased phagocytic ability have been reported in murine macrophages after dietary supplementation with n-3 FA (20,21).

Experimental studies indicate that EPA and DHA may have specific and partly different actions on cellular functions (22,23). Previous dietary studies have not investigated the effect of n-3 FA on monocyte phagocytosis, but have restricted their focus to neutrophils. We therefore examined the effect of dietary supplementation with highly purified EPA and DHA on monocyte phagocytosis and respiratory burst activity in humans ex vivo. Cryopreserved human monocytes isolated before and at the end of the supplementation period were used to avoid methodological variability (24), and the phagocytic properties were assessed with flow cytometry (FCM), allowing accurate measurements of single-cell functions on a large scale.

MATERIALS AND METHODS

Subjects and study design. Two hundred thirty-four healthy, nonsmoking men entered a double-blind parallel group intervention study lasting for 7 wk. Their mean age was 44 ± 5 yr.
(range 36–56). They did not take any drugs regularly, and consumed a traditional Western diet with less than four dishes of fish per week and did not expect to change their diet or lifestyle during the study period. Their total serum cholesterol was <8 mmol/L, diastolic blood pressure was <95 mmHg, and systolic blood pressure was <160 mmHg. The participants were randomly assigned to ingest either 4 g/d of 95% ethyl ester concentrate of EPA (20:5n-3), 4 g/d of 90% ethyl ester concentrate of DHA (22:6n-3), or 4 g/d of corn oil. The oils were given in indistinguishable soft gelatin capsules each containing 1 g oil and 3.0 IU vitamin E as antioxidant. Each person was asked to ingest four capsules a day. Drugs were manufactured by Pronova Biocare A/S (Oslo, Norway). Twenty subjects were randomly selected from each group prior to the intervention period and asked to give additional blood for isolation of mononuclear leukocytes before and after 7 wk of supplementation. Compliance was calculated as the percentage of the prescribed capsules taken. The study was approved by the regional board of research ethics and was performed according to Good Clinical Practice requirements (25). Written informed consent was obtained from all participants.

**Serum.** Autologous serum was obtained before and after 7 wk of supplementation with n-3 FA and prepared by clotting whole blood in sterile glass tubes for 1 h at room temperature and then centrifuged at 2000 × g for 10 min. Serum was frozen in aliquots of 0.5 mL and stored at −70°C.

**Preparation of peripheral blood mononuclear cells.** The isolation, freezing, and thawing procedures have been described previously (24). After overnight fasting, 120 mL blood was drawn from an antecubital vein into four 50-mL Falcon tubes (Becton Dickinson, Rutherford, NJ) containing EDTA as anticoagulant. Fifteen mL of the sample was mixed with 15 mL glucose-phosphate buffer and layered on top of 15 mL Lymphoprep (Nycomed Pharma, Oslo, Norway) and centrifuged at 301 × g for 30 min. The mononuclear cells at the interface were carefully pipetted off, transferred into another Falcon tube, and diluted before a second centrifugation step. The cell pellet was resuspended in 10 mL glucose-phosphate buffer and centrifuged slowly (20 × g for 15 min at 4°C) to remove the platelets. Thereafter the mononuclear leukocytes were resuspended and washed twice in RPMI 1640 (Biowhittaker, Walkersville, MD) containing 5% acid-treated (AT) fetal calf serum (FCS).

**Freezing procedure.** Mononuclear leukocytes suspended in 5% AT-FCS-RPMI were centrifuged, and the pellet was re-suspended in precooled RPMI 1640 with 50% AT-FCS. An equal volume of ice-cold RPMI containing 20% dimethyl sulfoxide dried (Merck, Darmstadt, Germany) was added dropwise over 3 min. Aliquots of 1 mL (4 × 10^6 mononuclear leukocytes) were dispensed into sterile cryo vials (Greiner Labortecchnik, Frickenhausen, Germany). The tubes were placed in an isopropylene box at room temperature, then frozen at −70°C for at least 2 h, and stored until further use at −135°C in a Bio-freezer (Queue Cryostar, Queue Systems, Parkenberg, WV).

**Thawing procedure.** The frozen cells were rapidly thawed with gentle and continuous agitation at 37°C in a water bath. The suspensions were transferred into 50-mL Falcon tubes in droplet form with 9 mL 20% FCS in RPMI 1640. The cells were centrifuged and finally resuspended in 5% heat-inactivated FCS in RPMI 1640. All experiments were performed 2 h after thawing. Mononuclear cell- and differential counts were made prior to and after thawing of the mononuclear leukocytes in a Coulter Counter S-plus STKR (Coulter Electronics, Harpenden, United Kingdom).

**Flow cytometry.** A Facsan flow cytometer (Becton Dickinson, San Jose, CA) with an excitation wavelength of 488 nm interfaced to a Hewlett-Packard 340 computer (Hewlett-Packard, Tokyo, Japan) was used for cell identification and measurements of monocyte phagocytosis and respiratory burst activity (24). By recording the fluorescence and light scattering of each cell, information relating to cell size and structure was determined. The resulting scattered light signals were given in a numerical scale and fluorescence signals in a logarithmic scale. Fluorescein isothiocyanate (FITC) fluorescence was measured at 530 nm, rhodamine at 500–530 nm, and right-angle light scatter at 488 nm. Calibration of light scattering and fluorescence was conducted daily with Calibre beads (Becton Dickinson).

**Bacteria.** Heat-inactivated Escherichia coli (serotype O102:K52:H6, hemolysine negative) isolated from blood cultures were labeled with FITC (Sigma Chemical Company, St. Louis, MO). The number of bacteria was adjusted to 1.5 × 10^8 per sample. Unlabeled bacteria from the same strain were used in the respiratory burst experiments.

**Phagocytosis.** Opsonization was performed by incubating the bacterial suspension (1.5 × 10^8) with 0.5% autologous serum for 7.5 min on a rock’n roller at 37°C. Monocytes (5 × 10^6) were added, giving a bacteria–cell mixture of 250 µL, and after 5 min the reaction was stopped by adding 250 µL ice-cold phosphate buffered saline with 0.05% EDTA. Mononuclear leukocytes were differentiated by FCM along the right angle- and forward light scatter, and gated separately. Anti-CD14 mAb (Immunotech, Marseille, France) was used to identify the monocyte population and to verify correct monocyte gating. The gate was set manually. Nonphagocytes and phagocytes were differentiated by simultaneous measurements of right-angle light scatter and FITC fluorescence by FCM. Phagocytes expressing fluorescence due to attached and/or internalized FITC-labeled bacteria were quantified as the percentage of the monocyte population (10,000 monocytes). The amount of attached and/or internalized bacteria was quantified as mean fluorescence intensity (MFI) (24,26). An FCM fluorescence quenching technique was used to distinguish between attachment and internalization of bacteria by addition of trypan blue to the cell suspensions (27). Extracellular FITC fluorescence representing attached bacteria was quenched, whereas the fluorescence of internalized bacteria remained unchanged.

**Respiratory burst activity.** Monocytes (5 × 10^6) in 250 µL RPMI 1640 with 5% FCS were mixed with 100 µL (10